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Title: Non-Celiac Gluten Sensitivity: All Wheat Attack is Not Celiac

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To The Editorial Staff

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We were very pleased to read that that the reviewers of our manuscript were very positive and we would like to thank them for taking the time to read through our review and for their excellent comments/suggestions.

We have addressed the reviewers' comments/suggestions as outlined in a point by point format below and highlighted in the review. We feel that the extremely helpful comments by the reviewers have helped shape our review into a more focused, informative and concise article on NCGS, for which we are grateful.

We hope that the incorporation of these changes (as specified by the reviewers) will enable us to now publish our article in WJG.

We also request that you add Moheb Boktor as one of the authors of this manuscript for his contributions in reviewing, revising and contributing to the final approval of this manuscript

Should you require any further information then please do not hesitate to contact me. I look forward to hearing from you and I thank you in advance.

Yours faithfully,

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Reviewer 1.

Comment 1: The authors have written a very good review on NCGS. It is up to date, and overall well written. I suggest the following revisions/modifications:

Response: We appreciate the reviewers' comment that our review is 'up to date and well written' and would like to respond to the points highlighted in this review.

Comment 2: It is well established that GFD is associated with better outcomes in celiac disease, including lower mortality, better bone health, and reduced risk of cancer.

Whether this is the case in NCGS is not known. Some recent data suggest increased cardiac mortality in patients who take GFD and who don't have celiac disease. The authors need to address that.

Response: We thank the reviewer for their excellent comment and we have now added the following paragraph in the discussion section:

"The importance of gluten has been outlined in recent research. Gluten consumption has been associated with a decreased risk of developing type 2 Diabetes Mellitus and also a decreased risk of coronary heart disease[62, 63]. Understanding these benefits of gluten beyond nutrition reflects the need for caution in the use of GFDs in patients without a proven diagnosis of NCGS. Although GFD seems to be the most important management strategy, it should be suggested only after careful examination and a definite diagnosis of NCGS."

Comment 3: The conclusion is too long. It should be reduced into a succinct summary.

Response: The reviewer is absolutely correct. We have made amendments to the conclusion section of the paper to reflect an abbreviated yet informative summary.

Comment 4: The English of the text needs some polishing as there are some misprints/mistakes.

Response: We thank the reviewer for their comment and we apologize the errors in the written language. We have now changed the manuscript accordingly.

Reviewer 2.

Comment 1: I read with interest the review MS "Non-Celiac Gluten Sensitivity: All Wheat Attack is Not Celiac" by J. Steven Alexander and coworkers. It deals with an emerging, intriguing issue, though often poorly addressed. The authors should be

commended for providing such a well-organized and structured review. No additional points on this side.

Response: We appreciate the reviewers' comment. Thank you.

Reviewer 3.

Comment 1: This is an interesting review by Igbinedion et al on NCGS.

Response: We thank the reviewer for their comment.

Comment 2: It was mentioned that NCGS presents with relatively non-specific set of symptoms which affects diverse organ systems. Symptoms of NCGS are often so vague and non-specific presenting as gastrointestinal and/or extra-intestinal symptoms. I disagree with this statement, as NCGS could be very disabling.

Response: We thank the reviewer for their comment and have changed the review as follows:

Previously:

"Beyond this, NCGS often carries a very wide and relatively non-specific set of symptoms which affects diverse organ systems[7]. Symptoms of NCGS are often so vague and non-specific presenting as gastrointestinal and/or extra-intestinal symptoms."

Added:

"Beyond this, NCGS often carries an extensive and relatively broad set of symptoms which affects diverse organ systems[7]. Symptoms of NCGS could be very disabling presenting as gastrointestinal and/or extra-intestinal symptoms[8]." The abstract portion of the manuscript has also been edited to reflect these changes.

Comment 3: Causal links between IBS and NCGS have been frequently suggested since most of the gastrointestinal symptoms in NCGS resemble IBS (similar Rome III criteria), including abdominal pain/discomfort, bloating, diarrhea and constipation[15]. There is also a debate as to whether a GFD can help symptom resolution in IBS after excluding CD as clinical trials have shown that GFD can reduce symptoms in patients with diarrhea-predominant IBS (IBS-D)[17]. Based on multiple RCT could say with confidence that there are no causal links in my opinion, it is simply mislabelling NCGS with IBS.

Response: We apologize for the structure of the sentence as it confuses the reader to think that there are causal links between NCGS and IBS. We were trying to identify how some of the similarity in symptoms between IBS and NCGS could affect prevalence estimates of NCGS. We have now corrected the epidemiology section as follows:

Previously:

"Causal links between IBS and NCGS have been frequently suggested since most of the gastrointestinal symptoms in NCGS resemble IBS (similar Rome III criteria), including abdominal pain/discomfort, bloating, diarrhea and constipation[15]. There is also a debate as to whether a GFD can help symptom resolution in IBS after excluding CD as clinical trials have shown that GFD can reduce symptoms in patients with diarrhea-predominant IBS (IBS-D)[17]. Although recent research has suggested that a low fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) diet regardless of gluten content improves symptoms in IBS[18]. Given the close symptomatic resemblance between NCGS and IBS, prevalence estimates may be unclear as patients with NCGS could be mistakenly diagnosed as IBS."

Added:

"An overlap between IBS and NCGS has been suggested since most of the gastrointestinal symptoms in NCGS resemble IBS (similar Rome III criteria), including abdominal pain/discomfort, bloating, diarrhea and constipation[15]. There is also a

debate as to whether a GFD can help symptom resolution in IBS after excluding CD, as clinical trials have shown that GFD can reduce symptoms in patients with diarrhea-predominant IBS (IBS-D)[17]. Although recent research has suggested that a low fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) diet, regardless of gluten content, improves symptoms in IBS[18]. Given the close symptomatic resemblance between NCGS and IBS, prevalence estimates may be unclear as patients with NCGS could be mislabeled as IBS."

Comment 4: The proposed diagnostic work-up includes 3 vital steps: Why the authors do not simply recommend Salerno expert criteria Ref 34? The authors suggest performing wheat specific IgE and skin prick test, and CD; IgA-tTG, IgG-DGP and IgA-EMA. What is the evidence behind doing all these tests? Should the tests not be advised according to presenting symptoms in each individual?

Response: We thank the reviewer for their excellent comments which we concur with. We reference the Salerno Criteria which advocates for the diagnosis of NCGS after ruling out WA/CD. The diagnostic criteria we propose is a simplified version of the Salerno Criteria with the figure highlighting the algorithm. We understand these tests could be cumbersome and entirely wasteful if patients' history and physical are not suggestive of the condition. Therefore, we now added the following in the diagnosis section:

"Of note, these tests can be tailored by the clinician based on the patients' presenting symptoms. The physician can bypass testing of CD or WA and proceed with the work-up for NCGS if patients' history and physical is not suggestive of the condition."

Reviewer 4.

Comment 1: This paper is a review of the current knowledge of NCGS, highlighting the remaining challenges and questions which may improve its diagnosis and treatment. The data are presented in a thorough and balanced manner, however there are corrections and issues that should be dealt with.

Response: We thank the reviewer for their comment and have addressed their concerns below.

Comment 2: Sapone et al. (21) observed in NCGS expression of TLR2, and to a lesser extent TLR1 but not TLR4, as was erroneously reported in the manuscript (Pathogenesis)

Response: We apologize for having erroneously reported the expression of TLR4 in NCGS correctly pointed out by the reviewer. We have now made changes in the pathogenesis section to reflect this update:

Previously:

"Because of the lack of evidence for T-cell involvement and the apparent contribution from toll-like receptors (e.g. TLR-4, TLR-2)[21], NCGS may be more of an innate rather than adaptive immune response."

Added:

"Due to the lack of evidence for T-cell involvement and the apparent contribution from toll-like receptors (e.g. TLR-2, TLR-1)[21], NCGS may be more of an innate rather than adaptive immune response." Other sections in this paper including figure 1 have now been edited to reflect this change.

Comment 3: Table 1 Comparison of Gluten Sensitivity Disorders, is confusing: remove IBS for their unclear relation to gluten ingestion, as authors themselves reported in the table

Response: We thank the reviewer for their excellent point. We highlighted in our report how some of the gastrointestinal symptoms in NCGS resemble IBS (similar Rome III

criteria). We also referenced a recent update by Aziz et al. (17) that shows how GFD can reduce symptoms in patients with diarrhea-predominant IBS (IBS-D). We have now changed the title of Table 1 from "Comparison of Gluten Sensitivity Disorders" to "Comparison of Gluten Related Disorders". This is to better identify the essence of the table. We highlighted IBS as a gluten related disorder in the table to give a brief distinction between IBS as an unclear gluten related disorder, and other clear gluten related disorders.

Comment 4: In NCGS we don't have diagnostic biomarkers, it was shown a positivity for AGA in approximately 50% of cases but low specificity and we can talk about mechanism potentially involved (innate immunity); with regard to diagnosis it must be reported for NCGS: Double-blind, placebo-controlled, cross-over trial, as the authors themselves reported in figure 2; As Management of NCGS patients, a gluten-free regimen should be considered; Please change: Colonic and extra-manifestations with intestinal and extra intestinal manifestation; Cytomorphology with duodenal histology; Immunophenotype with HLA Haplotypes (DQ2 and DQ8)

Response: We thank the reviewer for their comment and we have now changed the table to incorporate the suggestions.

Comment 5: With regard to the management of NCGS it is important to include the potential use of ancient wheat variants (diploid wheat species) as new dietary opportunities for NCGS patients both for their marked reduction of toxicity demonstrated in in vitro cellular assays (Gianfrani C et al. Immunogenicity of Monococcum wheat in Celiac Patients Am J Clin Nutr 2012;96:1338–44; Mazzarella G et al. Extensive in vitro gastrointestinal digestion markedly reduces the immune-toxicity of gliadin from ancient Triticum monococcum wheat: implication for celiac disease prevention. Mol Nutr Food Res. 2015 Sep;59(9):1844-54) as well as for their lower concentration of ATIs respect modern wheat (Zevallos VF et al. Nutritional Wheat Amylase-Trypsin Inhibitors Promote Intestinal Inflammation via Activation of Myeloid Cells.Gastroenterology. 2017 Apr;152(5):1100-1113.

Response: We thank the reviewer for their most interesting comment. This information was a very interesting revelation into a developing treatment strategy in NCGS. We have now added this paragraph below to this section describing the potential use of 'ancient wheat variants' as dietary alternatives in NCGS:

"The use of ancient diploid wheat species (e.g. *Triticum monococcum* ssp.) as compared with common wheat as a new treatment strategy is gaining ground[67]. Gianfrani et al. demonstrated the low toxicity of these wheat proteins in celiac disease patients following in vitro gastrointestinal digestion[67]. Newer studies have shown the distinction between these older wheat variants and modern wheat[68]. Older wheat variants were shown to have lower bioactivity and a lower concentration of ATIs in comparison with modern wheat[68]. Mechanistically, the modern wheats were observed to have high levels of TLR-4-activating ATIs which are highly resistant to intestinal proteolysis[68]. The application of these studies to favor the use of ancient wheat variants in the NCGS population would be a major step in the advancement of treatment strategies in this disease."

Comment 6: The discussion must be reviewed and reduced; some information may be reported in the respective paragraphs for example the information about the biomarkers as CD14 and LBP must be reported in Pathogenesis; the ex-vivo gluten challenge as a method for diagnosis must be removed because to date, there are no validated mucosal biomarkers that can differentiate CD from NCGS. Moreover, such technology requires a well-equipped laboratory therefore it is not at all a simplified diagnostic strategy for the clinician.

Response: We concur with the reviewer and we thank them for their comment. The conclusion/discussion section has now been edited to reflect an abbreviated yet informative summary. The changes have been made in the conclusion section to remove unclear points that were earlier stated as highlighted by the reviewer.

Comment 7: Finally, endoscopy for duodenal biopsies howsoever is invasive subjecting the patient to stress.

Response: We thank the reviewer for this great point, which we agree with. As such we have now included the following sentence in the review:

"If highly suspicious of CD, the physician can proceed with upper endoscopy (EGD) for duodenal biopsy, although this should not be routine testing for every patient".

Comment 8: ATIs engage TLR4 and not TRL2 as erroneously reported by the authors.
Response: We thank the reviewer for their excellent comment, and we agree that ATIs engage TLR4 and not TRL2. This change is now reflected in the pathogenesis section as follows:

Previously:

"The role of ATIs in mounting an immunological response has been shown in animal and human research models and is believed to be an important oral antigen both in CD as well as in NCGS[26]. This predominantly innate immune response involves macrophages, neutrophils and intestinal dendritic cells via activation of the TLR2 and to a lesser extent TLR1 and TLR4 complexes[21, 26]."

Added:

The role of ATIs in mounting an immunological response has been shown in animal and human research models and is believed to be an important oral antigen both in CD as well as in NCGS[26]. This predominantly innate immune response involves macrophages, neutrophils and intestinal dendritic cells via activation of the toll like receptor complexes[21, 26].

Comment 9: I suggest to assemble epidemiology and clinical presentation in a single paragraph.

Response: We thank the reviewer for this excellent suggestion. However, we respectfully disagree as we wanted to keep clinical presentation as a separate section to focus on the symptoms expressed in NCGS in comparison with other gluten related disorders like CD.