Author's response to reviews

Title: Anti-Outer membrane protein C and anti-glycoprotein 2 antibodies in inflammatory bowel disease and their association with complicated forms of Crohn's disease

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Author's response to reviews: see over
Dear Sirs,

Thank you for your kind comments to our article "Anti-Outer membrane protein C and anti-glycoprotein 2 antibodies in inflammatory bowel disease and their association with complicated forms of Crohn´s disease".

Detailed responses to the comments/questions are written at the base of this document.

We have made corrections required by the reviewers and we send our new version of the manuscript. Changes made in the manuscript are highlighted with the coloured (red) text.

The final text was also edited by an English native speaker.

Kind regards,

Darina Kohoutova, MD, Ph.D.
Responses to the reviewer 1:

Major criticisms:

1. In the results the Authors state there is a significant difference in anti-GP2 IgA between Crohn’s disease patients with or without extraintestinal manifestations, p=0.05. How did they find significant difference if p=0.05 rather than p<0.05? It does not seem plausible to be typing mistake, since they repeat “p=0.05” both in abstract (page 2, line 29) and in results (page 6, line 7). Please clarify it.

Thank you. Yes, we excluded this statement from our abstract and corrected it in Results from „statistically significant difference” to „trend towards statistically significant difference”, as p value was just 0.05.

2. Please add sensitivity, specificity, negative and positive predictive values of serum anti-OmpC IgA, anti-GP2 IgG and anti-GP2 IgA antibodies in all groups. Could you add them both in Table 1 and in “Results” sections?

We added a comment to Results with a reference to the Table 1. We point out the fact again, that the values of serum IgA anti-OmpC antibodies <20 U/mL were assessed as negative, values >25 U/mL were considered to be positive according to the manufacturer. Anti-GP2 IgG and IgA antibodies were with negative results <15 U/mL and positive results >20 U/mL. That means, that the presence of „grey zone“ was accepted by the manufacturers and therefore sensitivity, specificity, negative and positive predictive values can be misrepresented in this particular case.

Should the results of sensitivity, specificity, positive predictive value and negative predictive value be omitted in the Table 1, we can clarify and explain in the Results why we did so.

3. From page 5, line 30 to page 6, line 4: on the basis of values of Crohn’s disease patients with surgery, they look to be approximately the sum of B2 patients with B3 patients, as I expect. Therefore, if I am right, please remove this analysis, as it is already described in Table 2.

Yes, you are right. We omitted the analysis.

4. Page 6, line 8-11: Since anti-GP2 IgA are higher in Crohn’s disease patients with immunosuppressive therapy, please specify if these patients are responder or not. If there are both subgroups (responder and not responder) please split them and, accordingly, compare them with the group of Crohn’s disease patients without any treatment or treated with 5-aminosalicylates only. Moreover, please compare the other antibodies (anti-OmpC IgA and anti-GP2 IgG) between these three groups of Crohn’s disease patients.

Only responders to immunosuppressive therapy were included, therefore splitting into two groups is not applicable.

Another study would be interesting – assessment of anti-OmpC IgA (and other antibodies) in each patient at the beginning of a flare-up (if present) and after induction of the remission with immunosuppressive therapy (using pair t-test for statistics in each individual).
5. Page 20, line 20-22: you cannot declare that anti-OmpC and anti-GP2 antibodies are useful to identify Crohn’s disease patients, who are more likely to develop complicated forms of disease. In order to be state it, you should have a follow-up of Crohn’s disease patients from diagnosis to complication development in association with measurement of these antibodies, which – on the other hand - are increased once patients already have the complication (B2 and/or B3 phenotypes).

Thank you, we omitted this statement in our discussion.

**Minor criticisms:**

1. In the methods of abstract please delete number of men, women and age for each group recruited in the study, since this information is reported in the “Methods” section afterwards.

   Yes, we deleted this information in the methods of abstract.

2. In the methods of abstract please write which antibodies are tested in patients’ sera.

   Thank you, we added this.

3. The introduction is very short, only 178 words. Please expand it with major comments on papers cited (in particular from Reference 8 to 16) and with some observations regarding anti-OmpC and anti-GP2 antibodies, their target and what it is known about these antibodies in IBD.

   Yes, we expanded the introduction according to your recommendation.

4. In the “Methods” section please type a title of any method used in the study, for instance “Patients”, “ELISA”, “Statistical analysis” and so on.

   Yes, we added this.

5. In the “Methods” section please add duration disease and what treatments the patients are on at the time of blood withdrawal.

   We completed these data according to your recommendation.

6. Please calculate whether in UC patients there is a correlation between anti-OmpC IgA and anti-GP2 IgA values in UC patients, between and anti-GP2 IgG and anti- OmpC IgA values, between anti-GP2 IgG and anti-GP2 IgA as well as you did in CD patients (Page 5, line 1-3).

   It was calculated and added into the text.

7. What are the extraintestinal manifestations of Crohn’s disease patients enrolled in this study? Please specify them in “Methods” section.
We added and specified extraintestinal manifestations of CD patients in the „Methods“ section.

8. Page 6, line 7: Please compare the other antibodies (anti-OmpC IgA and anti-GP2 IgG) between Crohn’s disease patients with or without extraintestinal manifestations.

We included these results.

9. Page 7, line 15-16: please remove this statement, since Crohn’s disease patients with surgery look to be approximately the sum of B2 patients with B3 patients.

We removed this statement.

10. Page 8, line 11-12: please specify that even if the anti-GP2 IgA antibodies are higher in Crohn’s disease patients with extraintestinal manifestations, there is not significant difference.

Yes, we added this particular specification.

11. Graph 1 legend: please associate asterisk with p<0.001, as described in “Results” sections.

We added this information.
Responses to the reviewer 2:

1. The authors state that this is not a novel study but it is confirmatory of previous ones. However, the present study is difficult to read. The result section needs to be simplified and better explained.

The result section has been improved (also with respect to the recommendations of the second referee):
- first part of „Results“ describes values of anti-OmpC IgA, anti-GP2 IgG and anti-GP2 IgA antibodies in CD. Comparison between CD patients with the controls is stated here. Further, association of these antibodies with CD phenotypes (B1-B3 and L1-L3, also with references to the Table 2-3) is declared.
- second part of „Results“ describes the above mentioned antibodies in patients with UC.
- third part of „Results“ displays association of investigated antibodies with specific/complicated forms of CD (such as CD with extraintestinal complications) and further relationship of antibodies tested with age of onset of the disease, family history, perianal disease and therapy of CD patients.

2. Also the tables are rather chaotic. For example, reading the text, I assumed that tables 2 and 3 presented clinical data on the patients (n° of males and females, age at diagnosis, age at testing, etc) while they present antibody results. The antibodies results could be all presented in these tables, including statistical significance, and omitted from the main results section.

Yes, we changed the legends to the Table 2 and 3 (to make it clear). Precise characteristic of patients (number of patients, males, females, mean age, duration of disease) is written in the methods section. There is a lot of results provided and multiple tables had to be added (as statistics included different methods - non-paired t-test, Mann-Whitney rank sum test, Spearman rank order correlation and Pearson product moment correlation). Therefore we choose the most relevant results and have stated them in a simple form in the results section itself.

3. The authors write that this is a prospective study performed between 2010 and 2012. Since all the experiments were performed with ELISA techniques, I think they collected the serum samples, froze and stored them to be tested all together with the ELISA kits. If this should be the case, I am not sure that this is a prospective study.

This was a prospective study, definitely. It was planned in the year 2009, sera were collected between 2010 and 2012 with the aim to investigate anti-OmpC and anti-GP2 IgG and IgA antibodies. In practice, antibodies were tested approximately every 6 months (when appropriate number of sera (according to the antibody kit) were collected).

Quality of written English: Needs some language corrections before being published.

The final draft of our manuscript was edited by an English native speaker.