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Warszawa 13.08.2015

World Journal of Gastrointestinal Pharmacology and Therapeutics Editorial Board

Response to Reviewers

Dear Editors,

We would like to submit a revision of the manuscript entitled **"Clinical utility of quantitative multi-antibody Polycheck immunoassays in the diagnosis of coeliac disease"** by Ewa Konopka, Maciej Grzywnowicz, Beata Oralewska, Joanna Cielecka-Kuszyk, Ilona Trojanowska, and Bożena Cukrowska.

In this letter, we would like to respond to points raised by the reviewer. All suggestions were very valuable. As suggested by the reviewer appropriate changes and clarifications were included in the revised version the manuscript. We believed that changes has improved the manuscript. All responses are presented below.

We consider this paper to provide complex information about the utility of multi-antibody tests in the diagnosis of CD, which is highly relevant in the current approach to serological tests in CD. Therefore, we sincerely hope that you might find our manuscript appropriate for publication in *World Journal of Gastrointestinal Pharmacology and Therapeutics*.

Sincerely Yours,
Prof. Bożena Cukrowska.

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RESPONSE TO REVIEWER

Major comments

1. The author's state that included was preselected patients with biopsy proven celiac disease and with positive IgA anti-tTG or IgG anti-DGP antibodies and concludes that detection of celiac-specific antibodies with multi-antibody PCPs is effective and improves the diagnosis of celiac disease. With this preselection the results can be questioned. If the inclusion criteria were that the patients had positive levels of celiac-related antibodies, it is not surprisingly that all the patient in the present study also had increased levels of celiac-related antibodies with the Quantitative Polycheck immunoassay. To be able to actually evaluate the method there is a need to include:
   - Patients with villous atrophy and no circulating celiac-related antibodies (false negatives)
   - Patients with circulating celiac-related antibodies and normal mucosa (false positives)
   - Patients with circulating celiac-related antibodies and minor mucosal changes (if this category is excluded, the assay can't be better than the gold biopsy standard)

First of all, we would like to apologize for the confusing usage of "*improve*" term, which is discussed more in the response to major comment no.3.

The preselection of the patients was made on purpose to create the group of CD patients to test a new method efficacy and the negative group. It was based on the tTG IgA

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Elia Celikey results, and the histotological analyses of intestinal biopsies, thus by using such selected groups we would like to verify the sensitivity of the Polycheck method. It was not based on the other antibodies results, because they were not included into the routine diagnosis pathway.

We agree that addiction of proposed specific study groups would be beneficial for the better evaluation of the method, but in this case the study should be performed on unselected patients, and then histology of biopsies should be done. Only in this way we could select false negative, false positive and potential CD patients. Thus in this study we were unable to form such groups. However, the "false negative" and false positive patients very rarely occur in our practice (about 2 case in past few years) due to usage of the effective tTG IgA Elia Celikey kit (Thermo Fisher). That is why we decided to use this test as a reference one (also in ESPGHAN recommendation by Husby et al [1] this test was specified as one which has the highest specificity and sensitivity >90-95%).

Moreover, in our practice we often observe initially "false positive" patients who develop the celiac disease in later time (potential celiac disease cases). Similar patients with positive CD specific antibodies and minor mucosal changes (Marsh I) – potential CD patients when having HLA-DQ2/DQ8 genotype were not included to this study, but most of them were also positive when Polycheck tests were used.

We agree that presented data and selection of the group could not support the conclusion that the multi-antigen method is better than biopsy, and that was not the aim of the study.

1. Husby S, Koletzko S, Korponay-Szabo IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012;54(1): 1136-160.

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2. How many of the included children were younger than 2 years of age? Several reports have shown that some of young children lack anti-tTG. To state that this method should be able to replace the biopsy procedure, it is necessary to address this question.

In our study, 3 children under 2 years of age were included into the CD patients group. Two of them had exactly 2 years and one of them was younger than 2 years (22 months). All three children had positive anti-tTG IgA results of both Elia Celikey (Thermo Fisher) and Polycheck (Biocheck) methods. The characteristics of results for under 2 years old children is presented in Table 1 below.

The Table 1 in the main manuscript has been modified with an annotation about children under 2 years of age: "[#] *In the CD patients study group were 3 children 2 years of age*."

Table 1. Characteristics of children under 2 years of age

Sex	Month of birth	Year of birth	Age (months)	Month of testing	Year of testing	tTG IgA Elia Celikey	tTG IgA Polycheck Celiac IgA	tTG IgG Polycheck Celiac IgG	DGP IgA Polycheck Celiac IgA	DGP IgG Polycheck Celiac IgG	Biopsy 1-yes 0-no	Marsh III
m	8	2011	22	6	2013	32,4	37	0,47	21	77	1	1
w	8	2011	24	8	2013	>100	>100	5,2	0,43	38	1	1
w	12	2011	24	12	2013	>100	>100	3,4	48	98	1	1

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3. The authors conclude that: *the present assay could improve the diagnosis of celiac disease*. However, the present results do not support this statement.

We agree that term "*improve*" is exaggerated and it could be unfortunately misleading. The main focus of the presented paper was to evaluate the efficacy of the Polycheck method in contrast to routinely used in our hospital Elia Celikey (Thermo Fisher) method. We believed that the efficacy of Polycheck method was presented convincingly.

The appropriate section of the abstract was modified:

Before changes:

"*Conclusions: The present study showed that detection of coeliac-specific antibodies with multi-antibody PCPs is effective and it improves the diagnosis of CD.*"

After correction:

"*Conclusions: The present study showed that detection of coeliac-specific antibodies with multi-antibody PCPs is effective and efficacious in the diagnosis of CD.*"

Our results for 4 antibodies: anti-tTG-IgA/IgG and anti-DGP-IgA/IgG – with double or more positive tests required to determine positive results showed that 98% sensitivity and NPV, 100% specificity and PPV, with overall diagnostic accuracy 99% - even with included patients with selective IgA deficiency.

Using the combination of four antibodies (anti-tTG-IgA/IgG + anti-DGP-IgG/IgA) classified 49 from 50 children with CD as positive, 46 from 50 children from control group

as CD negative, and only 5 children from each group (n=100) were neither classified as CD positive nor negative with further verification needed.

Simultaneous performance of 4 antibodies could significantly reduce time of diagnosis and could make shorter waiting time for the introduction of a gluten-free diet. It could be important not only that to avoid of complications celiac diseases but also to alleviate a nuisance of disease for the child and mother. Such approach significantly simplifies the process of diagnosis, and that was on out mind by the "*improve*" usage.

Minor comments

1. The use of "*specific antibodies against a disease inducing factor*" as a description of deamidated gliadin peptides is confusing and should be avoided.

We agree that such a description could be confusing, therefore, the appropriate changes were introduced. Please, find below the changes made.

Before changes:

"*Objectives: Coeliac disease (CD) is a chronic, systemic gluten-depended autoimmune disorder characterized by presence of specific antibodies against a disease inducing factor (deamidated gliadin peptides - DGP), and autoantibodies against tissue transglutaminase (tTG).*"

After correction:

"*Objectives: Coeliac disease (CD) is a chronic, systemic gluten-depended autoimmune disorder characterized by presence of specific antibodies against deamidated gliadin peptides (DGP), and autoantibodies against tissue transglutaminase (tTG).*"

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2. What was the diagnosis of the control subjects? This could be clarified with a table.

The diagnosis of the majority of the control subject was classified as Functional Gastrointestinal Disorders (FGIDs). Two cases were classified as inflammatory bowel disease.

The description "*The diagnosis of the majority of the control subject was functional gastrointestinal disorders. Two control cases were classified as inflammatory bowel disease.*" was added into Material and Methods – Patients section.

3. Table 2 could be clarified.

The current organization of Table 2 was found to be most suitable to present the large portion of data. Additionally, the data regarding not effective or meaningful strategies were not included. Table 2 could be rearranged due to the review's remarks or be clarified by change of table lines format by *World Journal of Gastrointestinal Pharmacology and Therapeutics*.

Answer to chief editor:

Dear Fang Fang Ji,

Please find in the attachment the updated manuscript file with the added information about the follow up diet observation for children with confirmed celiac disease (CD).

The following sentences were added in the *Material and Methods – Patients section*:

Out of 50 children 46 were observed for at least one year after introduction of gluten free diet, and in all but one improvement of clinical syndromes and systematic decrease in specific CD antibodies were noticed. The child without serological and clinical improvement did not comply with dietary recommendations.

With kind regards,

Dr. Maciej Grzywnowicz