Responses to the Reviewers

We would like to thank the Reviewers for their valuable comments on our paper. The issues they raised had been addressed in the revised manuscript and the point-to-point answers are provided below.

Reviewer #03017551

We would like to express our sincere thanks for kind opinion on our work.

Reviewer #03474080

We would like to express our sincere thanks for kind opinion on our work.

Reviewer #03647328

ABSTRACT: Please, add a "s" to the term "asses".

The word has been corrected.

INTRODUCTION: Please remove the term "incurable"(surgery for UC could be considered curative).

The term has been removed. However, It is estimated that up to 50% IBD patients can suffer from at least one extra-intestinal manifestation (EIMs) and the presence and activity of them do not always reflect activity of intestinal inflammation (for instance ankylosing spondylitis and uveitis). One of the most dramatic EIMs is primary sclerosing cholangitis which may be complicated with cholestasis, cholangitis, cholangiocarcinoma and colorectal carcinoma. Moreover, there is no effective medical therapy which may change the course of the disease. In this respect, surgery for UC cannot be considered curative in all patients (Harbord M. et al. The First European Evidence-based Consensus on Extra-intestinal Manifestations in Inflammatory Bowel Disease. J Crohns Colitis 2016, 10, 239-254).

MATERIALS AND METHODS: The authors exclude patients with "indeterminate colitis". They probably refer to "unclassified colitis", because "indeterminate colitis" is referred to colectomy specimens, while "unclassified colitis" is used when diagnosis of UC or CD cannot be made based on standard clinical testing. Please change the term "indeterminate colitis" with "unclassified colitis".

The term "indeterminate colitis" has been substituted with "unclassified colitis" as suggested.

RESULTS: The authors consider anemia if Hb<13,5 in males. According to WHO classifircation of anemia, the cut off value is 13 gr. What's the reference used by the authors? Please clarify.

In Poland the reference values are provided by the laboratories conducting the analyses because they frequently depend on the population, method applied and the analyzer used therefore the laboratory is obliged to provide the reference values together with patient's results. Central Hospital Laboratory which was conducting the determination of hemoglobin for our patients is considering Hb≥13.5 as a normal value. Also, the same criteria are used by Mayo Clinic (http://www.mayoclinic.org/diseases-conditions/iron-deficiency-anemia/diagnosis-treatment/diagnosis/dxc-20266573).

However, please note that the statistically significant result we present in our manuscript concerns the inverse correlation between IL-9 and hemoglobin concentrations, which is independent from the set thresholds.

Upon the suggestion of another Reviewer, the information on the applied criteria for anemia and cachexia has been transferred into the Materials and Methods section.

DISCUSSION:

- *In the discussion UC is defined, uncorrectly, an autoimmune disease, while in introduction the authors consider it a "not classic autoimmune disease". Please correct.*

The sentence in question has been removed from the revised manuscript due to reduction of the Discussion length required by another Reviewer.

- *In the discussion CD is considered a Th1 condition, UC a Th2 disease, but this is an old distinction. Please consider the Th17 pattern.*

We agree that this is an old distinction that is why we stated that it was traditionally considered as such and that this classic paradigm has been recently challenged. The results on the pattern of IL9 correlations with Th1 and Th2 cytokines we present in our manuscript seem to confirm that the distinction is outdated. However, to avoid misunderstanding, we added the statement on IBD association with Th17 pattern to the revised manuscript. Unfortunately, we had to refrain from discussing the issue any further. Th17 cytokines were not analyzed in our study and we are obliged by one of the other reviewers to decrease the length of your discussion and focus it on the most important messages.

Reviewer #03658410

1. Please provide data on IL-9 levels in acute gastroenteritis and celiac disease to demonstrate its applicability in inflammatory diseases.

We are afraid that it has to be some misunderstanding, probably caused by the fact that we used the plural form of “inflammatory bowel diseases” (instead of inflammatory bowel disease) that might suggest we intended to exam also other inflammatory bowel conditions. The title has been corrected.

Based on study protocol and the decision of Ethical Committee we were allowed to conduct our study only on patients with Crohn's disease and ulcerative colitis and healthy volunteers. Although we sincerely agree that it would be of great interest to expand the study population to include other diseases such as celiac disease or acute gastroenteritis, there is no such possibility now when the study has been completed and closed.

2. Please decrease the length of your discussion focusing on the most important messages.

The Discussion has been shortened by approximately 350 words.

3. Please provide a scheme or graph correlating IL-9 with Mayo endoscopic score and CDAI score.

The requested graphs have been added to the revised manuscript.

Reviewer #00742314

Specific comments: Introduction:

On Line 14, the expression "Inflamed gut biopsies from UC patients have been found to overexpress IL9 both at mRNA and protein levels" needs reference.

The reference has been added.

- *On Line 32, the authors mentined that "Th1 and Th2 subset signatures" were analysed. It might be interesting to mention which cytokine belongs to each group.*

To clarify the issue, the analyzed cytokines have been described as coming from inflammatory, angiogenic and of Th1 or Th2 categories. Please note that the studied cytokines are representatives of categories and not the whole subset signatures.

Material and methods: - On Line 2, the expression "apparently healthy controls" needs a better explanation.

The exact inclusion and exclusion criteria for control group were provided at the end of the paragraph in the original manuscript.

On Line 8, more information about the "few exceptions" is necessary. For instance, How many patients?; which medication did they take? which medication did they take?

In our case there were five patients who have not been treated with derivatives of 5'-aminosalicylate (5'-ASA): one because of the adverse reactions and four with the disease limited to the small intestine. In this group, as a maintenance therapy, azathioprine was administrated.

- Line 16: How the "active inflammation" was measured?

All individuals from a control group self-reported their health condition and were further evaluated by the physician who screened them for any signs of fever, rush, swelling or local redness. Additionally, blood donors, who constituted majority of control group, underwent blood testing including WBC count and ESR. The original statement has been supplemented with "based on physical examination and medical history".

- *Please, inform in this section how cachexia and anemia groups were characterized.*

Definitions for anemia and cachexia used in current study have been transferred from Results to Materials and Methods section.

Results: - It might be interesting to show the pair comparisons in figure 1. For instance, the difference between inactive IBD X control group; - A better explanation about how the pair or the multiple comparisons between the groups is needed (figure 2). Moreover a better explanation about why grouping in active disease to compare against a single one is needed.

The proper way to conduct multiple comparisons is, as was done in our manuscript, to use one way ANOVA (or Kruskal-Wallis H test for non-normally distributed data) instead of a series of t-tests (or Mann-Whitney U test) for two-group comparisons. The reason for it is that with every t-test there is

5% chance of making a Type I error. By running two or more t-tests on the same data the error is accumulating. In case of three groups analyzed in pairs, just as a situation in case of our Figure 1, the error would be close to 15%, which is unacceptable. Using ANOVA, as we did, causes the error to remain at acceptable level of 5%. At the same time ANOVA allows for distinguishing pairs, between which there are statistically significant ($p<0.05$) differences. In the Figures, they are denoted by letters.

Line 09: please, specify what "endoscopic findings" are positively correlated with IL-9;

To clarify the issue, the sentence was re-phrased and "endoscopic findings" were replaced by "Mayo endoscopic score".

- *Line 10: Which criteria did you use to reach 53 UC patients and please, inform how many were active or inactive;*

Not all UC patients were subjected to endoscopy at the time of blood sampling for our study. As stated in the manuscript, data were available for 53 patients. Of these, 38 had active and 15 inactive disease.

- *The authors should comment the impact of IL-9 in the mucosal healing in the CD patients;*

We did not study IL9 association with mucosal healing in CD. Majority of the patients with Crohn`s disease had the inflammatory changes located outside the range of colonoscopy. Evaluation of the small intestine involvement is performed with MR or CT enterography. Both methods cannot be applied in mucosal healing evaluation.

- *The authors should add informations about sample collection and the management;*

The complete information on blood collection and handling was given in the first paragraph of Materials and Methods section (Analytical Methods) of the original manuscript.

- *The authors are encouraged to present the cytokines results;*

The study focus is on IL9. Presenting results for other cytokines in a spectrum broader than their interplay with IL9 will inevitably cause the loss of clarity and conciseness, for which our manuscript has been praised by other Reviewers.