Higher infliximab and adalimumab trough levels are associated with fistula healing in patients with fistulising perianal Crohn's Disease (Manuscript number 71392)

We thank Professor Subrata Ghosh, Professor Andrzej S Tarnawski and the reviewers for their excellent comments and feel that the paper is stronger now as a result. We have endeavoured to answer each of their comments to the best of our ability.

Reviewer #1:
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (General priority)
Specific Comments to Authors: In this retrospective study the authors assessed the relationship between adalimumab trough levels and clinical fistula healing in patients with fistulising CD. They showed that higher infliximab and adalimumab trough levels were associated with perianal CD fistula healing, with higher rates of healing in higher tertiles of infliximab and adalimumab levels, thus confirming the existed evidence that fistula healing improves with higher anti-TNF trough levels. However no association with fistula closure was observed. The authors included 114 (66 infliximab, 48 adalimumab) patients of whom 72.7% achieved fistula healing and 27.3% fistula closure. Concerning the results of adalimumab administration they showed that 77% achieved fistula healing and 35.4% fistula closure. However, in a previous abstract published on January 2020 the same group of authors included 123 patients (IFX = 72; ADA = 51) of whom 75.0% on maintenance IFX achieved fistula healing and 30.6% achieved fistula closure. (B Gu, K Venkatesh, A J Williams, W Ng, C Corte, S Ghaly, W Xuan, S Paramsothy, S Connor. P586 Higher infliximab and adalimumab trough levels are associated with fistula healing in patients with fistulising perianal Crohn's disease. Journal of Crohn's and Colitis, January 2020;14:S490–S491). What is the explanation for these differences?

With regards to the differences in numbers, the abstract published January 2020 had 123 patients and this paper had 114 as the data was re-reviewed when writing this manuscript – 9 patients were noted who did not have an anti-TNF trough level within 12 weeks of clinical assessment and were removed.

Reviewer #2:
Scientific Quality: Grade A (Excellent)
Language Quality: Grade A (Priority publishing)
Conclusion: Accept (High priority)
Specific Comments to Authors: The authors share their experience on anti-TNF agents in CD patients in Australia. The aim is stated clear. The authors stated clearly what study found and how they did it. The title is informative and relevant. The references are relevant and recent. The cited sources are referenced correctly. Appropriate and key studies are included. The introduction reveals what is already known about this topic. The research question is clearly outlined. The research question also justified given what is already known about the topic. The process of selection of the subjects was clear. The variables are well defined and measured appropriately. The study methods are valid and reliable. There are enough details provided in order to replicate the study. The data is presented in an appropriate way. The text in the results add to the data and it is not repetitive. Statistically

significant results are clear. It is clear which results are with practical meaning. Results are discussed from different angles and placed into context without being overinterpreted. The conclusions answer the aim of the study. The conclusions are supported by references and own results. The limitations of the study are not fatal, but they are opportunities to inform future research. Specific comments on weaknesses of the article and what could be improved: Major points - none Minor points - none

We thank the reviewers for their comments.

Reviewer #3:
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Minor revision
Specific Comments to Authors: The manuscript deals with an interesting topic concerning the effectivity of two anti-tumor necrosis factor-alpha agents in healing perianal fistulas that are usually presented in patients with Crohn's disease. Although there are other articles in the literature dealing with the use of anti-TNF for the treatment of fistulas, the current work stands out for being the largest study to assess the relationship between adalimumab trough levels and clinical fistula healing. I would like to make the following comments and suggestions:

1. In the abstract, methods session, data about the antibodies against infliximab and adalimumab measurement was missing.

The methods section of the abstract has been corrected and now reads:
"Data collected included demographics, serum infliximab and adalimumab trough levels (mg/L) within 12 weeks before or after their most recent clinical assessment, and concomitant medical or surgical therapy".

2. In the methodology section, page 6, item "Study Design and Patient Population", I suggest the authors to include the information on the moment when the blood collection was performed, taking into account the schedule of the anti-TNF injections. Moreover, I also suggest to provide more details concerning the type of samples that were used (is it serum?).

Blood collection was performed within 12 weeks before or after their most recent clinical assessment. All levels were taken as trough levels, i.e. immediately before the next dose of anti-TNF agent. Samples taken for anti-TNF agents were serum samples and were measured using a drug sensitive enzyme-linked immunosorbent assay (Grifols Promonitor for adalimumab; LISA-Tracker and Grifols Promonitor for infliximab). This was initially in a separate section of the methodology titled anti-TNF levels so we have moved it up to the end of the ""Study Design and Patient Population" for better clarity. This section now reads as follows:
"We included patients on maintenance infliximab or adalimumab with a documented perianal examination who had a serum infliximab or adalimumab trough level collected within 12 weeks before or after their most recent clinical assessment. Infliximab and adalimumab trough levels as well as antibodies to infliximab and adalimumab were

measured using a drug sensitive enzyme-linked immunosorbent assay (Grifols Promonitor for adalimumab; LISA-Tracker and Grifols Promonitor for infliximab). Infliximab and adalimumab trough levels were measured both in a proactive manner and reactive manner in patients failing treatment across the study sites. Patients who had been changed from infliximab to adalimumab or vice versa had relevant data included in both the infliximab and adalimumab groups."

3. The Montreal Classification should be added to the patient's characteristics table to better describe the patients included in the study. In addition, information on previous surgeries could also be included in this table.

We have added in rows to distinguish between A1, A2 and A3 to table 1; in addition to the median age that was present. The other aspects of the Montreal Classification including disease location (ileal, colonic, ileocolonic, no luminal disease, upper gastrointestinal involvement), disease behaviour (stricturing disease, penetrating disease) were already included on this table.

4. How long was the time between diagnosis (fistula) and the blood collection?

The time between fistula diagnosis and blood collection was not collected. This data was not available for many of the patients in this study.

5. Was a clinical intervention performed after the trough levels results?

No clinical intervention was performed after the trough level as part of this study as it was a retrospective study. Clinical interventions that were performed in response to the trough levels at the time of collection were not recorded in this study.

6. Previous studies have already demonstrated the advantage of acidifying samples pre-anti-drug antibodies ELISA, with the aim of breaking down the antibody immune complexes, which prevent an effective binding to the kit, thus increasing its sensitivity and avoiding false negatives. Did the authors perform this pre-treatment for the measurement of anti-drug antibodies? If not, I suggest them to include and discuss this information in the discussion section.

Acidifying samples prior to analysis by ELISA is currently a research technique which is under investigation. Antibody results obtained in this manner are not standardised, and the clinical significance of such results still requires validation. Testing of all samples in this study was performed according to assays that are currently available in diagnostic laboratories and approved for clinical diagnostic use by the Therapeutic Goods Administration in Australia, so this technique was not used or discussed in this paper.

7. In the results section, page 9, third line "Five patients had been changed from infliximab to adalimumab or vice versa and were included in both the infliximab and adalimumab groups". Can't the fact that these patients had already presented some loss of response to the medications influence the result? This information could have been mentioned in the discussion.

We have added this to the discussion as suggested.
"Five patients in this study had been changed from infliximab to adalimumab or vice versa and were included in both groups, however the anti-TNF level and anti-TNF antibody levels at the time of changing treatment were not collected. Reassuringly, there have been previous studies demonstrating that presence of infliximab antibodies does not decrease future response rates to adalimumab and vice versa"[30].

8. The assays for measuring the trough levels were different. Was there any difference in the results while comparing the tests that were used?

The data regarding which assay was used for each specific trough level was not collected so was unable to be compared.

9. Table 1 shows the patient demographics and disease characteristics. Were those variables between the groups statistically analyzed? Were the group under infliximab treatment and that one under adalimumab homogeneous considering their demographics and disease characteristics?

The patient demographics and disease characteristics were not compared between the infliximab and adalimumab groups as this was not the purpose of the study and did help better interpret the results. We have carefully considered the reviewer's suggestion and opted not to add this analysis in, as it was not the purpose of the study and may therefore add potentially confusing and irrelevant information.

Overall, it is a well written manuscript, but it needs revision.

4 LANGUAGE POLISHING REQUIREMENTS FOR REVISED MANUSCRIPTS SUBMITTED BY AUTHORS WHO ARE NON-NATIVE SPEAKERS OF ENGLISH
As the revision process results in changes to the content of the manuscript, language problems may exist in the revised manuscript. Thus, it is necessary to perform further language polishing that will ensure all grammatical, syntactical, formatting and other related errors be resolved, so that the revised manuscript will meet the publication requirement (Grade A).
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We have checked the manuscript for language and grammar errors.

5 ABBREVIATIONS

We have checked the abbreviations in the manuscript and ensured they fit the guidelines provided.

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