Barcelona, 28th July 2016

To the Editor,

We respectfully submit the revised version of our manuscript entitled *"Towards a new paradigm of microscopic colitis: incomplete and variant forms"* (Ref. 27436), which includes the suggestions provided by the reviewers, for your consideration to be accepted in *World Journal of Gastroenterology*.

Additionally, a point-by-point response to all the remarks made by referees is provided below.

I look forward to hearing from you soon.

Sincerely yours,

Dr. Danila Guagnozzi, MD, PhD

ANSWER TO REVIEWER 1:

"Nice document review of the iMC, you should break the paragraphs (many are way too long) accordingly and use """" for their multiple Pubmed search terms. You should also recommend the use of the term MC-unclassified, in accordance to the IBDU.

- Following the reviewer suggestions, we have now shortened the long paragraphs.
- Furthermore, we added the use of " " for our Pubmed search terms.
- A consensus to define the incomplete forms of microscopic colitis (MC) is still under debate. We used the incomplete MC term following the recommendation recently published by the working group of digestive diseases of the European Society of Pathology (ESP) and the European Microscopic Colitis Group (EMCG) *(**Lagner C**, et al; Working Group of Digestive Diseases of the European Society of Pathology (ESP) and the European Microscopic Colitis Group (EMCG). Histology of microscopic colitis – review with a practical approach for pathologists. Histopathology 2015; 66: 613-26).* However, we understand that the term and concept of "incomplete MC" is new and subject to controversy, in particular regarding the minimum criteria required for diagnosis. The other European Consensus on the histopathology of inflammatory bowel disease (IBD) states in the ECCO-ESP statement N. 27 that "*the term inflammatory bowel disease unclassified could be used for patients with chronic colitis who clearly have inflammatory bowel disease based on the clinical history but for which macroscopic and/or endoscopic biopsies show no definitive features of ulcerative colitis or Crohn's disease*" (***Magro F,** et al; European Society of Pathology (ESP) and the European Crohn's and Colitis Organization (ECCO). European consensus on the histopathology of inflammatory bowel disease. J Crohn's Colitis 2013; 7: 827-851*). However, it is important to stress that the previous statement refers only to IBD subtypes and not to MC subtypes, defined in the ECCO-ESP statement N. 31-37 without any definition of the incomplete MC forms. Bearing this in mind, it is still unknown whether we can define MC as a sub-group of IBD, however, as available evidence is still lacking, so we propose the use of the definition term *"incomplete"*, as recommended by the recently published international consensus on MC (***Lagner C** et al; Working Group of Digestive Diseases of the European Society of Pathology (ESP) and the European Microscopic Colitis Group (EMCG). Histology of microscopic colitis – review with a practical approach for pathologists. Histopathology* 2015; 66: 613-26).

ANSWER TO REVIEWER 2:

"The paper entitle "Towards a new paradigm of microscopic colitis: incomplete and variant forms" by Danila et al is a review of incomplete and variant forms of microscopic colitis. This is well-written and very complete review, on an interesting topic. I have two major concerns related to this paper: this is more a review on microscopic colitis than on incomplete forms, and most important, the definition of incomplete microscopic colitis (and variants) is not well established, is very heterogeneous between studies and this may limit the collection of the data for a review. Some information on the manuscript could be given on tables. More data on therapy and biopsy collection is missing. Minor correction "this study is already registered ans is in the process of subject recruitment" should be "already registered and is the…".

- We thank the reviewer for his/her comments and would like to also underline that data available on the review topic are really limited and heterogeneous, considering that the term and concept of *"incomplete"* MC is new and subject to controversy. However, we tried to limit the possible bias regarding data search by performing a comprehensive search on PubMEd, Cochrane, Medline and Scopus libraries using all terms to identify the possible incomplete and variant forms of MC (on page 4, lines 26-33).
- Following reviewer's suggestion, we have added table 2, which describes the incidence of incomplete forms (page 12, line 19).
- We have also corrected the sentence on page 17, line 13 to: "This study is already registered and is in the process of subject recruitment".
- We have also added further information on therapy and on sensitivity of biopsies collection in incomplete MC. In particular, we have added to the manuscript the following information: "The diagnostic sensitivity of biopsies from the right and left colon did not differ among MC subgroups including MCi, the latter having a sensibility of 91% (95% CI, 84-96) for the right colon and of 97% (95% CI, 91-99) for the left colon [74]" (page 13, lines 20-22); "It is important to stress that in MCi, some cases of spontaneous remission such as those observed in the classical subtype of MC have also been reported, however this was particularly evident for patients with MCi [74]"(page 18, lines 2-4).

ANSWER TO REVIEWER 3:

"This review provides a valuable overview of the different disease manifestations and the information is useful for those with special interest within the field. There are some comments:"

1. **The page numbers are wrong in the first 9 pages, instead of 1 and so on.**

   We have corrected the page number across the manuscript.

2. **Page 6, second paragraph, line 6: From 2007, MC was classified into…..-was it really a consensus or a suggestion?**

   We thank the reviewer for this important observation. The classification of MC into these five subtypes is a suggestion proposed by the authors of the article and not a consensus (***Falodia S**, et al. Spectrum of microscopic colitis in a tertiary care centre in India. Trop Gastroenterol 2007; 28: 121-5*). In order to clarify the message we have changed the sentence to: "some authors proposed classifying MC forms into five subtypes" (page 5, lines 26-27).

3. **Page 6, second paragraph, last part: MCi recently emerged….with a reference from 1999. "Recently" should maybe be more limited?**

   Following the given suggestion, we have removed "Recently" from that sentence.

4. **Page 7, first paragraph, last part: ileal inflammation was described. This is important. It could maybe be mentioned that the inflammation in colon is most pronounced in the right colon adjacent to the terminal ileum. This probably has some clinical significance although we do not yet recognise it.**

   We agree with the reviewer and have accordingly included the following sentence: "observing that generally the colonic inflammation is most pronounced in the right colon adjacent to the terminal ileum" (page 6, lines 29-30).

5. **Page 8, middle part, reference 25: a gold standard for the collagen band thickness is lacking. There has been some agreement about cut-off, see for example Bela Veress but also other authors that has suggested a cut-off that, to the best of my knowledge is commonly accepted. Than you for the observation.**

   Whereas the cut-off value to define a pathological thickness of the subepithelial collagenous band in colonic biopsies of patients with CC and CCi has been proposed and used, the gold standard to quantify the collagen band thickness is still lacking and not reported in a recently published international consensus. In particular, the article by Magro et al (European consensus on the histopathology of inflammatory bowel disease cited as reference N. 25 in our manuscript) states that: "There is also no agreement among pathologists about the "ideal method" for the assessment of the thickness of the collagen band: histologic evaluation,

conventional measurement using a calibrated micrometer scale or semiautomatic micrometer measurement" on page 843.

6. **Page 11, second paragraph, last part: IELs can be increased but it is not stated what part of the intestine that is referred to Celiac disease predominantly affects the proximal part of the small intestine and in that part HP infection and giardiasis (among others) could also contribute to an increased number of IELs. If it really is the colon that is discussed in this paragraph it should be clarified.**

   On page 11, lines 8-10 we specify that "several studies showed a heavy infiltration of CD8+ cytotoxic T-lymphocytes (CTLs) in the colonic mucosa of MC patients" because this disease affects predominantly the colonic mucosa. However, the level of involvement of the other parts of the intestine, including the small bowel is still unknown.

7. **Pages 13-14 about epidemiology: This description is correct. However, in view of the geographical variations for a number of other immune mediated GI diseases it could be worth mentioning the north-south gradient that is similar to that in IBD. See for example Vigrens article in WJG from 2012.**

   Following given suggestions, we have added the sentence: "…observing a north-south gradient only for CC [73]". In fact, in a recent meta-analysis published in 2015, a north-south gradient was identified for CC being 4.47 [CI95%: 2.41-5.94] in North America and 5.73 [CI95%: 3.66-7.8] per 100,000 person-year in North Europe compared to 2.63 [CI95%: 1.41-3.84] in Southern Europe. Moreover, a significant north-south gradient for LC has not been evidenced yet (reference N. 73: ***Tong J,*** *et al. Incidence, prevalence, and temporal trends of microscopic colitis: a systematic review and meta-analysis. Am J Gastroenterol 2015; 110: 265-76*) (page 12, lines 6-7).

8. **Page 14, second paragraph: The incidence is described in three articles by Björnbak, Fernandez-Bañares and Rasmussen from 2016, but how about the article by Rasmussen et al from 2012 in APT instead of the one from 2016?**

   We agree with the reviewer on the importance of the suggested publications, however, the article by Rasmussen MA and Munck LK entitled "Systematic review: are lymphocytic colitis and collagenous colitis two subtypes of the same disease – microscopic colitis?" and published in Alimenthary Pharmacology and Therapeutics (2012) is a systematic review and not an original article, which focuses on the calculation of incidence and/or prevalence of MC. Moreover, the article does specifically address the issue of incomplete MC. For these reasons we preferred to describe the three original articles addressing the incomplete MC epidemiology (***Fernández-Bañares F****, et al. Paucicellular lymphocytic colitis: is it a minor form of lymphocytic colitis? A clinical pathological and immunological study. Am J*

Gastroenterol 2009; 104; 1189-98; ***Bjornbak C****, et al. Microscopic colitis: clinical findings, topography and persistence of histopathological subgroups. Aliment Pharmacol Ther 2011; 34. 1225-34;* ***Rasmussen J****, et al. The temporal evolution of histological abnormalities in microscopic colitis. J Crohns Colitis 2016; 10: 262-8*).

9. **Page 15, 4 lines from bottom: define => defining.**

   Following given suggestions, we have changed "define" to "defining" on page 14, lines 13.

10. **Page 16, lines 6 and 11: Two sentences are stated as "finally". Perhaps it should be only the last.**

    Following given suggestions, we have eliminated "Finally" in the first paragraph, leaving it only in the last paragraph.

11. **Page 17, lines 5 from bottom: slight => slightly.**

    Following given suggestions, we have changed "slight" to "slightly" on page 15, lines 20.

12. **Page 19, second paragraph: Lactose malabsorption can be primary caused by a mutation and detected by gene analysis or secondary caused by any kind of GI disorder and then detected by oral testing. These two different entities have been mixed up in this paragraph. Secondary lactose malabsorption could be caused by MC but not the genetic mutation unless these should be some kind of linkage disequilibrium and that has not been suggested anywhere.**

    The clinical manifestation of primary or secondary lactose malabsorption depends on lactase activity but also on visceral hypersensitivity and anxiety level as observed in irritable bowel syndrome (IBS) patients. Following given suggestions we have clarified on page 16, line 26 that we referred to secondary lactase malabsorption: "to induce a secondary lactose malabsorption".

13. **Page 20, line 10-12: Inflamed lamina propria is more important than IEL for the diarrhoea. The line of thought is unclear to me. How can that conclusion be drawn (that lamina propria is responsible for the diarrhoea)? How about for example sodium channels? See Schulzke's works about this.**

    The mechanism by which the alteration of the mucosal immune response generates the dominant symptoms of the disease (diarrhoea) is still under investigation, with several studies showing that the diarrhoea in LC patients could have an inflammatory origin. Indeed, the severity of diarrhoea seems to be associated with the intensity of inflammation in the lamina propria in LC while in patients with CC it is not correlated to the thickness of the collagenous band (***Lee E****, et al. Subepithelial collagen table thickness in colon specimens from patients with microscopic colitis and collagenous colitis. Gastroenterology 1992; 103: 1790-6*). Furthermore, in those patients with CC, who had a temporary ileostomy, the

recurrence of inflammation in the lamina propria was the first histological change observed in the development of the symptomatic disease (***Munch A**, et al. Dynamics of mucosal permeability and inflammation in collagenous colitis before, during and after loop ileostomy. Gut 2005; 54: 1126-8*). Nonetheless, other studies indicate that osmotic and secretory components could also contribute to the development of diarrhoea in MC patients (***Bohr J**, et al. Effect of fasting on diarrhoea in collagenous colitis. Digestion 2002; 65: 30-34; **Bürgel N**, et al. Mechanisms of diarrhoea in collagenous colitis. Gastroenterology 2002; 123: 433-43; **Protic M**, et al. Mechanism of diarrhea in microscopic colitis. World J Gastroenterol 2005; 11: 5535-39*). Following given suggestions, we have changed the phrase on page 17, lines 25-29 to: "In fact, a recent study has evaluated the contribution of inflammatory mediators to water secretion in the sigmoid colon of patients with LC. The key effector cytokines TNFα, IFNγ and Il-15 inhibited γ-ENaC upregulation in response to aldosterone through a MEK1/2-mediated pathway, preventing ENaC from reaching its maximum transport capacity, leading to Na malabsorption, which directly contributes to diarrhoea [86] (***Barmeyer C**, et al. Dysregulation through activation of MEK 1/2 contributes to impaired Na+ absorption in lymphocytic Colitis. Inflamm Bowel Dis 2016; 22: 539-47*).

14. **Page 20, conclusions: … higher incidence of BAM and lactose malabsorption. BAM is correct but lactose malabsorption – can that conclusion be drawn?**

    We agree with the reviewer and have accordingly changed the sentence to: "MCi has clinical and histological features that support its classification as a form of MC. In fact,…" (page 18, lines 11-12).