Point-by-point response to Reviewers' Comments on the Manuscript

Computer-aided texture analysis combined with expert's knowledge: Improving endoscopic celiac disease diagnosis

Dear editors and reviewers,

Thank you very much for your valuable and constructive comments.
We have addressed all the comments as shown in the revised manuscript.

The following points have been changed with respect to the previous version of our manuscript (apart from smaller editorial changes related to specific reviewer comments):

Reply to Editor's Concerns

1) Please provide certificate letter by professional English language editing companies

   Attached, we provide the required professional English language certificate.

2) You need to provide grant application form(s) or certificate of funding agancy for every grand or we will delete the „Supported by..."

   The contract of funding for the project "Towards improved celiac disease diagnosis" numbered KLI 429-B13 signed by a representative of the governmental Austrian Science Fund (FWF) and Andreas Vécsei is supplied along with the revised version of our manuscript.

3) AIM: No more than 20 words, and start with „To ..."

   The text was changed to meet these requirements.

4) RESULTS: no less than 120 words

   The text was changed to meet these requirements.

5) Reference number format should be corrected

   The reference number format was corrected.

6) Abbreviations and acronyms are often defined the first time they are used within the main text and then used throughout the remainder of the manuscript

   Revising the manuscript, we focused on a consistent use of abbreviations. Changes were made with regard to the abbreviation "CD" (celiac disease). The abbreviation "DB" is never used again and was therefore removed. Only the abbreviations DB-1, DB-2 and DB-3 are used for identifying the three databases (Page 6).

7) References: DOI and PMID should be added

   In the revised version, these details were added.

8) Please provide the decomposable tables (Table 2)

   Incidentally, we inserted a figure instead of an editable table. In the revised manuscript we are now providing an editable table (Page 20, 21).

9) Please finish them (Comments: Background, Research frontieres,…)

We finished these headings (Page 15).

10) Please check that there are no repeated references, Please add PubMed citation numbers and DOI citation to the references list and list all authors. The authors should provide the first page of the paper without PMID and DOI.

We checked that there are no repeated references.
Additionally, PubMed as well as DOI citation numbers were added.

11) Please provide an Audio Core Tip

Attached, we provide an Audio Core Tip.

Reply to Concerns of Reviewer 00742022

1) How was the biopsy location correlated to the image processing location?

Before a specific site was biopsied, an endoscopic image was taken just from the same mucosal area with the intended biopsy site located at the center of the image. This comment was very useful to us and we have added this information in the methods section of the manuscript so that the correlation of image processing location and biopsy location becomes clearer. (Page 5, last paragraph and page 6, lines 1-2 and 14-16)

2) This image data was divided into three distinct image databases (DB) DB-1, DB-2 and DB-3 as outlined What are these databases and why?

Thanks for this question. The information was only available in Table 1, but not in the text. We added some details on the three image databases in the text:
„This image data was divided into three distinct image databases (DB) DB-1, DB-2 and DB-3 as outlined in Table 1 collecting images acquired with a specific imaging protocol into a separate database. DB-3 contains images acquired with narrow-band imaging whereas DB-1 and DB-2 contain images obtained with traditional white-light endoscopy. Images in DB-2 and DB-3 where obtained with newer endoscopes compared to DB-1 (see Table 1). The separation was performed in order to avoid bias in the results due to variations within the image data sets [22]."

3) A manual selection of image sections was introduced. How were the manual sections selected?

A highly experienced consultant selected the patches from endoscopic images according to quality assessment criteria, such as sharpness, appropriate exposure, visibility of features and low degree of degradations (Page 6, last paragraph).

4) Needs grammar improvement.

A professional English language editor proofread the revised manuscript and made the required correction to improve the grammar. Please find a language certificate letter among the resubmission documents.

5) All image data of one certain patient were merged Explain.

This sentence was re-phrased to improve clarity. Details on how the redundancy is exploited, is

provided by the consecutive paragraph („To obtain patient-based decisions, Hybrid Diagnosis was performed as described ...“) (Page 9, subsection: Patient-based Classification).

6) Table 1 - the three databases and how they were derived requires explanation.

   As explained in 2), we added some details on the databases in the text.

7) Table 2 is too busy. Needs to be changed into separate tables or less information.

   Table 2 was split into Table 2 and Table 3. Table 2 contains individual classification rates and Table 3 contains the average accuracies. These tables still are quite busy; however, we think that all of this data should be provided to the reader anyway. Additionally, the important information is separately presented in Fig. 3 which might be easier to interpret (Page 20, 21).

Reply to Concerns of Reviewer 00009417

1) In the introduction, histological staging of celiac disease is introduced as a subject of significant intra- and interobserver variability. Using the proposed binary score (Marsh 0 and Marsh 3) the variability would be dramatically decreased. this should be mentioned in the manuscript when histological and computer-aided approaches are compared.

   We agree with this reviewer and added this point to the discussion section (Page 14, lines 29-33): “One strength of computer-aided endoscopic diagnosis of CD is its observer independence. However, the significant intra- and inter-observer variability in the histological staging of CD described in the literature refers to only the use of the Marsh classification. This classification variability might be significantly less if pathologists also used a binary histological staging (normal mucosa vs. villous atrophy) instead of the Marsh classification.”

2) The diagnostic data of hybrid celiac disease diagnosis do not give the relevant information to omit the diagnostic biopsy. The hybrid approach is not useful in diagnosis of Marsh I and Marsh II. Importantly, the hybrid technique does not separate Marsh 0/ normal (healthy) and Marsh I (diseased). In conclusion, the hybrid technique does not substitute the biopsy and does not improve biopsy-avoiding diagnosis. The power of the hybrid technique is in tissue sampling to take the diagnostic biopsy. This statement should be clearly given and the manuscript should be re-written.

   We agree with this reviewer that the hybrid technique cannot diagnose Marsh-1 or Marsh-2 lesions. In such cases, where villous atrophy is not detected, biopsies and subsequent histologic evaluation will still be needed. However, we think that in the foreseeable future it will be possible that, when using the hybrid approach, biopsies are not needed in patients with endoscopically proved villous atrophy and positive celiac autoantibodies. Using a different new approach Cammarota et al. already demonstrated that his biopsy-avoiding strategy in diagnosing CD is highly sensitive and specific when villi were shown to be absent.

   To delineate the strengths and limitations of the hybrid method more clearly, we added the following comment in the discussion section (Page 14, lines 6-22) including a new reference:

   “The clinical relevance of the reported hybrid classification approach would be primarily to support endoscopists in identifying whether and where biopsies from the duodenum are to be taken. Especially in the case of a patchy distribution of villous atrophy in the midst of normal mucosa, the hybrid system could indicate areas with villous atrophy, thus targeting the biopsy. Subsequently, such a diagnostic approach including selective and targeted tissue sampling might improve the accuracy of CD diagnosis, especially for less experienced endoscopists. In the foreseeable future, it would be conceivable that with this reported hybrid approach, biopsies

could be avoided or reduced in some carefully selected scenarios, such as endoscopic evidence of villous atrophy in patients with positive celiac antibodies[36] or monitoring the histologic recovery of CD patients on a gluten-free diet[39] Hence, the hybrid approach could finally result in cost savings by reducing the number of biopsy specimens. However, one limitation is that with the hybrid approach, it is not possible to detect Marsh-1 or Marsh-2 lesions. Therefore, the hybrid approach is not suitable to completely substitute for diagnostic biopsy. In cases where villous atrophy is not detected, biopsies and subsequent histopathologic evaluation will still be indispensable. Biopsies should always be performed in the case of macroscopic wall abnormalities, which indicate CD-associated intestinal lymphomas."

[Cammarota G, Cuoco L, Cesaro P, et al. A highly accurate method for monitoring histological recovery in patients with celiac disease on a gluten-free diet using an endoscopic approach that avoids the need for biopsy: a double-center study. Endoscopy. 2007;39:46–51]

3) In the duodenum, celiac disease is frequently found with a patchy pattern of tissue damage. Using the hybrid approach a panel of images per patient is necessary to assist the diagnostic procedure. The fundamental link should be more clearly addressed.

   To indicate the need of a panel of images per patients to cope with a possibly patchy distribution of intestinal mucosa areas affected by CD in the midst of normal mucosa we have added additional information in the discussion section: (Page 14, lines 6-11)

   "The clinical relevance of the reported hybrid classification approach would be primarily to support endoscopists in identifying whether and where biopsies from the duodenum are to be taken. Especially in the case of a patchy distribution of villous atrophy in the midst of normal mucosa, the hybrid system could indicate areas with villous atrophy, thus targeting the biopsy. Subsequently, such a diagnostic approach including selective and targeted tissue sampling might improve the accuracy of CD diagnosis, especially for less experienced endoscopists."

Reply to Concerns of Reviewer 00002261

1) What is a "setting"?

   To improve understandability, we chanced the following sentence: „Compared to experts' decision, in 24 out of 27 classification settings (consisting of three imaging modalities, three endoscopists and three classification approaches), the best overall classification accuracies were obtained with the new hybrid approach." (Abstract/results).

2) How did you calculate accuracies to 94-100 %

   The classification accuracies obtained for one certain image patch are actually significantly lower (mostly between 80 and 90 % - see Fig. 3, green bars). However, as there is distinctly more than one image patch per patient available, by merging all available decisions of images of a certain patient, accuracies can be increased (Fig. 3, dark blue bars). The high impact of merging the information is supposed to be due to a quite high independence of e.g. image degradations. Patchwise misclassifications due to invisible markers can thereby be compensated effectively. Even if expert's decisions are fused in a similar way, the accuracies of up to 99.5 % are obtained. Nevertheless, as we are aware that rates can vary with respect to the image data and the operating expert, we investigated all combinations and additionally reported mean rates as well as standard deviations (Page 21, Table 3).

Reply to Concerns of Reviewer 00004594

1) However, I am concerned by the following points: - To my knowledge celiac antibodies such as IgA anti-transglutaminase (and anti-endomysium) are important for the diagnosis of CD. The interest of the endoscopy is to confirm the diagnosis and to show that, under gluten free diet, a normal mucosa is restored. The phenotypic characterization of lymphocytes (CD3+, CD8+) is also available with biopsies since can switch to celiac sprue and lymphoma. - The interest to perform biopsies of the duodenal bulb has been recently discussed in contrast to initial recommendation (Am J Gastroenterol 2016; 111:124–133). - In children, the European recommendation (ESPGHAN) is to not perform endoscopy but to use CD antibodies. If IgA anti-transglutaminase are ≥10 times upper limit of normal after positive HLA test and serum anti-endomysial antibodies, it is not necessary to perform a complementary endoscopy. This is not the case in adults. - It is not necessary to perform HLA DQ2-DQ8 if CD antibodies are positive. In contrast, HLA DQ2-DQ8 is recommended in patients already under gluten free diet which can negate CD antibodies and endoscopic biopsies. Indeed, if patients are DQ2- or DQ8-, they don't have CD.

   We agree with the point that celiac antibodies are important for the diagnosis of CD. However, biopsy stays a critical component in the diagnosis of CD. Neither biopsy nor celiac antibodies alone is sufficient to make a diagnosis. It is true that according to the criteria of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published in 2012, there is the option to diagnose CD without upper endoscopy and duodenal biopsies in very limited cases. But even in those situations antibodies are not sufficient and only a small proportion of patients fulfil the certain clinical, serological and genetic criteria needed for a biopsy-less diagnosis. Consequently, biopsies of the small intestine is still warranted in the vast majority of children. In our study these biopsies were taken from the bulb (at least 1 biopsy) and from the second and the third part of the duodenum (at least 4 biopsies) according to the recommendations then in force [European Society for Pediatric Gastroenterology, Hepatology and Nutrition guidelines for the diagnosis of coeliac disease]. Again, we agree that HLA DQ2-DQ8 typing is not necessary in all CD patients. It should be performed in patients with an uncertain diagnosis of CD. In our study all children were HLA-DQ2 and/or HLA-DQ8 positive to further reduce the possibility of false positive CD diagnosis.