Clinical utility of quantitative multi-antibody Polycheck immunoassays in the diagnosis of coeliac disease

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Abstract

AIM: To evaluate the clinical utility of multi-antibody strategies in the diagnosis of coeliac disease (CD), the new quantitative Polycheck immunoassays were analysed.

METHODS: Polycheck Celiac Panels (PCPs) are immunoenzyme screening assays for the quantitative measurement of coeliac-specific immunoglobulin class G (IgG) or class A (IgA) in serum. Lines of relevant antigens are coated together with five IgG or IgA standard lines used for the standard curve as positive control. PCP IgA consists of human recombinant human tissue transglutaminase (tTG) and deamidated gliadin peptides (DGP) as targets to detect IgA antibodies. PCP IgG consists of tTG, DGP and IF (intrinsic factor) antigens to detect antibodies in IgG class. PCPs were performed on 50 CD patients, including 6 cases with selective IgA deficiency, and 50 non-coeliac controls. CD diagnosis was performed according to the ESPGHAN recommendations: The presence of specific anti-tTG-
IgA or anti-DGP-IgG (in the case of IgA deficiency) antibodies, typical histopathological changes in duodenal mucosa described in Marsh-Oberhüller classification as at least grade 2. The diagnosis of the majority of the control subjects was functional gastrointestinal disorders. The PCP results were compared with reference EliA Celikey.

RESULTS: The usage of PCPs led to the correct identification of all CD patients. In our study, PCPs showed 100% agreement with the histopathological results. PCP IgA test showed a 98% concordance and correlated positively (R = 0.651, P = 0.0014) with EliA Celikey test. The highest specificity and positive predictive value (both 100%) were observed for the detection of Polycheck anti-tTG-IgA antibodies. The highest sensitivity and negative predictive value (both 100%) were achieved by Polycheck anti-DGP-IgG antibody detection. The best performance (98% sensitivity and negative predictive value, 100% specificity and positive predictive value, diagnostic accuracy - AU ROC 99%) was observed for the strategy of using both PCP IgA and IgG and determining positive outcomes of the test with two or more coeliac-specific antibodies detected. The majority of coeliac patients had multiple antibodies. All four antibodies were detected in 7 (14%) cases, 19 children (38%) were positive for three antibodies and 23 (46%) were positive for two antibodies.

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

Key words: Coeliac disease; Tissue transglutaminase; Deamidated gliadin peptides; Multi-antibody tests; Polycheck celiac panels

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Core tip: Detection of coeliac-specific antibodies has become a useful tool in the diagnostics of coeliac disease. Different serology test combinations have been found to improve diagnosis in comparison to a single antibody test. Recently, multi-antibody strategy has been implemented in immunoassays. In this study we have found that multi-parametric quantitative Polycheck immunoassay is reliable in reference to intestinal biopsy results and measurements of anti-tissue transglutaminase-IgA by a reference method. The best overall clinical performance was obtained by a combination of both IgA and IgG panels, with two and more positively detected antibodies, to determine the outcome.

INTRODUCTION

Coeliac disease (CD) is a chronic immune-based systemic disorder caused by intolerance to dietary gluten in individuals with genetic predisposition. Gluten is a storage protein in wheat, barley, and rye, which triggers an inflammatory state in the small intestine, leading to the induction of the cytotoxic intra-epithelial lymphocytes, reduction of villus height, hyperplastic cryptae and finally to complete villus atrophy. CD is characterised by the presence of specific antibodies, including specific ones against a disease inducing factor: Deamidated gliadin peptides (DGP), as well as autoantibodies against tissue transglutaminase 2 (tTG).

Assessing the levels of serum antibodies that were applied in the diagnosis of CD for over 40 years, starting from the determination of anti-gliadin and anti-reticulin antibody levels[11]. The discovery that a major target of autoantibodies in CD is tTG, which is related to deamidation of gliadin peptides by this enzyme, allowed to better understand the pathogenic pathway of events leading to CD development[12,13]. In recent years, the usage of native gliadin as the target of serology diagnostics of CD was withdrawn from the CD routine diagnosis due to inferior performance compared to a highly specific and sensitive anti-tTG tests[14]. Deamidation of gliadin peptides enhances their immunogenicity, which leads to a higher specificity and sensitivity of tests for anti-DGP-IgA and -IgG antibodies than native gliadin tests[14,15]. Studies on the performance of anti-DGP-IgG tests in the diagnosis of CD showed that it is comparable with anti-tTG-IgA tests[16,17]. In contrast to anti-tTG-IgA, anti-DGP-IgG tests are not affected by the presence of hemolysis in a tested sample[18] and are effective in the detection of CD in patients with selective IgA deficiency[19].

The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) published, in 2012[10], clinical guidelines including algorithms of CD diagnosis with the crucial role of serology tests. According to ESPGHAN recommendations, the initial approach to patients with suspected CD includes serological screening for anti-tTG-IgA and measurement of a total IgA level to exclude selective IgA deficiency, i.e., immunodeficiency which occurs in CD patients, with a relevance of 2%-8%[10]. The initial usage of anti-tTG-IgA tests is based on its both high sensitivity and specificity values[17]. In the case of confirmed selective IgA deficiency, anti-tTG-IgG, anti-DGP-IgG or anti-endomyosial IgG tests are recommended to detect CD[10]. An alternative approach, especially recommended for CD screening in at risk groups, consists of direct testing for
This study enrolled 50 paediatric patients with CD and 50 non-coeliac age and sex matched control children, treated in the Children's Memorial Health Institute, Warsaw, Poland, between January 2013 and September 2014. All patients underwent intestinal biopsy during endoscopy, with the histological examination of the small intestine specimens classified according Marsh-Oberhüner scale[11]. Serum samples collected from all children and stored at -20°C were used for antibody detection. CD diagnosis was performed according to the ESPGHAN recommendations: The presence of specific anti-tTG-IgA or anti-DGP-IgG (in the case of IgA deficiency) antibodies, typical histopathological changes in duodenal mucosa described in Marsh-Oberhüner classification as at least grade 2. 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. The patients's characteristics are presented in Table 1. Out of 50 CD patients, the selective IgA deficiency was detected in 6 children (12%). The diagnosis of the majority of the control subjects was functional gastrointestinal disorders. Two control cases were classified as inflammatory bowel disease. Written, informed consent was obtained from all patients with respect to the use of their blood for scientific purposes.

Detection of antibody by single-antibody immunoassay
The fluoroimmunoassay Elia Celikey IgA, and for patients with IgA deficiency Elia Gliadin DP IgG kits (Thermo Scientific, Phadia GmbH, Freiburg, Germany) were used for the detection of anti-tTG-IgA and anti-DGP-IgG antibodies. Single, well-based immunoassays were performed according to the manufacturer’s protocols using an automated Thermo Scientific Phadia 100 system (Freiburg, Germany). The antibody level > 10 U/mL was considered positive.

PCPs
PCPs are immunoassays designed as nitrocellulose membrane strips with different antigens placed in individual lines. Polychack Panel IgA consists of human recombinant tTG and DGP antigen lines as targets to detect IgA antibodies. Polychack Panel IgG consists of human recombinant tTG, DGP and IF (intrinsic factor) antigens to detect antibodies in IgG class. Antibody detection by Polychack Panels were performed according to the manufacturer’s protocol. Briefly, patients sera, diluted at 1:100, were incubated for 45 min at room temperature. In the next step, anti-human-IgG or -IgA monoclonal detection antibodies were added for 30 min. Finally, the substrates (5’bromo-4’chloro-3’ indolyolphosphate/4’ nitro-bluetetrazolium; BCIP/NBT) were added for 20 min, and the colour intensity of the specific lines corresponding to antibody concentration was scanned and the result was calculated according to the calibrator curve present in each cassette. For anti-tTG-IgA and anti-DGP-IgG, which allows to omit total IgA testing and reduces the number of tests that are needed to be performed[10].

The methods of detection of CD-specific antibodies have been based so far on various immunoenzymatic assays allowing individual measurements of one antibody type per blood sample. Recently, Polychack Celiac Panels (PCPs) have been introduced as a new diagnostic option in CD. PCPs represent a unique approach in measurement of coeliac-specific antibodies, by combining the detection of multiple antibodies in a single blood sample with a quantitative standard curve based immunoassay on the nitrocellulose membrane. ESPGHAN guidelines recommend a validation for every antibody test being used for CD diagnosis, by comparing the results of a novel test with results obtained from histopathological examination of small intestine specimens, and another reference serology test with high specificity and sensitivity[10]. The aim of this study was the assessment of the sensitivity, specificity and clinical utility of multi-antibody Polychack IgA and IgG immunoassays in the diagnosis of CD in reference to histology results and the detection of anti-tTG-IgA antibodies by a reference method.

MATERIALS AND METHODS

Study design
This is a retrospective study, which was designed to investigate the sensitivity and specificity of Polychack Celiac IgA and IgG (Biocheck GmbH, Muenster, Germany) quantitative, multi-parametric immunoassays in the diagnosis of CD. According to ESPGHAN recommendations, test results were validated with reference methods: Intestinal biopsy and EliA Celikey IgA method for anti-tTG-IgA detection, along with another serological test with known high sensitivity and specificity (Thermo Scientific, Phadia GmbH, Freiburg, Germany). Test results were validated in a group of children with biopsy-proven CD and a control group of non-CD children.

Patients
This study enrolled 50 paediatric patients with CD and

### Table 1  Characteristics of the patients

|                | Coeliac disease | Non-coeliac disease |
|----------------|-----------------|---------------------|
| No. of patients| 50              | 50                  |
| Females        | 28 (56%)        | 29 (58%)            |
| Males          | 22 (44%)        | 21 (42%)            |
| Mean age in years | 8.7 ± 4.7 (2.5-17.5) | 11.6 ± 4.8 (3-17.5) |
| Histopathological results1 | Marsh 0 | 0 (100%) |
|                | Marsh II | 6 (12%) |
|                | Marsh III | 44 (88%) |

1Biopsy results were classified according to the Marsh-Oberhüner classification[11]. In the coeliac disease patients study group were 3 children ≥ 2 years old.
the quantification of antibody concentrations, Biocheck Imaging Software (BIS) was used. The antibody concentration > 0.8 kU/L was considered as positive. The assays have an equivocal range defined, 0.3–0.8 kU/L. For statistical purposes, equivocal results were considered as negative in the analysis.

Statistical analysis
The diagnostic performance of Polycheck serological tests was determined by calculating the sensitivity, specificity, positive and negative predictive values (PPVs and NPVs), areas under the receiving operator characteristic curves (AU ROC) and likelihood ratios (LR). Data were analysed using Statistica 10 software (StatSoft, Poland). Correlations of results were computed with the Spearman rank correlation coefficient.

RESULTS

Sensitivity and specificity of antibody tests on panels
We analysed for the each antibody test: Sensitivity, specificity, PPV, NPV, AU ROC, and likelihood ratios for positive (LR+) and negative (LR-) results. Statistical performance of antibody tests is presented in Table 2.

The highest specificity and PPV (both 100%) were observed for the detection of anti-tTG-IgA antibodies. The highest sensitivity and NPV (both 100%) were calculated for anti-DGP-IgG antibodies detection. Anti-tTG-IgG and anti-DGP-IgA were specific (both 98% of specificity); however, they presented low sensitivity (48% and 30% respectively). Diagnostic accuracy determined by the AU ROC curve value for anti-DGP-IgG was 98%, for anti-tTG-IgA 93%, for anti-tTG-IgG 73% and for anti-DGP-IgA 64%.

Considering clinical value of tests, LR+ was significant for all detected antibodies detected antibodies. Due to 100% specificity, LR+ for anti-tTG-IgA was incalculable, however, indicating the highest value of performing a diagnostic test.

Two panels combination
Since PCPs were designed as multi-antibody assays, we verified the statistical value of them in the combination of two panels. Detection of anti-tTG (IgA and IgG) and anti-DGP (IgA and IgG) in the combination of both PCPs when two or more antibodies were positive showed the best statistical performance among the analysed tests (individual or in combination). The 98% of sensitivity and NPV, and 100% of specificity and PPV resulted in excellent diagnostic accuracy (AU ROC 99%). The value for LR+ was incalculable due to 100% specificity, which pointed the best reliability of the positive result. For the negative result LR- was 0.02. Values for the combinations are summarised in Table 2.

Validation against the reference
Polycheck tTG-IgA results showed significant correlation with EliA Celikey IgA results: \( R = 0.651, P = 0.0014 \) \( (n = 21) \). Both assays matched anti-tTG-IgA positive results in 43 out of 44 cases (98% agreement). One of the children, who had positive anti-tTG-IgA antibodies measured with the EliA Celikey IgA kit, was negative for the Polycheck IgA immunoassay, but the level of anti-tTG-IgA was 0.48, which is a borderline value for PCPs. Simultaneously, this child had the single positive result for anti-DGP-IgG antibody, determined by Polycheck IgG. All children with biopsy-proven CD have positive antibodies detected with PCPs (100% agreement with the histopathological results).

Antibody profiles in the study group
The majority of coeliac patients had multiple antibodies detected. Selective IgA deficiency has been described for 6 out of 50 CD patients. All four antibodies were detected in 7 (14%) cases, 19 children (38%) were positive for three antibodies and 23 (46%) were positive for two antibodies. Only the patients with a normal IgA level had single positive anti-DGP-IgG; however, anti-tTG-IgA was borderline in these cases. All CD patients had positive anti-DGP-IgG antibodies. All but one CD patients with normal IgA level had positive anti-tTG-IgA. All 6 children with selective IgA deficiency had both positive anti-DGP-IgG and anti-tTG-IgA. In a non-coeliac control group, 4 children had single positive results, but none had multiple antibodies detected. Summary of obtained antibody profiles for both CD and

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### Table 2 Statistical sensitivity and specificity of Polycheck Celiac IgA and IgG tests for single antibody and for selected multi-antibody combinations

| Specific coeliac antibody | Sensitivity % | Specificity % | PPV % | NPV % | AU ROC | LR + ² | LR- |
|--------------------------|--------------|--------------|-------|-------|-------|-------|-----|
| Single antibody positivity |             |              |       |       |       |       |     |
| Anti-tTG-IgA¹            | 97.7%        | 100.0%       | 100.0%| 98.0% | 98.9% | -     | 0.23|
| Anti-tTG-IgG             | 48.0%        | 98.0%        | 96.0% | 65.3% | 73.0% | 24,000| 0.531|
| Anti-DPG-IgA             | 34.1%        | 98.0%        | 93.8% | 62.8% | 68.1% | 17,045| 0.673|
| Anti-DPG-IgG             | 100.0%       | 96.0%        | 96.2% | 100.0%| 98.0% | 25,000| 0.000|
| Combination of two or more positive antibodies |             |              |       |       |       |       |     |
| Anti-tTG-IgA/-IgG + anti-DGP-IgG/-IgA | 98.0% | 100.0% | 100.0%| 98.0% | 99.0% | -     | 0.020|

²Calculations after excluding patients with selective IgA deficiency; ³For strategies, where specificity of a test/combination was 100%, the likelihood ratio for a positive result could not be calculated. PPV: Positive predictive value; NPV: Negative predictive value; AU ROC: Area under a receiving operator characteristic curve; LR+: Likehood ratio for a positive result; LR-: Likehood ratio for a negative result.
non-CD controls are presented in Table 3.

**DISCUSSION**

The traditional golden standard in the diagnosis of CD is the intestinal biopsy. However, morphological changes of intestinal mucosa are not CD-specific and they might be caused by other pathological conditions\[^{12}\]. The biopsy is an invasive procedure, and the histopathological results are strongly dependent on the experience of the pathologist\[^{13,14}\]. Therefore, usage of serology tests to detect coeliac-specific antibodies has been increasing simultaneously with the improvement of the methodology, i.e., replacing non-human tTG with a human recombinant tTG and introduction of DGP.

This study was retrospective with preselected patients with biopsy proven CD and with positive single anti-tTG-IgA or anti-DGP-IgG antibodies, which were used as references to validate the performance of any new immunoassays. PCPs detect CD specific multi-antibodies and follow ESPGHAN recommendations stating that novel anti-tTG and anti-DGP tests should produce quantitative, numerical values, expressed in arbitrary units\[^{10}\]. Obtained results show that PCPs fulfil ESPGHAN requirements by achieving 98% agreement with the reference Elia Celkey IgA test and 100% agreement with biopsy results when using Polycheck Celiac IgG and IgA tests alone, or in combination. There was one discrepancy in the anti-tTG-IgA testing, and this was where a CD child had negative anti-tTG-IgA results with the Polycheck IgA, but positive with the Elia Celkey IgA test. However, the result, classified as negative, has fitted the equivocal range, and this patient had the positive anti-DGP-IgG antibodies. Therefore, the child would have been correctly diagnosed as coeliac-positive with combination of both IgA and IgG PCPs.

Recently, a limited number of strategies using different combinations of tests detecting simultaneously more than the single coeliac-specific antibody were developed in an attempt to achieve better clinical performance, and to define applicable approaches, allowing the omission of a biopsy during CD diagnosis\[^{15-17}\]. PCPs are the multi-antibody detecting system, therefore, their performance could be considered in several ways. Each panel measures either IgA or IgG specific coeliac-antibodies from a single serum sample, which is a novel approach, not utilising any combinations of separate tests, e.g., immunoenzymatic tests\[^{4}\]. Our study has shown that the detection of anti-tTG-IgG and anti-DGP-IgG has the best performance in the diagnosis of CD, and that anti-DGP-IgG has a comparable diagnostic value as anti-tTG-IgA. This result is concordant with earlier studies\[^{15,16,17}\]. The diagnostic accuracy of anti-tTG-IgG was better than anti-DGP-IgA, which is concordant with previously made meta-analysis\[^{17}\]. Considering the overall characteristics, the best performance was observed for the combination of two PCPs (4 antibodies: Anti-tTG-IgA/IgG and anti-DGP-IgA/IgG) with double or more positive tests required to determine a positive results.

This strategy showed excellent 98% sensitivity and NPV, 100% specificity and PPV, with overall diagnostic accuracy of 99%. Our results were comparable with previous studies, showing that a combination of more than one antibody test creates the better CD diagnostic opportunity than single antibody testing\[^{15-17}\]. Multi-antibody testing might lead to the lower sensitivity in exchange for higher specificity\[^{14,16,17}\]; however, in this study, the multi-antibody strategy achieved still higher sensitivity (98%), which is only slightly reduced sensitivity when compared to the anti-DGP-IgG test. Multiple strategies with any positive antibody used in determination of positivity were expected to have a lower specificity and higher sensitivity than any single antibody test\[^{4}\], which was observed in this study as well. Our results indicate that the most beneficial for CD diagnosis is multi-antibody testing with two and more positive antibodies.

The IgA deficiency is a CD associated condition more common among CD patients than in the general population, therefore, we found it to be suitable to include IgA deficient patients in the characterised study group. The prevalence of selective IgA deficiency was 12% in our CD patients. The observed high sensitivity of anti-tTG-IgA (97.7%) decreased to 86% after including IgA deficient patients (data not shown). The significant advantage of the presented multi-antibody combination was the highest diagnostic accuracy without discriminating on normal and IgA deficient patients. Performing simultaneous multi-antibody detection in both IgA and IgG classes might provide one-step, time-saving diagnosis of CD, independent from selective IgA deficiency.

In this study, we have calculated the likelihood ratios for CD in the studied population. The LR for the positive results is the ratio of the probability of a coeliac-positive patient acquiring a positive test result to the probability that non-coeliac patients acquires positive test results, while LR for the negative result describes the opposite situation. It was pointed that LRs are useful in the clinical interpretation of CD antibody tests, since they are independent from the prevalence of CD\[^{17}\]. We found that the highest LR was observed for double positive tests strategies; however, the precise value could not be calculated because of 100% of PPV in those combinations. The lowest LR for negative results was obtained for double negative tests strategies with the IgG panel alone or quadruple negative tests in combination. Similarly, Vermeeursch et al\[^{17}\], observed the highest LR for the positive result in CD testing for the strategy with double positive outcomes for coeliac antibodies, and the lowest LR for the negative result for double negative tests. Sugai et al\[^{15}\] described the comparable test results for LR for positive results; however, for CD exclusion, the lower LR was observed for triple negative than for double negative results.

ESPGHAN guidelines\[^{10}\] allow, under certain circumstances, to omit a biopsy as a confirmatory procedure and to base it on the serology results and genetic background...


In conclusion, we found Polycheck Celiac IgA and IgG panels to be reliable immunoassays in CD diagnosis. Recently, several studies have evaluated the performances of different combinations of antibody tests and their utility in avoiding intestinal biopsies [15-17]. It was found that multi-antibody screening in CD could be successfully used to increase the overall performance of serology diagnostics [15-17]. Our aim was to assess how quantitative, screening Polycheck immunoassays would perform in the diagnosis of childhood CD, since that approach might become more popular in practice. In this study, we have shown that the significant majority of CD children (49/50) have more than one positive coeliac-specific antibody, and that 46 out 50 children with no CD have been recorded as negative for all four antibodies (Table 3), and the best diagnostic accuracy was achieved with the combination of two or more positive antibodies. With defining an outcome as positive with two or more detected antibodies, and as negative with all four non-detected antibodies, it could allow to avoid 98% of intestinal biopsies with no missed CD cases in the analysed study group. Sugai et al. [16], showed that combinations of two different assays would allow the avoidance of 92%-98.7% of all intestinal biopsies in the high-risk group, and 92.1%-99% in the low-risk group (3-5 missed CD cases), which is compatible with our findings. It is worth emphasising that no CD case was missed with the combination presented in this study. Bürgin-Wolff et al. [15], with usage of three assays (anti-tTG-IgA + anti-DGP-IgG/-IgA) would avoid 78% of biopsies; however, IgA-deficient cases were excluded in that study. The combination presented in this study could be used regardless of IgA deficiency. The limitation of this study is the preselection of the study group; therefore, future validations of the presented strategy are required.

In conclusion, we found Polycheck Celiac IgA and IgG panels to be reliable immunoassays in CD diagnostics. Both panels presented very good clinical performance, which was even better in the combination with double or more detected antibodies as positive results, meeting criteria set by the ESPGHAN guidelines [15]. They showed 100% agreement with biopsy results and provide numerical, quantitative results. The detection of IgG coeliac antibodies by PCPs, especially with the well-defined performance of anti-DPG-IgG, determined high clinical utility in the group of patients including cases with selective IgA deficiency. The combination of both panels is reliable and effective in CD diagnosis, regardless of any selective IgA deficiency.

### Table 3 Antibody profile in celiac disease patients and non-celiac disease controls

| Anti-tTG-IgA | Anti-tTG-IgG | Anti-DGP-IgA | Anti-DGP-IgG | CD patients n = 50 | Non-CD controls n = 50 | Total n = 100 | Classification in combination (anti-tTG-IgA/IgG + anti-DGP-IgG/IgA) |
|-------------|-------------|-------------|-------------|--------------------|--------------------|-------------|---------------------------------------------------|
| +           | +           | +           | +           | 7 (14%)            | 0                  | 7           | 49 positives                                      |
| +           | +           | -           | -           | 11 (22%)           | 0                  | 11          |                                                   |
| +           | -           | +           | -           | 8 (16%)            | 0                  | 8           |                                                   |
| +           | -           | -           | +           | 17 (34%)           | 0                  | 17          |                                                   |
| -           | +           | +           | -           | 6 (12%)            | 0                  | 6           |                                                   |
| -           | -           | +           | +           | 1 (2%)             | 2 (4%)             | 3           | 5 not classified                                  |
| -           | -           | -           | +           | 0                  | 1 (2%)             | 1           |                                                   |
| -           | -           | -           | -           | 0                  | 1 (2%)             | 1           |                                                   |
| -           | -           | -           | -           | 0                  | 46 (92%)           | 46          | 46 negatives                                      |

1 Patients with confirmed selective IgA deficiency. +: Antibody present; -: Antibody absent; Using the combination of four antibodies (anti-tTG-IgA/IgG + anti-DGP-IgG/IgA) classified 49 children as CD positive, 46 as CD negative, and 5 were neither classified as CD positive nor negative with further verification needed. CD: Celiac disease; DGP: Deamidated gliadin peptides; tTG: Tissue transglutaminase.

### COMMENTS

**Background**

Serological testing of coeliac specific antibodies: Anti-tissue transglutaminase (tTG) and deamidated gliadin peptide [deamidated gliadin peptides (DGP)] immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies has become increasingly important for the diagnosis of coeliac disease (CD), starting to rival the biopsy results. The discovery that a major target of autoantibodies in CD is tTG, which is related to deamidation of gliadin peptides by this enzyme, allowed to understand better the pathogenic pathway of events leading to CD development. Introduction of the human tissue transglutaminase into diagnostic methods was the major breakthrough in the diagnosis of CD. In recent years, the usage of native gliadin as the target of serology diagnostics of CD was withdrawn from the CD routine diagnosis due to inferior performance compared to a highly specific and sensitive tests anti-tTG IgA and anti-deamidated gliadin IgG. It was found that anti-DGP of the IgG class might be useful for the identification of coeliac disease in children under 2-3 years of age and in patients with IgA deficiency. The European Society for Paediatric Gastroenterology, Hepatology, and Nutrition published in 2012 clinical guidelines pointed that the serology diagnostic of coeliac disease is crucial in determination of following diagnostic steps. It also allows to omit the biopsy under certain conditions based only on coeliac specific antibodies and genetic background. Finally, it points an alternative approach, especially recommended for CD screening in at risk groups, consisting direct testing for anti-tTG-IgA and anti-DGP-IgG, what allows to omit total IgA testing and reduces a number of tests needed to perform.

**Research frontiers**

Since accuracy of coeliac-specific serological tests have improved and multi-antibody approaches have occurred, the effectiveness of such strategies is the focus of up-to-date studies, often as potential alternative to biopsy results. Additionally, the evaluation of clinical use of new markers such as anti-DPG is the important part of the research field.

**Innovations and breakthroughs**

The aim of this study was the evaluation of the clinical utility of new multi-antibody quantitative Polycheck Celiac Panels immunoassays in the diagnosis of CD, since several strategies detecting coeliac-specific antibodies are being recently investigated. The study has shown concurrent with earlier studies results that the detection of anti-tTG-IgA and anti-DGP-IgG has the best performance in the diagnosis of CD, and that anti-DGP-IgG has a comparable diagnostic value as
anti-TG-IgA. In study, the best performance was observed for the combination of two polycheck celiac panels (4 antibodies: anti-TG-IgA/IgG and anti-DGP-IgA/IgG) with double or multiple positive tests required to determine a positive result. This strategy showed excellent 98% sensitivity and NPV, 100% specificity and PPV, with overall diagnostic accuracy of 99%. These results were comparable with previous studies, showing that a combination of more than one antibody test creates the better CD diagnostic opportunity than single antibody testing.

Applications
The use of multi-antibody strategy, like use of polycheck celiac panels, which consists coeliac-antibodies in both IgG and IgG classes, could be beneficial in patients with coexistent to CD selective IgA deficiency. In contrary to cascade approach, multiple serological tests might increase the sensitivity of the diagnostic strategy and lead to faster diagnosis, since serology several markers are tested simultaneously.

Terminology
DGP. Gliadin peptides deamidated by tissue transglutaminase 2 enzyme, which leads to great increase of their immunogenicity; Multi-parametric, multi-antibody serology tests: The tests which allows to measure more than one coeliac-specific antibody from one serum sample in a single measurement test; Quantitative test: Test which produces the quantitative results with exact concentration of antibodies being measured.

Peer-review
In this study the authors have explored the utility of quantitative multi-antibody Polycheck immunoassays in the diagnosis of celiac disease.

REFERENCES
1. Seah PP, Fry L, Rossiter MA, Hoffbrand AV, Holborow EJ. Anti-reticulin antibodies in childhood coeliac disease. Lancet 1971; 2: 681-682 [PMID: 4105712 DOI: 10.1016/S0140-6736(71)92248-3]
2. Dieterich W, Ehnius T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997; 3: 797-801 [PMID: 9212111 DOI: 10.1038/nm0797-797]
3. Jabri B, Soldi LM. Tissue-mediated control of immunopathology in coeliac disease. Nat Rev Immunol 2009; 9: 858-870 [PMID: 19935805 DOI: 10.1038/nr8577]
4. Rashtak S, Ettorre MW, Homburger HA, Murray JA. Comparative usefulness of deamidated gliadin antibodies in the diagnosis of celiac disease. Clin Gastroenterol Hepatol 2008; 6: 426-432; quiz 370 [PMID: 18304884 DOI: 10.1016/j.cgh.2007.12.030]
5. Prause C, Ritter M, Probst C, Daehrich C, Schumlierger W, Konorowski L, Lieske R, Richter T, Bauer AC, Stern M, Uhlig HH, Laass MW, Zimmer KP, Mothes T. Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease. J Pediatr Gastroenterol Nutr 2009; 49: 52-58 [PMID: 19465869 DOI: 10.1097/MPG.0b013e31819fdae3]
6. Sugai E, Vázquez H, Vázquez H, Nachman F, Moreno ML, Hwang HJ, Cabanne A, Crivelli A, Nachman F, Vázquez H, Niveloni S, Argonz J, Mazure R, La Motta G, Furlano R, Siddler MA, Mulder CJ, Goeres MS, Mearin ML, Bieber T, Giersiepen K, Branski D, Catassi C, Treiber A, Zimmer KP. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012; 54: 136-160 [PMID: 22197856 DOI: 10.1097/MPG.0b013e318212a3d0]
7. Lewis NR, Scott BB. Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease. Aliment Pharmacol Ther 2010; 31: 73-81 [PMID: 19664074 DOI: 10.1111/j.1365-2036.2009.04105.x]
8. Arguelles-Grande C, Norman GL, Bhatag G, Green PH. Hemolysis interferes with the detection of anti-tissue transglutaminase antibodies in celiac disease. Clin Chem 2012; 56: 1034-1036 [PMID: 22244046 DOI: 10.1373/clinchem.2009.141242]
9. Villalta D, Tonutti E, Prave A, Koletzko S, Uhlig HH, Vermeersch P, Bossuyt X, Stern M, Laass MW, Ellis JH, Ciclitira PJ, Richter T, Daehrich C, Schumlierger W, Mothes T. IgG antibodies against deamidated gliadin peptides for diagnosis of celiac disease in patients with IgA deficiency. Clin Chem 2010; 56: 464-468 [PMID: 20022984 DOI: 10.1373/clinchem.2009.128132]
10. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Sharma R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgemann M, Miki M, Ribes-Koninckx C, Ventura A, Zimmer KP. Multi-antibody immunoassays in the diagnosis of celiac disease. Eur J Gastroenterol Hepatol 2014; 11: 655-663 [PMID: 25266110 DOI: 10.1038/euroastro.2014.162]
11. Collin P, Kaukinen K, Vogelsang H, Korponay-Szabó I, Sommer R, Schreier E, Volta U, Granito A, Veronesi L, Mascart F, Ocmand A, Ivarsson A, Lagenqvist C, Bürgin-Wolff A, Haddiselimovic F, Furlano R, Siddler MA, Mulder CJ, Goeres MS, Mearin ML, Kaukinen K, Vázquez H, Niveloni S, Argonz J, Mazure R, La Motta G, Furlano R, Siddler MA, Mulder CJ, Goeres MS, Mearin ML, Bieber T, Giersiepen K, Branski D, Catassi C, Treiber A, Zimmer KP. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease: a biopsy-proven European multicentre study. Eur J Gastroenterol Hepatol 2005; 17: 85-91 [PMID: 15647647 DOI: 10.1097/00042737-200501000-00017]
12. Husby S, Murray JA. Diagnosing celiac disease and the potential for serological markers. Nat Rev Gastroenterol Hepatol 2014; 11: 655-663 [PMID: 25266110 DOI: 10.1038/euroastro.2014.162]
13. Collin P, Kaukinen K, Vogelsang H, Korponay-Szabó I, Sommer R, Schreier E, Volta U, Granito A, Veronesi L, Mascart F, Ocmand A, Ivarsson A, Lagenqvist C, Bürgin-Wolff A, Haddiselimovic F, Furlano R, Siddler MA, Mulder CJ, Goeres MS, Mearin ML, Kaukinen K, Vázquez H, Niveloni S, Argonz J, Mazure R, La Motta G, Furlano R, Siddler MA, Mulder CJ, Goeres MS, Mearin ML, Bieber T, Giersiepen K, Branski D, Catassi C, Treiber A, Zimmer KP. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease: a biopsy-proven European multicentre study. Eur J Gastroenterol Hepatol 2005; 17: 85-91 [PMID: 15647647 DOI: 10.1097/00042737-200501000-00017]
14. Mubarak A, Nikkel P, Houwen R, Ten Kate F. Reproducibility of the histological diagnosis of celiac disease. Scand J Gastroenterol 2011; 46: 1065-1073 [PMID: 21668407 DOI: 10.3109/00365521.2011.589471]
15. Bürglin-Wolff A, Mauro B, Faruk H. Intestinal biopsy is not always required to diagnose celiac disease: a retrospective analysis of combined antibody tests. BMC Gastroenterol 2013; 13: 19 [PMID: 23343249 DOI: 10.1186/1471-230X-13-19]
16. Sugai E, Moreno ML, Hwang HJ, Cabanne A, Crivelli A, Nachman F, Vázquez H, Niveloni S, Argonz J, Mazure R, La Motta G, Canigia ME, Smecuol E, Chopita N, Gómez JC, Mauriño E, Bái JC. Celiac disease serology in patients with different pretest probabilities: is biopsy avoidable? World J Gastroenterol 2010; 16: 3144-3152 [PMID: 20593499 DOI: 10.3748/wjg.v16.i25.3144]
17. Vermeersch P, Ghebros K, Marien G, Hoffman I, Hiele M, Bossuyt X. Serological diagnosis of celiac disease: comparative analysis of different strategies. Clin Chem Acta 2012; 413: 1761-1767 [PMID: 22771970 DOI: 10.1016/j.cca.2012.06.024]

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