Author’s response to reviews

Title: Autoantibody Detection is Not Recommended for Chronic Pancreatitis: A Cross-sectional Study of 557 Patients

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Responses to the Reviewers’ Comments

Dear Dr. Vishal Sharma


Thank you very much for the decision and advice. We have carefully studied the comments and made the appropriate corrections. The modifications are highlighted in the revised manuscript and we use the MS mode to tack the changes. Our point-by-point responses to the reviewers’ comments are listed below in this letter. We have had the language edited by professional company and uploaded the proof.

We hope that this resubmitted version is acceptable for publication in your journal. We look forward to receiving your reply.

With kind regards,

Sincerely yours,

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Editor Comments:
1) Provide reasons for admission of these patients

Response: Thanks for your reminding. As a tertiary referral center, Changhai Hospital has an annual volume of >800 CP patients for diagnosis and treatment from all over China. The main reasons for admission of these patients were recurrent abdominal pain. For CP patients who did not experience pain, interventions were performed only when complications such as biliary stricture, pancreatic portal hypertension etc had occurred, whereas diabetes mellitus and/or steatorrhea was not an indication for invasive treatment of CP(1, 2). Corrections were made in Methods section, line 3, paragraph 2, page 3.

References


2) The limitations like selection bias, and the possible problems with the choice of controls, lack of estimate on causality as the samples were tested once disease had occured need to be highlighted in the discussion

Response: Thank you very much for the correction. There are several limitations of the present study. Firstly, the observational study design (cohort study) is inherent to selection bias(1). And we chose control group through literature search in PubMed due to difficulty for large-scale collecting the serum of healthy people to detect all antibodies in clinical, but the historical healthy controls may increase the inaccuracy of target autoantibodies’ frequencies as there may be differences among different races, countries, regions and nations. Secondly, this study lack of estimate on causality between autoantibody and CP occurrence as the samples were tested once disease had occurred. Adjustments were made accordingly in Discussion section, line 1, paragraph 2, page 11.

Reference


3) Was normality of the distribution tested prior to using parametric tests. If no, need to redo the statistical analysis. If yes, please state so

Response: Thank you very much for your reminding. The distribution tested prior to using parametric tests of all autoantibodies was normal, and their sensitivity and specificity were pretty
high in preliminary validation tests. Adjustments were made accordingly in Methods section, line 4, paragraph 3, page 4.

4) When the authors report univariate logistic regression analysis do they mean univariate analysis or univariate analysis screening

Response: Thank you very much for your reminding. The univariate logistic regression analysis means univariate analysis screening. We selected P value less than 0.15 from univariate analysis screening as candidates for multivariate logistic regression analysis. Adjustments were made accordingly in Methods section, line 6, paragraph 2, page 6.

5) The term healthy controls alone may not suffice better to use historical healthy controls

Response: Thank you very much for your reminding. The term healthy controls were replaced by historical healthy controls in manuscript and table.

6) Table 3 is confusing. Please provide independent tables for univariate and multivariate LR analysis for each of the four antibodies separately. Also tables in the Supplementary file dealing with antibodies are not clear. Please separately provide results of univariate (comparison between positive and negative CP patients). If predictors of positivity of a given antibody in CP patients are to be estimated data from positive and negative cases should be compared for univariate analysis and then variables be chosen for multivariate LR. It is not clear why data for Healthy controls has been used for this analysis

Response: Thank you very much for the correction. Univariate analysis results (comparison between positive and negative CP patients) of four antibodies were showed in newly established Table 3 and Table S3. Univariate and multivariate LR analysis results of four antibodies were illustrated in Table 4 and Table S4-S6. Data for healthy controls were only used to compare the positive rate of autoantibodies between CP patients and healthy controls (Table 2), from which we screen out four differential expressed autoantibodies (β2-GPI, anti SS-B, SMA and ACL antibody). Then we investigated the related factors of four autoantibodies in CP patients through univariate analysis and multivariate LR analysis, thus data for healthy controls has not been used for this analysis of related factors.

Reviewer Comments:

Reviewer #1:

Major:

1. Why this auto antibody was done in these patients as diagnosis of chronic pancreatitis is imaging based. If it was done suspecting AIP then what are the frequency of auto antibody most
frequently seen in AIP patients like ANA, rheumatoid factor, anti lactoferrin, anti carbonic anhydrase?

Response: Thank you very much for the reminding. Autoimmune is regarded as the major definite pathogenesis of AIP(1-4), but whether autoimmune factor plays a role in non-AIP CP or not was unknown(5, 6). In the past, patients suspected of CP admitted to our center for the first time would be received serum autoantibody detection for etiology identification and differential diagnosis. Patients in our study had been detected ANA antibody spectrum includes anti-ds DNA, anti-ss DNA, anti-SM, anti-RNP, anti SS-A, anti SS-B, anti-Jo-1 antibody, anti-Scl 70, anti-proliferating cell nuclear antigen, anti-nucleosome, anti-ribosomal, anti-PM-Scl antibody, etc. Rheumatoid factor is one of specific antibody for rheumatoid arthritis, and not all patients in this study were detected rheumatoid factor, so it was not included in our study. Anti lactoferrin antibody and anti carbonic anhydrase antibody are two rare antibodies, and our center can't test them yet.

References


2. Four antibody are less frequently associated with study group compared to healthy control, are they protective against development of CP? No mention in discussion
Response: Thank you very much for the reminding. The current study showed that the frequencies of serum β2-GPI and anti SS-B antibody in patients with CP were significantly higher than that in historical healthy controls, and the frequencies of serum SMA and ACL antibody in patients were significantly lower than that in historical healthy controls (all $P < 0.05$). Until now, the diagnosis of CP is mainly based on the clinical manifestation and imaging findings. Although this current study identified four differentially expressed autoantibodies between non-AIP CP patients and healthy controls, they had limited value in diagnosing non-AIP CP, and could not help the differentiation of non-AIP CP from other pancreatic diseases because of low specificity(1). And there is no research to confirm that these four autoantibodies are protective against development of CP up to now. Adjustments were made accordingly in Discussion section, line 2, paragraph 1, page 11.

Reference


3. Uniformity of detection of antibody is not there like some by ELISA and some by IIF?

Response: Thanks for your good question. The detection of antibody could be achieved through a variety of methods. In this present study, 22 common autoantibodies kits were all purchased from EUROIMMUN Medical Laboratory Diagnostics Stock Company and detected through EUROLINE, ELISA and IIF. Although the methods of some kits were different, their sensitivity and specificity were pretty high in preliminary validation tests (Table S1).

4. When there is definite diagnosis of etiology of CP, why these antibody were done in alcohol etiology of CP. Is it done for prognostication, no mention in discussion.

Response: Thank you very much for the reminding. At present, CP is regarded as a disease with multiple etiological factors including alcohol, autoimmunity, biliary tract diseases, etc(1). And alcohol and autoimmunity factors may coexist. In the past, patients suspected of CP admitted to our center for the first time would be received history taking, physical examination, imaging examination and laboratory examination (including serum autoantibody detection) for diagnosis, etiology identification, treatment guidance and prognosis evaluation. Through this present study, we indicate that autoantibodies detection is not recommended conventionally unless suspected of AIP due to limited significance for diagnosis and treatment of CP. Adjustments were made accordingly in Discussion section, line 1, paragraph 2, page 10.

Reference

5. Discussion should be focused association of autoantibody with CP. No need of discussing about antibody or its association with other etiologies.

Response: Thank you very much for the reminding. Adjustments were made accordingly in Discussion section in Discussion section, line 6, paragraph 1, page 9.

6. What is the explanation for DM in first/second/third degree relatives as protective factor for beta 2-GPI and DM, CBD stricture as risk?

Response: Thank you very much for the reminding. This present study preliminarily investigated the autoantibody detection result and their clinical significance in patients with non-AIP CP. Result showed that β2-GPI antibody was the most frequent, and DM, DM in first/second/third degree relatives and CBD stricture were the independent related factors. But there is no previous study to confirm the relationship between β2-GPI antibody and family history of DM, CBD stricture. Only a few studies have showed that β2-GPI antibody may participate in the occurrence and development of DM(1-3). Adjustments were made accordingly in Discussion section in Discussion section, line 6, paragraph 3, page 9.

References


MINOR

1. Few spelling mistakes need to be corrected
Response: Thank you very much for reminding. Few spelling mistakes had been corrected.

Reviewer #2: This manuscript describes the investigation of the usefulness of autoantibodies to diagnose chronic pancreatitis. The authors determined serum levels of several kinds of autoantibodies in 557 patients with chronic pancreatitis. However, the authors failed to find useful autoantibody biomarkers. These findings seem not to be worth for publication for many gastroenterological researchers.

Response: Thanks for your review. This present study screened out four differential expressed autoantibodies (β2-GPI, anti SS-B, SMA and ACL antibody) between non-AIP CP patients and healthy controls. Due to limited significance for diagnosis and treatment of CP, autoantibodies detection is not recommended conventionally unless suspected of AIP. Although these autoantibody biomarkers may not be very useful for diagnosis and treatment of CP up to now, we provided a reference value for clinical practice, and helped avoiding unnecessary laboratory examination to save medical resources.