Author's response to reviews

Title: Potential blood-based markers of celiac disease

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Version: 2
Date: 4 September 2014

Author's response to reviews: see over
Cover letter
Dear Editor,

We would like to thank the referees for their reviews that have provided us with valuable comments and suggestions, and given us an opportunity to improve our manuscript. The manuscript has been copy edited by a professional language editing service, and formatted according to the journal style.

The size of Table 2 has been changed to fit a portrait format by merging column “Age” with column “Gender”, and column “Marsh grade” with column “Gluten-free diet”.

Line 30:
“n = 13” has been changed to the “n = 14”.

Lines 144-146:
“In order to avoid zero values, results below the detection limit of the assay were substituted for the detection limit divided by two.” was removed and replaced with “In order to distinguish results below the detection limit of an assay from missing data, the former were replaced with the detection limit divided by two.”.

Lines 176-178:
“To avoid zero values, results below the detection limit of the assay were substituted with the lowest detected value divided by two.” was removed and replaced with “In order to distinguish results below the detection limit of an assay from missing data, the former were replaced with the lowest detected value of the assay divided by two.”

Otherwise our response has been itemized in connection to the comments from each of the referees.

Reviewer's reports:
Referee 1:
Minor revisions:
1. Genes, but not proteins, must be in italics. Eg DQ2 (DQA1*05/DQB1*02, without any spaces).

All gene names have been italicized.

Lines 403-405:

Referee 2:
Major revision
A couple of points - The correlation between the potential novel markers and Marsh grade was moderate at best and appears to be inferior to the correlation between Marsh grade and current standard serology. "Significant correlations were observed between Marsh grade (all cases) and levels of 224 CXCL11 protein (Fig. 2; Spearman rank correlation coefficient (rs) = 0.50), and IL21 and IL15 225 mRNA (rs = -0.46 for both, data not shown)." Can the authors explain this? Was there a cut off value that could reliably diagnose coeliac disease?

As noted by the referee, the correlation between Marsh grade and the novel markers was inferior to that between Marsh grade and current serology. Although the novel markers exhibited a general increase in expression with respect to Marsh grade there were also a considerable overlap between for instance grade 0 and grade 3A (e.g. CXCL11, Fig. 2). Therefore, based on this relatively small set of patients, a cutoff value that reliably diagnosed celiac disease was not apparent. However, the diagnostic performance has now been investigated further using ROC curve analysis (see below).

Providing a sensitivity/specificity analysis of current serology is necessary. Could the authors potentially provide a ROC curve analysis of the novel markers (as well as current serology) as this may provide the best analysis of the differences between the tests.

Sensitivity and specificity of both novel and current markers have been illustrated and analyzed using ROC curves, both for the individual markers and for logistic regression models of marker combinations. These results have been integrated into the manuscript as follows:

Figure 4 has been added to the manuscript.

Lines 35-38:
Finally, the diagnostic performance of anti-TG2, potential blood-based CD markers, and logistic regression models of combined markers was evaluated using receiver operating characteristic (ROC) curve analysis.
ROC curve analysis revealed a slight, non-significant increase in the area under the curve for the combined use of anti-TG2 and different constellations of potential blood-based CD markers compared to anti-TG2 alone.

The diagnostic performance of individual assays and logistic regression models of assay combinations was evaluated using receiver operating characteristic (ROC) curve analysis (MedCalc Statistical Software version 13.1.2, MedCalc Software, Ostend, Belgium).

**ROC curve analysis**

ROC curve analysis of discrimination between cases with active CD and without CD (Fig. 4A and 4B) revealed a larger area under the curve (AUC) for anti-TG2 (AUC = 0.97) in comparison to CXCL11 protein (AUC = 0.81), TNFSF13B mRNA (AUC = 0.85), and a logistic regression model based on CXCL11 protein and TNFSF13B mRNA (AUC = 0.91). A logistic regression model based on anti-TG2, CXCL11 protein, and TNFSF13B mRNA resulted in the highest AUC (0.98). However, compared to anti-TG2 alone, this improvement was not significant (p = 0.54). ROC curve analysis of discrimination between cases with active CD and normalized CD (Fig. 4C) revealed an AUC of 0.90 for anti-TG2 and 0.78 for TNFRSF9 mRNA. Compared to anti-TG2 alone, a logistic regression model based on anti-TG2 and TNFRSF9 mRNA resulted in an increased AUC of 0.93 (p = 0.54).

ROC curve analysis showed that, as a single test, the already established anti-TG2 assay outperformed the new potential blood-based CD markers. However, it might be possible to increase the diagnostic performance by considering several assay results jointly. Adding new markers to the anti-TG2 assay produced a slight, though non-significant, increase in diagnostic performance.

The abbreviations “ROC, receiver operating characteristic” and “AUC, area under the curve” have been added.

**Figure 4 - Receiver operating characteristic (ROC) curves**

ROC curve analysis of discrimination between cases with active celiac disease (CD) and without CD (A and B) or between cases with active CD and normalized CD (C). ROC curves in (A) correspond to CXCL11 protein (solid line), TNFSF13B messenger RNA (mRNA) (dashed line), and a logistic regression model of CXCL11 protein and TNFSF13B mRNA (dotted line). ROC curves in (B) correspond to tissue transglutaminase autoantibodies (anti-TG2) (solid line) and a logistic regression model of anti-TG2, CXCL11 protein, and TNFSF13B mRNA (dashed line). ROC curves in (C) correspond to anti-TG2 (solid line),
TNFRSF9 mRNA (dashed line), and a logistic regression model of anti-TG2 and TNFRSF9 mRNA (dotted line).