Author’s response to reviews

Title: Microscopic features of small bowel mucosa of patients with Crohn’s disease

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Author’s response to reviews:

Dear Vishal,

We greatly appreciate the interest of you and the reviewers in our manuscript (Microscopic features of small bowel mucosa of patients with Crohn’s disease, BMGE-D-19-00517). The reviewers’ comments have been very insightful and constructive, allowing us to address several issues that have been overlooked.

We responded to each of the reviewer’s comments and have incorporated all of their suggestions into the revised manuscript. Our point-by-point responses are given below.

We appreciate very much your further consideration of our manuscript and look forward to hearing from you.

Sincerely yours,

Xiao-yu Chen, M.D., Ph.D.
Dear Dr. Rizwan Ahamed,

We greatly appreciate your careful reading of our manuscript and your pertinent comments. Our point-by-point responses are given below.

Q1. It was very interesting to read the different types of granulomatous lymphangitis discovered in these set of patients. Can you make it clear if the types mentioned are actually separate entities or belong to the same spectrum of the disease pathology?

Answer: It was indeed very interesting to find granulomatous lymphangitis only on mucosa of Crohn's patients, which to some extent was a coincidence with Dr. Van Kruiningen’s report. We agree that granulomatous lymphangitis is the essential change of Crohn's disease. Our study mainly described the morphology of granulomatous lymphangitis and pointed out that there were different forms of granulomatous lymphangitis on small bowel mucosa of Crohn's patients. We consider it is a certain stage during the development of Crohn's disease and belong to the same spectrum of the disease pathology, but not a separate entity. All these forms of granulomatous lymphangitis showed that macrophages accumulated in lymphatics and it could be see as a manifestation of macrophages’ dysfunction while the latter is an important aspect of CD’s pathogenesis.

Q2. I understand this study was mainly intended as a pathological analysis, however some more data could have been presented on the clinical status of these patients. For instance, the Crohn's Disease Activity Index (CDAI) of these set of patients have not been mentioned.

Answer: We appreciate that you give a good suggestion to our study. Though the Crohn's disease activity index (CDAI) is the gold standard for evaluating the clinical condition of Crohn's disease (Crohn's), it will take a week to observe the parameters. Commonly we use the Harvey-Bradshaw index to evaluate the CD patients in our hospital. It was reported that results from the Crohn's Disease Activity Index were correlated with those from the Harvey-Bradshaw Index. As supplementary, we will add the Harvey-Bradshaw indices and other aspects of CD patients as “Additional table 1” in the manuscript (Result section, page 6, line 129-131).

Q3. Did all the patients undergo double balloon enteroscopy irrespective of their clinical presentation?

Answer: A good question. Actually in our hospital, not all the patients undergo double balloon enteroscopy irrespective of their clinical presentation. Commonly ileocolonoscopy with multiple biopsy specimens is the first-line procedure for diagnosing CD. When there is severe, active disease, the value of full colonscopy is limited by a higher risk of bowel perforation. In these circumstances initial exible sigmoidoscopy is safer and ileocolonoscopy should be postponed until the clinical condition improves. Capsule endoscopy and enteroscopy with biopsy are
tolerated well and useful procedures for the diagnosis of CD in selected patients with suggestive symptoms after failure of radiological examinations.

Q4. What was the indication for enteroscopy in the patients? How was small bowel Crohn's diagnosed?
Answer: We conducted the enteroscopy according to the Chinese guideline for the clinical application of enteroscopy. Here we listed the indications for enteroscopy in our country.

1. Potential small bowel hemorrhage
2. Suspected Crohn's disease
3. Unexplained diarrhea or protein loss
4. Suspected malabsorption (such as celiac disease)
5. Suspected small bowel tumor or proliferative lesion
6. Small bowel obstruction with unknown cause
7. Abnormal conditions (such as bleeding, obstruction, etc.) after intestinal surgery
8. Clinical examination suggested the possibility of organic lesions in small bowel
9. Review after treatment of confirmed small bowel lesions (e.g. Crohn's disease, polyps, vascular malformations, etc.)
10. Treatment of small bowel disease: polypectomy of small bowel, removal of small bowel foreign bodies (such as capsule endoscopy), treatment of small bowel vascular diseases, dilatation of small bowel, etc
11. Postoperative changes in the anatomical structure of the digestive tract resulted in the failure of ERCP by duodenoscopy.

There is not a single gold standard for the diagnosis of small bowel Crohn's disease. The diagnosis is confirmed by clinical evaluation and a combination of endoscopic, histological, radiological and/or biochemical investigations. Genetic or serological testing is currently not used for routine diagnosis of CD. Our standard for the diagnosis of Crohn's disease is consistent with the 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn’s Disease 2016.

Q5. How was the protocol of taking biopsies decided? Were they random biopsies or based on mucosal changes noted on endoscopy?
Answer: As for the patients being considered or suspected of Crohn’s Disease, the protocol of taking biopsies is according to “the 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn’s Disease 2016” and “Proposal of standardized pathological
diagnosis for inflammatory bowel disease through biopsies” of China. Generally at least two biopsies from five sites around the colon (including the rectum) as well as from the ileum were obtained. In other conditions, at least two biopsies were taken based on mucosal changes noted on endoscopy. Generally the clinicians did not take biopsies randomly.

To Reviewer 2 (Professor HJ Van Kruiningen)

Dear Professor HJ Van Kruiningen,

We greatly appreciate your careful reading of our manuscript and your pertinent comments. Before we start writing this manuscript, we have read many of your papers about Crohn's disease. We feel the same way that granulomatous lymphangitis are fundamental lesions in Crohn's disease. Actually before we start this study, we have noticed this interesting phenomenon (granulomatous lymphangitis) on the small mucosa of Crohn's patients for a long time. But the detailed morphological description of granulomatous lymphangitis especially on the small bowel mucosa and the diagnostic significance of this pathologic phenomenon have not been well illustrated yet. A lot of papers which included sections about the pathologic features of Crohn's disease even didn’t mention this changes whereas granuloma was the most frequently used pathologic feature. Granulomatous lymphangitis must be neglected or still have not been well recognized. We think it is not excessive to make more efforts to clarify its morphology and significance. Our point-by-point responses are given below.

Q1. “The authors have gone to extremes to quantify things that are subjective or that vary from one section to the next, such as numbers of these cells vs those cells, intra-epithelial neutrophils, basal plasmacytosis, crypt abnormality, goblet cells etc.”

Answer: That’s true. To some extent, the pathologic morphologies are subjective and it may result in the discrepancy among pathologists. Till now, it is still a problem afflicting pathologists on both tumorous and inflammatory lesions. That’s why we need to set control groups both in observational and experimental studies. Even though all the 21 pathologic parameters we selected in our study were not specific to Crohn's disease, the significant difference of the certain features between CD and the matched control groups meant something. Furthermore, these pathologic features in our study were not the first time to be used and they have already been analyzed in many other literatures. Here we take five recent literatures for examples.


Q2. “they have not acknowledged that Crohn's disease is a submucosal and intramuscular disease. Overly quantifying cells of the mucosa and aggregates of them is not helpful; cuts of resection specimens would be much more relevant, but also impossible to quantitate.”
Answer: On the contrary, we agree with you that Crohn's disease is a submucosal and intramuscular disease. We also paid special attention to the pathologic changes in submucosa. Firstly we compared the biopsy depth including mucosa, muscularis mucosa and submucosa among three groups. Next we analyzed the inflammation depth, submucosal lymphocytes aggregation, submucosal fibrosis, ganglion cells and so on because we know that many pathologic features of Crohn's disease were beyond the mucosa. However the appropriate way to evaluate submucosal changes was on resection specimens but not on biopsies. Even though we tried to get more information on the biopsy specimens, the findings in submucosa were limited. As you mentioned, counting cells of mucosa and aggregates of them may be not helpful. Our aim is to highlight that the number of macrophages in granulomatous lymphangitis may decrease to just a few. It should not be neglected in this condition. We consider this kind of illustration could help others recognize granulomatous lymphangitis more clearly.

Q3. Suspected CD is also too subjective. You need to study clearly defined CD, established by several methods, and forget about suspected material.
Answer: Actually the term of suspected CD is use by clinicians. We never use this term in pathologic diagnosis. It is true that the diagnosis of Crohn's disease is based on several other methods including clinical presentation, image examination and enteroscopy examination. The diagnostic criteria for Crohn's disease in our study were consistent with the 3rd European evidence-based consensus on the diagnosis and management of Crohn’s disease (2016). The key of our study is to describe the detailed morphologies of granulomatous lymphangitis and point out its significance in diagnosis of Crohn's disease. As the data showed in our study, one patient with granulomatous lymphangitis was originally clinically diagnosed as suspected Crohn’s disease. If we forget the case in this group, it would not reflect the reality and might be unobjective.

Q4. To give statistical significance to "uneven inflammation" is the epitome of subjectivity; foolish.
Answer: Anyway, we greatly appreciate your serious comments. It is generally known that ulcerative colitis is a continuous lesion and Crohn's disease is a kind of segmental lesion. In addition to the discontinuity of the lesion site, the inflammatory pattern is not continuous in Crohn's disease. Specific to the biopsy mucosa, the inflammatory pattern among biopsies, even on the same tissue, may be different, focal or uneven. In ulcerative colitis, the biopsy mucosa generally showed continuous or even inflammatory pattern as chronic colitis without normal
mucosa except treated cases. We can’t deny the subjectivity of all these pathologic features and we try to be objective to compare their occurrence in matched control groups.

To Reviewer 3 (Prof Kaushal)

Dear Professor Kaushal,
We greatly appreciate your careful reading of our manuscript and your approval of our research.

To Reviewer 4 (Arvind Ahuja, M.D.)

Dear Dr. Arvind Ahuja,
We greatly appreciate your careful reading of our manuscript and your pertinent comments. Our point-by-point responses are given below.

Q1. Van Kruiningen's have shown granulomatous lymphangitis in 53% of resection specimens out of 34 cases studied. In the present study granulomatous lymphangitis was present in approx 24% of biopsies with Crohn's disease. However, further large studies on resection specimens are required to validate this finding.

Answer: It is a good suggestion to validate this finding on resection specimens. Actually we have already started to study it on resection specimens. The preliminary data showed that granulomatous lymphangitis could present in all layers of the bowel wall including mucosa, submucosa, muscularis propria and subserosa. In small bowel, the most common site is villi. But in colon, the most common site is submucosa. 53% is a total rate in Dr. Van Kruiningen's research. We will analyze it in each layer of the bowel wall. In the daily pathologic work, we encountered more biopsies than resection specimens with Crohn’s disease. Therefore, to know well with this structure on biopsy specimens is important and meaningful which can be much helpful in diagnosis and differential diagnosis of Crohn’s disease. We hope this study could help others better understanding the microscopic characteristics of Crohn’s disease and find more clues pointing to Crohn’s disease, especially before surgery. So we consider it is necessary to pay more attention to the mucosal changes and it might be better to deal with the data on biopsy specimens as a whole. For the research on resection specimens, we haven’t finished yet. We hope you can review our data on resection specimens and the comparison with biopsy specimens in the near future.

Q2. Granulomatous lymphangitis may be a non specific finding and it is likely to be present in infection related ileitis; however again it needs to be studied in large cohort.

Answer: We searched on the pubmed database using granulomatous lymphangitis as the key word and found that granulomatous lymphangitis was indeed related to infectious disease, especially from animal cases. It is reported that murine norovirus (MNV)-4 infection could induce granulomatous lymphangitis and ileitis that similar to Crohn’s disease in mice. But few studies about the relationship between granulomatous lymphangitis and infection for example tuberculosis in human have been reported. No evidences can rule out this possibility either. At
the present, granulomatous lymphangitis may be a non-specific finding and we also consider its possible relationship with infection in human, so we discuss its value in the manuscript and it indeed needs more studies in large cohort.

Q3. In a review by Kedia et al concluded that micogranuloma are important histological features in CD but in tuberculosis endemic regions the diagnosis of Crohns disease is always clinic-pathological, endoscopic and radiological.
Answer: We agree that micogranuloma are important histological features in CD. In tuberculosis endemic regions for example in China, the differential diagnosis of CD and intestinal tuberculosis is still a challenge job. There is not a single gold standard for the diagnosis of Crohn's disease. Our standard for the diagnosis of Crohn's disease is consistent with the 3rd European Evidence-based Consensus on the Diagnosis and Management of Crohn’s Disease 2016. The diagnosis is confirmed by clinical evaluation and a combination of endoscopic, histological, radiological and/or biochemical investigations. Chest CT and T-SPOT was conducted as routine. Tuberculosis and the possibility of it need to be ruled out before the diagnosis of Crohn's disease.

Q4. The IHC pictures are not clear and I am not able to see the positive reaction (Fig 2D,E).
Answer: Here we post only Fig 2D and E and add the arrow symbol in the picture to show clearly the immunohistochemistry positive cells (see the supplementary material ). The arrow symbol was also be added in Fig 2D and E in the manuscript. In Fig 2D, the arrow is pointing to CD68+ macrophages in the lumina of a lymphatic vessel. In Fig 2E, the arrow is pointing to the endothelial cells of a lymphatic vessel with D2-40 expression in the center of a villus. The corresponding revision for figure legends was shown at figure legends section, page 19, line 365-367. We can see here that the structure of granulomatous lymphangitis is very tender. As we discussed in the manuscript, the morphology of granulomatous lymphangitis on biopsies including the number of cells can vary even between different levels of the same tissue. Therefore to know the histopathological morphologies of granulomatous lymphangitis on hematoxylin and eosin-stained sections is more important than the immunohistochemical result.

Q5. Authors should include important clinical and endoscopic findings based on which they diagnosed CD in 137 patients.
Answer: We appreciate that you give a good suggestion to our study. We agree that the clinical and endoscopic findings are important information when we diagnose CD. So we collected the data and added these information and other aspects of CD patients as “Additional table 1” in the manuscript (Result section, page 6, line 129-131).

Q6. It will be interesting to know in how many patients lymphadenopathy was seen out of 24 patients with granuloma.
Answer: We analyzed the rate of lymphadenopathy in all 137 CD patients and added these data in “Additional table 1” in the manuscript (Result section, page 6, line 129-131). Additionally in the 24 patients with granuloma, it is 54.17% (13/24).