Format for ANSWERING REVIEWERS

September 29th, 2014

Dear Editor,

please find enclosed the edited manuscript in Word format (file name: 13115-review.doc).

Title:HUMAN CYTOMEGALOVIRUS AND EPSTEIN-BARR VIRUS INFECTION IN INFLAMMATORY BOWEL DISEASE: NEED FOR MUCOSAL VIRAL LOAD MEASUREMENT

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First of all, we would like to thank the Editor and the Reviewers for their helpful criticism and suggestions, which have greatly contributed to the improvement of the manuscript, as follows:

1 The format has been updated

2 Revision has been made according to all the reviewers'suggestions, as specified in the following point-by-point letter

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Yours sincerely,

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#1. Reviewer 00034127

Excellent original work on the role of human CMV and EBV in mucosal lesions in refractory IBD.

We would liketo express our sincere thanks to the Reviewer for her/his positive consideration of our work.

#2. Reviewer 00041288

Congratulations on a well written and thought provoking study and manuscript.

We warmly thank the Reviewer for her/hispositive consideration and helpful comments,which contributed toimproving the quality and clarity of our work. Here below, please, find our point-by-point response to all the issues raised.

Comments below.

Abstract - Change "the risk factors" for "its risk factors"done. - Should it be ...endoscopic colon mucosal sample? Yes, it should. Now it hasbeen better specified.- Would change to: No correlation between peripheral blood values and immunohistochemistry was identifieddone. - Would change to: Steroid use was identified as a significant risk factor for both HCMV (P=0.018) and EBV (P=0.002) colitisdone. - Change to ...of mucosal specimens (throughout the manuscript)done.

Key words - Change refractoriness for refractorydone.

Core tip - Change "among" for "between"done. - Delete "Moreover"done. - Change to: ...loads, which correlated... done- Change to: ...activity. EBV infection was most prevalentdone. - Change to: Steroid therapy was identified as a significant risk factor for viral colitisdone.

Introduction - Change to: …localized to the gastrointestinal tractdone. - Change to: …majority of studiesdone.

Methods - Please change "blind pathologist" to "pathologist blinded to patient diagnosis and clinical disease status"done. - Was a power analysis performed? The number of positive cases at immunohistochemistry in each group was too low and the positive cells were too rare in the mucosa to justify a statistical analysis.

Results - Simply placing "(Table 1)" would suffice.Following the suggestion of another Reviewer, we left this short section on study population almost intact, while the data on control subjects were insertedinto Table 1. - The tables should be placed at the end of the manuscript, not embedded in the textdone. - In table 1, an extra column should be added for the respective P valuesdone. - The "mucosal viral load" section is confusing. Please use more periods and avoid long and confusing sentences. - I would try to shorten and focus the results section and place most of the numbers and statistics in the tables. At times, I would get lost amongst the P and correlation values, scores, etc. Try to keep it easy to digest for the reader. We fully agree with this remark, and we apologize for the unsatisfactory clarityof the original version, which has now been completely revised and a Table has been added to keep the text free from all the numerical values.- Were there any correlations between viral loads and the diseased anatomic site (i.e. ascending, descending, rectum, etc)? We are grateful to the Reviewer for raising this important issue. In this regard, we would like to specify thatdespite our early impression on the presence of a decreasing gradient of the viral load from the left to the right colon, as the number of

observations increased, this finding disappeared. However, the number of cases enrolled in this study is too small to allow definitive conclusions. - Were both HCMV and EBV positive cells and DNA load >10^3 copies/10^5 cells considered as positive markers for active viral colitis? In contrast with the current opinion (see the ECCO guidelines - ref. n.38),which state that "histopathologycombined with immunohistochemistry are highlyspecific and sensitive for verifying CMV infection in tissue orbiopsies" and that only in the presence of acute steroid-resistant colitis with positive cells, the diagnosis of viral end-organ disease may be assessed,we clearly demonstrated thatonly a mucosal viral load greater than 10^3 copies/10^5 cells is indicative of a superimposed active viral colitis. Moreover, thepresence of positive cellsat immunohistochemistry does not give any information about the status of the infection (reactivation? - end-organ disease?).- What is the significance of positive serology and DNA load >10^3 in asymptomatic, healthy patients? This should be clarified in the discussion. Actually, none of the asymptomatic, healthy patients had a mucosal viral load >10^3 copies/10^5 cells. If you mean a mucosal viral load >10^2 copies/10^5 cells in responder IBD patients, we suggest that it may be considered a grey area where the best thing to do is to avoid the use of steroids, while closely monitoringthe patient, as already specified in the Discussion section.If, on the other hand, you mean the presence of both a high mucosal viral DNA load plus detectable levels of viral DNA in peripheral blood, we believe that this situation represents the spread of the virus from the colon to systemic circulation. This hypothesis is supported by the evidence of a positive correlation between the values in the two compartments.- Replace "both viruses" with "HCMV/EBV colitis"done. - I would suggest replacing "Systemic steroids emerged as the main risk factor for" for "Systemic steroid use was identified as a significant risk factor for"done. - Replace "biological agents and topical steroids resulted positively related only to EBV" for "biologic agent and topical steroid use were significantly related to EBV colitis"done. - In general, data not shown should not be part of the results nor the discussion.As requested, we have added this information to the revised manuscript. - Why did only 1 patient receive Rituximab? Rituximab is not an anti-viral agent,thus we tentatively used this strategyin only one patient as an off-label prescription in order to destroy the B-cells that host the virus. However, the patient's poor outcome, despite a transient reduction in the viral load, discouraged us from using this therapeutic option in further cases.- Change to: "worsening of the patient's"done. - What were the indications for colectomy? In all refractory patients who underwent surgery, the main indication was bloody diarrhea with severe anemia and malnourishment orelectrolyteimbalance, except for two patients whodecided to go to other tertiary centers, where they underwent colectomy.

Discussion is good.We thankthe Reviewer for her/his appreciation. **Figures** are great!Again, we thank the Reviewer for this positive comment!

#3. Reviewer 00503513

First of all, we want to warmly thank the Reviewer for her/his positive reaction to our manuscript, and for her/his helpful comments aimed at improving the quality of our work.

The authors investigated CMV and EBV in tissue specimens of refractory and non-refractory mixed IBD patients by quantitative real-time PCR and immunohistochemistry. Additionally, the whole colon was mapped in order to correlated viral loads to endoscopic lesions. The main findings are: all refractory patients had viral loads over 1000 copies/10000 cells At least in INTRO additional risks of primary infections in immunocompromised subjects such as EBV-related lymphomas or the macrophage activation syndrome for both, CMV and EBV should be mentioned. We completely agree with this remark and thus we have added specific comments in the revised version of the manuscript. The paper is well written and, despite the small numbers of patients, should be published.We are grateful to the Reviewer for her/his favorable consideration. Minor points: Page 6, line 17: I would prefer the term smoking habits.We have now corrected thisaccordingly. Page 15, line 30: …target is T cells should read target are T-cells. As requested, we have now corrected this.

#4. Reviewer 00503587

This manuscript focuses on the presence of viral agents in patients with and without IBD. First of all, we warmly thank the Reviewer for her/his constructive comments aimed at improving the quality of our manuscript. Here below, please find our point-by-point response.

Specific Comments:

1.The first sentence of the Abstract could be more precise. Do the authors mean in the pathogenesis of IBD or in the exacerbation of disease or other? We fully agree with this remark, and we apologize for the unsatisfactory explanation given in the original version, which has now been correctedalso in the Introduction and at the beginning of the Discussion section.

2. The word correspondence in the Abstract may be better replaced with correlation.As requested, we have now replaced this word.

3. There are some errors of English language use in the Abstract and elsewhere that should be corrected.We apologize for the presence of such errors in the manuscript, which has now undergonefurther language revision.

4. In the Methods why is the word habitus in italics? We apologize for the typing error, which has now been corrected.

5. On page 6 (Methods) the word macroscopic is used incorrectly. This should read endoscopic - as the authors did NOT look at the surface of the bowel with their naked eyes, rather via the endoscope. We fully agree with this remark, and we apologize for our imprecision, which has now been corrected.

6. The definition of refractory is difficult to follow, and may incorporate many variables. We understand this criticism;however,as stated,we followed specific guidelines to define refractoriness in each patient on the basis of her/his current treatment and disease.

7. The authors define taking biopsies from involved and non-involved areas - how did they manage confluent disease (with no non-involved areas)? In this regard, we would like to specify that we did not harvest any mucosal specimens from border areas between involved and non-involved areas. However, it may be considered an interesting issue for further investigation.

8. Further, how did the authors consider pure ileal CD (with no colonic involvement)? It should be

noted that this kind of localization was present only in two non-refractory patients in which mucosal specimens were taken also in the terminal ileum as well as from all the colonic segments, where the mucosa appeared with some scars and erosions, and the viral load for both HCMV and EBV viruses were $<10^3$ copies/10^5 cells.

9. The Results should have subheadings throughout (the first sections lack these). As requested, we have now added the subheadingsto the first section too.

10. The details of the control subjects could be added to the Table 1 and taken from the text area. We fully agree with this suggestion and now the clinical features of controls have been added to Table 1.

11. According to Table 1, patients were assessed as to their L4 involvement - yet according to the Methods no patients underwent upper endoscopy. This should be corrected/clarified. As shown in Table 1, no patient had upper gastrointestinal tract localization of Crohn's disease, which had been assessed before the study commenced. This is why we did not mention the upper endoscopy in the Methods section since it was part of the diagnostic work-up carried out before enrolling subjects in the study.

12. Did the authors consider a role for stool testing for these viral agents? This would be more rapid and less invasive. We are grateful to the Reviewer for raising this important issue. In this regard, we have now added a specific comment in the Discussion section. Similarly, were there any changes on peripheral bloods that were helpful (lymphocyte count etc)?Again, wethank the Reviewer for this comment. With regard to this, we had already searched for any modification of peripheral blood white cell populations, and we did not find any significant difference between refractory and responder IBD patients.