Author’s response to reviews

Title: Ultrasonographic detection and assessment of the severity of Crohn's disease recurrence after ileal resection

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Version: 2 Date: 1 April 2010

Author’s response to reviews: see over
Dear Doctor Rachel Neilan,

We thank the two Reviewers for their in depth analysis of our work. Many of the queries have been appreciated and several suggestions are useful to improve the revised text.

We submit the revised manuscript conformed to the journal style, that takes into consideration the comments and suggestions of the reviewers. Changes have been underlined to facilitate the Editors. In addition we have addressed the ethical approval, the statement of competing Interests, and Authors’ contributions. All authors read and approved the final manuscript.

Enclosed is the point by point response to the Reviewers’ comments.

On behalf of all contributing Authors,

With best regards

Prof Enrico Corazziari

Point by point response

Reviewer OH Gilja

1. The introduction is OK and to the point and refers to previous relevant work, but could be better if it incorporated some of the points that should be cut from the start of the Discussion

As suggested some points of the discussion has been incorporated in the introduction

2. Of the four study questions posed, question 1 is poorly formulated. What does limited and minimal involvement really mean? Furthermore, if one wants to detect the initial/early recurrence, the method described is not appropriate. The patients are examined from 6 months up to 2 years after resection and the author has no way of knowing if the changes seen are early or initial. One would expect that the changes became more severe the further away from the operation date the patients were examined. Actually this is also shown by data in this study as Median time from surgery increases significantly with increasing Rutgeert score (Table 2). The title indicates that this method is used for early detection of recurrence of Crohns disease after ileal resection while the study is not designed for this. It should be more in the line of "Detection of recurrence....and so forth"

In Question 1 there is no mention of the word “early” but it reads “ initial, i.e. with minimal and limited involvement, recurrence of CD lesions”. Initial refers to the initial or low-grade manifestation of the CD recurrent inflammatory lesion that are limited to ICA. It does not refer to the time elapsed since the operation. Thus an initial low-grade involvement of ICA or neoterminal ileum may occur at any time after operation. As a matter of fact, although the progression of the disease is time related, the onset and rate of the progression may be extremely variable in any individual patient. Indeed in the present study 17% of patients had no recurrence (i.e. score 0) and 5% initial minimal lesions (i.e score 1) at follow-up period after surgery of 50±23 months (range 12-72) and 52±30 months (range 18-72), respectively. So the great majority, but not all recurrent
inflammatory lesions become more severe as time from operation progresses. The evidence that SICUS is able to detect initial or minimal/low-grade manifestation of CD recurrence is in the results of this study where an ICA wall thickness equal or greater than 3.5 mm completely discriminates endoscopic grade 1 (with \( \leq 5 \) mucosal aphtae) from grade 0. So we maintain question 1 and delete from the title the word “early” as it may generate confusion between initial/minimal lesion and a short time interval since the operation. We rephrased the question according to the Reviewer suggestion. Nonetheless there is no doubt that a relevant finding of this study is that a minimal involvement of the ICA precedes extension of the CD recurrent inflammation in the neo-terminal ileum and at SICUS an ICA wall thickening is detected earlier than the US modality used until now to assess wall thickness at level of the neo-terminal ileum.

For the reasons indicted by the reviewer the expression transmural is not correct unless the lesion is in fact transmural. The adjective intramural, i.e.” being within the substance of the wall” seems more appropriate since there is no commitment to which part of the wall the thickening is referred to. The text has been revised accordingly.

3. The expression “transmural” is introduced in the first paragraph of the introduction is used through the article as synonymous to wall thickening on SICUS. This is not correct as thickening of the gastrointestinal may be detected on ultrasound even though the pathological changes are not transmural. The thickening can be solely caused by edema in the mucosa and submucosa without involvement of the proper muscle and would then not be considered as

The patients evaluated were consecutive patients. The sentence has been correctly rephrased. During the follow-up period 58 CD consecutive patients referred to our IBD center underwent surgery and were thereafter followed until nowadays and then submitted to repetitive SICUS and endoscopy evaluations. In the analysis we included all the paired SICUS and ileocolonoscopy examinations performed within a 2 week interval during the follow-up period. Since not all patients underwent colonoscopy at the scheduled time, 18/58 patients performed 1 SICUS-ileocolonoscopy paired examination, during a follow-up period of 35±33 months (range 6-100), 25/58 2 examinations during a follow-up period of 44.6±24.3 months (range 12-99), 13/58 3 examinations during a follow-up period of 59±20.5 months (range 32-94) and 1/58 4 examination during a follow-up period of 56 months for a total of 111 paired evaluations.

4. The patient population in the material and methods (M&M) section is poorly and insufficiently described (Page 3, paragraph 6). There is no explanation as to how and when the patients were recruited, if this was consecutive from a cohort or on a case by case basis.

5. How many patients were excluded and for what reasons?
No patient who underwent surgery was excluded. Analysis of SICUS and endoscopy was limited to the paired SICUS-ileocolonoscopy examinations performed in a two week time frame. Some of the patients for inability or refusal did not perform at scheduled time the colonoscopy and this behaviour explains why not all patients were examined at the same time interval from the operation.

6. It is not clear in the M&M what is actually measured in the anastomosis (Page 4, paragraph 5)
Each ileal and colonic limb were separately measured from the mucosa to the outer rim of the muscularis propria, as indicated in Fig 1A were the calipers are positioned for the measurements, respectively 1.9 mm and 1.2 mm, reported in the figure itself that does indeed depict the ICA and not a fold. A sentence has been added to better clarify the modality of measurement.
7. Definition of bowel stenosis

This definition has been previously published and the measure of <1 cm is based on the results of a previous study performed with identical SICUS method in healthy subjects (See Reference 5). The sentence has been also rephrased.

8. I wonder why a higher frequency probe was not used

The aim of the study was not to assess in details the structure of intestinal wall at the level of ICA and neo-terminal ileum but just the thickness and extension of increased wall thickness at the level of neo-terminal ileum. For this purpose in presence of oral contrast distending the intestinal lumen allowing an optimal visualization of wall and an apparatus that can detect a BWT variation of 0.1 mm, the intestinal wall is properly delineated with the 3.5 MHz and 5 MHz probes. As correctly stated by the Reviewer the normal value of intestinal wall may be only of 1 mm. In the present study SICUS has been performed with the same technique and probes used to define normal values of small bowel wall thickness in healthy subjects (Pallotta et al Lancet 1999, Pallotta et al Ultrasound Med Biol 1999, Pallotta et al J Ultrasound Med 2000). Based on this normal values SICUS has been proven to accurately measure the extension of increased wall thickness as demonstrated by comparative surgical findings (Pallotta N et al IBD 2005). Furthermore several Authors used, even in absence of oral contrast, the same probes (Gashe G et al Gut 1999;44:112-117, 3.5 MHz and 5-10 MHz; Maconi G et al AJG 2003; 98:1546-1555. Parente F et al Gut 2002;50:490-495 Parente F et al Gut 2004;53:1652-1657 3.5 MHz and 7.5 MHz; Di Sabatino A et al IBD 2004;10:573-577 Castiglione F et al IBD Rispo A et al IBD 2006;12:486-489 5-7.5 MHz). A sentence has been added to better clarify the propriety of US equipment

9. There is no indication as to why the author would choose to dichotomise the wall thickness data with a cut off of 3.5 mm with a reference to previous studies in the text (page 5, paragraph 4)

The standardized definition of pathological wall thickening of the small bowel including the ileum is 3.0 mm. The normal wall thickness of the ICA has not been reported before this study. The wall thickness was dichotomised with a cut-off of 3.5 mm based on preliminary analysis showing that this cut-off value was the one better discriminating those with score 0 from those with score>0. In fact, about 93% of scores (i.e., 0 and 1-4) were correctly classified with this cut-off. This choice was not based on any previous reference. Text was revised to better explain this choice

10. It is not clear from the descriptive data when all the 111 different examinations were performed or if some patients were examined more than twice. And I wonder if Fischer's exact test be performed on variables which contain a mix of dependent an independent values? Regarding the results from the multiple regression analysis I would leave it to a statistician to evaluate if the criteria for using multiple regression and ordinal logistic regression have been met.

The 111 examinations refer to the 58 patients (see response #4). Methods section was revised to better explain the study design. All statistical methods reported in this manuscript took into account the repeated measurements adjusting the standard errors for the repeated measurements at different times for the same patient (see reference 11). However, we realized that a sentence (page 5, last paragraph) erroneously remained from a previous version of the manuscript and this does not refer to any analysis of the
current version of the manuscript. This paragraph has been deleted in the new version of the manuscript. All analyses were performed by a Statistician (Patrizio Pezzotti)
Dstat (previously misspelled Dste) is the Italian acronym for Doctor in statistics

11. Previous relevant work is acknowledged, but the author states that MRI does not have resolution to show early recurrence while supporting the claim with a reference on CT enterography

We acknowledge the mistake in the citation. It has been properly corrected.

12. Furthermore, limitations are not discussed properly Inter-observer variation is mentioned briefly, but the author argues that this may be amended by reducing intra-observer variability, which is not the case. I could think of a number of weaknesses beside this.

We did not argue that a low intra-observer variability can amend the inter-observer variability. Probably the sentence as written may create confusion and it has been now rephrased.

13. The patients are for instance examined at different time instances. This means that the authors can not be sure if the findings represent early or initial...

See our response to query #2. A sentence has been added in the discussion to highlight that at SICUS assessment of ICA wall thickness can detect earlier postoperative recurrence than assessment of the neoterminal ileum. Results of the study indicate that SICUS can detect minimal recurrent lesions and also early lesions if one considers that minimal lesions score 1 were detected in 31% of the patients at the first observation performed 6 months after surgery.

Major compulsory revision.
See response to previous queries. Manuscript revised in accordance

Minor essential revisions

1. The figure clearly shows the structure and the relevant findings properly indicated. Calipers show the effective measurements performed in this image.

2. Figure 3: The cut off values that offer the best sensitivity and specificity should be added to the ROC curves.

The Reviewer cites Figure 3 instead of Figure 4 that shows two ROC curves. The ROC curves reported refer to the results of a logistic model with two independent continuous variables: the extension of increased wall thickness at the level of the neo-terminal ileum and wall thickness values at the level of the ileo-colonic anastomosis. In this case, compared to a classical non-parametric ROC curve evaluating the accuracy of a single predictor on predicting a dichotomous outcome, there is not a cut-off value that can be identified and we can only show the predicted probabilities varying one of the two variables having fixed the other one.

Reviewer L Guidi

1. Did the 58 patients experience a colonoscopy every 6-12 months after the first follow-up after surgery?
2. How long have been the patients followed? According to the statement in the second paragraph of the results section, the longest follow up was 24 months, so how do the Authors explain the median interval from surgery and evaluation reported in table each Rutgeerts score (33.3/36/59.6 months respectively)?

During the follow-up period 58 CD consecutive patients referred to our IBD center underwent surgery and were thereafter followed until nowadays and then submitted to repetitive SICUS and endoscopy evaluations. In the analysis we included all the paired SICUS and ileocolonoscopy examinations performed within a 2 week interval during the follow-up period. Since not all patients underwent colonoscopy at the scheduled time, 18/58 patients performed 1 SICUS-ileocolonoscopy paired examination, during a follow-up period of 35±33 months (range 6-100), 25/58 2 examinations during a follow-up period of 44.6±24.3 months (range 12-99), 13/58 3 examinations during a follow-up period of 59±20.5 months (range 32-94) and 1/58 4 examination during a follow-up period of 56 months for a total of 111 paired evaluations.

Note that the longest follow up was not 24 months. The statement in the second paragraph of the RESULTS section was misleading since it referred to the first observations performed after surgery. The sentence has been properly revised.

3. How were selected the 111 pairs of SICUS and colonoscopy included in the analysis?

4. How many patients had sequential evaluations included in the analysis? (A table of results per single study interval could probably be helpful)

No patient who underwent surgery was excluded. Analysis of SICUS and endoscopy was limited to the paired SICUS-ileocolonoscopy examinations performed in a two week time frame. Some of the patients for inability or refusal did not perform at scheduled time the colonoscopy and this behaviour explains why not all patients were examined at the same time interval from the operation.

5. In fig 2B the asterisk relative to the significant difference between score 4 and score 1 (p<0.001) is missing.

We acknowledge the mistake in the figure. It has been properly corrected.