Author’s response to reviews

Title: An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity

Authors:

Umberto Volta (umberto.volta@aosp.bo.it)
Maria Teresa Bardella (mariateresa.bardella@yahoo.com)
Antonino Calabrò (a.calbro@dfc.unifi.it)
Riccardo Troncone (troncone@unina.it)
Gino Roberto Corazza (gr.corazza@smatteo.pv.it)

Version: 3 Date: 26 March 2014

Author’s response to reviews: see over
Reply to Editorial Board and Reviewers

RE: MS 2102786192122448 - "An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity" by Umberto Volta et al.

Editorial Board Comments:

Before deciding how to proceed, the Editorial Board asked us to:

1. Upload, as an additional file, the full questionnaire with all 60 items

Response: Done

2. Address the following two points within the revised manuscript as well as in your covering letter

   If you could add these details to your methods section, that would be great.

   a) It is unclear if the total number of patients (12,255) represent the total number of patients seen in the time period by the participating centers. Were these patients from the endoscopy lists or from consultation lists? or maybe both?

   Response 2a: Agreed. We specified in the Methods section (see page 6, line 5) that the study was designed by the 5 members of A.I.C/F.C. scientific Board for NCGS, but it was carried out by all the 38 investigators in charge of the participating centers. All patients, seen at the outpatient clinics, were evaluated by skilled investigators who identified subjects with suspected NCGS. As now specified in the text (see Methods sections, page 7, lines 1-2), all the 38 participating centers are recognized referral centers, included in the register of the Italian Health Ministry for the diagnosis of gluten related disorders. Moreover, as specified in the text (see Methods section, page 8 lines 15-20), 28 out of the 38 participating centers provided, together with the total number of suspected NCGS cases, the total number of new CD diagnoses and of patients seen in the period of the study. We have also specified that all the 12,255 patients observed in these referral centers were clinically evaluated by the investigators and underwent a thorough diagnostic work-up based on blood tests and invasive procedures (when needed) in order to identify or rule out NCGS and CD. This definitely clarifies that this study has been centered on a large number of subjects clinically evaluated and thoroughly investigated and that it is not based merely on endoscopy list or outpatient register.

   Response 2b: Agreed. In response to this observation, we clearly specified in the Discussion section (page 13, lines 12-15) that one of the strengths of the present study is represented by the high skill of the physicians working in the referral centers involved in this survey. All the patients were prospectively evaluated from a clinical point of view by experts who carried out the diagnostic work-up based on blood tests and endoscopy (when appropriate) to identify CD or NCGS.

Could you clarify the author list of this manuscript? You have included the “Study Group for non-celiac gluten sensitivity” in this list, who are listed on page 2 of the manuscript. Please could you ensure that these individuals are indeed considered as authors?

Response: All the people included in the “Study Group for non-celiac gluten sensitivity” gave a significant contribution to this paper. As experts in the field of gluten related disorders, they
diagnosed and enrolled patients with celiac disease (CD) and non-celiac gluten sensitivity (NCGS) as well as filled the questionnaires for each patient. Moreover, all of them supervised and approved the paper. Therefore, according to the guidelines of BMC Medicine, they must be regarded as authors of the manuscript. In the revised version of the paper we added this information in the section Author’s contribution.

Reviewer: Geoffrey Holmes
Reviewer #1 - In this Italian prospective study carried out over a period of one year, 486 patients with suspected non-celiac gluten sensitivity (NCGS) were identified from 38 centres (34 adult and 4 pediatric) with the aim of clarifying clinical features and establishing prevalence relative to celiac disease. Some interesting information has emerged with regard to symptoms, associated conditions, reactions to gluten and prevalence. A questionnaire was used to record the required information.

First of all, we wish to thank the Reviewer for his insights to our manuscript and the kind comments about the interesting information generated by our work.

Major revisions
1. Three forms of sensitivity to gluten have been proposed – celiac disease, wheat allergy and gluten sensitivity (ref 11 in the paper). NCGS is regarded as a reaction to gluten in which allergic and autoimmune mechanisms have been excluded. Serological tests for celiac disease are negative although antigliadin antibodies may be present. The duodenal mucosa is grossly normal. In this study 22% had IgE-mediated allergy so strictly speaking should not be included under the term NCGS as presently understood. In the Discussion page 13 these are regarded as a subgroup of NCGS. On page 14 of the Discussion the authors write that there are no biomarkers for NCGS, apart from AGA but include IgE positive patients in this group. The authors ought to make clearer what they mean by NCGS and how this differs from wheat allergy. The problem at the moment is the nomenclature for these conditions but as long as the authors define what they are talking about the difficulty should be solved.

Response: We thank the reviewer for his insightful comment which allows us to clarify an important aspect of our work. As rightly recognized by the reviewer, NCGS is defined as a reaction to gluten in which wheat allergy and celiac disease (CD) have been previously excluded. The exclusion of wheat allergy does not imply that subjects with NCGS cannot suffer from IgE-mediated reactions to allergens other than gluten. On this line, at least 16% of CD patients show allergy to mites, graminaceae and other pollens (Ciacci C, J All Clin Immunol 2004). In conclusion, the finding of a NCGS subset with IgE-mediated allergy (other than wheat allergy) is not surprising and does not contrast with NCGS diagnosis.

2. Duodenal biopsy was only performed in 302 (62%) of cases so why assume that some of these patients did not have celiac disease? This deserves a comment.

Response: We agree with the reviewer. None of the 302 biopsied patients with suspected NCGS had villous atrophy, thus excluding CD diagnosis in more than half of our patients. As noted by the reviewer, we cannot exclude that some of the remaining unbiopsied patients (n=184) with suspected NCGS might have CD, although the probability for CD is quite unlikely since the prevalence of seronegative CD is < 2% and several previous studies from our (J Clin Gastroenterol 2012) and other groups (Sapone et al, BMC Medicine 2012) did report no case of villous atrophy consistent with CD in patients identified as NCGS. We do not think that this comment must be added to the text since it is well-established that a negative serology rules out CD without need for duodenal biopsy except for very few cases.
3. How was IBS defined?

**Response:** IBS has been defined according to Rome III criteria as follows: symptoms of recurrent abdominal pain or discomfort and change in bowel habit for at least six months, with symptoms experienced on at least three days of at least three months. Two or more of the following must apply for the diagnosis: pain relieved by bowel movement; onset of pain related to a change in frequency of stool; onset of pain related to a change in the appearance of stool. These aspects have been summarized in the revised version (see page 10, line 7).

4. On page 12 of the Discussion the authors admit great limitations of their study. These should be stated in detail and how these impact on the conclusions drawn. The strengths of the study are worth emphasizing.

**Response:** Agreed. As suggested by the reviewer, we detailed the limitations and the strengths of our study in the discussion section. This part has been included in the revised version (page 13, lines 6-15).

---

**Minor essential**

1. Peer on page 6 should be pear

**Response:** Done

2. Familiarity on page 8 should be – family history of CD

**Response:** Done

3. On page 9 not well being should be lack of well being – same applies to Fig 2.

**Response:** Done

**Discretionary**

1. Add to the summary that only a proportion of cases had a biopsy

**Response:** Done

2. In the Background section it might be better the write - in recent years….world wide report…..spelt causes.

**Response:** Done

**Quality of written English:** Needs some language corrections before being Published

**Response:** English form has been revised

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

N/A
Reviewer: Knut E A Lundin

Reviewer #2- The paper by dr. Volta and colleagues describes a prospective effort to evaluate the frequency of non-celiac gluten sensitivity (NCGS) in a multi-center national setting. It is important that such efforts are undertaken, because adoption of a restricted diet has gained considerable proportions in the Western population. In the study, a total of 12255 patients were assessed. They found a possible NCGS diagnosis in 3.19% of their patients, compared to a celiac disease diagnosis in 2.77% of their patients. Although interesting, there are some comments to be made.

First of all, we wish to thank the reviewer for his insights and kind comments about the findings emerged by our work.

1. It is unclear if the total number of patients (12255) represent the total number of patients seen in the time period by the participating centers. Were these patients from the endoscopy lists or from consultation lists – or maybe both?

Response: We thank the reviewer for giving us the possibility to specify this important point. All patients were clinically investigated at the outpatient clinic of the 38 referral centers for the diagnosis of celiac disease and gluten related disorders. All of them were studied by the investigator in charge of the respective center, who identified subjects with suspected gluten disorders. Each subject enrolled in this study underwent a thorough diagnostic work-up based mainly on blood tests (serology, absorption tests, HLA typing if needed) and upper gastrointestinal endoscopy (duodenal biopsy). After the exclusion of CD and wheat allergy, physicians filled the questionnaire reporting all clinical information and other requested data for patients with suspected NCGS. Moreover, 28 out of the 38 participating centers also provided, together with the total number of suspected NCGS cases, the total number of new CD diagnoses and of patients seen in the period of the study in order to establish the ratio NCGS/total patients seen, CD/total patients seen and NCGS/CD. On the whole, 12,255 consecutively observed patients were evaluated by the investigators and underwent a thorough diagnostic work-up based on blood tests and invasive procedures (when needed). This has been specified by adding a sentence in the Methods section (see page 8, lines 18-20). So, we think to have definitely clarified that our study has been centered on a large number of subjects clinically evaluated and studied by means of appropriate tests and that it is not based merely on endoscopy list or outpatient register.

2. It is unclear if the study was done by dedicated clinicians taking part of the study or if this is a summary of all the activity by a number of clinicians. Obviously, this could influence the results.

Response: Thanks for the insightful comment. The study was designed by the restricted number of main authors (U V, MT B, A C, R T, GR C), but it was carried out by all the 38 investigators in charge of their own referral centers for the diagnosis of gluten-related disorders. These centers are included in the register of the Italian Health Ministry and recognized referral centers for these diseases. Two sentences have been added in the Methods section (see page 6, lines 1-2 and lines 5-9). See also the previous answer.

3. Data on the questionnaire used is missing. Thus, it is difficult to evaluate the data validity.

Response: As specified in the text, the NCGS questionnaire, including 60 items / questions, has been conceived by the Italian Association for NCGS Board, coordinated by Umberto Volta and Gino Roberto Corazza. The majority of the items are related to the presence or absence of specific symptoms (gastrointestinal and extraintestinal) which are reported as percentage in the figures 1 and 2. Other relevant information is clearly explained at page 8 (lines 5-11) and includes: 1) symptom frequency after gluten ingestion and time interval between the ingestion of gluten and symptom
occurrence; 2) who was the first to suspect NCGS; 3) associated disorders, 4) positivity for serum specific IgE (or Prick tests) to allergens (food and inhalants); 5) family history of CD; 6) IgG and IgA antigliadin antibodies of first (AGA) and second (deamidated gliadin peptide, DGP) generation; 7) other biochemical abnormalities; 8) HLA typing; and 9) intestinal biopsy (when performed). As suggested by the Editor, the questionnaire has been uploaded as a supplementary file.

4. A definition of the criteria for giving the patients a NCGS diagnosis is missing. It is not stated if the centers were monitored from the study organizers as part of the study.

Response: Agreed. The diagnostic criteria for NCGS were based on the Consensus Conference on Gluten Related Disorders, held in London, February 2011 and published in BMC Medicine in 2012 (reference 11). During this Consensus Conference a panel of experts defined the criteria adopted for the diagnosis of suspected NCGS (see Methods section, page 7, lines 10-14 and page 8, lines 1-2). These criteria have been extensively quoted in all papers dealing with NCGS.

5. It is stated that many NCGS patients were considered to have irritable bowel syndrome, but it is not stated that this diagnosis was made after Rome criteria. I further miss data on gastrointestinal symptom severity (e.g. by GSRS).

Response: Agreed. We added in the text that IBS patients were diagnosed according to Rome III criteria (see Results section, page 10, line 7) (see also answer 3 to reviewer 1). The design of the study did not include the use of Gastrointestinal Symptom Rating Scale (GSRS) and therefore we cannot provide this piece of information.

6. The frequency of CD patients could be biased because of positive serology taken by the general practitioners. This could explain much of the high frequency of CD diagnosis in the children. Did they collect data on prior serology?

Response: All CD diagnoses were prospectively established and verified in the referral centers based on both serology and histology.

7. The data on other immune disorders and biochemical alterations in the NCGS group is interesting and should be highlighted.

Response: Agreed. A sentence has been added in the discussion to underline the importance of associated immune disorders (see page 15, lines 5-6) and biochemical abnormalities (see page 15, lines 12-13) in NCGS.

8. The text is rather long and would improve from being shortened. However, the discussion is good and the authors make a number of good observations.

Response: We take the reviewer's comment, however we feel that shortening some sections would hamper the message of this paper by loosing precious info. The longest part of the paper is the discussion, but, as underlined by the reviewer, it contains a number of good observations which can provide useful information to the reader on this topic.

9. It is stated that a short-coming of the paper is that a blinded challenge was not done in all patients. Was it done at all? Any data?
Response: We thank the reviewer for allowing us to clarify this point. None of the patients with suspected NCGS underwent a blinded challenge as clearly specified now in the text (see page 13, lines 8-11).

10. They advise the readers to do a 6-week challenge to rule out coeliac disease in NCGS patients. This is a long challenge and more recent guidelines and papers suggest that a shorter challenge can be performed. The role of HLA typing in this setting should be mentioned. It was reported that coeliac disease is infrequent among NCGS patients (Brottveit et al 2011). Similar data exist from Italy (Biagi et al). This could be mentioned.

Response: Agreed. As indicated by the reviewer, a recent report (Brottveit M, Am J Gastroenterol 2011- ref. 23) suggested that a very short gluten challenge may be exploited to demonstrate CD by means of HLA-DQ2-tetramer staining for gluten specific T cells in patients with self-prescribed GFD (see page 13, lines 14-16). Nonetheless, we would like to underline that the traditional 6-weeks gluten challenge remains a routine tool to detect clear-cut celiac histological lesions in patients starting GFD without previous exclusion of CD. Finally, we specified the negative predictive role of HLA-typing for CD (see page 14, lines 16-18).

11. The figures would improve from better lay-out.

Response: Done

Quality of written English: Needs some language corrections before being published

Response: Done

Statistical review: No, the manuscript does not need to be seen by a statistician.

N/A