Feb. 2, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16283-Review.docx).

Title: Pancreatic cancer serum biomarker PC-594: Diagnostic performance and comparison to CA19-9

Author: S. Ritchie *et al*.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16283

We appreciate the detailed responses from the reviewers. We have revised the manuscript according to their suggestions, or addressed their specific concerns below:

Reviewer 00742509

The reviewer states that the experiments employed are likely supportive of the intended outcome, but suggests that we should evaluate PC-594 levels in subjects with inflammation.

Author response: We appreciate the reviewer's comment, and agree that investigating inflammation (among other pancreatic conditions) is important. However, this is not a question that can be adequately answered by a simple case-control study, and we strongly feel that the work required adding it to the current paper would be out of scope. The primary goals of this paper were to confirm that the PC-594 reduction was not purely a Japanese phenomenon and could be reproduced in North American subjects, and to report on the comparison between PC-594 and CA19-9. Our next phase of research will involve examining not only inflammation, but a more robust evaluation of various indraductal papillary mucinous neoplasms and PanIN lesions, along with further *in vitro* investigations into PC-594's anti-inflammatory mode of action. These analyses will form the bases of a separate publication. We have, however, modified the discussion (pg. 15) to emphasize the importance of addressing both inflammatory and non-inflammatory cysts in future studies.

The reviewer's second point is whether all subjects with a PC-594 deficiency will definitively show pancreatic cancer, and how to manage PC-594 deficient subjects in the clinic.

Author response: The answer is no, not all subjects with a PC-594 deficiency will have pancreatic cancer. A PC-594 deficiency represents an *increased risk* of having (or developing) pancreatic cancer. In fact, most people among the general population with a PC-594 deficiency will not have pancreatic cancer. It is important to point out that PC-594 is not intended to be used a diagnostic test for pancreatic cancer. Rather, a PC-594 deficiency is a risk factor, similar to family history, smoking or other factors. The clinical utility, which we published extensively on in our prior publication (Ritchie et al BMC Cancer 2013) by doing decision curve net clinical benefit analysis, is in the use of PC-594 to identify a subset of the population that is expected to have pancreatic cancer incidence high enough to warrant further screening, such as imaging. In light of the reviewer's comment, we have further emphasized potential clinical utility in the discussion (pg. 13-14), including our opinion that one of the next clinical issues that needs to be addressed is the prospective evaluation of PC-594 among subjects with various pancreatic lesions using transabdominal ultrasound.

Reviewer 02683167

The reviewer commented that the outcome of the findings is very interesting and relevant to the field. Their first comment regarded clarification on the novel aspects of this work in light of our previous publication.

Author response: Our previous paper focused on the metabolomics discovery, using high resolution mass spectrometry, followed by verification by tandem MS for several metabolic systems. The discovery and validation was primarily performed in Japanese patient samples, with the exception of a very small North American cohort of 14 patients, which was not highly powered. We also did not report any CA19-9 comparison data, which was the focus of the current paper. It is critical that biomarkers be evaluated across different geographic and ethnic backgrounds. Although we had preliminary evidence that PC-594 appeared to be valid in North American patients, this is the first report to address the issue with a well-powered patient cohort, and provides critical justification for further developing the marker a clinical decision tool. As requested by the reviewer, we have indicated the population used and the comparison to CA19-9 in the aim of the abstract, and the core tip.

The reviewer's second comment concerned details in the methods, specifically the source of the PC-594 standard and the method used to determine CA19-9 levels.

Author response: Per the request of the reviewer, we have added detailed methods on how the PC-594 method was isolated and purified, and how the CA19-9 levels were determined (pgs. 7-9).

The reviewer's third comment was to provide a reference for the general population incidence of CRC.

Author response: We have added the reference accordingly.

Reviewer 02682232

The reviewer thanked us for a good manuscript. We had difficulty understanding the comments of this reviewer, however we think the question centered on whether PC-594 is affected across all ethnicities, and whether this could be addressed by increasing the number of control subjects. The specific comment was "Any way, it be increased in all ethnicity or only in Caucasian Patient and group and delete in another ethnicity. So, you work upon one more ethnicity or many,by increase in Number of patient and control group but More than patient Number."

Author response: If we understand correctly, the reviewer is wondering whether PC-594 levels differ among control groups of different ethnicities. If so, this is a very good question that we are currently addressing. Due to changes in the PC-594 method, it was not possible to compare the levels in the previous Japanese controls to the North American controls. We have added a point to the discussion (pg. 13) to emphasize the point that geographic-specific cut-offs might be required based on the optimal trade-off between sensitivity and specificity.

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

Sincerely yours,

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