Author’s response to reviews

Title: Follow-up of pediatric celiac disease: Value of antibodies in predicting mucosal healing, a prospective cohort study.

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Author’s response to reviews: see over
Dear Dr. Denyer,

We uploaded a revised version of our manuscript, "Follow-up of pediatric celiac disease: Value of antibodies in predicting mucosal healing, a prospective cohort study." Below you will find a point by point response to the reviewers' comments. The changes we made in the manuscript are highlighted in yellow in our point by point response to the reviewers. We very much appreciate the careful study given to our manuscript and we have accepted all of the reviewers’ suggestions. We hope the paper is now acceptable for publication in BMC Gastroenterology.

Sincerely,

Andreas Vécsei

Point by point response to Carlo Catassi

Comment 1: Page 6 line 15: authors should explain here the criteria for re-biopsy group B patients: were they unselected and chosen by willingness to participate in the study, were they the less compliant to the treatment according to dietary interview, and so forth;

Response to comment 1:
As suggested, we specified the criteria for re-biopsy in group B patients as follows below. This additional explanation was added at the end of the paragraph with the subheading "Study design and subjects":

Within the 18-month study period, a total of 79 children presented for routine CD follow-up. All of these children were invited to participate in the study independent of the presence of symptoms or their adherence to the GFD according to dietary interview. As
such they were unselected and only chosen by their willingness to undergo follow-up endoscopy. In this context, 26 of 79 eligible children opted out of the study.

Comment 2: Page 9 line 4: the result of the small intestinal biopsy in these 3 patients should be described (were they all Marsh 0?)

Response to comment 2:
We agree and added this information in the third paragraph of the result section with the subheading “Performance of antibody tests in group A”:
Within group A2, positive EMA were found in 3 children (specificity 0.94; 95% CI 0.86 to 0.99) who all had Marsh 0 as the result of the small intestinal biopsy.

Comment 3: Page 9 line 22, it is not clear whether the duration of GFD was on average lower in subjects with EMA-positivity despite mucosal healing

Response to comment 3:
we are particularly grateful for this objection and added the information at the end of the section with the subheading “Performance of antibody tests in group B”:
Comparing the duration of the GFD in group B children with mucosal healing, EMA-positive children had been on the GFD for a shorter period of time (median age in years [IQR, min to max]: 1.5 [0.8, 1.0 to 3.0]) than the EMA-negative children (median age in years [IQR, min to max]: 2.3 [5.5, 1.0 to 12.9]) \( (P = 0.008) \).
And in the second paragraph of the "Discussion" we added:
Twelve of these EMA-positive children showed mucosal healing, a finding that reflects faster mucosal recovery than EMA-seroconversion. Indeed, EMA-positive children exhibiting mucosal healing had been on the GFD for a significantly shorter period of time than the EMA-negative children.

Comment 4: Page 10 line 12: authors should comment on the surprisingly low percentage of children with treated CD and Marsh 1 at biopsy (1.8%). Could it be related to the somewhat high cutoff for increased IEL count (30)?
Response to comment 4:
We agree with Carlo Catassi's assumption and added a corresponding comment at the end of the third paragraph of the "Discussion":

Surprisingly, a very low frequency (1.8%) of isolated increase of intraepithelial lymphocyte count (Marsh 1) was found among group B children. This finding could be related to the somewhat high cut-off for an increased intraepithelial lymphocyte count used in the study (≥ 30 lymphocytes/100 epithelial cells).

Comment 5: Page 11 line 15: authors assume that the finding of EMA in CD subjects showing mucosal healing indicate a “false” positivity. However adherence to the dietary treatment was not evaluated in this study nor were IgA deposits in the small intestinal mucosa. It is well possible that serum EMA positivity is more sensitive than gross histological damage to detect minor dietary transgression.

Response to comment 5:
We agree and changed the second paragraph of the "Discussion" and added a new reference [20]:

Twelve of these EMA-positive children showed mucosal healing, a finding that reflects faster mucosal recovery than EMA-seroconversion. Indeed, EMA-positive children exhibiting mucosal healing had been on the GFD for a significantly shorter period of time than the EMA-negative children. Moreover, 9 of 12 developed EMA-negativity on further follow-up. This delayed seroconversion might partially explain positive EMA in subjects showing mucosal healing [10]. However, adherence to the dietary treatment was not evaluated in this study nor was small intestinal mucosa examined for IgA deposits [20]. Therefore, we cannot rule out that serum EMA-positivity is more sensitive than gross histological damage to detect minor dietary transgressions.

Point by point response to **Antonio Picarelli**

**Comment 1:** *which were the clinical reasons that led to the performance of endoscopic duodenal biopsy?*

Response to comment 1:
We added the clinical reasons/indications for endoscopy in the Methods section at the end of the paragraph with the subheading "**Study design and subjects**":

The predominant complaints in group A1 children were abdominal pain (31.3%), failure to thrive or short stature (18.8%), chronic diarrhea (6.3%), flatulence (6.3%), recurrent headache (6.3%) and constipation (3.1%). A first-degree relative with CD (18.8%), IgA-deficiency (3.1%), autoimmune thyroiditis (3.1%), and iron deficiency anemia (3.1%) were the remaining reasons for CD screening in group A1.

The predominant complaints in group B were abdominal pain (15.1%), constipation (1.9%) and aphthous stomatitis (1.9%). Within group B, 79.2% of children were symptom-free.

**Comment 2:** *Why are they used different commercial tests? Are there differences between them in their diagnostic capability?*

Response to comment 2:
To clarify why we were using different commercial tests we added the following changes to the Methods section under the subheading "**CD serology**":

CD serology kits from different companies were used because Eurospital TG2-IgA and Orgentec EMA had been our routine test kits since 2005 onwards and on request Werfen Austria, Diagnostic Divisions, was willing to complete the armamentarium of current CD antibodies by providing Inova antibody kits free of charge during the study.
Comment 3: RESULTS: page 9 and ten, it is claimed that Ema were detected in 18 children from group B. Twelve of these EMA-positive children showed mucosal healing. To explain about what that statement was based.

Response to comment 3:
To explain the basis of this statement, we changed the beginning of the third paragraph of the subsection "Performance of antibody tests in group B" in the Results section as follows: However, within group B, positive EMA were detected in 18 children, 12 of whom exhibited mucosal healing. Of these children with EMA-positivity despite mucosal healing, all 12 had Marsh 0 as the result of the small intestinal biopsy; 9 (75%) became EMA-negative on further follow-up within the next 20 months.

Comment 4: Children still had positive EMA ranging from 1:5 to 1:80 despite Marsh of class 0. clarify the correlation, your data, among EMA, TG2-and DGP with the different degree of the Marsh lesion. Recent literature has shown a different interpretation of these cases by the use of duodenal mucosa cultures.

Response to comment 4:
To indicate the frequency of positive CD antibodies in children with Marsh 0 on rebiopsy we changed the end of the subsection "Performance of antibody tests in group B" in the Results section as follows: Overall, of the group B children exhibiting Marsh 0, 4 had positive TG2-IgA, 15 positive TG2-IgG, 12 positive EMA, 16 positive DPG-IgA, and 10 positive DPG-IgG. All 35 EMA-negative group B children with mucosal healing (34 children with Marsh 0 and one with Marsh 1) also had negative TG2-IgA.

Additionally we changed the second paragraph of the "Discussion" to mention a possible role of IgA deposits in monitoring dietary adherence and added a new reference [20]:
Twelve of these EMA-positive children showed mucosal healing, a finding that reflects faster mucosal recovery than EMA-seroconversion. Indeed, EMA-positive children
exhibiting mucosal healing had been on the GFD for a significantly shorter period of time than the EMA-negative children. Moreover, 9 of 12 developed EMA-negativity on further follow-up. This delayed seroconversion might partially explain positive EMA in subjects showing mucosal healing [10]. However, adherence to the dietary treatment was not evaluated in this study nor was small intestinal mucosa examined for IgA deposits [20]. Therefore, we cannot rule out that serum EMA-positivity is more sensitive than gross histological damage to detect minor dietary transgressions.