Dear Dr. Sorrentino,

Thank you for submitting your manuscript to World Journal of Gastroenterology, a peer-reviewed, online, and open access journal. We are pleased to inform you that one of the peer reviewers has completed his/her review of your manuscript.

1 Details of your submission

Journal title: World Journal of Gastroenterology

Manuscript NO: 52378

Title: Fecal lactoferrin accurately reflects severity and extent of mucosal inflammation in inflammatory bowel disease

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2 Peer-review report

<u>Reviewer #1:</u> I think that endoscopic parameters of SES-CD and endoscopic component of the Mayo score for UC could be more descriptive. For example: Endoscopic findings of UC: 0 - normal mucosa or inactive disease; 1 - Mild activity: erythema decreased vascular pattern, mild friability; 2- Moderate activity: marked erythema, lack of vascular pattern, friability, erosions; 3 - Severe activity: spontaneous bleeding, large ulcerations. More complete description could help readers that are not familiar to endoscopic scoring in IBD. SES-CD already includes surface involved by disease and surface involved by ulcerations. I really don't feel necessary to further include an analysis with a simple score system providing one point for each colonic segment. This analysis could add more bias.

Thank you for this comment. We have added the description of the scoring systems as supplemental tables. We agree with you that the SES-CD already includes includes the surface affected. However the Mayo score does not – and for our purposes it is very important to have such measures in ulcerative colitis. We have added a sentence in Methods (page 5) to reflect this point and avoid confusion – as you rightly suggest.

The cutoff reference value of "normal" fecal lactoferrin (0 - 7.24 UG/ML TECHLAB, Blacksburg,) could interest for better comprehension.

We are unsure regarding this comment. The normal lactoferrin values were established by large studies by TechLab – as customary in these cases.

The number of patients with CD described in abstract is different from table 1: 92 patients versus 131 patients.

Thank you. It has been corrected.

Of note most of the patients had severe activity for both UC and CD. No patient with ulcerative colitis was in remission. This is not unusual at IBD reference centers.

We agree.

<u>Reviewer #2</u>: This manuscript reports the findings from a retrospective study conducted by the authors in which they found that fecal lactoferrin accurately reflects severity and extent of mucosal inflammation in inflammatory bowel disease (IBD). The findings reported in this study are interesting, suggesting that fecal lactoferrin is a potential non-invasive biomarker for monitoring mucosal inflammation in IBD. Overall, the manuscript is clearly written. However, the followings should be corrected:

1. Page 3. Abbreviations for Crohn's disease and ulcerative colitis were already used in the first paragraph of the Introduction. However, in the second paragraph, the full names were still used. Please change them to abbreviations.

Corrected. Thank you.

2. Page 4. CRP and WBC: please provide full name when first mentioned in the manuscript.

Full name provided. Thank you.

3. Page 5: Biomarker testing: FL is from fecal sample, CRP and WBC are from blood samples. Please include this information in the manuscript.

Information included. Thank you.

4. Table 2: Please include P values in Table 2.

P values added

5. Figure legends: in the figure legends, please include the statistical analysis method used. The patient numbers in each group should also be included. The current Figure legends particularly Figure 1 and Figure 2 are just titles/subtitles.

The statitstical methods used and a description of the findings have been added to the figure legends.

<u>Reviewer #3</u>: This is a retrospective cohort study in a IBD center in Roanoke, VA, USA, including 131 CD and 57 UC patients. In general, the language quality (style, grammar, and spelling) of this paper is good. The article also showed complete and well-constructed logicality. However, there are some questions that might need the authors to illuminate:

1. This study depicted the known conclusion among IBD patients that FL showed a close correlation with the involved mucosal surface and with disease extent and was more closely correlated to endoscopy. But this article didn't point out the exact FL level that can accurately reflect the endoscopic assessment, and didn't answer that if FL could be the substitution of endoscopy for diagnosis and monitoring of IBD.

Thank you for this insighful comment. One of the main limitations in establishing a minimal FL cut-off level for inflammation is the fact that low FL levels might be associated with small bowel disease activity but with minimal, if any, colonic disease activity. This could be related to the different surface area of the two intestinal tracts - whereby a small extent of disease activity is relatively more significant in the small bowel than in the colon. However the precise explanation for this finding (as well as the establishment of a FL cut-off level for inflammaton in the colon vs small bowel) must await a dedicated prospective study. This has been discussed in the revised version of the manuscript (pages 9 and 10). FL can certainly be a tool to monitor the disease activity but it cannot be used for diagnosis – since the latter requires pathology.

2. Methods: authors didn't point out the time of this retrospective study and why?

This information has been added in Methods (Patient population)

3. The number of CD patients in the abstract was inconsistent with that in the results.

This has been corrected – thank you

4. One of the results showed that FL showed a higher correlation to SES-CD and DAI when it had been tested before the procedure compared to when it had been tested after the procedure in patients given effective fast acting medications (steroids and biologics). And authors gave the explanation: FL is a more timely indicator of disease activity than endoscopy. But why only in patients given steroids and biologics? Does it common in patients given other medications such as immunosuppressants (AZA)? The medications of these patients were not showed in the patients baseline characteristics.

Immunosuppressants (such as AZA) are not fast acting agents (they might take up to 6 months to become effective) and as such we did not expect any change in inflammation in a relatively short period of time (30 days). However, based on the comments of reviewer #5 we have now changed this statement.

<u>Reviewer #4</u>: The manuscript "Fecal lactoferrin accurately reflects severity and extent of mucosal inflammation in inflammatory bowel disease" is a retrospective study which evaluates the fecal lactoferrin in IBD. The results of this study were already published as an abstract in:
American Journal of Gastroenterology: October 2018 - Volume 113 - Issue - p S402 and
in Gastroenterology April 2017 Volume 152, Issue 5, Supplement 1, Page S777 The results are from 2016 and are not innovative.

Patient cohort included data up to 2018 so the patient cohort size and analyses have significantly expanded since the abstract in 2016. Most importantly, previous publications in abstract form are allowed by the journal.

<u>Reviewer #5:</u> In this single center retrospective cohort study the Authors aim at investigating the correlation between fecal lactoferrin (FL) levels and the degree of mucosal inflammation, disease location and disease extension. To be included in the study, patients had to have an endoscopy performed within 30 days of FL measurement. The degree of mucosal inflammation was assessed by validated endoscopic scores, disease extension and disease location were assessed with colonoscopy plus imaging in CD patients. Observation reported in the result section are: 1. A significantly different median FL level between specific levels of disease activity as assessed by endoscopic scores. 2. A slightly stronger correlation, expressed as Spearman score, between FL and endoscopic disease activity compared to CRP in patients with CD but not in patients with UC. 3. Higher median FL levels in patients with more than one inflamed colonic segment. 4. FL levels obtained before colonoscopy had a better correlation with SES-CD and DAI than levels measured after the colonoscopy in patients given steroids and biologics in between marker determination and the procedure. Based on this observation the Author conclude that: 1. FL is able to separate different levels of disease activity 2. There is a positive, significant correlation of FL with SES-CD and DAI and such correlation is not seen for WBC and is weaker for CRP 3. FL increase with the number of colonic segments involved and it might be an accurate indicator of the total disease burden. 4. FL variation in response to an immunomodulating drug is faster than mucosal macroscopic change thus FL is a more timely indicator of disease activity than endoscopy. Major issues:

1. FL is able to separate different levels of disease activity. A measure of test accuracy should be provided to be able to draw this conclusion.

We do not fully understand the nature of this comment. Is the referee implying that the lab test used for FL might not be accurate in quantifying FL? Or that we need a measure of sensitivity/specificity for each activty level? Figures 1 and 2 do provide in our opinion very good evidence that different dissease activities are associated with different levels of FL – which is the message of our paper.

2. There is a positive, significant correlation of FL with SES-CD and DAI and such correlation is not seen for WBC and is weaker for CRP. The statement contradicts the results presented in Table 2.

We agree with the referee here. The sentence has been changed to "Overall, both FL and CRP showed the highest Spearman correlations to SES-CD and DAI scores for all assessed patients (Table 2). WBC had a very weak correlation to both the SES-CD and DAI scores" in the Results section (page 7).

3. FL increase with the number of colonic segments involved and it might be an accurate indicator of the total disease burden. This conclusion cannot be drawn based on the results. Authors provide median FL levels according to disease extension in the colon. However, no p-value is provided nor a measure of test accuracy.

We agree with the referee. We have used the Kruskal Wallis test followed by pairwise Mann-Whitney comparisons. This test is considered a direct comparison of the medians in Table 4. This has been added to the Methods and reported in Results (page 8).

4. FL variation in response to an immunomodulating drug is faster than mucosal macroscopic change thus FL is a more timely indicator of disease activity than endoscopy. This conclusion cannot be drawn based on this observation. To be able to state so, an endoscopy should have been repeated at the time of FL collection. In patients with UC, MH can be achieved as early as week 6 after the introduction of an anti-TNF thus mucosal changes reasonably start occurring within days after an effective immunomodulating therapy is introduced

We agree with the referee here that we only provide indirect evidence that FL is a more timely indicator of endoscopy of changes in inflammation and that proving the latter would require ad hoc studies. Thus we have changed the sentnce to "The most likely explanation of this observation is that FL is a timely indicator of disease activity changes after therapy. FL concentration in feces is proportional to neutrophil translocation to the mucosa of the GI tract – a process that is quickly modulated by the activity of the inflammatory process (19)."