Author's response to reviews

Title: Limited utilization of serologic testing in patients undergoing duodenal biopsy for celiac disease

Authors:

Homer O Wiland IV (wilanh@ccf.org)
Walter H Henricks (henricw@ccf.org)
Thomas M Daly (dalyt@ccf.org)

Version: 3 Date: 17 June 2013

Author's response to reviews: see over
To the editor:

We thank the reviewers for their constructive comments on our article “Limited utilization of serologic testing in patients undergoing duodenal biopsy for celiac disease” (BMC Gastro MS 185043337930732). We have incorporated your suggestions into the revised document, and detailed responses to the reviewers’ comments are included below. We appreciate your continued consideration of our manuscript, and look forward to your reply.

Sincerely,

Thomas M Daly, MD

Cleveland Clinic, Dept of Pathology
9500 Euclid Ave
Cleveland OH, 44195
Ph: 216-444-4547
FAX: 216-444-4414
e-mail: dalyt@ccf.org

Referee 1:

1. Chart review: you revised clinical data only from the 161 patients who had positive results for either serology or duodenal biopsy. Did you consider if among patients negative for serology/biopsy there were celiacs on gluten free diet (GFD)? We recognize that there are likely a subset of patients with negative serology and biopsy who represent patients with CD who have been treated successfully on a gluten free diet, but for the purposes of our study, this information was not required to answer our main question (i.e. How often is serology being performed in patients biopsied to rule out CD, and if performed, with what results and what point in relation to biopsy?). By definition, our secondary study question (When performed, how can serologic results potentially help guide biopsy strategies), only relates to patients with abnormal laboratory findings, and chart review was performed on all patients within this group.

2. Chart review: you considered patients to be affected by CD if they had both positive serologic and biopsy results although adults with CD may also have negative antibodies. We agree, and chart review was performed on all patients with either abnormal serology or pathology results, with the final classification of CD based on these results coupled with clinical findings (response to gluten-free diet, extra-intestinal symptoms, previous history, etc). Therefore, antibody-negative CD patients with abnormal duodenal histology would have been identified during the chart review. We have revised the language in the methods section to make this clearer, and also included a flow chart of the data analysis process (new Figure 1).

3. Results: page 7, lines 18-21# Recent findings suggest that CD could be limited to the duodenal bulb or have a so called “patchy” pattern. Performing only one biopsy could lead to miss diagnosis. Did you consider the biopsy site? Information on the
number and location of duodenal biopsies is not routinely specified for samples submitted for pathologic review and therefore the proportion of biopsies from bulb vs. other duodenal locations cannot be accurately determined. In practice, rarely if ever is only one fragment of duodenal mucosa provided per specimen jar submitted, suggesting routine sampling of multiple sites.

4. **Discussion**: page 9-10, lines 2-4

Partial villous atrophy (PVA) is considered as a **CD related histological finding**. We realized upon receiving the reviews that we had made a significant nomenclature error in the methods section which mis-identified PVA as “partial villous atrophy”. In fact, PVA-IEL in our reporting system stands for “preserved villous architecture with increased epithelial lymphocytes,” an entity which is thought to be a relatively non-specific finding (Arch Path Lab Med 130:1020). Because this issue was raised by multiple reviewers, we have changed the nomenclature in our paper to IVA-IEL (intact villous architecture with increased epithelial lymphocytes) to minimize potential confusion for the readers, and have also inserted two additional references describing this histologic entity and its relation to celiac disease.

5. **In Table 1 you should consider PVA as CD (1.6% + 6.2% and 1% + 4.4%).** See response to comment 4 above.

**Minor**

1. **Introduction**: page 3, lines 1# insert “space” between is one. Done.

2. **Introduction**: page 3, lines 9# correct disease with diseases. Done.

3. **Introduction**: page 3, lines 11-20# you wrote that “diagnostic options for celiac disease can be divided into three categories […]”. However what you discussed later are not “options” but different steps through the diagnosis of CD. We disagree slightly with the reviewer’s comment. The three types of testing we mentioned (serology, genetics, and biopsy) are not necessarily sequential steps through CD diagnosis, but rather different testing options that may or may not be employed in a given patient. For example, high-risk patients who present with classic symptoms may progress directly to biopsy without genetic testing, while family members of affected patients may potentially be ruled out by genetic testing without the need for serology or biopsy. As such, we believe that “options” is an accurate term.

4. **Introduction**: page 4, lines 3-5# please indicate performances of each autoantibodies (tTG, dGDN, EMA). The methods used to perform the assays are described in the methods section, and the diagnostic characteristics of each autoantibody are described in the fourth sentence of this paragraph in the introduction. In addition, we have included references for studies which look at serology performance in more detail.

5. **Materials and Methods**: page 5# please provide patients sex, age range and median age. Done.
6. Materials and Methods: page 5 # please provide autoantibodies positive patients divided as tTG +, dGDN + or EMA +. We have included this information in the methods section.

7. Materials and Methods: page 5 # please check total serum IgA titer in order to identify patients with serum IgA deficiency. Total IgA levels were a standard part of the celiac panel in use at the time of this study, and we have added a sentence in the methods to explicitly state this.

8. Results: page 8, lines 3-5 # We are not surprised about that as extra-intestinal findings in CD have already been studied. Please compare your results to others. We have inserted a comment in the results stating the similarity of these findings to previously published work, along with an appropriate reference.

9. Table 2: please provide percentage values. Done.

10. Table 2: PVA results should be unified with CD results. See response to comment 4 above – PVA-IEL is actually a separate entity.

Referee 2:

Could the authors clarified if the biopsies were oriented and how many fragments were taken from each patient and from which part of duodenum? In order to reduce confounding factors, I would also suggest to use a standardized classification to categorize duodenal histology (e.g. modified Marsh or Corazza Villanacci Classifications). As stated in response to reviewer 1 (question 3), this type of information is not routinely specified for duodenal biopsy submissions. The histology laboratory at our institution is quite experienced in orienting biopsies, and pathologists either do not render diagnoses if sample orientation was inadequate or indicate as such in the report. To make a diagnosis this would have been noted in the report, and no such information was seen upon review of the pathology reports. Because the pathologic findings in various stages of CD are not specific, Marsh staging is not routinely reported at our institution, and for the purposes of our study differentiation between stages of CD was not important.

It is relatively unclear if the authors focus their work only on the low adherence of their institution to published guidelines or also on the presence of other diseases in non-duodenal samples (page 5) regardless of celiac serology. As they state in the discussion, other non-duodenal histological findings are common in CD patients, but it’s known that it is not mandatory multiple sampling. Thus, could the authors better explain the value of those data? Are they suggesting to perform more biopsies in serological positive patients? A little bit of both. As stated at the end of the introduction, the primary goal of the study was to determine the underlying cause of the high rate of negative biopsies sent for “rule out celiac” despite the availability of
relatively good screening tests (serology). This ultimately was found to be due to the majority of duodenal samples being submitted as part of a multi-site survey. Given that, we evaluated whether the use of serology could help to direct the choice of biopsy sites in such a survey based on the presence of other disease in either duodenal or other GI locations (table 2) We have included additional text in the conclusion (discussion, final paragraph) to help clarify these points.

Could the authors please clarify what they mean with “partial villous atrophy and increased intraepithelial lymphocytes”? PVA-IEL actually stands for “preserved villous architecture with increased epithelial lymphocytes”; please see response to comment 4 of referee 1 for detailed explanation and revisions made.

Referee 3:

The ABSTRACT is misleading as only pathology reports were reviewed. We have modified the abstract to clarify that the study was based on a review of pathology reports (1st sentence, methods portion of abstract).

We need a CONSORT flow chart of the number of patients/biopsies selected and reviewed. We have included a flow chart describing the data analysis plan for the study (new Figure 1).

Were any of the patients tested prior to being seen at the Cleveland Clinic. If this is not known then we need that as a limitation of the study. As the reviewer points out, this is an inherent limitation to this type of EMR-based study, and we have included a statement to this effect (1st paragraph, discussion).

Why were only 161 charts reviewed? We need a chart to demonstrate where patients were included/excluded in this study as requested above. Chart review was limited to patients with abnormal results for either biopsy or serology, which totaled 161 patients as shown in the flow chart requested above. We have also added a statement to this effect in the methods section.

How was the celiac group different from the PVA-IEL group. Surely many of these patients had celiac disease? PVA-IEL would be a Marsh IIIa celiac classification. This was due to a nomenclature error in the original submission - PVA-IEL actually stands for “preserved villous architecture with increased epithelial lymphocytes”; please see response to comment 4 of referee 1 for detailed explanation and revisions made.

How many biopsies were taken (biopsy pieces) in each group and How many subjects had duodenal bulb biopsies taken? In how many were the diagnosis made only in the bulb biopsies. Both according to whether serologies were performed and according to diagnoses. Information on the number of fragments submitted is not routinely collected – please see the response to reviewer 1 (question 3) for a more detailed description.
How many of the original biopsies were reviewed by the investigators? The surgical pathology service at our institution is a subspecialty based model, and all biopsies were originally reviewed and signed out by subspecialists in GI pathology. Biopsies were not additionally reviewed by the authors.