

January 15th, 2014

Dear Editor,

With reference to our correspondence regarding language certification for non-native English speakers, I hereby re-submit the manuscript after it has been edited and certified by the American Journal Experts editing service. Please find enclosed the edited manuscript of this date in Word format (file name: 6295-review.doc) along with the language certificate. Please also use this letter of today and not the letter of December 29th 2013 for page references regarding amendments suggested by your reviewers.

Title: Routine diagnosis of intestinal tuberculosis and Crohn's Disease in Southern India

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers, and all comments have been attended to.

We would have wished for more complete data sets with regards to qualitative variables and follow-up investigations. However, as our aim was to describe current practice in Southern India, the reality differs from the "gold standard".

In the revised manuscript, text deleted from the original manuscript has been lined over (~~lined over~~), while any new text added to the manuscript is highlighted with yellow.

Reviewer 1 (Reviewer No. 00227582):

Answers to general comments:

- The study was not done blinded and therefore bias could have interfered. The study was performed in a clinical setting, describing current practice of diagnosing intestinal tuberculosis (ITB) and differentiating it from Crohn's Disease (CD) in Southern India. Still, we believe that including four different centres does reduce bias to some extent. The nature of the study has been clarified in the revised manuscript on page 6, lines 7-10: "(...) were prospectively included from four South Indian medical centres in a consecutive manner (...). Centre investigators identified patients with ITB or CD. The inclusion/exclusion criteria were thoroughly discussed at meetings and clarified in the study protocol".
- We agree that some of the variables could have been quantified to a greater extent. This has been eluded on page 7, lines 36-37: "Weight measurements at examination revealed lower

body weight in the ITB patients (median: 52 kg, IQR 13) than in the CD patients (median: 59 kg, IQR 12), $P=0.001$.”; page 10 lines 5-9: “Weight loss was reported more frequently in ITB patients, and their body weight at examination was lower than in the CD patients. Data of our patients’ weight prior to receiving care was unavailable. Thus, whether the ITB patients’ baseline body weight prior to illness was lower than in the CD patients is unknown.”; and page 11, lines 23-27: “Of the four variables found to be predictive of disease, “pain” and “nodularity” could not be further sub-classified. Conversely, “weight loss” and “number of involved intestinal segments” allowed for objective estimation: a significant difference in body weight was found between the groups; and the endoscopists scored the number of involved segments according to pre-defined anatomic locations.”

Answers to specific comments:

- Page 7, lines 12-14: “We considered our study to be an exploratory analysis, therefore $P < 0.05$ was considered statistically significant and we did not perform any correction for multiple testing.” This is a valid justification according to our biomedical statistician (MC Småstuen, co-author).
- We are not quite sure what the reviewer means by classifying the variables in a “2, 3 or 4-dimensional space”. If inclusion of more variables in the multiple regression analysis is addressed, we are afraid that the relatively small cohorts of 37 or 38 cases do not allow for testing more than two variables at a time, considering the response numbers in the data sets. Having said that, we have tried to group patients according to values of all the mentioned variables but have not found any other variable sets with statistical significant difference between the groups. At last, three of the variables showing significant difference between the groups in the univariate models are mentioned in the revised manuscript on page 8, lines 32-33: “Variables such as malaise, nausea and watery diarrhoea were excluded from the models due to their confounding potential.”

Reviewer 2 (Reviewer No. 00058696):

- Comment 1: “The authors have not clearly outlined a hypothesis”.
As this study is descriptive we do not find it right to state a hypothesis or investigate for any causal relationships.
- Comment 2: “The authors do not summarize inclusion or exclusion criteria for selection of patients, and the authors do not describe the treatment regimens used during the study.”
In the revised manuscript we have listed the inclusion and exclusion criteria used for selection of patients, c.f. page 19, Table 1. The treatment regimen used for the ITB patients is in the revised manuscript referred to on page 6, lines 12-14: “All ITB patients were prescribed ATT for six months according to the Indian Revised National Tuberculosis Control Program”. As for the CD patients, therapy was individualised and could not be systematically sub-classified to give any additional information in our limited cohort of 37 patients.
- Comment 3: “The authors do not mention the lack of intended follow-up and high dropout rate as a limitation.”
Lack of follow-up and dropout rate have been discussed on page 7, lines 25-29: “In total, 24 of 75 patients were lost to follow-up clinical evaluation: 19 ITB and 5 CD patients. Follow-up endoscopy was only achieved in the minority of the patients, as many either refused repeat endoscopy or were lost to follow-up after clinical evaluation at 2 months. Two patients initially treated with ATT had their diagnosis revised to CD after endoscopy at 6 months. None of the CD patients had their diagnosis revised to ITB.”; and on page 11 lines 33-35: “. Despite repeated attempts to contact patients for scheduling appointments,

one third of the patients were lost to follow-up. Hence, we are unaware of their clinical course and could not confirm their diagnosis”.

- General comments:

“The authors frequently alternate single and double-spacing after paragraph breaks. We suggest that they be consistent with spacing, using only one or the other.”

In the revised manuscript we have consistently used single spacing after paragraphs.

- Specific comments:

“Page 9, lines 14-29: consider grouping together to form one paragraph instead of breaking to form new paragraphs that contain only one sentence. Page 9, lines 26-27: move period to end of sentence, after “(Appendix, Figure 1)” Page 10, line 6: move period to end of sentence, after “(Table 3)” Page 10, line 21: move period to end of sentence, after “(Appendix, Table 4a)” Page 11, line 5: remove period between “CI 1.7-20.6” and (Appendix, Table 4b)” Page 12, line 18: Replace “Alike” with “Unlike” Page 13, lines 19-24: Consider consolidating these two paragraphs into one paragraph. Page 14, lines 1-8: Consider consolidating these three paragraphs into one paragraph. Page 15, lines 12-19: Consider consolidating these two paragraphs into one paragraph.”

All suggestions have been taken into account, except for Page 12, line 18: “Alike” has been replaced with “As in” (revised manuscript page 9, line 34).

Reviewer 3 (Reviewer No. not available, reviewed on 2013-11-19 22:34):

- General comment:

“Several earlier studies have attempted to evaluate differences between intestinal TB and Crohn's, in order to facilitate differential diagnosis. Such studies suffer from a common problem: circular reasoning. That is, the investigators make a diagnosis, then check out the differences between the two diagnosed groups, then state that the differences help to make a diagnosis.”

In the revised manuscript, we refer to this problem and stress that currently known differences do not necessarily help to make a diagnosis, c.f. page 12 lines 28-34: “Clinical features distinguishing between the diseases may overlap, and a range of other diseases may present identically to ITB and CD. Simple, inexpensive and rapid diagnostic modalities for diagnosing and differentiating between these diseases, applicable in populations of both high and low TB endemicity are needed for the differentiation and diagnosis of ITB and CD”

- Specific comments:

1. “What were the precise criteria by which the initial diagnosis was made in each patient, especially since the authors have started treatment for Crohn's in an equal number of patients in a country where intestinal TB is commonly believed to be more common than Crohn's?”

In the revised manuscript, inclusion criteria have been listed in a new Table 1 on page 19.

2. “What were the specific follow-up features that made the investigator confirm or change the diagnosis?”

Follow-up and confirmation of diagnosis is discussed in the revised manuscript, page 6, lines 30-31: “Treatment response was evaluated on clinical grounds, with improvement in signs and symptoms being regarded as confirmatory for diagnosis.”

3. How many patients did not have a follow-up colonoscopy? How was the diagnosis confirmed in these patients?

Sixty-five patients did not have a follow-up colonoscopy. Many patients refused repeated

invasive investigations. Diagnosis was confirmed by clinical evaluation of symptoms and signs.

4. A third of the patients were lost to follow up. The authors do not discuss this group -- what measures were taken to contact them, why did they drop out, etc. Could they have not improved or actually worsened on treatment, making them drop out? That casts a shadow on the initial diagnosis.

We agree that the limited follow-up casts a shadow on the final diagnosis and have discussed this in the revised manuscript on page 11, lines 33-35: ". Despite repeated attempts to contact patients for scheduling appointments, one third of the patients were lost to follow-up. Hence, we are unaware of their clinical course and could not confirm their diagnosis."

5. The authors state that some of the traditional methods used to diagnose TB are now obsolete. In the list they include AFB studies and MTb culture. In fact, I would believe that these tests have a strong specificity and positive predictive value. I would have liked it if the authors had included these tests in their battery.

The expression "obsolete" has been modified in the revised manuscript on page 5, lines 33-34: "Traditional diagnostic modalities: (...); are often negative in extra-pulmonary TB". As the aim of the study has been more clearly addressed in the revised manuscript we would like to convey that the study describes the diagnostic methods currently applied in the region and not necessarily the "gold standard" of ITB diagnosis: Page 6, lines 2-4: "In this prospective multi-centre study, we evaluated routine clinical, endoscopic and laboratory variables currently applied for diagnosing ITB in Southern India. Using these variables we evaluated the ability to differentiate ITB from CD"; and in more detail on page 6, lines 8-10: "Although generally recommended in the literature, *M.tb* specific microbiologic diagnostics were not routinely applied as our study was descriptive and sought to reflect clinical practice in the region." Furthermore, we have discussed the traditional methods in more detail in the revised manuscript, page 11, lines 36-37 and page 12, lines 1-3: "As we sought to describe the current clinical practice in Southern India, only diagnostic modalities routinely available were evaluated. Although positive acid-fast staining and/or culturing of *M.tb* from intestinal biopsies have high positive predictive values for TB diagnosis, these methods were not applied, possibly due to their low yield shown in previous studies [1,5,14]. Additionally, it may be unfavourable to leave a patient untreated pending the result of a slow growing *M.tb* culture."

6. Chest radiography could reveal associated pulmonary TB. CT or MR enterography provides valuable information in these diseases, e.g., necrotic nodes, mesenteric stranding, number and length of segments involved. Why didn't the authors consider these tests, which are commonly done in such situations?

Chest radiography has been discussed in the revised manuscript on page 12 lines 3-5: "As negative chest radiographs may not rule out ITB, routine chest x-ray was not performed in our cohort. Chest symptoms were recorded in four of our 38 ITB patients."

Indeed, CT or MR enterography may bring valuable information to aid in ITB or CD diagnosis. Even so, these investigations are regarded as quite costly and require experienced staff not readily available in the region. Hence, as stated on page 12, lines 5-6: "CT or MR enterography was not applied in the participating centres during the study period."

7. The Introduction and Discussion tend to be verbose. The authors should highlight how

their study achieves anything different from the several other studies from the same country addressing this issue - each one coming up with different features that they say help to differentiate intestinal TB from Crohn's

The introduction has been shortened in the revised manuscript. As we have tried our best to answer the reviewers comments, the discussion has been slightly extended in the revised manuscript.

We have commented on the similar disease characteristics found in other studies on ITB and CD from the region in the revised manuscript on page 12, lines 23-24: "The features of our patients are consistent with previous descriptions of ITB and CD from the Indian subcontinent." This is followed by a suggestion on the rise of CD in the region, c.f. page 12, lines 24-26: "Interestingly, nearly as many CD patients as ITB patients were recruited from the secondary and tertiary centres of this study. This result suggests that CD is increasing in South India."

3 References and typesetting were corrected according to the revision policies of BPG.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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