July 15, 2021

RE: Manuscript NO: 66628 "EUS Features of Autoimmune Pancreatitis: The difference between the diffuse type and focal type"

Dear Distinguished Professor Professor Subrata Ghosh, Professor Andrzej S Tarnawaaski, Editors, and Reviewers:

Thank you so much for your time and energy in reviewing this manuscript. We sincerely thank all Editors and Reviewers for their valuable and constructive comments and suggestions, which really helped us in improving this manuscript significantly. We have fully addressed all comments in a point-by-point manner. We appreciate your patience while we made all necessary revisions.

We are submitting a revised manuscript. All authors have read and approved the final revised version. Please refer to the point-to-point response below. We hope the revised manuscript could be more suitable for your consideration of publication in the journal of *World Journal of Gastroenterology*.

Comments by the reviewers:

Reviewer #1

1. The authors described that patients were enrolled prospectively. Was this stud prospective or retrospective? IRB approval was obtained in 2021. Did the investigators obtain written informed consent after IRB approval?

Reply: Thank you so much for your important suggestion. This is a retrospective study which included the AIP patients enrolled in an observative program in our department since January 2012. The informed consents which was generally applicable in our hospital for possible future study of the existing data, documents and records in such a manner that subjects cannot be identified, directly or indirectly were previously collected. We have corrected the corresponding part in the manuscript (Page 7 Line 13-14; marked with highlighting) to avoid misunderstanding.

2. Please add detailed methodology of the image selection and blinding. Who selected the images from the database and how two investigators were blinded? How to treat disagreement between two investigators?

Reply: Thank you for your important suggestion. We have added the detailed methodology of image selection and blinding in the manuscript (Page 8 Line 6-10; marked with highlighting).

3. For MPD dilation, please clarify its location. Was it observed in the area without AIP involvement?

Reply: Thanks for your inspiring and important question. We indeed ignored showing the location of the MPD dilation in submitted manuscript. As you pointed out, the MPD dilation often located proximally to the AIP affected area in which the MPD or surrounding parenchyma was involved, so it could be observed in the area without AIP involvement. We have added the above content in the manuscript (Page 15 Line 7-8; marked with highlighting).

4. Please clarify how the investigators differentiate DHA vs. FHA on EUS?

Reply: Thank you for your important comments. We are sorry for the ignorance of expression of this important definition. We divide the pancreas into three parts: head, body, and tail. If “hypoechoic area” of AIP involves more than one part of pancreas on EUS examination, the investigators will diagnose diffuse hypoechoic area (DHA), or else will diagnose focal hypoechoic area (FHA). We have updated the above content in the manuscript (in Supplement Table 1, Page 32 Line 1-3, marked with highlighting).

5. Finally, how can you apply the study results in the diagnosis of diffuse and focal AIP? Any suggestions?

Reply: Thank you for this helpful suggestion. In this study, we tried to interpret the EUS features of the AIP patients and demonstrated the differences between the diffuse type and focal type in pancreatic parenchyma, pancreatic duct and common bile duct. We also performed multivariate regression for the diffuse AIP. We did not try to apply these EUS differences for the diagnosis of diffuse and focal AIP, which is basically based on the CT/MR findings or EUS assessment after the diagnosis of AIP. The EUS features of the focal AIP will be helpful to differentiate it from the pancreatic cancer, which has been addressed in our published study.[1] We have updated the above content in the manuscript (Page 16 Line 1-3; marked with highlighting).

1. Guo T, Xu T, Zhang S, et al. The role of EUS in diagnosing focal autoimmune pancreatitis and differentiating it from pancreatic cancer. *Endosc Ultrasound*. 2021. Jun 26. doi: 10.4103/EUS-D-20-00212. Epub ahead of print.

Reviewer #2

My major concerns regarding:

1. The title of the manuscript does not reflect the aim of the study that should be better exposed.

Reply: Thanks for your suggestive comments. The present title does not reflect the aim of the study, so we have changed it to "EUS features of autoimmune pancreatitis: the typical findings and chronic pancreatitis changes" for better covering the contents of the manuscript. We have updated the short title as well.

2. The 32.3 % of patients are "non-diagnostic" so they underwent to EUS-FNA with only the 39.1% received level 1 and 2 histological evidence. The reference 19 of the manuscript cites a prospective, multicentric study in which the authors showed that the 78% and the 45% of patients were diagnosed with level 1 or 2 of type 1 AIP, respectively with the use of 22-gauge Franseen needle and of 20-gauge forward-bevel needle. Also, a recent metanalysis of Facciorusso et al. showed that the overall diagnostic accuracy of EUS tissue acquisition was 54.7% with a clear superiority of FNB versus FNA. In the manuscript a better explanation of the reasons regarding the "no use" of FNB needles is advised.

Reply: Thank you so much for the important comments. As you pointed out, there is overt evidence that FNB needles are superior to FNA needles in overall diagnostic accuracy in recent years. The long time period during which all patients are included in the study (since 2012; our center did not have FNB needles until 2015) might

explain the reason that we did use the FNB needles (22G Procore and 20G Procore) but in a low proportion (12% in diffuse AIP patients and 29.9% in focal AIP cases; see Supplement Table 4), and so that the low diagnosis accuracy is understandable. Nowadays, just like what you suggest, we use 20G Procore needles for most cases with a suspected diagnosis of AIP in our center, for better tissue core yielding and diagnosis accuracy. Also, though the FNA needles seemed not to supply enough tissue for definite pathological diagnosis of AIP, they did have the clinical significance of ruling out malignancy in AIP patients.[2] We have added the above content in the manuscript (Page 17 Line 11-15; marked with highlighting).

2. Sugimoto M, Takagi T, Suzuki R, et al. Endoscopic Ultrasonography-Guided Fine Needle Aspiration Can Be Used to Rule Out Malignancy in Autoimmune Pancreatitis Patients. J Ultrasound Med. 2017; 36: 2237-44.

3. The EUS features chosen by the author (parenchymal, cholangitis-like and peripancreatic changes). I think that one of the most important missing data is about the characteristic of the main pancreatic duct, cited only in the context of chronic pancreatitis via Rosemont criteria. In fact, in the ICDC criteria the ductal imaging is described, such as, long strictures or focal narrowing without marked upstream dilation. It would be interesting in your AIP patients, especially in the subset of focal type, to find typical EUS features of main pancreatic duct in the same way of bile duct characteristics.

Reply: Thank you for the important comments. As you pointed out, in the ICDC criteria, the main pancreatic duct imaging includes long (>1/3 length of the main pancreatic duct) or multiple strictures without marked upstream dilatation, or segmental/focal narrowing without marked upstream dilatation (< 5 mm), which are usually evaluated by endoscopic retrograde pancreatography. These are both typical MPD images for AIP. We described the MPD changes of AIP following the Rosemont criteria (such as stone/calculi, dilation, focal stenosis, diffuse stenosis/irregularity), which was also used in the study of Hoki N et al. In a way, the conception of “diffuse stenosis/irregularity” (diffuse uneven or irregular outline and ectatic course) in this study partially overlapped with ICDC duct imaging, and we may employ the description of ICDC in the future study.

4. The use of ancillary techniques such as contrast enhanced-EUS and elastography could be helpful in the differential diagnosis between focal type AIP and pancreatic cancer, this aspect should be better exposed in the manuscript. In my opinion this retrospective study is original, but its role is purely descriptive, and the clinical implications are not so clear.

Reply: Thank you for your important comments. Contrast-enhanced-EUS (CE-EUS) and elastography both could help to distinguish between focal AIP and pancreatic cancer, but sometimes the sensitivity and specificity were not that satisfactory. In the

study, limited patients received CE-EUS (1 cases) and elastography (9 cases), so we did not include these aspects in the manuscript.

The main aim of this study is to fully describe the EUS features of in the high number of untreated AIP cases (which is a simple but important issue, but only interpreted in limited cases; see Supplement Table 3), and to show the EUS features differences between diffuse and focal AIP cases; both issues have not been illustrated by other studies, and we believe that our data is valid and add new ideas in the field.

Reviewer #3

Major issues

1. I agree the importance of EUS features of AIP. However, the authors described only its characteristics of diffuse and focal type of AIP. What is the strongest point in this study? What findings were the most important for differentiating diffuse and focal type of AIP? For example, was EUS superior to other modalities such as dynamic CT? The authors should describe the novelty of their study and discuss further perspective of EUS for the management of AIP.

Reply: Thank you so much for your important comments. We think that the strongest point in this study is to fully describe the EUS features of in the high number of untreated AIP cases, and to show the EUS features differences between diffuse and

focal AIP cases; both issues have not been answered in previous studies. To differentiate the diffuse and focal AIP is not that difficult if AIP diagnosis was confirmed according to the ICDC, which will depend on the dynamic CT/MR or EUS itself for the involving parts of pancreas (the whole pancreas is divided in to 3 parts: head, body and tail; cases with more than one part of pancreas involved are diagnosed as diffuse AIP). The EUS typical features of AIP (especially the cholangiopathy-like features) can help to differentiate the diffuse AIP from classic chronic pancreatitis and differentiate the focal AIP from pancreatic cancer as is shown in our published study.[1] We have updated the above content in the manuscript (Page 16 Line 1-3; marked with highlighting).

1. Guo T, Xu T, Zhang S, et al. The role of EUS in diagnosing focal autoimmune pancreatitis and differentiating it from pancreatic cancer. *Endosc Ultrasound*. 2021. Jun 26. doi: 10.4103/EUS-D-20-00212. Epub ahead of print.

2. The definition of ‘diffuse’ and ‘focal type’ is obscure. The authors mentioned that ‘diffuse’ is defined as ‘more than 1/3 pancreas involved’. What modality did you use for this definition? Did all patients receive a dynamic CT scan prior to EUS examination? Or did the authors apply EUS findings for differentiation of diffuse and focal type? If the authors used EUS findings to distinguish between two types, it seems obvious that DHA is more common in ‘diffuse type’, and FHA is more common in ‘focal type’.

Reply: Thanks for your important comments. We are deeply sorry for this unintentional mistake made during drafting this manuscript, and actually it would be "the pancreas is divided into three parts: head, body, and tail. Cases with more than one part of pancreas enlarged are defined as "diffuse enlargement" and grouped as the "diffuse type"". We have corrected the description in the Method section accordingly (Page 8 Line 12-16; marked with highlighting).

In this study, all patients received dynamic CT or MR before EUS examination.

Though we found predictors for the diffuse AIP (Table 3), we did not solely apply EUS findings to distinguish the two types, because with the diagnosis of AIP established the differential diagnosis of diffuse and focal type can be readily made through modality such as CT/MR.

Indeed, the EUS feature of DHA is more prevalent in "diffuse AIP group", and FHA is more common in the "focal group", which is consistent with the definition of "diffuse" and "focal" type (enlarged part of pancreas is the affected area of AIP, so is the "hypoechoic" area). Note that DHA also exists in limited cases of the focal type, that is "diffuse" hypoechoic area in the "focal" enlarged pancreas; we did not define these cases as "diffuse type" to avoid confusion with the ICDC which emphasizes the diffuse or focal "enlargement" of pancreas.[3] We have added the above content in the manuscript (Page 14 Line 3-5; marked with highlighting).

3. Shimosegawa T, Chari ST, Frulloni L, et al. International Consensus Diagnostic Criteria for Autoimmune Pancreatitis. Pancreas. 2011; 40: 352-8.

3. In table 2, the author compared EUS findings between the diffuse and focal type of AIP by univariate analysis. I recommend performing a multivariate analysis to distinguish between two types.

Reply: Thanks for your inspiring suggestion. We have performed the logistic regression analysis for the EUS findings between the diffuse and focal type of AIP and try to find the predictors of the diffuse AIP. The result is shown in Table 3 (updated in the Result section; Page 12 Line 6-10, marked with highlighting) and the Discussion section is also updated (from Page 13 Line 16-19; marked with highlighting).

4. In table 1, the levels of ALT and CA19-9 were significantly higher in the diffuse type than that in the focal type. Were they affected by jaundice or cholestasis?

Reply: Thanks for your comments. We have checked the data and found that the total bilirubin level and jaundice cases proportion in both groups showed no significant difference. The elevated ALT may be due to the hepatitis caused by the IgG4 related disease, and the elevated CA 19-9 level may be explained by the pancreatitis and IgG4 related cholangitis which is more prevalent in the diffuse group.

5. In table 2, the proportion of patients with bile duct wall thickening in diffuse group was significantly higher than that in focal group. Is this difference just due to proportion of head involvement? The authors should add the number of patients with head involvement to table 2.

Reply: Thank you for the important comments. As you pointed out, Bile duct wall thickening resulting from IgG4-associated cholangitis was more frequently seen in the diffuse group (pancreatic head was involved in almost all diffuse AIP cases) and focal AIP cases with pancreatic head involved. We have updated the above contents in the manuscript (from Page 14 Line 7-15; marked with highlighting).

6. Was there a difference in the number of involved organs (extrapancraetic lesions of AIP) between diffuse and focal type? I recommend adding this information to table 1.

Reply: Thank you for the important comments. We have added these contents in Table 1 accordingly.

Minor issues

1. On page 6, line 5, 'no study' seems to be a mistake of 'No study'.

Reply: Thanks for your reminding. We are deeply sorry for this unintentional mistake made during drafting this manuscript and have corrected the mistake accordingly.

2. There are two punctuation marks on page 9, line 22; ‘carbohydrate antigen 19-9 which were higher in the diffuse group..’.

Reply: Thanks for your reminding. We have corrected the mistake accordingly.

3. On page 10, line 17, 'than in the focal group' seems to be a mistake of 'than in the diffuse group'. Please check and correct it.

Reply: Thanks for your reminding. We have corrected the mistake accordingly.

4. I recommend that the authors use ‘mg/dl’ as the unit for IgG and IgG4.

Reply: Thanks for your reminding. We have changed the unit for IgG and IgG4 in Table 1.

Reviewer #4

1. Was your intention to compare CP features in both groups, to demonstrate the predilection and rate of progression to CP between Diffuse and focal groups ? This has to be stated in you study.

Reply: Thanks for your important comments. Actually, we wanted to compare the chronic pancreatitis change level (or rather CP features) in both groups. We have stated this in the Introduction section (Page 7 Line 5-6; marked with highlighting).

2. There are many of the calculations that don't add up in your study. For example, "For the typical AIP features: there were significantly more patients with DHA in the diffuse group (92.1% vs. 22.5%, P < 0.001), while there were significantly more patients with FHA in the focal group (0 vs. 83.1%, P < 0.001)" - The statement above is rather confusing as the numbers don't add up to 100%. When you say 0 vs 83.1%, what are you comparing?

Reply: Thanks for your important reminding. We are sorry for the unclear expression in the manuscript. We are comparing the proportion of different EUS features in the two groups, and the proportion of a common feature in each group may not add up to 100%. We have changed the expression as "there were significantly more patients with DHA in the diffuse group (197 of 214 cases, 92.1% vs. 16 of 71 cases, 22.5%, $p < 0.001$), while there were significantly more patients with FHA in the focal group (0 of 214 cases, 0% vs. 59 of 71 cases, 83.1%, $p < 0.001$)" for more distinct expression.

We have corrected all proportion comparation expression in the Result section as described above to avoid misunderstanding.

3. “For MPD changes, there were significantly more patients with MPD dilation in the focal group than in the focal group (14.0% vs. 25.3%, P = 0.03).” - There is a mistake in this statement. Please correct it.

Reply: Thanks for your reminding. We have corrected the mistake accordingly (Page 12 Line 3, marked with highlighting).

4. In the discussion segment, it would be also advisable to touch on the additive value of EUS findings in comparison to other imaging modalities.

Reply: Thank you for your constructive suggestion. Compared to other image modalities, EUS can find the early fibrosis parenchymal changes of CP (like HF, HS, and peripancreatic hypoechoic margin) in AIP cases, which change dynamically after corticosteroid therapy. As the tool for accessing the fibrosis degree of pancreas, the EUS findings of CP may be used as a tool for predicting the pancreatic atrophy and diabetes exacerbation, which need further investigation in the future.[4] We have added the above contents in the Discussion section (from Page 16 Line 21 to Page 17 Line 1-4; marked with highlighting).

4. Yamada Y, Masuda A, Sofue K, et al. Prediction of pancreatic atrophy after steroid therapy using equilibrium-phase contrast computed tomography imaging in autoimmune pancreatitis. *JGH Open*. 2020; 4: 677-83

5. Supplement Figure legend • The supplement figure legend is rather confusing. The authors should attempt to simplify it. For example - Non-diagnostic AIP has been divided into EUS FNA and response to steroids. These are 2 completely different variables and can't be lumped under the same heading. Alternatively you could use 2 separate tables to illustrate your findings. - The response to steroids for the definitive AIP (Diffuse group) has not been mentioned.

Reply: Thanks for your important reminding. We are sorry for the unclear expression and misunderstanding in Supplement Figure. Actually, some of the non-diagnostic cases chose to take the diagnostic steroid trial, the others chose to receive the EUS-FNA; other than LPSP level 1 pathological finding, cases who received FNA will also take the diagnostic steroid trial. Cases who responded to the diagnostic steroid trial would be finally diagnosed with AIP. We have added the above contents in the manuscript (Page 30 Line 2-8; marked with highlighting).

All the Above was our point-by-point Reply to All the comments of Reviewers. And we had made all necessary modifications and updating in the revised manuscript

meanwhile. These valuable comments and suggestions really helped us to improve our manuscript a great deal.

Hopefully, Editors and Reviewers can review our revised manuscript for further consideration of its acceptance to the journal of *World Journal of Gastroenterology*.

If you have any questions or suggestions, we shall always be happy to address them all and improve the manuscript further.

All the best with you and all your staff,

Yours sincerely,

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