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POINT BY POINT ANSWER TO REVIEWERS

TO THE EDITOR

Dear Editor, we appreciated all comments of the Reviewers and accepted all their suggestions.
Here we report our point by point answer to the comments of the Reviewers.
All corrections are in **RED** in the Text (marked revision).

Best regards
Raffaella Tortora

REVIEWERS' COMMENTS TO AUTHOR:

Reviewer 1

Comment from Professor Colin Howden (Associate Editor):

- Please indicate how the use of a gluten-free diet was determined. How long were participants required to be off a gluten-free diet.

Adherence to Gluten free diet was determined by measuring a-tTG serum levels in our patients one year after they start this dietary regimen. This point was better clarified in the RESULTS section. Moreover, we added a sentence in the DISCUSSION section and a new reference [23] about this topic.

Reviewer 2

- The Title must attract readers and be very clear. What about "The presence of anti-endomysial

antibodies and the level of anti-tissue transglutaminases can be used to diagnose adult coeliac disease without a duodenal biopsy".

According to Reviewer's suggestion, we changed the Title of our paper. In view of the length of such a title we let the final decision to the Editorial Office (in case of acceptance of our paper).

- All proprietary test kits should have their details provided very precisely at first mention – (Manufacturer, Town, Country).

Anti-tissue transglutaminase (a-tTG) IgA antibodies were measured by ELISA (Enzyme-Linked Immunosorbent Assay, automated system; Delta Biologicals SRL, Rome, Italy). Tests for anti-endomysial (EMA) IgA antibodies were identified by using immunofluorescence on a section of monkey esophagus (Delta Biologicals SRL, Rome, Italy).As requested, we added more details to test kits used. In particular, we added these features in the METHODS section (see "serology").

- A ROC curve may satisfy statisticians, but can you provide a figure with the concentration of each patient's anti TGA, and with different symbols for positive/ negative EMA, against the histology grades. The question is, how many false positives and negatives did you find?

As suggested by the Reviewer, we added more "readable" figures showing the concentration of anti-tTG in each patient in accordance with the Marsh grade (for diagnosing CD and detecting atrophy). Thus, Figure 1 and 2 were modified adding a dot plot diagram (Figure 1B, Figure 2B). The main strength of our paper was the maximization of the specificity (100%). As a consequence the use of this a-tTG cut-off determined the fact that no false positive cases were found in our population. On the contrary, when using a a-tTG cut-off of 45 U/mL, the number of false negative cases was 109 (35%) . This concept was clearly underlined in both Results and Discussion Sections.

- The legends to figures and tables must be informative – such that the reader can look at the figure or table, and understand it without having to check for details in the text of the article. Imagine your

figure or table being pasted into a lecture slide – will the audience be able to understand it? Not least, the number of patients in the groups.

We appreciate this comment and improved the legends of our figures and tables.

- In the past few years, AP&T has published several relevant papers about the diagnosis and management of coeliac disease, which could augment the Discussion, and these papers are readily available to our Readers.

As suggested by this reviewer, we commented several relevant papers about diagnosis and/or management of CD in the DISCUSSION section. In particular, we added these references:

[22] Lewis NR, Scott BB. Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease. Aliment Pharmacol Ther. 2010 Jan;31(1):73-81.

[23] Dipper CR, Maitra S, Thomas R, et al. Anti-tissue transglutaminase antibodies in the follow-up of adult coeliac disease. Aliment Pharmacol Ther. 2009 Aug;30(3):236-44.

[31] Cammarota G, Cesaro P, Martino A, et al. High accuracy and cost-effectiveness of a biopsy-avoiding endoscopic approach in diagnosing coeliac disease. Aliment Pharmacol Ther. 2006 Jan 1;23(1):61-9.

Reviewer 3

The study addresses the topic of how to correctly diagnose celiac disease by possibly avoiding the duodenal biopsy in adults. While evidence-based recommendations are available from many studies in pediatric age, there is relative paucity of data fin the adult literature.

This reviewer finds this prospective study well conducted and the data well presented. Only minor criticisms:

- Perhaps in the discussion section it should be emphasized that the sensitivity of the proposed

diagnostic cut-off is quite low, so that physicians should be aware of the need to consider celiac disease even when TTG and/or EMA levels are not high.

We appreciate this comment and added a sentence in the DISCUSSION section. In particular, we underlined that the use of this a-tTG cut-off point determined the fact that the number of false negative outcomes was relative high (109 cases; 35%).

- The discussion is very thorough, but it gets to be lengthy and it could (should) be shortened to make it more easily accessible to the reader.

The Authors tried to shorten (with the limitation of answering also to the considerations of the other Reviewers) the Discussion section.

Reviewer 4

Comments for Transmission to the Authors

This paper attempts to assess the predictive value of a-tTG level for diagnosing celiac disease. The authors found that a value of at least 62.4 U/ml predicted Marsh 3 at histology, in 100% of the cases. The authors conclude that patients with a-tTG above this cut-off value could avoid duodenal biopsies if they are less than 50. In addition they estimate cost savings in this population of patients.

The authors recognize the limitations of their study: all patients were EMA -positive and had suggestive symptoms and/or strong risk factors of celiac disease.

I have several issues.

- Who read the duodenal biopsies : was it centralized or not ?

The analysis of biopsies was centralized at our Pathology. This was better clarified in Methods Section.

2. Could you detail the methodology for IEL count (i.e., immunohistochemistry) ?

Immunohistochemistry was used by our pathologists to determine IEL count. We added this sentence in the METHODS section (“Endoscopy and Histology”).

3. Was the Marsh classification made by the authors retrospectively or by the pathologists on their report?

In all cases, our expert pathologists made the Marsh classification on their report. This point was better clarified in the Methods section.

4. Were the duodenal biopsies interpreted in a blinded fashion, i.e., without knowledge of EMA and a-tTG results?

The pathologist was blinded about serology of patients. This matter was better defined in the Methods section.

5. How do you define an expert operator for EMA antibody reading ?

Tests for anti-endomysial (EMA) IgA antibodies were performed by operators confident with immunofluorescence procedures. According to Reviewer’s observation we deleted the term “expert”.

6. Does the cost-saving calculation take into account the false negative results and the need for upper GI endoscopy in patients above 50?

We re-evaluated the cost-saving when considering the exclusion of patients above 50 years (28.823,00 € ($ 39.561)). These corrections were made in Results and Discussion Sections.

- Could you describe further the patients who have a-tTG lower than 62.4 U/ml and Marsh 3? Are they older than other patients?

As suggested, we analyzed the features of patients having a-tTG lower than 62.4 U/ml and Marsh 3. In our hands no relevant differences about age and/or other variables were evident between the two groups of CD patients (mean age 34.1±12.4 vs 32.1±12.8; p=0.2). We did not report these findings in the Results and Discussion sections to shorten the paper.

- At least some of these patients had duodenal biopsies after GFD. Did the authors test for correlation between Marsh classification and a-tTG titers in this setting ?

No patient underwent further endoscopy/biopsy during and at the end of the study period. This was clarified in the Results section.