July 25, 2014

Dear Editor,

Title: Epstein-Barr virus is related to 5-ASA, tonsillectomy, and CD19+ cells in Crohn's disease.

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The manuscript has been improved according to the suggestions of reviewers:

Specific Comments:

1. The work includes a very small group of subjects: these numbers are further limited when subgroup analyses are attempted. This needs to be very carefully acknowledged

Reply: This fact has been indicated in the material and methods section, as well as, in the results and discussion sections.

2. There are numerous errors of English language usage or grammar. These must all be corrected

Reply: The manuscript has been corrected by a fluent English language speaker.

3. The METHODS section of the ABSTRACT is essentially the same as the AIM just beforehand

Reply: The sentence has been deleted and rewritten according to the indications of the reviewer.

4. The results section of the ABSTRACT mentions CD19 cells, without having mentioned this at all earlier

Reply: The detection of CD19 subpopulations has been included in the Methods section of the abstract.

5. The first sentence of the INTRODUCTION needs referencing. In the second sentence of the INTRODUCTION, the authors refer to an earlier report - this report must be referenced after this sentence (not subsequently)

Reply: We have included an adequate reference in the first sentence of the Introduction (**Thompson AI**, Lees CW. Genetics of ulcerative colitis. *Inflamm Bowel Dis*. 2011; **17**:831–48. [PMID: 21319274 DOI: 10.1002/ibd.21375]). In the second sentence of the Introduction the number of the reference has been changed accordingly.

6. The METHODS details the number of subjects in each group. This would be expected to be located in the RESULTS section.

Reply: This has been added in the results section

7. The patients with newly diagnosed CD are said (METHODS) to have presented after diagnosis. This doesn't make sense - if they were diagnosed, then they must've presented already.

Reply: "or shortly after" has been deleted

8. The subgroup analyses (tonsilectomy etc) leads to very small group sizes.

Reply: This has been indicated in Materials and Methods, Results and Discussion sections.

9. The Methods suggest that the VCA data was to be anaylsed according to negative/equivocal/positive status, but this was not included in the RESULTS

Reply: For the interpretation of the results, values higher than 12 U/mL were considered as positive. This has been corrected in the material and methods section.

10. The RESULTS section comments on the features of the CD group, but not the control group. Also, this data detailing the features of the two groups may be better to be included in a Table.

Reply: Both groups were paired by sex and age ± 5 years, so that the characteristics of the control group did not differ significantly in age and sex. In addition, the inclusion criteria of healthy controls group were: absence of acute infections, inflammatory, autoimmune or immunodeficiency diseases; and no immunosuppressive or antibiotic treatment or any kind of vaccine during the previous year.

11. Page 7 of the RESULTS comments that the newly diagnosed patients were "presumably" not on therapy - was this not known?

Reply: "As presumably" has been deleted.

12. The data shows lower levels in the newly diagnosed CD group than in any of the other three groups. This pattern is not clearly explained. If higher levels relate to therapies, then why would the control group have higher levels? If higher levels are related to underlying immunodeficiency, then all the CD groups should be higher than controls.

Reply: Figure 1 shows the levels of anti-EBV IgG in the three clinical scenarios and the control group without differentiating whether or not to take treatment. Patients in treatment reached levels of anti-EBV IgG of 144.3 than were higher than the observed in the control group (129.1).

13.Why should just 5-ASA be associated with higher levels? Why not the immunosuppressive drugs? Did the authors combine the results from immunosuppressive therapies versus others?

Reply: In the discussion section the effects of 5-ASA experimentally observed were described (ref 17 to 21). These immunomodulatory proprieties of the drug could explain the results obtained by us in the present study.

14. Similarly, it is hard to explain why high levels would be seen in people with one drug, but lower levels in those on two or three drugs

Reply: This fact could be due to the antibiotic activity of one of the drugs used in the treatment of some patients. This group of patients showed the lower levels of IgG anti-EBV. This could be due to an elimination of some infectious agent that perhaps was the cause of the immune dysfunction.

15. Did the differences observed still exist when other multiple variables were taken into account?

16. How about co-morbidities (smoking, other drugs, etc)??

Reply: the small group of patients not allowed us to do subgroup analysed including other multiple variables or co-morbidities.

17.The legend for Table 1 does not have a legend present.

Reply: the legend of Table 1 is below

Significant differences in IgG anti-Epstein-Barr virus (EBV) antibodies between 1 and 3 drugs/patient (P = 0.028). Values are expressed as means ± SD. P value, Mann-Whitney U Test. 5-ASA:

5-Aminosalicylic acid (Mesalazine); TNF: Tumor necrosis factor.

18. The use of the abbreviation T and the term T-bars is confusing in Fig 3. Control populations would be expected to be given on the left side of the graph

Reply: The Figure has been corrected according to reviewer suggestion

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

Sincerely yours,

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