ANSWER TO REVIEWERS

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9412-review.doc).

Title: Plasma cathepsin L: A prognostic marker for pancreatic cancer

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

Response to 1st Reviewer's Comments:

Comment: The pathological diagnosis of this article is not well provided. And therefore "pancreatic cancer" may be more reliable.

Response: As suggested by the reviewer, the title has been modified.

Comment: It may be more exact if expressed as "plasma", because the "blood" is complex and include many types of cells among which cathepsin is expressed in macrophages.

Response: The word "blood" has been replaced by "plasma" at all the suggested places.

Comment: The evaluated value?

Response: As suggested by the reviewer, the abstract has been revised to include the Cathepsin L levels.

Comment: In fact, anti-tumor therapy of pancreatic cancer also include chemotherapy, radiotherapy, et al.

Response: We differ with the reviewer on this issue. For pancreatic cancer, chemotherapy and radiotherapy are adjuvant therapies or used as palliative therapy. The only CURATIVE therapy is surgery.

Comment: What about the level of cathepsin in serum/plasma of other pathological state not only in tumors?

Response: In this study, we have measured plasma Cathepsin L levels in patients with chronic pancreatitis (benign pancreatic disease) as well as healthy individuals and found both to be lower than in pancreatic cancer.

Comment: It is better to provide the epidemiological data for the patients.

Response: Ours is a tertiary referral center and in this study, all patients with pancreatic cancer coming to our department between 2007 and 2011 were included. Hence, the epidemiological data

for these patients would not be reflective of the true picture as would have been in a community based study. In light of this, we feel that the epidemiological data is not relevant for this paper.

Comment : The detail of diagnosic method for each patients is strongly suggested, for the fact that sometimes pancreatic cancer and pancreatitis are so similar in CT/MRI scan. And we wonder if the diagnosis of pancreatic cancer is confirmed by the MRI. According to NCCN guideline, CT is used for screening for pancreatic cancer and MRI is used for confirmation and differential diagnosis.

Response: More details about the diagnostic methods for both pancreatic cancer and chronic pancreatitis have been added in the revised manuscript. For diagnosis of pancreatic cancer, a Contrast Enhanced CT scan performed using pancreatic protocol is used whereas chronic pancreatitis diagnosis is based on ultrasound investigations along with typical clinical history. Only in cases of chronic pancreatitis with inflammatory head mass, an EUS-guided FNAC is needed to discriminate from pancreatic cancer.

Comment: It is better to provide the epidemiological data for the patients. And the diagnosic method of pancreatitis is also required.

Response: As suggested, the method used for diagnosing pancreatitis has been included in the revised methods section on page 7.

Comment: The readers would appreciate if the demographic data was provided as supplement material.

Response: Demographic data (mean age and sex ratio) as well as summary of clinical parameters of the pancreatic cancer patients has already been given in the text and table 1. Please clarify if extra information is required.

Comment: What about the demographic data of the controls?

Response: In the revised manuscript, the demographic data for healthy controls and chronic pancreatitis patients has been included in the results section on page 10.

Comment: pancreatic cancer

Response: The phrase "carcinoma of the pancreas" has been replaced by "pancreatic cancer" at all the suggested places.

Comment: The ROC analysis of patients with the resectable tumors is suggested, because pathological diagnosis of these patients was reliable.

Response: Only 25 patients had resectable tumors. When these were divided into 2 groups based on their survival status (outcome), the number was too small to do an ROC analysis. Hence, ROC analysis of patients with resectable tumors could not be done.

Comment: Pancreatic duct carcinoma is not confirmed by the pathology. The conclusion is controversial.

Response: The last sentence refers to the protease levels in the tumor tissues versus non-neoplastic tissues (all of which have been confirmed by pathology). Hence, this sentence has been rephrased to clarify that it refers to tissue levels.

Response to 2nd Reviewer's Comments:

Comment: The authors have investigated 127 consecutive patients with pancreatic cancer, 26 healthy controls and 25 patients with chronic pancreatitis. Why these numbers were choosen remains elusive as a power calculation for investigating these numbers is lacking. Hence a power calculation based on earlier reports (Niedergethmann M, et.al. Pancreas. 2004 Oct;29(3):204-11) should be included in the methods section.

Response: A pilot study which included 10 samples in each group was done at our center. Based on the results of that pilot study, the sample size was decided. We enrolled more patients of pancreatic cancer to assess the prognostic value of plasma cathepsin L levels.

Moreover, the earlier report by Niedergethmann et al. could not be used for calculating sample size since they did not measure plasma cathepsin L levels (only tissue expression was studied in that report).

Comment: Moreover, the authors should mention their earlier report about this topic (Singh N et.al. Cancer Invest. 2013 Aug;31(7):461-71) and if figures are reused this should be mentioned in the figure legend.

Response: The said paper has been mentioned in the revised text (page 14). The figures have not been reused.

Comment: The authors state that 20 fields were scored for cathepsin L staining. The magnification should be mentioned.

Response: The magnification has been mentioned in the revised material and methods section.

Comment: The resection criteria should be mentioned in the methods section and the Cathepsin L levels should be shown in a dot plot for the following categories: patients who were resectable, patients who were peroperatively irresectable and patient who were pre operatively irresectable due to locally advanced disease and patients who had metastasis at presentation.

Response: There is a general consensus regarding the definition of localized resectable pancreatic cancer. It is one where the lesion is restricted to the pancreas and its draining lymph nodes without involvement of the superior mesenteric artery, a patent superior mesenteric/ portal venous system and no distant metastasis. This has been added in the revised text at page 7.

The dot plot of the cathepsin L levels in various categories of patients as mentioned above has been included as supplementary figure 3.

Comment: The cathepsin L levels of patients with pancreatic cancer, chronic pancreatitis and healthy individuals should be given in a dot plot.

Response: The dot plot has been prepared and included as supplementary figure 1.

Comment: The cathepsin L levels of patients with stage I, II, III and IV disease should be shown in a dot plot.

Response: The dot plot has been prepared and included as supplementary figure 2.

Comment: An important advantage of Cathepsin L over CA 19.9 could be the relation of CA 19.9 with cholestasis. Hence, the prognostic value of Cathepsin L and CA 19.9 should be investigated in cholestatic patients seperately.

Response: It is a good suggestion by the reviewer but unfortunately we do not have the data for cholestasis in all cases as it was not part of our objectives. Hence, the analysis suggested by the reviewer cannot be done in this study.

Comment: In addition to the Kaplan Meier Curve shown for Cathepsin L, a Kaplan Meier Curve for

CA 19.9 and tumor stage should also be given.
Response: Kaplan Meier curve for tumor stage has been included as Figure 1C and that for CA19.9 as supplementary figure 4.
Comment: In the operated patients information should be given regarding the presence of cholestastis to exclude the possibility that the disappearance of cholestasis might explain the decrease in Cathepsin L levels.
Response: Again, as mentioned above, we do not have the required data to do this analysis.
Comment: Table 3 should be aligned.
Response: The table has been aligned in the revised manuscript.

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

Sincerely yours,

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