REPLY to REVIEWERS

Reply to reviewer one : We thank the reviewer for taking the time to assess our paper and his positive comments. We agree to his suggestion and changed the wording of the conclusion to provide further clarity. Minor grammar and punctuation errors were also corrected . This study is the first of its kind to implement MIC-1/GDF15 as a screening tool in an asymptomatic population with a genetic predisposition of developing pancreatic cancer. Part of a national screening program it has taken us 8 years to prospectively recruit these asymptomatic patients at risk of pancreatic cancer. Our study is a feasibility study and we hope our results will start a new wave of research (larger, multicentric, prospective trials) into investigating the role of this biomarker in early detection of neoplastic tumours to validate our finding and provide further characterisation of this biomarker.

Reply to reviewer 2. We thank the reviewer for taking their time to assess our paper and his positive comments. This study is the first of its kind to implement MIC-1/GDF15 as a screening tool in an asymptomatic population with a genetic predisposition of developing pancreatic cancer. Part of a national pancreatic cancer screening program it has taken us 8 years to prospectively recruit these 120 asymptomatic patients at risk of pancreatic cancer. It would take us 15 years to prospectively recruit 200 asymptomatic patients on our own, hence it is important to publish our finding to stimulate further research into the role of MIC1. Our study is a feasibility study and we hope our results will lead to multicentric, prospective trials into investigating the role of MIC1 in early detection of neoplastic tumours , to validate our finding and provide further characterisation of this biomarker. In our cohort we screen for malignancy hence amylase, lipase and faecal elastase are not elevated and will not bring any new information. Studies have shown that amylase and lipase are not elevated in premalignant or malignant pancreatic conditions such as pancreatic cysts, BDIPMN. Minor grammar and punctuation errors were corrected.

Cover Letter in Response to Reviewer's Comments to *The World Journal of Gastroenterology*

Re manuscript number 53164 entitled 'Macrophage inhibitory cytokine-1/growth differentiation factor-15 in premalignant and neoplastic tumours in a high-risk pancreatic cancer cohort

The authors would like to firstly thank you for the time taken to review the submitted manuscript and the comments made. Please find below a point-by-point response to questions in BLUE with reviewer's comments in italics.

Reviewer 1 comments,
This manuscript has interesting information in pancreatic cancer research. However, case numbers are too small for ROC analysis. Although I commented this point in the first review, the authors did not improve the manuscript.

The authors of the presented manuscript thank the reviewer for their analysis and comment on the presented manuscript. The main critique is the small number of malignancies detected, not the study itself. This we cannot change but want to show you this is comparable with previous studies on this topic and the study has multiple merits and why it should be published and how it can help other researchers. We will provide references to support our statements.

This is the only study of it's kind that has been undertaken in high-risk patients for pancreatic cancer internationally and therefore we believe that it has merit. This study has merit in that it is a prospective study detailing data collected **over an 8 year period** of patients that have been deemed to high risk of pancreatic malignancy using pre-determined criteria. The study population is 120 well, healthy individuals which is a suitable number for a pancreatic cancer screening program and the follow up is one of the longest. Langer[1] et al published on 76 patients in their PC screening program followed up over 5 years, Verna[2] et all had 51 patients followed up for 3 years, Sud[3] et all had 30 patients follow up for 3 years, Poley[4] et al 44 patients over 2 years, Ludwig[5] et all had 109 patients follow up for 7 years, Canto[6] et all had 216 individuals followed up for 28 months. All these studies were published in very reputable journals Gut, Gastroenterology, American Journal of Gastroenterology, Pancreas. Our number is 120 patients followed up over 8 years which is well above the average of the already published studies.

Pancreatic malignancy is a rare entity, and pancreatic cancer attributable to a genetic predisposition is even more scarce. Therefore patient recruitment and subsequent malignancy detection is respectively low. This study was able to identify two pancreatic malignancies and three extra-pancreatic malignancies in an asymptomatic population undergoing screening, in which MIC-1 was deemed to be predictive on ROC curve analysis. There is variability internationally in the incidence of pancreatic malignancy detection in screening, however this study demonstrates a 2.5% incidence

of malignancy diagnosed on EUS, and 4.2% diagnosed implementing additional imaging techniques available through the screening program. This is consistent with international published figures(Sud et all 2 malignancy/30 screened, Verna 1/51, Lange 0/76, Poley 1/44) and higher than a recent meta analysis that show the yield of screening to be 0.76 (Coral et al 2019) [7].

This is not a study of MIC-1 in patients with a disease where you can accumulate any numbers you want for ROC analysis to look good, this is a long term follow up of HEALTHY Individuals at risk of pancreatic cancer to see if this biomarker over time increases as cancer develops to potentially use a blood test as a screening test. As the study title shows this study is MIC 1 in" premalignant and malignant lesions" and correlations with MIC-1 levels. Premalignant lesions and its subtypes are detected by EUS and this results are also presented and equally important to the malignant results. Providing information about abnormal EUS and MIC-1 value will help further researchers to use our data as a starting point and take these results further.

The authors understand the comments made regarding ROC curve analysis, however considering this study assessed the predictive capacity of MIC-1 in pancreatic malignancy and malignancy in general in an asymptomatic population deemed high risk for developing pancreatic cancer and was able to present significance, it provides preliminary evidence that is in support of the capacity of MIC-1 as a serological marker predictive of malignancy. The authors note that previous studies have utilised small cohort sizes in the analysis of MIC-1/GDF15 as a serological biomarker. Sugimoto[8] *et al.* was able to demonstrate through analysis of a cohort of 23 patients with biliary tract cancer that serum MIC-1 in combination with CA19-9 was useful for screening biliary tract cancer, and that biliary MIC-1/GDF15 was effective in the diagnosis of biliary tract cancer. The same study emphasised their cohort size as a limitation, a similar problem the authors of the presented study have mentioned in the submitted discussion. In addition to this, Zhou[9] *et al.* were able to demonstrate that MIC-1/GDF15 had diagnostic capability in distinguishing between pre-pancreatic cancer and normal controls using a cohort of 20 patients with pre-pancreatic cancer. This is similar for prostate cancer, where Kagohara[10] *et al.* were able to demonstrate that MIC-1/GDF15 was a strong candidate biomarker for cancer screening and disease aggressiveness using a cohort of 50 patients diagnosed with prostate cancer, comparing it to controls. Fisher[11] *et al.* were also able to demonstrate significant predictive capacity for MIC-1/GDF15 utilising small cohort sizes for Barrett's oesophagus (N=37) and Barrett's oesophagus with low grade dysplasia (N=16). Similar studies in assessing the ability for MIC-1/GDF15 to predict distant metastases in colorectal cancer patients have also been performed with limited cohorts. Jakubowska[12] *et al.* was able to demonstrate that MIC-1/GDF15 was a significant discriminator of patients with distant metastasis with a cohort of 25 patients. These studies, although small, highlight that there is merit associated with preliminary evidence for the predictive capacity of MIC-1 in malignancy, and additionally in predicting metastatic disease.

We ,as doctors involved in pancreatic cancer screening for over 10 years and researchers , have a duty to present our positive and negative findings. Larger research community needs to hear our results and perform further studies so we can

reach a significant result with joint efforts much sooner than each of us individually. We fully acknowledge in the study limitations that we need more numbers but this can only achieve through international collaboration for which we need to present interim results. As we cannot change the numbers and this is the only critique, we have added throughout the manuscript that this is a PILOT study. Hence the readers will understand this is proof of concept study, first of its kind in the world and cannot have large number. We can also add " a pilot study" in the title if the editor thinks this is appropriate.

Reviewer 2 comments , SAME PERSON AS REVIEWER 1

This manuscript describes the importance of macrophage inhibitory cytokine-1/growth differentiation factor-15 as detecting pre-malignant pancreatic lesions and neoplastic tumors in an asymptomatic high-risk cohort part of Australian Pancreatic Cancer Screening Program. The authors investigated 120 participants and 47 participants (39.2%) had an abnormal EUS and five participants (4.2%) were diagnosed with neoplastic tumors, three by EUS (two pancreatic and one liver) and two by MRI/CT (breast cancer, bladder cancer) done for follow up of abnormal EUS. However, there are some problems in this manuscript as described below. Although AUC value is high, ROC is not beautiful because total numbers are small. Therefore, the authors should investigate much more cases. The authors should compare pancreas-specific inflammation parameters such as amylase, lipase, and elastase1 to macrophage inhibitory cytokine-1/growth differentiation factor-15.

As this is the same reviewer as reviewer 1 , please see above answer. As previously explained amylase, lipase are markers of pancreatitis and do not go up in pancreatic cancer . Faecal elastase is a marker for pancreatic insufficiency and cancers do not present with pancreatic insufficiency. None of these markers are suitable for a pancreatic cancer screening program.

Reviewer 3 comments

The manuscript entitled "Macrophage Inhibitory Cytokine-1/Growth Differentiation Factor-15 in premalignant and neoplastic tumours in a high-risk pancreatic cancer cohort by Robert Sean O'Neill et al is an interesting prospective study and the authors have collected a unique dataset using standard methodology. The paper is generally well written and structured. Overall, this is a clear, concise, and well-written manuscript. The introduction is relevant and theory based. Sufficient information about the previous study findings is presented for readers to follow the present study rationale and procedures. However, in my opinion the paper has some shortcomings in regards to results and conclusions. The authors indicate that "MIC-1/GDF15 has predictive capacity for neoplastic tumours in asymptomatic individuals with a genetic predisposition for PC and further imagining may be warranted in patients with a normal EUS and raised MIC-1. Larger prospective studies are required to further define the role of MIC-1/GDF15 as a serological biomarker in pre-malignant pancreatic lesion". In agreement with the authors, this study needs to be validated with larger prospective studies (including healthy

volunteers and non-malignant pathologies in multicenter studies) for finding false positive and false negative values with higher precision.
SEE ALSO COMMENT to reviewer 1. We note this reviewer commented ACCEPT and we thank you for this. The authors understand that the results of presented manuscript are preliminary and, although the study cohort is small and the incidence of malignancy detection was small, the significant results obtained on ROC curve analysis demonstrate preliminary evidence that MIC-1/GDF15 has significant predictive capacity for malignancy detection in an asymptomatic cohort undergoing screening for pancreatic malignancy. The authors hope this study will start a new wave of collaborative research so more number can be achieved and these results validated in a much larger cohort.

We hope that you find the submitted manuscript acceptable for publication. Thank you once again for your consideration.

Yours sincerely,

Alina Stoita MBBS FRACP
Staff Specialist Gastroenterologist, St Vincent's Hospital Sydney
Assistant Lecturer, The University of New South Wales, Sydney, Australia
Department of Gastroenterology, St Vincent's Hospital, Sydney, 390 Victoria Street, Sydney, 2010, Australia
Email - alina.stoita@svha.org.au

References

[1] Langer P, Kann PH, Fendrich V, Habbe N, Schneider M, Sina M, Slater EP, Heverhagen JT, Gress TM, Rothmund M, Bartsch DK. Five years of prospective screening of high-risk individuals from families with familial pancreatic cancer. Gut 2009; 58: 1410-1418 [PMID: 19470496 DOI: 10.1136/gut.2008.171611]
[2] Verna EC, Hwang C, Stevens PD, Rotterdam H, Stavropoulos SN, Sy CD, Prince MA, Chung WK, Fine RL, Chabot JA, Frucht H. Pancreatic cancer screening in a prospective cohort of highrisk patients: a comprehensive strategy of imaging and genetics. Clin Cancer Res 2010; 16: 5028-5037 [PMID: 20876795 DOI: 10.1158/1078-0432.CCR-09-3209]
[3] Sud A, Wham D, Catalano M, Guda NM. Promising outcomes of screening for pancreatic cancer by genetic testing and endoscopic ultrasound. Pancreas 2014; 43: 458-461 [PMID: 24622079 DOI: 10.1097/mpa.0000000000000052]
[4] Poley JW, Kluijt I, Gouma DJ, Harinck F, Wagner A, Aalfs C, van Eijck CH, Cats A, Kuipers EJ, Nio Y, Fockens P, Bruno MJ. The yield of first-time endoscopic ultrasonography in screening individuals at a high risk of developing pancreatic cancer. Am J Gastroenterol 2009; 104: 2175-2181 [PMID: 19491823 DOI: 10.1038/ajg.2009.276]
[5] Ludwig E, Olson SH, Bayuga S, Simon J, Schattner MA, Gerdes H, Allen PJ, Jarnagin WR, Kurtz RC. Feasibility and yield of screening in relatives from familial pancreatic cancer families. Am J Gastroenterol 2011; 106: 946-954 [PMID: 21468009 DOI: 10.1038/ajg.2011.65]

[6] Canto MI, Hruban RH, Fishman EK, Kamel IR, Schulick R, Zhang Z, Topazian M, Takahashi N, Fletcher J, Petersen G, Klein AP, Axilbund J, Griffin C, Syngal S, Saltzman JR, Mortele KJ, Lee J, Tamm E, Vikram R, Bhosale P, Margolis D, Farrell J, Goggins M. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. Gastroenterology 2012; 142: 796-804; quiz e14-e15 [PMID: 22245846 DOI: 10.1053/j.gastro.2012.01.005]

[7] Corral JE, Das A, Bruno MJ, Wallace MB. Cost-effectiveness of Pancreatic Cancer Surveillance in High-Risk Individuals: An Economic Analysis. Pancreas. 2019;48(4):526-36.

[8] Sugimoto M, Takagi T, Konno N, Suzuki R, Asama H, Watanabe K, Nakamura J, Waragai Y, Kikuchi H, Takasumi M, Sato Y. The efficacy of biliary and serum macrophage inhibitory cytokine-1 for diagnosing biliary tract cancer. Scientific reports. 2017 Aug 23;7(1):1-6.

[9] Zhou YF, Xu LX, Huang LY, Guo F, Zhang F, He XY, Yuan YZ, Yao WY. Combined detection of serum UL16-binding protein 2 and macrophage inhibitory cytokine-1 improves early diagnosis and prognostic prediction of pancreatic cancer. Oncology letters. 2014 Nov 1;8(5):2096-102.

[10] Kagohara LT, Li J, Pavlovich CP, Davis C, Mangold L, Zhu G, Morrissey C, Partin AW, Mandecki W, Veltri RW. MIC-1 and Endoglin are protein serum biomarkers capable of increasing the clinical diagnostic specificity of the PSA test.

[11] Fisher OM, Levert-Mignon AJ, Lord SJ, Lee-Ng KK, Botelho NK, Falkenback D, Thomas ML, Bobryshev YV, Whiteman DC, Brown DA, Breit SN. MIC-1/GDF15 in Barrett's oesophagus and oesophageal adenocarcinoma. British journal of cancer. 2015 Apr;112(8):1384-91.

[12] Jakubowska K, Pryczynicz A, Dymicka-Piekarska V, Cepowicz D, Jagodzińska D, Lewczuk Ł, Lebelt A, Ozimkiewicz M, Kiszło P, Guzińska-Ustymowicz K. The growth differentiation factor-15 (GDF-15) can be useful in the detection of distant metastases in sera of colorectal cancer patients. Progress in Health Sciences. 2016;6(1):40-8.