Author’s response to reviews

Title: Symptoms and signs in individuals with serology positive for celiac disease but normal mucosa

Authors:

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Version: 2 Date: 3 June 2009

Author’s response to reviews: see over
Reviewer: Vincenzo Villanacci

Reviewer: The paper is well written with useful notices in the field of diagnosis and treatment of coeliac disease. I suggest to accept the paper.
Authors: We would like to thank Prof Villanacci for these kind comments.

Reviewer: I ask to the authors only a specification about the number of intraepithelial lymphocytes because at moment the normal value is 25-30 IEL for 100 epithelial cells and in the classification of Marsh-Oberhuber was used the value of 40/100 epithelial cells. It is important in the text of the paper this specification. In the bibliography I suggest to enclose two papers on the argument.


Authors: We agree this is an important topic. In an earlier paper published by BMC Gastroenterology [1], we examined the cut-off for IEL among Swedish pathology departments and found that 19/23 pathology department (83%) reported using 30 IELs per 100 as their cut-off for "increased IEL count", while one department reported using 25 and three departments used 20 as their cut-off.

In the revised paper we have cited papers by Hayat et al [2] and Veress et al [3]; and added the following lines to the discussion:
“Most Swedish pathology departments consider >30 intraepithelial lymphocytes per 100 as abnormal[1] (and not >40 as was suggested in older literature[4]. Recent data indicate that a cut-off of 25 per 100[2] or 30 per 100 is appropriate [3]."
Ludvigsson et al: Symptoms and signs in individuals with serology positive for celiac disease but normal mucosa
Revision. 1st June 2009.

Reviewer 2: Kamran Rostami

Reviewer: …difficult to read… language needs editing
Authors: We have revised and edited the manuscript to improve the quality of the language.

Reviewer: …confusions on the definition of some terminology like latent CD.
Authors: In the first version of our paper we defined latent CD as “positive CD serology in patients with a normal mucosa” (trying to adhere to the definition of the NIH; page S3 “…a positive serology but no villous atrophy on biopsy.” [5]. However, in consideration of the comments by Prof Rostami we now describe our patients (“positive CD serology and normal mucosa”) instead of using the term “latent CD”. We hope that use of this description is unambiguous, but we will happily clarify the definition further if requested.

Reviewer: The treatment approach toward this subgroup of patient is different in this study compared to what was recommended in previous guidelines…. I don’t understand; based on what 13% (8-21%) of patients with normal histology have been treated with GFD?... Latent CD are not usually the best candidates for Gluten Free Diet…. Authors: Although, previous guidelines suggested that only patients with villous atrophy suffered from CD and should receive a gluten-free diet, we found that some Swedish patients with positive CD serology but normal mucosa had nevertheless received a gluten-free diet. One reason may be that a large proportion of our patients had symptoms ((Table 2); and it has previously been shown that individuals with only minor abnormalities (not VA) in the small intestinal mucosa may benefit from GFD. We have cited the paper by Tursi et al in our discussion [6]. Clearly there will be some heterogeneity of dietary change and compliance in this group.

The figure “13%” is a description of clinical reality, mirroring that physicians will not always follow national or international guidelines.
We have omitted the figures “8-21%” so as not to confuse the reader. They otherwise constituted the exact 95% confidence interval of the proportion “13%”.

Reviewer: As mentioned in discussion, AGA is far less sensitive and specific for CD. Latent CD and the way is described in this study is confusing the clinician. Latent refers specifically to patients who have abnormal antibody blood tests for celiac disease and normal small intestines but no signs or symptoms of celiac disease. I think
Ludvigsson et al: Symptoms and signs in individuals with serology positive for celiac disease but normal mucosa
Revision. 1st June 2009.

the title is not representing the findings of this study and should be changed.

**Authors:** We have changed the title. It now reads: “Symptoms and signs in individuals with serology positive for celiac disease but normal mucosa”

**Reviewer:** How the authors are confident that the antibodies are not due to other comorbidities especially those with positive AGA.

**Authors:** In Table 4 we listed comorbidites mentioned in the biopsy reports. E.g. only some 0.4% of patients had an indication of IBD (review of biopsy reports). In the patient chart review, 2/112 patients (<2%) with available data had IBD (both had positive TTGA); and also in that review were other comorbidities rare. We therefore feel confident that positive CD serology in the large majority of patients with normal mucosa cannot be explained by other comorbidity.

**Reviewer:** The best would be to use the AGA positive group as AGA positive not latent CD! And compare the clinical presentation with tTG and EMA positive group!

**Authors:** In the revised manuscript we have compared symptoms and signs (and calculated p-values) according to the type of CD serology (EMA+, TTGA+ and AGA+).

Our comparison of symptoms and signs were however post-hoc analyses. Such exploratory analyses carry an increased risk of chance findings, simply due to multiple testing (see citation from statistical textbook by Armitage at the end of this response). We therefore used the Bonferroni correction to adjust for multiple testing, since this constitutes best statistical practice [7]. The use of Bonferroni correction was also discussed with biostatistician Ass. Professor Fredrik Granath (a number of Granath’s publications are listed at the end of this response [1, 8-10]).

Using the Bonferroni correction only p-values <0.003 were statistically significant (0.05 divided by the number of analyses; n=18). Only one analysis was statistically significant by this definition: year of biopsy. Within “year of biopsy” there were statistically significant differences between TTGA+ and EMA, and between TTGA+ and AGA, but not between AGA+ and EMA+.

In addition it should be noted that there were no consistent differences regarding symptoms and signs according to CD serology subtype. “Any GI symptom” was most frequently seen in TTGA+ patients; diarrhea most often seen in AGA+ patients; and weight loss and constipation most often seen in EMA+ patients.

For the convenience of the reader we have presented all p-values (chi-2 test) below. These have not been included in the paper instead we have added the following lines to the legend of Table 2:

“*Only for year of biopsy was there a statistically significant difference according to CD serology subtype when we corrected for multiple testing (Bonferroni correction [7]). There was no statistically significant difference in prevalence of any symptom or sign according to CD serology subtype.”
Background data

<table>
<thead>
<tr>
<th>Background data</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>0.970</td>
</tr>
<tr>
<td>Median age at first biopsy; range (years)</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>Year of biopsy, median</strong></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reported heredity for CD</td>
<td>0.225</td>
</tr>
</tbody>
</table>

Other diseases

<table>
<thead>
<tr>
<th>Other diseases</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes Mellitus, type 1</td>
<td>0.423</td>
</tr>
<tr>
<td>Depression</td>
<td>0.043</td>
</tr>
<tr>
<td>Thyroid disease</td>
<td>0.153</td>
</tr>
<tr>
<td>Liver disease or increased liver enzymes</td>
<td>0.883</td>
</tr>
</tbody>
</table>

Symptoms

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any gastrointestinal symptom*</td>
<td>0.676</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0.787</td>
</tr>
<tr>
<td>Weight loss / growth failure</td>
<td>0.835</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0.480</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.214</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.908</td>
</tr>
</tbody>
</table>

Laboratory data

<table>
<thead>
<tr>
<th>Laboratory data</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia or iron deficiency</td>
<td>0.087</td>
</tr>
<tr>
<td>Folic acid deficiency</td>
<td>0.133</td>
</tr>
<tr>
<td>B12-deficiency</td>
<td>0.538</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate, increased</td>
<td>0.232</td>
</tr>
</tbody>
</table>

Reviewer: Result: This statement is just inappropriate; We identified 3,736 individuals with latent CD! As mentioned above not every patient with positive serology means latent celiac disease! Please revise; you may say that you identified 3,736 individuals with positive serology and normal biopsy instead!

Authors: Done. Throughout the text we now talk of “normal biopsy with a positive CD serology”.

Reviewer: This is very confusing; In 90% (95% CI=83-95%; 103/114) of individuals undergoing validation, latent CD could be confirmed through patient charts. Or 46% (36-55%) had diarrhea, while 26% (18-35%) had anemia 90%, 46% and 26% of whom? You perhaps are talking about 114 selected cases. Please clarify for the readers! There are some confusing numbers used; in abstract 120 latent CD in results 114 in discussion 112 and in table/flowchart 118!!!

Authors: N=120. Overall this validation was based on 120 patients. This number is given since it is important to present the patients eligible for validation.

N=114. When we assessed the degree of misclassification (i.e. the proportion of individuals who despite being identified as having “latent CD” through register
Ludvigsson et al: Symptoms and signs in individuals with serology positive for celiac disease but normal mucosa
Revision. 1st June 2009.

matching did not fulfill the following criteria: “(a) positive CD serology and (b) normal mucosa but (c) never had villous atrophy or inflammation)” (Figure 2). N=112. Symptoms and signs were evaluated in 112 patients.

In the abstract we have only listed the figure “112”, “120” and “114” have been explained in the main text of the paper. However, only “112” appear in the abstract. We also believe that the percentages within brackets in the abstract may be confusing. These are 95% confidence intervals, but have been omitted from the revised abstract.

Authors: We have added an appendix (Appendix II) with the Swedish SnoMed classification and its relationship to the Marsh classification.
We have cited the paper by Prof Rostami in Appendix II, as well as the paper by Rostom et al [11].

Reviewer: What does all these % 46% (36-55%), 26% (18-35%) ,,..
Authors: These are 95% confidence intervals of proportions (i.e. percentages).
Estimating confidence intervals are described in Armitage et al [7], and has also been discussed with biostatistician Ass. Professor Fredrik Granath. We have clarified in the text that the proportions in parentheses are 95% confidence intervals.

Reviewer: #Discussion is not focused and the message is unclear and doesn’t appear to come across the way it should! In discussion the authors need to justify why this strategy is effective? Why should we look for latent CD?
#In contrast to the authors’ finding Latent CD have no symptoms. However, the reason for biopsy and serology in this study group was based mainly on their symptoms. How do the authors explain this?
Authors: In the revised paper we no longer use the term latent CD. For this reason we have not discussed why one should look for latent CD. In patients with symptom suggestive of CD it is natural to investigate CD; and CD serology then constitutes the first step. We have edited the discussion to give it more focus.

Reviewer: #The authors could bring more life in their discussion by discussing the possibility of microenteropathy and microscopic enteritis as a sole explanation for this fashion of presentation. We know that relying on villous atrophy would result in missing a significant number of cases. We also know that inflammatory anaemia and inflammatory malabsorption would happen within normal looking mucosa.
Ludvigsson et al: Symptoms and signs in individuals with serology positive for celiac disease but normal mucosa
Revision. 1st June 2009.

# The authors may emphasize in conclusion that CD may present in a great deal with microscopic enteritis and symptomatic seropositive cases might benefit from a gluten free diet as absence of villous atrophy is not evidence of absence of CD.

Authors: #We agree with the reviewer that microscopic enteritis is an underestimated entity. Although our previous validation found that Swedish pathology departments use CD3-staining, we cannot rule out the possibility that some of the patients with normal mucosa and positive CD serology in our study had microscopic enteritis. Neither can we rule out that some patients with “normal mucosa” did not have altered enterocytes, and affected microvilli (as described in table 1 of your paper [12]).

#We have discussed microscopic enteritis in both the discussion and the conclusion.

Discussion:
“Neither can we rule out the possibility that some patients classified as having Marsh 0 had in fact sub-microscopic changes (microscopic enteritis), and we lack individual-based data on immunohistochemistry for our patients [12, 13]. Microscopic enteritis has increasingly been recognized as an important cause of CD-like symptoms including malabsorption [12]. These patients have a low intraepithelial lymphocyte count but nevertheless show altered enterocytes and affected microvilli [12]. Microscopic enteritis is also associated with other autoimmune diseases.”

Conclusion:
“A proportion of individuals with normal mucosa (Marsh 0) and positive CD serology may in fact have microscopic enteritis[12].”

Reviewer: Conclusion is not conclusion at all! It is a repeating version of results. Please delete or change!
Authors: We have changed (and shortened) the conclusion. The revised version does not repeat the results of the paper.

USE OF BONFERRONI CORRECTION
Citation from Armitage et al [7]

..consider the problem in multiple testing. Suppose each of the p variables is tested by a univariate method... Then some variables may differ significantly between groups whilst others may not. A difficulty in interpretation is that p tests have been carried out so that, even if the overall null hypothesis that none of the variables differ between the groups is true, the probability of finding at least one significant difference is higher than the nominal significance level adopted. ...

The Bonferroni procedure is sometimes used to correct for this. If p independent comparisons are carried out at a significance level of alpha, then the probability that one of more are significant is (1- (1-alpha)exponent p, and when alpha is small this is approximately p x alpha. Therefore setting alpha to alpha/p ensures that the probability of finding one or more significant effects does not exceed alpha.” [7]
REFERENCES


