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Title: Non-invasive prediction of persistent villous atrophy in celiac disease

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Dear editor,

Comments to the review report are fulfilled into the text in *Italic.*

Reviewer #1: This paper has the aim of finding markers which are predictive of villous atrophy in patients affected by celiac disease in treatment with gluten free diet. The conclusions are that a combination of anti-transglutaminase and anti-gliadin antibodies evaluated by an appropriate cut-off value with ultrasonographic intestinal picture may reach an optimal value of sensitivity and specificity. Main comments are:

• Was villous atrophy that Authors found in these patients expression of a lack of response to gluten free diet or of gluten assumption? This aspect is relevant since encloses the real clinical purpose of this study.

Explained in text

• "the study protocol was approved by the local ethical committee University Hospital Brno", more specific details are requested.

supplemented

• "Lab kits for analyses were provided by TestLine Clinical Diagnostics ltd". The location of the manufacturer is lacking. This is arelevant aspect expecially because it indicates the population who was tested for cut-off value validation.

Specified in text

• Ultrasonographic aspects were referred only to a report of literature. Is it enough?

Expanded

• "The calculated cut-off values were 13.4 U/ml and 6.7". How did they differ from that suggested by the manufacturer? How Authors explain this difference (if there is)? Could an "in situ validation" be useful?

Explained in text

Reviewer #2: - Introduction. "Mucosal healing is a main endpoint of this therapy; however, this goal is achieved only in about 60% of patients after one year of GFD" Define what do you mean for mucosal headlining, since in many patients Marsh I damage persist, but this is not considered a problem

Specified in text

- Do you advice to look for VA (invasively or not invasively) only in symptomatic patients or in asymptomatic patients too? If the second case, with what time-point?

Specified in text

- "In our retrospective cohort study, we included patients who had been on the GFD for at least one year and for whom data on follow-up duodenal biopsy and quantitative evaluation of aTTG and/or aDGP using the enzyme-linked immunosorbent assay (ELISA) method was available as well." Why some of your patients (and others no) underwent to biopsy-follow up?

Explained in text

- "Abdominal ultrasonography was available in subgroup of the patients." Why some of your patients underwent to ultrasonography follow-up examination?

Specified in text

- "All the selected patients underwent esophagogastroscopy with biopsy from the distal duodenum" Do you perform duodenal biopsied in DIII-DIV?

specified

- Statistical analysis Declare the statistically significant level you used. Specify the software.

specified

- Table 1: do not report mean and median, report one of two according to statistical distribution

corrected

- "The most frequent clinical symptoms and laboratory signs of malnutrition at the time of follow-up biopsy were diarrhea (23.2%), abdominal pain (20.7%), weight loss (9.8%), sideropenia (26.8%), vitamin D deficiency (20.7%), and anemia (11.0%)." In clinical practice, the majority of CD patients respond well to GFD: how do you explain these number?

Expanded in text

- "However, non-invasive abdominal ultrasound is widely available" Abdominal ultrasound is widely available, but bowel ultrasound, at least in the majority of the country, is performed by only few specialists

Specified in text

Reviewer #3: In the retrospective study sensitivity, specificity, and negative predictive value of aTTG and

aDGP serology to predict persistent villous atrophy was investigated in 82 patients. The authors conclude that combination of bowel ultrasound examination and improved serology may achieve better accuracy. As a normative value the authors use histological examination of mucosal biopsies and the graduation system Marsh/ Oberhuber. Comments

1. Histology is used as the normative to judge serology and bowel ultrasound. This correlation should be more clearly given in the abstract and in the body of the manuscript.

corrected

2. Results; Page 7: Oberhuber instead of Obenhuber

corrected

3. Results; percentages should given 22.2% ; 37.2% ; 29,3% instead of 22,2%; 37,2%; 29,3%

corrected

4. Graphs for correlation of Marsh/ Oberhuber classes with serology and ultrasound could help to better visualize the association.

Authors think graphs are not useful and clear for presentation of this data.

Reviewer #4: This clinical study considers the evaluation of possible predictor factors of villous atrophy in celiac disease, like anti-tissue transglutaminase antibodies, anti-deamidated gliadin peptide antibodies, and abdominal ultrasonography. This study makes an additional contribution to studies which help to improve non invasive diagnostic possibilities and find reliable widely available non-invasive marker of persistent villous atrophy (VA), in celiac disease patients. The study is set up correctly. The material studied allows to drawn the conclusions. The paper is written well, the Introduction give a good overview about the study background and the authors raised clearly the hypothesis of the study. The description of material studied is accurate. The aim of the study is fulfilled. The material studied is large enough and allows to drawn the conclusions. The Results are presented clearly and have been discussed well. The 7 tables give good overview about the results. The authors find that a combination of serology with ultrasound imaging increased positive predictive value and specificity to 88.9% and 98% for aTTG IgA and to 90.0% and 97,8% for aDGP IgA to predict persistent atrophy. The combination of serology and experienced bowel ultrasound examination may achieve better accuracy in the detection of atrophy. Asymptomatic patients with lower levels, particularly of both aDGP IgA and IgG, do not need to undergo the follow-up duodenal biopsy to evaluate persistent VA. However, the following point needs to be considered:

1. In Material and Methods in paragraph concerning Duodenal sampling and assessment of histological findings the authors mentioned that "Immunohistochemistry was used for identification of intraepithelial lymphocytes (CD3 and CD8 expression), macrophages (CD68 expression), and plasmocytes (CD138 expression) evaluation in the stroma." However, there is no description of immunohistochemical method used in this study and any results of evaluation above mentioned cells.

Taken out, not relevant for this study.

Reviewer #5: In this retrospective study, the Authors investigate the accuracy of different non-invasive

tools (antibodies, ultrasonography, symptoms) to assess the persistance of villous atrophy (VA) in Celiac Disease patients (CD). They have shown that none of the above tools per se is sufficiently accurate to predict VA persistance as desirable in clinical practice, but the combination of serology and US can increase the overall accuracy. The study is interesting yet shows some major flaws:

1. Why only 82/190 patients had available biopies? If the clinical pattern of patients was ruling the decision to go for as biopsy, you may have selected patients with a more severe disease and hence more prone to show persistent atrophy

explained in text

2. What is the need to show persistent atrophy beyond clinical or laboratory response? Your data do not seem to support this need. Please expand

expanded

3. Results : "Autoantibodies aTTG were positive (cut-off value 18U/ml recommended by manufacturer) in 18 cases (22,2%), aDGP were positive (cut-off value 20U/ml determined by laboratory) in 29 cases (37,2%)." Was this observed at diagnosis, at follow-up? If at FU, the high proportion of positive antibodies may show inadequate adherence to GFD or refractory disease

Explained, expanded

4. Is US a validated technique for the diagnosis and follow-up of CD patients? Not to the best of my knowledge. Please expand or take out

Expanded, specified in text

5. Data should be either in the text or in tables

corrected

6. A calculation of the sample needed to reach statistical significance should be given

corrected