Author's response to reviews

Title: Celiac disease: A comprehensive current review

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Point-to-point reply to Reviewers

We wish to thank the reviewers for their insightful comments, which will improve the quality of our manuscript. A point-by-point reply to the comments / criticisms / suggestions raised by referees is reported below:
Reviewer #1 Concepción Nuñez

The authors review the current knowledge about celiac disease, covering important aspects related to the disease and trying to include some novel discoveries, some of them from their own group.

It is a quite complete and well-written review. There are only some minor points that I consider that must be clarified or included.

1. At the end of the abstract, the authors conclude that the future challenge is related to the identification of new treatments. Even though it is an important point, many efforts should be also focused on diagnosis. Seronegative cases and those with mild histological lesions constitute an important challenge nowadays. Despite the great advances in pathophysiology and diagnosis, at present celiac disease is still a clearly underdiagnosed condition.

Response: We agree with the reviewer, a statement on the importance of research aimed to clarify seronegative and minimal changes celiac disease has been added in the Abstract (which has been thoroughly re-written according to Reviewer #4 comments). See page 2, lines 16-19.

2. In the part related to Genetics the authors mention the roles of HLA class II genes, saying: "Also common to other autoimmune diseases is the relevant role of human leukocyte antigen (HLA) class II genes, specifically DQ2 and DQ8" (page 6 line 34). Although DQ2 and DQ8 are the main predisposing factors, they are not genes. HLA-DQA1 and HLA-DQB1 are the main genes involved in celiac disease. Depending on the alleles present in both genes the DQ8 haplotype or DQ2 haplotype or genotype can be present, leading to the DQ8 and DQ2 heterodimers, respectively, that are involved in CD pathogenesis.

Response: Agreed. We changed the text accordingly. See Genetics paragraph, page 5, lines 3-4.

3. The sentence "The erroneous adaptive immune response consequence of a highly specific interplay between selected gluten peptides and MHC class II HLA-DQ2/8-restricted T-cell antigens plays a paramount role in CD pathogenesis" (page 8, lines 56-60) needs to be rewritten. The interplay is between selected gluten peptides (which are indeed the HLA-DQ2/8 restricted antigens) and MHC class II HLA-DQ2/8 heterodimers. The authors probably meant: "…MHC class II HLA-DQ2/8-antigen restricted T-cells".

Response: Agreed. We changed the text accordingly. See page 7, line 4 from the bottom.
4. I find very interesting the inclusion of two terms absent in the Oslo definitions: seronegative CD and GFD non-responsive CD. However, regarding the definition of seronegative CD, it is noteworthy that lesions milder than Marsh 3a are more frequent in subjects with low titer or negative antibodies. Thus, I think seronegative CD should include the presence of milder lesions than atrophy (Marsh 1 and Marsh 2), although in those cases the differential diagnosis is more complicated due to the increased number of potential causes of milder enteropathy.

Response: We thank the reviewer and take her thoughtful comment on this very intriguing topic. However, we would like to point out that patients with seronegative CD (i.e. absence of detectable antibodies against transglutaminase, deamidated gliadin peptides and endomysium) should have an atrophic lesion at the histology that recover after gluten withdrawal. The introduction of Marsh 1 and Marsh 2 patients in this category is questionable since, as indicated by the Reviewer, other conditions may interfere with a final appropriate diagnosis. Thus, to avoid misleading interpretations, we humbly suggest maintaining the text as it is currently written.

5. Related to this point, the authors consider CD only in presence of atrophy, including milder enteropathy with positive antibodies as potential CD. According to the ESPGHAN (J Pediatr Gastroenterol Nutr. 2012 Jan;54(1):136-60): "According to the Marsh classification, lesions include infiltrative, hyperplastic, and atrophic patterns... IELs in numbers >25/100 epithelial cells suggest an infiltrative lesion". Although it is also said "only 10% of subjects presenting infiltrative changes have CD", the possibility exists and if the authors prefer to exclude those cases, at least they should include a comment about it. In this context, consider to reference the paper "Intestinal intraepithelial lymphocyte cytometric pattern is more accurate than subepithelial deposits of anti-tissue transglutaminase IgA for the diagnosis of celiac disease in lymphocytic enteritis". F Fernández-Bañares et al. PLoS One. 2014; 9(7): e101249.

Response: We understand the reviewer’s comment; however, we did consider milder enteropathy as potential CD provided that this subset shows a serological profile compatible with the condition. Regarding the 10% of subjects mentioned by the reviewer, we do agree that intraepithelial lymphocyte cytometric pattern is more accurate than subepithelial deposits of anti-tissue transglutaminase IgA for the diagnosis of celiac disease in lymphocytic enteritis. A sentence (and the related reference) has been added in the revised text (see page 17, lines 2-4).
6. Following the same topic, in page 15 line 26, the authors used villous atrophy as one of the five criteria needed for CD diagnosis according to the rule "four out that five". I consider that Marsh 1 and Marsh 2 should be included, as in the original article: celiac enteropathy at the small bowel biopsy (Including Marsh-Oberhuber 3 lesions, Marsh-Oberhuber 1-2 lesions associated with positive celiac antibodies at low/high titers).

Response: Actually, the reviewer is right. The ‘four out of five rule’ does include Marsh 1 or 2. For a better understanding of the text, we have introduced a note indicating the current point raised by the reviewer. See page 13, line 14.

7. Include references about the test for monitoring the GFD (page 20 line 53).


8. Table 3. It is confusing why anti-DGP antibodies are named DGP-AGA, being AGA the term usually used for anti-gliadin peptides, please correct. Also in table 3, which papers have been used to obtain the diagnostic accuracy of the presented serological tests? To see better values for specificity and PPV for anti-DGP than for anti-tTG seems surprising. Please revise.

Response: Agreed. Both indications have been corrected in Table 3. Thanks for catching them up.


Table 5 should include Marsh 3a and 3b under grade B1.

Response: Done.

9. Figure 1 needs better resolution.

Response: Agreed. Figure 1 has been changed.
10. Is Figure 2 referred to adult patients? Please, specify.

Response: Yes, correct. The Figure refers to adult patients. This has been clarified in the figure legend.

11. Figure 3. Please, delete "(continuous gluten exposure, slow response to gluten-free diet and refractory celiac disease)" of the legend, it can be seen in the Figure.

Response: Done.

12. Legend of Figure 4 is misleading. The diagnosis is based on serological screening, but it is not a serological diagnosis since it uses biopsy for final decision. Please, modify.

Response: Absolutely agree. We have amended the figure legend.

13. Reference 66 does not deal with potential CD (page 18 line 41).

Response: Thank you for catching this typo; reference 69 is the right one.
Reviewer #2 Kamran Rostami

This is a comprehensive review by very well-known and respected authors in this field. This paper review many aspect of CD and overall it is an excellent addition to the current literature. The authors interpretation of pathogenesis and reviewing the current literature is valuable especially since they had substantial contributions in creating those evidence themselves.

I have few suggestions and recommendations:

1. Histology as the gold standard is controversial even though the authors mention that ESPGHAN criteria is not accepted everywhere yet. The authors criteria for diagnosing CD is valuable. However, the current evidence reliably suggest, some children could safely be diagnosed based on serology and the elements proposed by ESPGHAN.

Response: We take the reviewer’s comment about this very debated issue. Currently, two major views challenge physicians as to whether biopsy should (or not) be taken in the pediatric age. Both pros and cons views to biopsy children with suspected celiac disease hold true; however, most pediatric cases, especially those cases with low-medium anti-tTG2 titers, require histopathological assessment to confirm celiac disease diagnosis. This sentence has been added in the text. See page 13, line 5-7.

2. Introduction: CD has undergone a true metamorphosis! This might need to be formulated differently. We are capable to diagnose the atypical forms of disease and it is unlikely that CD has changed in anyway. The authors also explain that beautifully in the following sentences.

Response: Agreed. The reviewer is right. Celiac disease has undergone a true metamorphosis, which is the result of a better knowledge of the risk groups through the implementation of the serological tests. See Introduction, page 3, lines 7-8.

3. Some of the information overlap and repeated in different sections especially on serology.

Response: Agreed. Sentences with possible overlap have been deleted.
4. Histology

The recent consensus studies on histology of coeliac disease have been overlooked. The Oberhuber classification has been heavily criticized by Marsh and it is considered unsubstantiated. I recommend the authors taking these in consideration when discussing the histology:


Response: Thank you for this comment. The following sentence has been added along with your paper: “Even if it is well-established that coeliac patients always display IEL ≥25%, a recent paper stressed the importance of a high IEL count for CD diagnosis underlining that the mean IEL count in untreated CD was 54 ± 18/100 enterocytes, whereas in non-CD patients the value was 13 ± 8 (Rostami K et al., Gut 2017; 66: 2080-2086 ).” See page 17, lines 4-8.


Response: Agreed. The indicated ref along with a sentence (see page 17, lines 12-16) have been added, as suggested by the reviewer.


Response: Agreed. The indicated ref along with a sentence (see page 17, lines 12-16) have been added, as suggested by the reviewer.

5. Follow up

The first follow-up should include a screening of antinuclear antibodies (ANA/ENA) and non-organ specific autoantibodies

ANA is non-specific and may cause unnecessary concerns. The screening with ANA should be reserved for selected patients at high risk for autoimmunity.

Response: We agree with the reviewer that ANA at low titer are unspecific and might give rise to unnecessary concern. However, we suggest to define the ANA pattern and titer. Should this test reveal a high titer (along with ENA positivity), then this information could be useful to detect other autoimmune associated diseases, e.g. primary biliary cholangitis and Sjögren syndrome. See changes made in the text at page 25, line 8-10.
6. I’m unsure if an ultrasound would be necessary for every coeliac patient. There is no enough evidence to justify that.

Response: We agree. Ultrasonography is indeed not required in any patient with celiac disease, however in patients with suspected hyposplenism or liver abnormalities or with possible underlying complications, this tests is useful, not expensive and non invasive. We would humbly suggest to maintain this exam in the list of diagnostic tests for celiac disease.

7. In my personal experience, most of symptoms that do not resolve after GFD is related to additional lactose intolerance rather than the whole FODMAP list. Secondary lactose intolerance is common in CD patients and this is not IBS.

Response: We agree with the Reviewer that lactose as well as FODMAPs intolerance may contribute to symptom generation in most cases of non-responsive celiac disease. However, it should be pointed out that lactose belongs to the list of FODMAPs and therefore it is difficult to distinguish one from another in terms of individual contribution to non-responsive celiac disease.

8. Also repeating the duodenal biopsy in patients with good response to GFD without micronutrient deficiency is wasting of time and resources. It would not be necessary to be undertaken routinely.

Response: Agreed. A second biopsy after GFD should be recommended only in those patients with persisting symptoms and demonstrable laboratory deficiencies of micronutrients.
Reviewer #4 Justine Turner

This is a wonderful state of the art comprehensive review by leaders in the field and I agree with the authors its timely.

We wish to thank the Reviewer for his valuable comments which certainly increased the quality of our manuscript.

The only concern I have is the length of review and as such most of my minor suggestions below are to reduce text for brevity and increase readability.

Response: We thank the reviewer for helping us to shorten the manuscript which has been reduced in length accordingly.

I also suggest the title could be changed something like "Celiac disease: a comprehensive current review" or similar.

Response: We take the Reviewer’s suggestion and changed the title as indicated.

I would like to see the Abstract markedly reduced to a brief paragraph (or two at most of a few sentences each only) that mainly indicates why the authors feel this review is necessary at this time and what it will cover.

Response: The abstract has been significantly reduced.

Page 4: line 21, please insert comma after identified', even in geriatric patients'; please delete "outbreak" and line 38 change 'extreme raise' to 'increase'; line 41, suggest delete 'often criticized, leaving' and change to '...the real cause of the risk in CD diagnoses remains unknown.'

Response: The suggested amendments / changes have been introduced in the revised text.
Page 5: line 6, delete human and change 'disorder' to 'disorders'; line 14 delete '(so called invisible...iceberg); line 60, change to '... environmental changes that have reduced our exposure to pathogens.'

Response: All suggested amendments / changes have been done.

Line 41, would the authors like to comment here on the DQ8 mouse model of celiac disease? (Verdu and others)

Response: We take the Reviewer’s comment on the HLA-DQ8 transgenic mouse model. However, we feel this model mainly focuses on GI functional aspects inherent to disturbed gut physiology observed in patients with CD. These aspects are far beyond the purposes of the present review and would take a considerable explanation before introducing the main data of the transgenic model. Thus, we suggest to not mention the HLA-DQ8 mouse.

Page 10: please add a reference line 6 after ecosystem; line 26 Firmicutes is a phyla and should not be in italics.

Response: All suggested amendments / changes have been done.

Page 11: line 16, suggest begin new paragraph after reference 70 and change to '...CD is more ...'; line 36, suggest new paragraph after reference 2.

Response: All suggested amendments / changes have been done.

Page 13-14: line 60, suggest change to '...European study showed diagnostic accuracy of ESPGHAN...'

Response: All suggested amendments / changes have been done.

Page 14: add reference line 9 after anti-tTG assays; line 45, change 'Most' to 'Many'.

Response: All suggested amendments / changes have been done.
Page 15, delete lines 16-24 'Macroscopically evident... and lymphoma.' I personally do not feel the current data supports DGP as being widely validated in comparison to EMA and TTG - would you consider to remove?

Response: We humbly disagree with the Reviewer. Those lines are in our mind useful to define the importance of hyposplenism in CD.

Concerning DGP, in principle we are in line with Reviewer as current evidence indicated that an isolated positivity of DGP antibodies detects CD only in 15% of cases. However, although limited we feel DGP should be mentioned in the spectrum of CD-related serology. Also, the other Reviewers asked us to mention DGP.

Page 16, add new paragraph line 11 after reference 110.

Response: Done.

Page 17, line 11 suggest delete 'Despite it might...' so start at 'Histology remains...'; line 19 change 'Current indications' to 'Current recommendations are for four...'; line 45, start new paragraph after references 122-124.

Response: Done.

Page 20, suggest you delete lines 29-33 as repetitive 'On the other hand...'; line 60 replace the comma at the end of the sentence with a full stop.

Response: Done.

Page 21, I think the paragraph line 21 beginning 'Conversely, ...' flows well with the prior paragraph and does not need to be new.

Response: Absolutely right. Done.

Page 22, line 53, suggest new paragraph after '...improvement.'

Response: Done.
Page 23, line 60, can you include the background risk for context.

Response: We take the Reviewer’s comment. The indicated suggestion has been already covered in the text as follows: “A diagnosis of RCD should always be suspected by persistent villous atrophy despite a strict, one-year GFD, negative serology (some cases may show the persistence of low-titer CD-related antibodies), the exclusion of other causes of persistent villous atrophy and phenotyping of the intestinal lymphocytic population aimed to confirm the presence (type 2) or absence (type 1) of a monoclonal rearrangement of T cell receptor (TCR).” See last lines of page 22 and beginning fo page 23.

Page 24, line 46, can limit ORs to one decimal place.

Response: Done.

Page 25, I do not think the header should be restricted to adults, children also need careful follow up as you immediately outline in the fist paragraph. However, on page 26, line 38 you might then want to add '... follow up duodenal biopsy in adults in order...' as the data is so limited for children.

Response: Agreed. The term “adults” has been added; also, a short paragraph on follow-up in children has been added. See page 26.

Page 26, I would like you to comment more on GIP testing as this is very controversial and current.

Response: Agreed. A sentence on GIP has been added.

Page 27, line 21, suggest new paragraph after reference 165.

Response: Done.

Page 28, the new paragraph beginning IL-15 could easily follow on from the former.

Response: Done.
Page 29, line 14, can you add a reference to '... remain undiagnosed.' this is such an important issue to highlight and only really comes up here, at least direct the reader to primary sources.

Response: Done.

Regarding Table I cannot see where they are referred to in the text; this may need to be added. Again for brevity I suggest you delete Table 2 and 3.

Response: We absolutely agree with Table 1, which has been inserted in the text. We humbly feel that Tables should be kept in order to better summarize some key aspects of this review.

Figures again for brevity I suggest you delete Figure 2 and 3.

Response: Likewise Tables, we would prefer to maintain the current Figures (also we have been asked by the Editor(s) to have Figures).