We would like to thank the reviewers for their comments on our manuscript which we feel have helped us to improve the present manuscript. Below you find our point-by point replies to the five reviewers' comments**.** All changes in the manuscript are clearly marked in red color.

Reviewer #1:

1- The Mayo's scoring system presented in the Table is unnecessary, already widely known.

Answer: *We thank for the suggestion. Yes, we agree and have deleted that from Table 1, page 26.*

2- Number of cases in subgroups is very small, and this may compromise the validity of the findings for a global readership to apply. This is particularly serious with regard to collagenous colitis histopathological remission.

Answer: *Recruitment of microscopic colitis patients in this study took place from October 2008 till September 2011. A potential limitation of this study is the small cohort of MC patients. There are several difficulties in recruiting MC patients: MC can only be diagnosed by histopathological examination and the patients with confirmed diagnosis are usually not undergoing repeated colonoscopy. Therefore, the patient collection will take time to accomplish. Nevertheless, to be able to cure the disease rather than simply relieve their symptoms, studies like this one are necessary to understand the immunopathogenesis despite the low number of patients available.*
In case of collagenous and lymphocytic colitis patients in histopathological remission, they came to our clinic due to ongoing clinical symptoms with a previous diagnosis of CC or LC. To make sure they were not suffering from any other intestinal inflammatory disease, they agreed to undergo an additional colonoscopy. This is normally not performed and in that respect the findings of a few patients no longer fulfilling the histopathological criteria for CC or LC were novel and interesting. We therefore felt that they should be included in the study, but as a separate group, despite these patients being quite rare. We have acknowledged these limitations in the Discussion, from page 17 line 28 till page 18 line 5.

3- The manuscript should benefit from language editing by one of the co-authors with clinical experience.

Answer: *We have read through the manuscript again carefully and made a number of editorial corrections throughout the manuscript, which are all marked in red.*

Reviewer #2:

1- General comments: This is very nice study about expression of the cytokines in the GUT in ulcerative colitis and microscopic colitis.
2- Specific comments:
    a. Discussion is very well organized.
    b. References: are appropriate, relevant, and updated.
    c. Tables and figures: are appropriate, well structured.
3- Therefore I classified the manuscript Differential Expression of IL-1/TLR Signaling Regulators in Microscopic and Ulcerative Colitis into grade B.
4- According to the language evaluation the revised article is evaluated as grade B.
5- I conclude that the authors should make only minor changes in the article regarding English language, so I think that you should accept it.

Answer: *We thank for the positive comments. We have read through the manuscript again carefully and made a number of editorial corrections throughout the manuscript, which are all marked in red.*

Reviewer #3:

In my opinion it is very interesting and very original study shedding a new light on the pathophysiology of MC. The only limitation is the low number of patients included in this study.

Answer: *We thank for the encouraging comments. Recruitment of microscopic colitis patients in this study took place from October 2008 till September 2011. A potential limitation of this study is the small cohort of MC patients. There are several difficulties in recruiting MC patients: MC can only be diagnosed by histopathological examination and the patients with confirmed diagnosis are usually not undergoing repeated colonoscopy. Therefore, the patient collection will take time to accomplish. Nevertheless, to be able to cure the disease rather than simply relieve their symptoms, studies like this one are necessary to understand the immunopathogenesis despite the low number of patients available. We have acknowledged these limitations in the Discussion, from page 17 line 28 till page 18 line 5.*

Reviewer #4:

The authors present a differential expression of IL-1/TLR regulators in microscopic and ulcerative colitis. The major objection of this study is the low number of patients that involves to the results presented and for this reason do not provide sufficient experimental evidence or data to draw firm scientific conclusions.

Answer: *Recruitment of microscopic colitis patients in this study took place from October 2008 till September 2011. A potential limitation of this study is the small cohort of MC patients. There are several difficulties in recruiting MC patients: MC can only be diagnosed by histopathological examination and the patients with confirmed diagnosis are usually not undergoing repeated colonoscopy. Therefore, the patient collection will take time to accomplish. Nevertheless, to be able to cure the disease rather than simply relieve their symptoms, studies like this one are necessary to understand the immunopathogenesis despite the low number of patients available. We have acknowledged these limitations in the Discussion, from page 17 line 28 till page 18 line 5.*

1- The authors should consider in their discussion and conclusions the frequent association of LC with other autoimmune disorders (thyroid disease, diabetes mellitus, celiac disease, psoriasis, and rheumatoid arthritis), inflammation in the lamina propria with increased intraepithelial lymphocytes and the fair response to steroids. In this sense infectious agents, drugs, or food antigen such as gluten may be precipitating factors

Answer: *We thank the reviewer for pointing out this. Yes, we are aware of the association between MC and autoimmune diseases. One of the CC patients had Sjögren's syndrome. One CC and one LC patient had hypothyroid disease. In this study, in total four patients on budesonide treatment were included. Overall, we did not detect any significant correlations between these diseases or the budesonide treatment and expression levels of the TLR signaling regulators investigated in these patients. Nevertheless, we cannot exclude that the low number of patients may have disguised possible effects of the drugs on the parameters investigated. We agree that these points should be clarified better and we have now included this information in the Materials and Methods section, page 7 lines 1 to 2 and in the Discussion, page 14 lines 11 to 19.*

2- The authors should more data about if they noted complete histological and/or symptomatic remission in the patients and also if there is other patients that reverted back into UC, because others authors suggesting that LC could present as a continuum of UC. Are the patients completely asymptomatic?

Answer: *We did not have any patients having conversion between LC and UC. This information has now been added to the Materials and Methods section, page 7 lines 16 to 17. In terms of CC and LC histopathological remission patients, they had clinical symptoms, i.e. chronic diarrhea with more than 3 bowel movements per day, whereas the histopathological examination did not fulfill the diagnostic criteria for MC.*

3- The induction of IRAK-M is necessary to limit pathologic inflammation and cytokine secretion. The authors should consider that IRAK-M has varying roles in immunopathology depending on the disease context. For example, in the setting of chronic inflammatory diseases, IRAK-M expression is desirable because it can limit

excessive immune responses. In contrast, IRAK-M expression may prevent proper innate immune clearance of pathogens in the setting of immunodeficiency. Moreover, deficiencies in IRAK-M may predispose individuals to inflammatory bowel disease (IBD), as IRAK-M is on the genetic susceptibility locus for IBD. In ulcerative colitis patients, an association between caspase recruitment domain (CARD15) mutant patients and IRAK-M was found, suggesting a possible impairment in the negative regulation of TLR-signaling causing IBD. IRAK-M has a well-established role in reducing immune responsiveness to continuous pathogen exposure. By negatively regulating TLR signaling, IRAK-M inhibits production of pro-inflammatory mediators and contributes to the induction of endotoxin tolerance.

Answer: *We thank for these explanations. We are aware of the study demonstrating three Crohn's disease-associated mutations in CARD15 as increased risk factors also for UC patients compared to patients without CARD15 mutations (Weersma et al 2007 Scandinavian J Gastroenterology). Due to the mutations, a possible impaired IRAK-M production was suggested, but was not shown experimentally in that study. To the best of our knowledge, our study is the first demonstrating altered mRNA expression of IRAK-M in UC and MC patients. Therefore, we believe that our results contribute to the knowledge of IRAK-M in human inflammatory diseases. We realize that this information would be beneficial for the readers; and we therefore included the explanations in the Discussion part, from page 14 lines 25 till page 15 line 3 and lines 8 to10.*

4- The authors should better explain and interpret the results obtained. In this sense what it is the significance the increased expression of miR-155 in active UC, active CC and LC in remission? What is the interpretation the enhanced expression of miR-21 (anti-inflammatory) in active CC, but no in remission and increased in LC remission but no in active?

5- How is possible that in active CC, active UC and LC in remission patients present increased expression of miR-155 and increased expression of miR-21, if the up-regulation of miR-21 decrease miR-155 expression?

Answers for 4 and 5: *miR-155 is regarded a pro-inflammatory microRNA, and therefore it could contribute to disease activity. Compared to controls, we detected significantly enhanced expression levels of miR-155 in active UC and active CC patients. We found significantly increased miR-155 expression in LC patients in histopathological remission (LC-HR) compared to controls but we did not observe any differences compared to active LC patients. Our results might suggest that the role of miR-155 is different between UC and CC versus LC with regard to the pathogenesis of these diseases in active and remission stages. Since active CC, LC-HR and active UC patients had increased expressions of both miR-155 and miR-21 in our study, it is possible that miR-21 expression is increased in order to decrease miR-155 expression. Moreover, an anti-inflammatory mechanism is usually initiated to decrease a pro-inflammatory response in order to keep homeostasis and therefore one might see both pro-inflammatory and anti-inflammatory responses increased simultaneously (as compared to controls). We realize that this interpretation was not presented clear enough in the Discussion and it is therefore rewritten accordingly, page 16 lines 7 to 8 and lines 21 to 24.*

6- The discussion should rewritten, in which interpret and discuss better the results obtained.

Answer: *We thank for the suggestion. We have now included the following explanations in the Discussion:*

- *Page 14 lines 11-19: Previous studies have frequently suggested that MC shows associations with other autoimmune diseases and with non-steroidal anti-inflammatory drugs [3]. The present study included four patients on budesonide treatment. Overall, we did not detect any significant correlations between the TLR signaling regulators investigated and other autoimmune diseases, nor did we detect any correlations between these regulators and current medications including budesonide. Nevertheless, we cannot exclude the possibility that low numbers of patients may have disguised possible effects of the drugs on the parameters investigated.*
- *From page 14 line 25 till page 15 line 3: IRAK-M has varying roles in immunopathology, depending on the disease context. In chronic inflammation, IRAK-M expression is likely desirable in order to limit excessive immune responses, though it may prevent proper innate immune clearance of pathogens. Moreover, a possible impaired IRAK-M production has been suggested as a risk factor for UC patients carrying three Crohn's disease-associated mutations in the caspase recruitment domain 15 (CARD15) compared to patients without mutations, but this has not been demonstrated experimentally.*
- *Page 15 lines 8-10: By negatively regulating TLR signaling, IRAK-M and miR-146a inhibit production of pro-inflammatory mediators and contribute to the induction of endotoxin tolerance.*
- *Page 16 lines 7-8: miR-155 is regarded as pro-inflammatory microRNA, therefore it could contribute to disease activity.*
- *Page 16 lines 21-24: An anti-inflammatory mechanism is usually initiated to decrease a pro-inflammatory response and maintain homeostasis, and therefore one might expect to see both pro-inflammatory and anti-inflammatory responses being increased simultaneously.*
- *From page 17 line 28 till page 18 line 5: A potential limitation of this study is the small cohort of MC patients. There are several difficulties in recruiting MC patients: MC can only be diagnosed by histopathological examination, and patients with a confirmed diagnosis do not usually undergo repeated colonoscopy. It will therefore take time to accumulate appropriate patients. Nevertheless, to be able to cure the disease rather than simply relieve the symptoms, studies like this one are necessary to understand the immunopathogenesis despite the low number of patients available.*

Reviewer #5:

The manuscript by Gunaltay and colleagues focuses on key TLR regulators in colitis. Overall comment: The conclusions able to be clearly drawn from this work is limited by the small samples sizes, and the bias from variations within each subgroup (esp. treatment effects). These limitations must be more clearly acknowledged.

Answer: *We would like to thank for the suggestion. We have now included the following explanations in the Discussion:*

- *From page 17 line 28 till page 18 line 5: A potential limitation of this study is the small cohort of MC patients. There are several difficulties in recruiting MC patients: MC can only be diagnosed by histopathological examination, and patients with a confirmed diagnosis do not usually undergo repeated colonoscopy. It will therefore take time to accumulate appropriate patients. Nevertheless, to be able to cure the disease rather than simply relieve the symptoms, studies like this one are necessary to understand the immunopathogenesis despite the low number of patients available.*

- *Page 14 lines 11-19: Previous studies have frequently suggested that MC shows associations with other autoimmune diseases and with non-steroidal anti-inflammatory drugs [3]. The present study included four patients on budesonide treatment. Overall, we did not detect any significant correlations between the TLR signaling regulators investigated and other autoimmune diseases, nor did we detect any correlations between these regulators and current medications including budesonide. Nevertheless, we cannot exclude the possibility that low numbers of patients may have disguised possible effects of the drugs on the parameters investigated.*

Specific Comments:

1- There are minor errors of English grammar and/or sentence construction that require correction

Answer: *We have read through the manuscript again carefully and made a number of editorial corrections throughout the manuscript, which are all marked in red.*

2- The word MACROSCOPICALLY is used incorrectly in at least three places in the manuscript. This should be corrected to read endoscopically (as macroscopically refers to examination with the naked eye).

Answer: *We would like to thank for the notification, we made the corrections in these three places: page 4 line 5, page 6 line 10 and page 7 line 22.*

3- Crohn's disease was an exclusion factor for the patient groups. Was this excluded just on the basis of colonoscopy or was upper endoscopy and small bowel imaging also completed to definitively exclude CD?

Answer: *Thank you for pointing out the necessity for clarification on this subject. The patients were excluded if they had a history of Crohn's disease and/or fulfilled the criteria according to endoscopic observations, small bowel imaging and histopathological examinations. This is now clarified in the Materials and Methods section, page 6 lines 4 to 8.*

4- In the methods section (page 5), the text reports that patients diagnosed with MC underwent tests because of three symptoms. This implies that all patients had all three symptoms - is this correct?

Answer: *We would like to thank the reviewer for pointing out the necessity to clarify this point. All MC patients had watery diarrhea and some of them also had abdominal pain and/or weight loss. These parts have been clarified in Materials and Methods section, page 6 lines 12 to 14.*

5- As per above comment, a number of patients were being treated with various drugs at the time of assessment: how have the authors considered the effects of these therapies?

Answer: *The present study included four patients on budesonide treatment. Overall, we did not detect any significant correlations between the TLR signaling regulators investigated and other autoimmune diseases, nor did we detect any correlations between these regulators and current medications including budesonide. Nevertheless, we cannot exclude the possibility that low numbers of patients may have disguised possible effects of the drugs on the parameters investigated. We agree that these points should be clarified better and we have included this information in the Discussion, page 14 lines 13 to 19.*

6- Where were the study biopsies obtained? One place indicates that they were sourced from the hepatic flexure, whilst another indicates proximal colon. This should be consistent and clear.

Answer: *The biopsy specimens from MC patients and controls were taken from the hepatic flexure, whereas biopsies from UC patients were collected from endoscopically affected areas of the colon. In addition, the routine biopsy specimens were obtained from the proximal, transverse and distal colon for confirmation of diagnosis through histopathological examination of the paraffin embedded slides by an experienced gastropathologist. This has been now clarified in the Materials and Methods section, page 6 lines 12 to 13.*

7- Further, were the study biopsies always obtained from involved areas? (e.g. in those with UC)

Answer: *Yes, the biopsies from UC patients were collected only from involved areas i.e. in the distal colon. This has been now clarified in the Materials and Methods section, page 6 line 10.*

8- Some of the details of the patients (as covered in Table 1) are results rather than methods, and would be better located in the Results section

Answer: *We agree and have now replaced Table 1 into the Results part, page 10 line 5.*