Peer Review File

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Reviewer A

Comment 1: the paper is well structured and statistics is good. However, a major drawback of the paper is the IgG food intolerances; this is not validated by scientific studies and not supported by professional societies. In this regard, I suggest removing this analysis and keeping only the nutritional assessment of Crohn's disease patients.

Reply 1: Thank you for your comment. We do agree with this comment. But we don't want to delete them for the moment. Although there are few articles related to food intolerance, recent study have shown that controlling diet through food intolerance can improve the quality of life in IBD patients (Doi: 10.1093/ibd/izy110). It is a common test in China, We think it's worth doing this test.

Changes in the text: not change yet

Comment 2: With respect to nutritional parameters, it would be good to add (if available) subgroups analysis on serum iron, calcium, magnesium, ferritin, B12 and folate, vitamin D. Also, oral/enteral intake of patients should be taken into account in the setting of malabsorption (if available, according to a validated nutritional risk questionnaire such as NRS 2002).

Reply 2: Thank you for your suggestion. We have add subgroups analysis on serum iron, calcium, magnesium, ferritin, B12 and folate, vitamin D. Limited by retrospective study design, we didn't have data about oral/enteral intake of patients and validated nutritional risk questionnaire.

Changes in the text: see Page 6, line 134-138, Supplementary table 18-24.

Comment 3: Discussion section should be expanded. Exocrine pancreatic insufficiency in the setting of small bowel disease should be mentioned.

Reply 3: Thank you for your suggestion, exocrine pancreatic insufficiency is important, we add it into our manuscript.

Changes in the text: see Page 8-9, line 181-185.

Comment 4: The manuscript requires minor language polishing, otherwise language is good.

Reply 4: Thank you for your suggestion.

Changes in the text: we make a language polishin for manuscript.

Reviewer B

Comment 1: In this study, more than half (59.72%) of patients had L4 lesions, which was much higher than in previous studies. Are L4 lesions and their subtypes mutually exclusive without ileocolic lesions? Previous studies have shown that isolated upper gastrointestinal tract involvement is rare in Crohn's disease. Selection bias was suspected in this study.

Reply 1: Thank you for your suggestion. In our study, L4 patients may have L1-L3 lesions. We also describe definition of disease locations in the method. We dad strict inclusion and exclusion criteria, and the data collectors didn't know the detail of the study, they were only informed to collect relevant data. At the same time, we use flow chart to show our research design.

Changes in the text: see Page 3, line 63-64. Page 4, line 74-76. Table 1, Supplementary Figure 1.

Comment 2: Body weights and albumin levels were higher in L4 patients compared with non-L4 patients. These results showed that the nutritional status of L4 patients was better than non-L4 patients. The explanation of the results was not enough.

Reply 2: Thank you for your suggestion. For nutritional status, we supplementary add subgroups' analysis on BMI, serum iron, calcium, magnesium, ferritin, B12 and folate, vitamin D. We used exocrine pancreatic insufficiency to make discussion expanded.

Changes in the text: see Page 6, line 134-138, Supplementary table 11-24. see Page 8-9, line 181-185.

Comment 3: Perianal lesions were more in non-L4 patients than in L4 patients according to Tables 1 and 2. However, the author said that the L4 phenotype was more common in those with perianal lesions (line 259).

Reply 3: Thank you for your suggestion. It is a mistake, perianal lesions were more in non-L4 patients than in L4 patients.

Changes in the text: see Page 9, line 201-204.

Comment 4: When food allergies are investigated in a subgroup of the study population, it is recommended to perform a propensity score match to determine the relationship between the food

allergy and the L4 phenotype.

Reply 4: Thank you for your suggestion. Propensity score match is really a good analysis . But there are enough data on food intolerance in our groups, which may not be necessary to perform a propensity score match.

Changes in the text: not change yet.

Comment 5: Line 14: intolerance to albumin and 14 foods? I think you should change the sentence as "albumin, and intolerance 14 foods"

Reply 5: Thank you for your comment, we have change it.

Changes in the text: see Page 1, line 18-20.

Reviewer C

Comment 1: *Cohort selection

The authors report a high prevalence of L4 disease. It must be ensured that this is not merely the result of selection bias.

The manuscript should include a detailed description and/or flow-chart of how many patients were identified by the initial data search, and how many patients were subsequently excluded for specific reasons. What proportion of all patients diagnosed with Crohn's Disease at this center could be included in this study?

Inclusion criteria were: availability of both an endoscopic procedure AND imaging, OR surgery. This suggests a selection of patients at high risk for L4 disease, and may not be representative for the entire CD population. What were the indications for these procedures? How many patients were excluded for not having undergone these procedures?

Exclusion of patients positive for H. Pylori may cause selection bias due to its high prevalence.

Reply 1: Thank you for your suggestion. we use flow chart to show our research design. The description of inclusion criteria is controversial. This is our actual inclusion criteria: (1) Patients who

were first diagnosed with CD in our hospital. (2) availability of gastroscopy, ileocolonoscopy, double-balloon enteroscopy or capsule endoscopy and imaging (computer tomography, or magnetic resonance imaging) data, and (3) If patients were diagnosed with CD by operation, a complete operation reports of patients were needed. The inclusion criteria (2) and (3) were ensured that the including patients had a clear disease locations. Only 36 patients had positive H. Pylori, which unlikely can cause selection bias.

Changes in the text: See Flow chart: Supplementary Figure 1. For definition of history of surgery, see Page 4, line 80-83.

Comment 2: *Timeline

The aim of the study is to "evaluate clinical characteristics of L4 CD phenotype and its subgroups AT DIAGNOSIS" (line 73). However, "all complications were recorded during the disease or hospitalization (line 118)". In Table 1, "mean disease course" is 29.65 months (is this disease duration? Or months since onset of symptoms?). This is contradictory.

The manuscript does not provide data on specific timing of data-collection, procedures, etc. Meanwhile, patients diagnosed with CD were included between 2013 and 2019, thus allowing for 7 years of follow-up for some patients versus cross-sectional data only for others.

If the outcome (L4 disease) could indeed occur during follow-up, time-to-event analyses should have been performed (Kaplan Meier curves and Cox regression analyses) instead of logistic regression.

This is important, because for example the association between stricturing disease ~ L4 disease can be explained by

A) symptomatic strictures leading to more diagnostic procedures, resulting in a higher detection rate of L4 disease during follow-up OR

B) L4 disease genuinely being a risk factor for developing strictures later on OR

C) a higher co-occurrence of L4 disease and B2 disease at the time of diagnosis.

Reply 2: Thank you for your suggestion.For "all complications were recorded during the disease or hospitalization (line 118)". In Table 1, "mean disease course" is 29.65 months (is this disease duration?

Or months since onset of symptoms?). The description here is not accurate. We have corrected it.

For the follow-up data, We have no follow-up data at present. The main purpose of our study was to observe the disease characteristics of patients at the time of diagnosis, and in this study design , our patients were all diagnosed in our hospital.

Changes in the text: For complications: see Page 4, line 80. For disease course: see Page 4 line 77-78.

Comment 3: *Perianal disease

The authors conclude that perianal disease is a risk factor for L4 disease, while the data shows that perianal disease is protective for (i.e. inversely associated with) L4 disease (OR 0,63, table 1).

Reply 3: Thank you for your suggestion. It is a mistake, perianal lesions were more in non-L4 patients than in L4 patients. Table is right.

Changes in the text: see Page 9, line 201-204.

Comment 4: *Food intolerance

It is highly remarkable that ~40% of the CD patients included in this retrospective study had data available on food intolerance (ELISA assays). Please explain the indication for testing, and when this was performed (prior to diagnosis, at diagnosis, after diagnosis).

Moreover, >50% of the patients with available ELISA tests were found to be intolerant of tomatoes, soybeans, rice and eggs. Please explain if this was expected (e.g. compared to the general population) and, if possible, whether this correlated with symptomatic food intolerance. Is it possible that this is a result of L4 disease activity (caused by disruption of the mucosal barrier, resulting in increased antigen exposure?), rather than a risk factor for developing L4 disease?

Please explain if the association between tomato intolerance and L4 disease could be result of multiple testing and perform Bonferroni correction if indeed appropriate, as 14 food categories were tested for an association with L4 disease.

Reply 4: 1. IBD patients often have food intolerance. This test should be performed on all patients diagnosed with IBD. But it's not a mandatory inspection in our hospital, We only have data for patients

who would like to have the test.

2. It is not expected compared to the general population.

3. This test is performed when the patient is diagnosed with CD.

4. It is possible that this is a result of L4 disease activity. To our knowledge, It is thought that classic food intolerance is caused by food allergies based on IgE-mediated antibody responses; however, immediate allergic reactions are rare in IBD. Therefore, a delayed immune response mediated by IgG antibodies following exposure to a particular antigen may account for adverse food reactions in IBD (PMID: 25083057, 17918636, 1499910).

5. For the comparison of food intolerance between L4 and non L4, the tomato intolerance and L4 disease could not be result of multiple testing.

Changes in the text: not change yet.

Comment 5: *Higher weight and albumin levels in L4 disease

The authors conclude that L4 patients had higher weight and higher albumin levels at the time of diagnosis (contrary to what is expected). The authors conclude that this is possibly related to food intolerance and disease activity. It seems very unlikely that increased presence of food intolerance and increased inflammation would indeed explain higher body weight and albumin levels.

The authors reference two articles describing weight gain upon treatment with anti-TNF, but this is not comparable to the context of this study (presence of L4 disease at diagnosis, at which point one would expect malnutrition).

Reply 5: It is really not easy to explain. We also conducted a subgroup analysis of BMI, and L4 patients also had higher BMI, we used the exocrine pancreatic insufficiency to indeed explain higher body weight and albumin levels.. We had deleted the “This is consistent with a recent study showing that CD patients with markers of greater disease severity were more likely to gain weight(25,26)”.

Changes in the text: Delete references of 25 and 26, and corresponding sentences. For discussion: For BMI: See page 6, line 134-135. Supplementary material data Table 11-17 and 25. For add discussion about contradictory finding: see Page 8-9, line 181-185.

Comment 6: Minor comments

Comment 6.1: "Associations" would be a more appropriate descriptor of the relationship between L4 disease and the parameters studied than "risk factors".

Reply: we had change the word "Risk factors" into "Associations" and "relevant factors".

Changes in the text: see page 1, line 14 and 16. See page 2, line 38. See page 139, line 139, 143 and 146.

Comment 6.2: Please review the entire manuscript (including title, tables and figures) for grammar and style, as it contains misspellings and unfinished sentences.

Reply: Thank you for your suggestion. We have revised it.

Changes in the text: Thank you for your suggestion. We have revised it.

Comment 6.3: Please provide a definition for "disease course".

Reply: Thank you for your suggestion. We have provided a definition for "disease course".

Changes in the text: see Page 4 line 77-78.

Comment 6.4: Please provide a definition for "disease active period" and report the unit (days? Months?) in which it is reported in table 1.

Reply: The word "disease active period" is ambiguity. we change it into word (disease active state). simultaneous evaluation CDAI score of patients when they diagnosis with CD, CDAI score ≥ 150 was considered as disease active state.

Changes in the text: see Page 4 line 78-80, table 1 and table 2.

Comment 6.5: In table 1, mean body weight is 53 kg. What was the length and BMI?

Reply: thank you for your suggestion. We have provided BMI and analysis BMI on subgroups.

Changes in the text: see Page 6 line 134 -135, Supplementary material table 11 to 17.

Comment 6.6: Non-normally distributed data should be reported as medians (IQR) instead of mean (SD) and be tested with Mann-Whitney U tests.

Reply: Thank you for your suggestion. We have revised it.

Changes in the text: see Page 4 line 90 -92. Table 1 and Table 2, Fig 1B and Fig 2A.

Comment 6.7: This is a retrospective cohort study, not a case-control study.

Reply: Thank you for your suggestion. We have revised it.

Changes in the text: see Page 2 line 42.

Comment 6.8: In table 1, please report the numbers of L4 patients with concomitant L1,L2 or L3 disease

Reply: Thank you for your suggestion. We have revised it.

Changes in the text: see table 1 and table 2.

Comment 6.9: Figure 1B is confusing as the y-axis represents years for Age at onset and months for "disease course"

Reply: Thank you for your suggestion. We have revised it.

Changes in the text: see Figure 1B

Reviewer D

Comment 1: Abstract: It is not recommended to use unclear abbreviation (eg. EGD) in abstract.

Reply 1: Thank you for your suggestion, we have change it. Due to the frequent occurrence of upper gastrointestinal, we still intend to use abbreviations(L4) .

Changes in the text: See page 1, line 10.

Comment 2: Definitions: It is not clear for me if patients with two upper GI localisations (eg EGD and jejunum) were include in the study. And if yes, in which group were they involved.

Reply 2: We didn't make a clear description to this part. We have add it into manuscript. each patient can only enter one group. L4 - EGD was defined patients who had EGD lesions with/without any other gastrointestinal lesions. L4–jejunal was defined as jejunal but no with EGD involvement, and L4 - proximal ileal subtype included patients with proximal ileal involvement without that of jejunal or EGD.

Changes in the text: See page 3, line 63-64, page 4, line 74-76. For flow chart: Supplementary Figure 1.

Comment 3: FFood intolarence: Is there any information regarding gluten sensitivity and lactose intolerance? In my view these are clinically more significant states than eg tomato intolerance.

Furthermore, I wonder if patients had any clinical symptoms of food intolreance or they just had positive test results.

Reply 3: We didn't have any information regarding gluten sensitivity and lactose intolerance. To our knowledge, few patients had clinical symptoms of food intolerance, majority of patients just had positive test results. To our knowledge, It is thought that classic food intolerance is caused by food allergies based on IgE-mediated antibody responses; however, immediate allergic reactions are rare in IBD. Therefore, a delayed immune response mediated by IgG antibodies following exposure to a particular antigen may account for adverse food reactions in IBD (PMID: 25083057, 17918636, 1499910).

In our study, Food intolerance defined as serum IgG antibodies against tomato, rice, corn, egg, wheat, milk, pork, chicken, beef, crab, codfish, soybean, shrimp and mushroom. IgG levels > 50U/ml was considered positive.(using specific ELISA kits according to the manufacturer's instructions (Biomerica, Inc. USA)

Changes in the text: not change yet.

Comment 4: Characteristic: My mean problem with this article, that it is based on body weight. Body weight without taking into account eg. body height is NOT informative parameter of nutritional status. BMI is not even mentioned in this article (although BMI is still not the optimal marker of nutritional status). Calculation based on body weight is meaningless in this situation, without correction (eg. BMI) further conclusions cannot be drawn from the results.

Reply 4: Thank you for your suggestion, It is important. We have add the BMI into our manuscript.

Changes in the text: See page 6, line 134-135. Supplementary material data Table 11-17 and 25.

Comment 5: I did not find information about disease activity, medical and nutritional therapy . As these may modify significantly nutritional status they should be taken into account..

Reply 5: We preliminarily compared the disease activity of patients with CDAI score in table 1 and supplementary material table 1-6. For medical and nutritional therapy, patients may have had nutritional and medical treatment in other hospitals, but they have not been confirmed CD in those

hospitals.

Changes in the text: See table 1 and supplementary material table 1-6.

Comment 6: Authors should specify what surgical interventions were performed (due to stenosis, upper GI or lower GI, abscess etc.)

Reply 6: Thank you for your suggestion. We added it into table.

Changes in the text: see table 1 and table 2.

Comment 7: Risk factors: it is not clear whether eg. body weight is a cause or a consequence of the risk factors indicated. I suggest re-think of these factors..

Reply 7: we had change the word “Risk factors” into “Associations” and “relevant factors” as Reviewer C suggested.

Changes in the text: see page 1, line 14 and 16. See page 2, line 38. See page 139, line 139, 143 and 146.

Comment 8: Discussion, Line 236-239. In my view the explantation does not explain enough the contradictory finding. Further interpretation is needed

Reply 8: It is really not easy to explain. We also conducted a subgroup analysis of BMI, and L4 patients also had higher BMI, we re-discussed the results in the discussion.

Changes in the text: For add discussion about contradictory finding: see Page 8-9, line 181-185.