Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

Reliability of Autoantibodies Against a Tissue Transglutaminase Neo-Epitope for the Diagnosis of Pediatric Celiac Disease

Jiri Nevoral [1], Ondrej Hradsky [1], Jan Lastovicka [2], Eva Neubertova [3], Jindra Reissigova [4], Jiri Bronsky [1]

[1] *Department of Pediatrics, University Hospital Motol, 2nd Medical School, Charles University, Prague, Czech Republic*
[2] *Department of Immunology, 2nd Medical School and University Hospital Motol, Charles University, Prague, Czech Republic*
[3] *BIOLAB Praha, S.R.O, Prague, Czech Republic*
[4] *Institute of Computer Science of The Czech Academy of Sciences, Czech Republic*

SUMMARY

Background: **This study aimed to assess the declared benefits of the new test using antibodies against tissue transglutaminase in complex with gliadin representing a neo-epitope in the IgA and IgG class of immunoglobulins compared with currently used tissue transglutaminase antibodies in the IgA class of immunoglobulins among children.**
Methods: **In the cross-sectional study (P1 study, n = 406) and two small-size prospective observational studies (P2 study, n = 59 and P3 study, n = 12), serum samples from all children were simultaneously tested for endomysial antibodies, IgA tissue transglutaminase antibodies, and antibodies against tissue transglutaminase in complex with gliadin in the IgA and IgG class of immunoglobulins. The exact McNemar test, Wilcoxon test, and Spearman's correlation coefficient were used to analyze the data.**
Results: **We found a significant asymmetry of the tissue transglutaminase antibodies test compared with the antibodies against tissue transglutaminase neo-epitope test (P1). More patients (1.5%) had tissue transglutaminase antibodies positive and antibodies against tissue transglutaminase neo-epitope negative results, whereas no patients had tissue transglutaminase antibodies negative and antibodies against tissue transglutaminase neo-epitope positive results. Of 59 children with tissue transglutaminase antibodies and/or endomysial antibodies positive results (P2), one (1.7%) did not have celiac disease. In agreement with the P1 study, four patients (6.8%) with confirmed celiac disease were tissue transglutaminase antibodies positive and antibodies against tissue transglutaminase neo-epitope negative. In this group, the sensitivity of the antibodies against tissue transglutaminase neo-epitope test for diagnosis of celiac disease was 91.4% (95% confidence interval, 81.0 - 97.1%). Among children diagnosed with functional gastrointestinal disorder (P3), all had negative serological test results, and none was diagnosed with celiac disease.**
Conclusions: **The results do not indicate that antibodies against tissue transglutaminase neo-epitope test would be an unambiguously better test than the currently used tissue transglutaminase antibodies.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.190530)

Correspondence:
Ondrej Hradsky
University Hospital Motol and
2nd Medical School
Department of Pediatrics
V Uvalu 84
150 06 Prague 5
Czech Republic
Phone: +420 224432298
Fax: +420 224432020
Email: ondrej.hradsky@lfmotol.cuni.cz

KEY WORDS

antibody, celiac disease, diagnostics, children

Manuscript accepted June 27, 2019

INTRODUCTION

Celiac disease (CD) is currently defined as a systemic autoimmune disease caused by prolamins and occurring in genetically susceptible individuals who are carriers of HLA-DQ2 or HLA-DQ8. The disease is characterized by various combinations of clinical manifestations that are dependent on gluten prolamins, including gastrointestinal and extraintestinal symptoms, elevated levels of CD-specific autoantibodies, and enteropathy in the small intestine [1]. Currently, antibodies against recombinant human tissue transglutaminase type 2 in IgA class (anti-TG-IgA) are considered more versatile and specific for CD diagnosis compared with histological changes of the mucosa of the small intestine [2]. High levels of antibodies specific for CD in children have been shown to have excellent predictive value for the presence of small intestine villi atrophy, which make the diagnosis of CD possible in a clearly defined group of pediatric patients without biopsy. Evidence suggests that this is realistic, and approximately one third of patients do not need a biopsy [3,4]. Anti-TG-IgA has a very high sensitivity and specificity for CD [5] and has therefore become a fundamental serological screening test for the condition. Recently, reports have been published regarding a new serological test, wherein tissue transglutaminase in complex with gliadin represents a neo-epitope resulting from the enzyme-substrate interaction. Antibodies against this neo-epitope, called anti-TG-neo, were studied in adults and children separately in IgA and IgG classes and simultaneously in both immunoglobulin classes [6-11]. In 95 children with CD, the new antibodies against tissue transglutaminase neo-epitope in the IgA and IgG class of immunoglobulins (anti-TG-neo-IgA + IgG) serological test was declared to have greater sensitivity (97%) and similar specificity (99%) for CD compared with those of previously used anti-TG-IgA antibodies [12]. This study aimed to compare both tests and assess the declared benefits of anti-TG-neo-IgA + IgG antibodies for CD diagnosis in children compared with the currently used anti-TG-IgA test.

MATERIALS AND METHODS

Study design

The first study was designed as a cross-sectional study (P1). The study involved all children (age, 1 - 18 years) who were screened with anti-TG and EMA as part of a wider differential diagnosis in 2017 and 2018 (n = 406; Table 1). At the same time, all children were simultaneously tested with anti-TG-neo-IgA + IgG. Consecutive blood samples were sent to the central immunology laboratory (in University Hospital Motol) by the physicians of various pediatric disciplines and evaluated anonymously. Subjects with IgA deficiency were excluded from the analysis (IgA values < 0.2 g/L). The second study was a prospective observational study (P2). The study consisted of children (age, 2 - 17 years; n = 59) indicated for endoscopic entero-biopsy based on positivity of anti-TG-IgA and/or EMA. These patients were referred by pediatricians for examination on various symptoms or screening in allergy clinics and in families with CD in 2015 and 2016 (Table 2). None of the children adhered to a gluten-free diet before the examination. These children were tested for anti-TG-neo-IgA + IgG at the time of biopsy. Subjects with IgA deficiency were excluded from the study.

The third study was also designed as a prospective observational pilot study (P3) that involved children (age, 7 - 17 years; n = 12) who underwent gastroscopy in 2015 and 2016 at the Department of Pediatrics due to symptoms suggestive of functional gastrointestinal disorders without suspicion of CD. Gastroscopy and serological testing (EMA, anti-TG-IgA and anti-TG-neo-IgA + IgG) were performed in all patients.

Laboratory methods

Antibodies

The serum samples were tested for anti-TG-IgA using an IgA-tTG ELISA Kit (Biosystems S.A., Barcelona, Spain) and for anti-TG-neo by AESKULISA CeliCheck New Generation IgA + IgG (AESKU Diagnostics, Wendelsheim, Germany). The cutoff value was set at 12 arbitrary units/mL by IgA-tTG ELISA Kit and 24 U/mL by CeliCheck, in accordance with the manufacturer's instructions. The samples were further tested for the presence of IgA-endomysial antibodies (EMA) by using indirect immunofluorescence on monkey distal esophagus cryostat sections. The serum was diluted to 1:20 (Biosystems S.A., Barcelona, Spain).

Evaluation of "boundary finding" indicates faint but noticeable fluorescence, and "weakly positive" assessment indicates distinct but weak fluorescence.

Biopsy

The samples of the mucosa of the small intestine were obtained by using an endoscopic procedure. Based on the ESPGHAN recommendation, biopsy specimens should be obtained from the second/third portion of the duodenum (four samples), and at least one biopsy specimen should be obtained from the duodenal bulb [1]. Histological findings were evaluated based on the Marsh classification [13].

Statistical analysis

The asymmetry of the screening tests for CD was analyzed using the exact McNemar test. The difference and correlation between continuous variables were evaluated using the Wilcoxon rank-sum test and Spearman's correlation coefficient, respectively. We considered $p < 0.05$ as statistically significant. All data were analyzed using R statistical software version 3.5.1 [14].

Ethical consideration

The ethics committee of University Hospital in Prague Motol approved the study, with reference number EK-272/16. Informed consent was obtained from a parent or

legal guardian for all study participants before endoscopic procedures and laboratory analysis.

RESULTS

P1 study

Out of 406 children examined, 206 (50.7%) were boys. There were no significant differences in age ($p = 0.989$), serum levels of anti-TG-IgA ($p = 0.765$), and anti-TG-neo-IgA + IgG ($p = 0.548$) between boys and girls (Table 1). The correlations between age and anti-TG-IgA ($r = 0.015$, $p = 0.761$) and age and anti-TG-neo-IgA + IgG ($r = 0.009$, $p = 0.852$) were insignificant.

Figure 1 shows the association between the tests for CD. Although the results of the anti-TG- IgA and anti-TG-neo-IgA + IgG tests were the same at 98.5%, the results were significantly asymmetric in the remaining 1.5% ($p = 0.031$). No patient was anti-TG-IgA negative and anti-TG-neo positive. Six anti-TG-IgA positive patients were anti-TG-neo-IgA + IgG negative.

The asymmetry of the assay was even greater when the EMA results were compared with the results of anti-TG-neo-IgA + IgG ($p < 0.001$). Some children (5.4%) had anti-TG-neo-IgA + IgG negative and EMA positive results; however, none had anti-TG-neo-IgA + IgG positive and EMA negative results.

P2 study

Table 2 shows the characteristics of the children suspected with CD and with positive anti-TG-IgA and/or EMA test results. Similar to the P1 study, no significant differences were found in age ($p = 0.271$) and serum levels of anti-TG-IgA ($p = 0.263$) and anti-TG-neo-IgA + IgG ($p = 0.601$) between boys and girls. The correlation between age and anti-TG-neo-IgA + IgG was insignificant ($r = -0.168$, $p = 0.203$). However, a significant negative correlation was found between age and anti-TG-IgA ($r = -0.355$, $p = 0.008$).

Figure 2 shows the results of tests for CD in children who underwent entero-biopsy. Of 59 children suspected with CD selected based on the anti-TG-IgA and/or EMA positivity, one (1.7%) did not have CD (Marsh 0). This patient was EMA negative, anti-TG-IgA positive, and anti-TG-neo-IgA + IgG negative. One patient (1.7%) was anti-TG-IgA negative, anti-TG-neo-IgA + IgG negative, and EMA positive, and histological finding corresponded to the Marsh 3a classification. Four (6.8%) patients with confirmed CD were anti-TG-IgA positive and anti-TG-neo-IgA + IgG negative.

The results of the anti-TG-neo-IgA + IgG and anti-TG-IgA tests were the same at 91.5%. The sensitivity of anti-TG-neo-IgA + IgG for the diagnosis of CD in this selected group of patients was 91.4% (95% confidence interval [CI], 81.0 - 97.1).

P3 study

The characteristics of 12 children without suspicion of CD are presented in Table 3. Among these patients, all had negative serological test results (anti-TG-neo-IgA + IgG and anti-TG-IgA), and none was diagnosed with CD (all had Marsh 0, except for one that had Marsh 1). The final diagnosis in all included children was functional gastrointestinal disorder.

DISCUSSION

Antibodies specific for CD, i.e., EMA and anti-TG-IgA, are used for screening and diagnosis. Based on previously published data EMA with a sensitivity of 93.0% (95% CI, 92.1 - 93.8) and specificity of 99.7% (95% CI, 99.5 - 99.8) has a very high predictive value for CD [16, 17], and its measurement is therefore used as the reference standard for the detection of antibodies specific for CD. EMA also has a very low rate of false positivity. Anti-TG-IgA specific antibodies can be measured only in relative units, so numerical values are kit specific and show considerable variations. However, most tests can distinguish slightly (4 times ULN), moderately (6 times ULN), and highly (10 times ULN) positive anti-TG levels (1).

Measurement of anti-TG-IgA is nevertheless currently considered the best CD screening test for widespread use, and its positive predictive value can be increased by simultaneous EMA positivity [21]. To apply the new ESPGHAN guidelines [1], clinicians must understand the performance of their celiac serology tests [22].

We considered that a neo-epitope resulting from TG in complex with gliadin could lead to improvements in the specificity and sensitivity of the test and reduce false positive results. This led us to assess the detection of antibodies against the TG-neo-epitope simultaneously in both IgA and IgG immunoglobulin classes. Experience with the evaluation of anti-TG-neo antibodies has been published in several studies. For example, in the study by Rozenberg [8] in a cohort of 112 children, anti-TG-neo-IgA + IgG had 98% sensitivity and 80% specificity for CD testing at the cutoff level recommended by the manufacturer.

In another study, anti-TG-neo-IgA and anti-TG-neo-IgG were evaluated separately in a group of 10 children and 31 adult patients with CD [11]. In the still commonly used anti-TG-IgA assay, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for CD were 100%, 98.82%, 95.35%, and 100%, respectively. When anti-neo-TG-IgA was tested with a cutoff level of > 20 U/mL, the sensitivity, specificity, PPV, and NPV were 100%, 97.04%, 89.13%, and 100%, respectively. These values were the same or lower than those of the original anti-TG-IgA assay.

The sensitivity and specificity of anti-TG-IgA, anti-TG-neo-IgA, anti-TG-neo-IgG, and anti-TG-neo-IgA + IgG were compared in 95 children with CD and in the control groups of children with abdominal pain and healthy children [12]. The results are surprising for very low anti-TG-IgA sensitivity of 55.6%, at a specificity of 100%. The reason for such low sensitivity of the test

Table 1. Characteristics of patients in the cross-sectional study (P1 study) [1].

Variable	n	Mean	SD	Median	Minimum	Maximum
Age (years)	406	10.7	4.5	11.0	1.0	18.0
Anti-TG-IgA (units/mL) [2]	397	5.4	11.9	2.4	1.3	90.6
Anti-TG-neo IgA + IgG (U/mL)	406	29.3	154.6	5.28	3.6	1,685.0

[1] Children screened for CD in the departments of endocrinology, pneumology, gastroenterology, immunology, hematology, neurology, rheumatology and general pediatrics.
[2] In 9 of 406 children, the anti-TG-IgA values were unknown but were > 100.

Table 2. Characteristics of patients in the prospective observation study (P2 study) [1].

Variable	n	Mean	SD	Median	Minimum	Maximum
Age (years)	59	8.7	3.7	8.0	2.0	17.0
Anti-TG-IgA (units/mL) [2]	55	351.4	434.4	208.0	11.5	2,487.0
Anti-TG-neo IgA + IgG (U/mL)	59	1,320.6	1,280.0	884.0	4.0	3,412.0

[1] Exhibiting symptoms: allergic diseases (39.0%), CD family screening (13.6%), abdominal pain (10.2%), growth failure (11.9%), anemia (5.1%), weight loss (3.4%), anorexia (3.4%), type 1 DM (3.4%), diarrhea/bloating (3.4%), fatigue (1.7%), other: delayed puberty, cystic fibrosis, elevated liver function tests (5.1%).
[2] In 4 of 59 children, the anti-TG-IgA values were unknown but were >100.

Table 3. Characteristics of patients in the prospective observation study (P3 study) [1].

Variable	n	Mean	SD	Median	Minimum	Maximum
Age (years)	12	13.4	3.2	14.5	7.0	17.0
Anti-TG-IgA (units/mL)	12	3.4	2.2	2.7	2.2	10.3
Anti-TG-neo IgA + IgG (U/mL)	12	4.1	1.9	3.5	3.1	10.0

[1] Children exhibiting symptoms of functional gastrointestinal disorders.

used to measure anti-TG-IgA is unclear, and data in the literature regarding its explanation are inadequate [5]. For the anti-TG neo-IgA + IgG test, the sensitivity and specificity were 97% and 99%, respectively.

To appropriately compare both tests (anti-TG neo-IgA + IgG vs. anti-TG-IgA), the optimal design of the study would be to prospectively evaluate both tests on a large-population based cohort. However, this is extremely difficult to perform in clinical practice. Thus, we focused on evaluation of anti-TG neo-IgA + IgG advantages declared previously in scientific literature in the setting of three studies to test asymmetry of both tests and to evaluate their reliability for the diagnosis of CD in patients with and without suspicion of CD.

We found a statistically significant asymmetry of the anti-TG-IgA test currently used compared with the anti-TG-neo-IgA + IgG (P1). In all cases when anti-TG-neo-IgA + IgG was positive, we also found anti-TG-IgA positivity. On the contrary, there were situations when only anti-TG-IgA was positive. Anti-TG-neo-IgA + IgG positivity was less common: anti-TG-IgA was positive overall at 8.4% and anti-TG-neo-IgA + IgG at 6.9%. The asymmetry of the assay was even greater when the EMA results were compared with the results of anti-TG-neo-IgA + IgG. EMA was positive overall at 12.3%.

We also compared the performance of both tests when the information on the gold reference standard (CD diagnosis confirmed by biopsy of the intestinal mucosa) was available. We found false positivity of anti-TG-IgA (slightly increased titers) in one patient (n = 59) without CD (Marsh 0). This patient was both EMA and anti-TG-neo-IgA + IgG negative (P2).

Anti-TG-IgA evaluated in a meta-analysis as a screen-

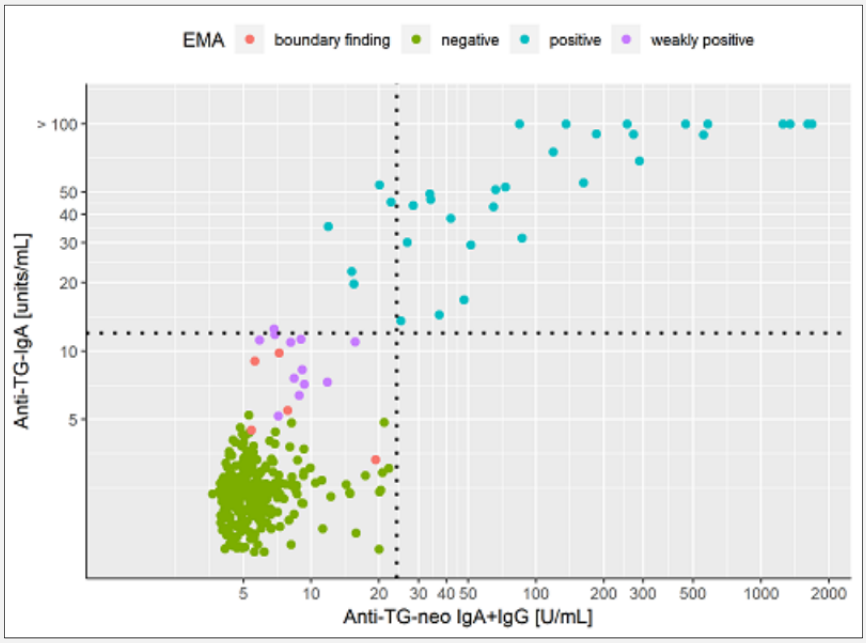

Figure 1. Results of the cross-sectional study comparing results of anti-TG-IgA, anti-TG-neo-IgA + IgG, and EMA (n = 406, P1 study).

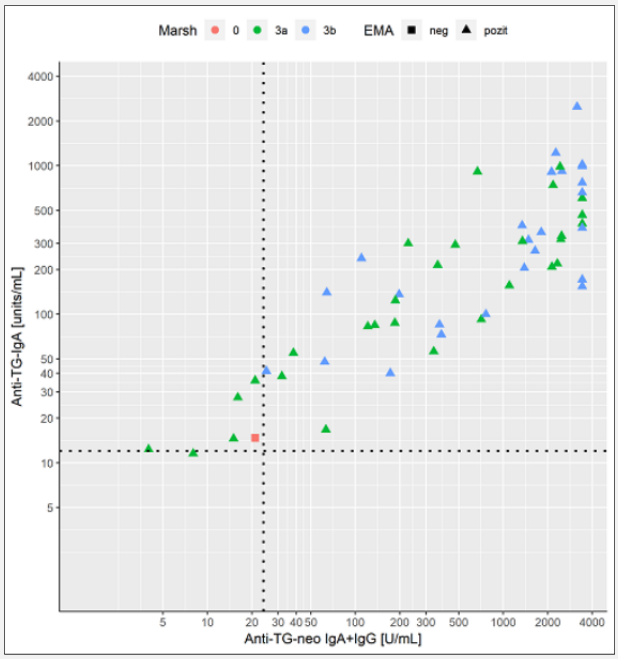

Figure 2. Biopsy findings and anti-TG-neo-IgA + IgG results in anti-TG-IgA- and/or EMA-positive patients (n = 55, P2 study).

ing test in 3,110 pediatric patients (1,876 with CD, 1,234 without CD) yielded sensitivities and specificities of ≥ 90% and ≥ 90%, respectively [5]. However, slightly increased titers of anti-TG-IgA are also found in a number of diseases unrelated to CD, such as autoimmune diseases, tumors, myocardial injury, liver disease, psoriasis, and EBV infection [19]. Transient increases in non-specific anti-TG-IgA have also been observed in acute febrile infectious diseases [20]. The EMA in such situations is often negative, which was also the case of our patient. This is explained by the fact that EMA is a special subset of CD-specific antibodies against tissue transglutaminase, where the TG protein is offered as the antigen bound to fibronectin. Antibodies against TG unrelated to CD, arising from tissue damage or inflammation for example, are directed against intracellular TG or hidden epitopes negative in EMA tests [2]. HLA-DQ typing is helpful in identifying false-positive anti-TG-IgA patients negative for EMA under screening conditions and unmasks possible misdiagnoses of CD [21]. Here, 58 patients had anti-TG-IgA and/or EMA positivity and CD confirmed by biopsy (P2), and four (6.9%) patients were anti-TG-IgA positive and anti-TG-neo negative. This result, in fact, is in accordance with the P1 study. Hence, the sensitivity of anti-TG-neo-IgA + IgG was lower (91.4%) in this group (95% CI, 81.0% -97.1%). Despite the wide confidence interval (a larger sample-size study is needed) and the estimated sensitivity for the selected population (not general), it prevents us from confirming the previously published superiority of anti-TG neo test in the diagnosis of CD. It should be noted that the sensitivity of anti-TG-neo-IgA + IgG was estimated in children with CD with anti-TG-IgA and/or EMA positivity, and therefore, the study design did not allow us to estimate the sensitivity of anti-TG-IgA. The specificity of tests was explored in pediatric patients with functional abdominal pain (P3). It is just a model example wherein duodenal biopsy was performed, followed by anti-TG-IgA and anti-TG-neo-IgA + IgG antibody testing. In all of these children, the mucosa was normal, and both tests were negative without false positivity. To further investigate specificity, a study with greater power is needed. For these reasons, conducting an extensive population study showing histological findings along with values of two different tests in the general population is warranted to assess the diagnostic value of both tests correctly in relation to the degree of intestinal mucosa atrophy. Nevertheless, owing to ethical problems with endoscopies in asymptomatic subjects, conducting such a study is practically difficult.

CONCLUSION

Anti-TG-IgA and anti-TG-neo-IgA + IgG tests are significant asymmetrical tests, i.e., they did not provide the same results in children without IgA deficiency. The results do not indicate that the anti-TG-neo-IgA + IgG test would be an unambiguously better test than the currently used anti-TG-IgA test. However, a larger sample size is required to support this finding.

Acknowledgment:
Supported by Ministry of Health, Czech Republic-conceptual development of research organization, Motol University Hospital, Prague, Czech Republic 00064203, project GA UK No. 136215, No. 246216 and No. 3646 17 by the Charles University in Prague.

Declaration of Interest:
J. Nevoral reports personal fees and non-financial support from AbbVie, Nestlé, Nutricia, outside the submitted work; O. Hradsky reports personal fees and non-financial support from AbbVie, MSD, Nestlé, Nutricia and Ferring outside the submitted work; J. Lastovicka has nothing to disclose, E Neubertova has nothing to disclose. J. Reissigova has nothing to disclose, J. Bronsky reports personal fees and non-financial support from AbbVie, MSD, Nestlé, Nutricia, Ferring, Walmark, Biocodex outside the submitted work.

References:

1. Husby S, Koletzko S, Korponay-Szabó IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease. J Pediatr Gastroenterol Nutr 2012;54:136-60 (PMID: 22197856).

2. Korponay-Szabó IR, Troncone R, Discepolo V. Adaptive diagnosis of coeliac disease. Best Pract Res Clin Gastroenterol 2015;29: 381-98 (PMID: 26060104).

3. Klapp G, Masip E, Bolonio M, et al. Celiac disease: the new proposed ESPGHAN diagnostic criteria do work well in a selected population. J Pediatr Gastroenterol Nutr 2013;56:251-6 (PMID: 23111763).

4. Nevoral J, Kotalova R, Hradsky O, et al. Symptom positivity is essential for omitting biopsy in children with suspected celiac disease according to the new ESPGHAN guidelines. Eur J Pediatr 2014;173:497-502 (PMID: 24233405).

5. Giersiepen K, Lelgemann M, Stuhldreher N, et al. Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. J Pediatr Gastroenterol Nutr 2012;54:229-41 (PMID: 22266486).

6. Matthias T, Neidhöfer S, Pfeiffer S, Prager K, Reuter S, Gershwin ME. Novel trends in celiac disease. Cell Mol Immunol 2011; 8:121-5 (PMID: 21278765).

7. Matthias T, Pfeiffer S, Selmi C, Eric Gershwin M. Diagnostic challenges in celiac disease and the role of the tissue transglutaminase-neo-epitope. Clin Rev Allergy Immunol 2010;38:298-301 (PMID: 19629760).

8. Rozenberg O, Lerner A, Pacht A, et al. A novel algorithm for the diagnosis of celiac disease and a comprehensive review of celiac disease diagnostics. Clin Rev Allergy Immunol 2012;42:331-41 (PMID: 21279475).

9. Lytton SD, Antiga E, Pfeiffer S, et al. Neo-epitope tissue transglutaminase autoantibodies as a biomarker of the gluten sensitive skin disease-dermatitis herpetiformis. Clin Chim Acta 2013;415: 346-9 (PMID: 23142793).

10. Barak M, Rozenberg O, Froom P, et al. Challenging our serological algorithm for celiac disease (CD) diagnosis by the ESPGHAN guidelines. Clin Chem Lab Med 2013;51:e257-9 (PMID: 238398 14).

11. Porcelli B, Ferretti F, Vindigni C, Terzuoli L Assessment of a Test for the Screening and Diagnosis of Celiac Disease. J Clin Lab Anal 2016;30:65-70 (PMID: 25385391).

12. Lerner A, Jeremias P, Neidhöfer S, Matthias T. Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable then anti-tTg for the diagnosis of pediatric celiac disease. J Immunol Methods 2016;429:15-20 (PMID: 26684936).

13. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol 1999;11:1185-94 (PMID: 105 24652).

14. R Core Team. R: A Language and Environment for Statistical Computing. 2016. www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing

15. Khatib M, Baker RD, Ly EK, Kozielski R, Baker SS. Presenting Pattern of Pediatric Celiac Disease. J Pediatr Gastroenterol Nutr 2016;62:60-3 (PMID: 26111294).

16. Korponay-Szabó IR, Kovács JB, Lörincz M, Gorácz G, Szabados K, Balogh M. Prospective significance of antiendomysium antibody positivity in subsequently verified celiac disease. J Pediatr Gastroenterol Nutr 1997;25:56-63 (PMID: 9226528).

17. Lewis NR, Scott BB. Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibody tests). Aliment Pharmacol Ther 2006;24:47-54 (PMID: 16803602).

18. Korponay-Szabó IR, Laurila K, Szondy Z, et al. Missing endomysial and reticulin binding of coeliac antibodies in transglutaminase 2 knockout tissues. Gut 2003;52:199-204 (PMID: 125244 00).

19. Szondy Z, Korponay-Szabó I, Király R, Fésüs L Transglutaminase 2 dysfunctions in the development of autoimmune disorders: celiac disease and TG2-/- mouse. Adv Enzymol Relat Areas Mol Biol 2011;78:295-345 (PMID: 22220477).

20. De Leo L, Quaglia S, Ziberna F, et al. Serum anti-tissue transglutaminase antibodies detected during febrile illness may not be produced by the intestinal mucosa. J Pediatr 2015;166:761-3 (PMID: 25722272).

21. Händel N, Mothes T, Petroff D, et al. Will the Real Coeliac Disease Please Stand Up? Coeliac Disease Prevalence in the German LIFE Child Study. J Pediatr Gastroenterol Nutr 2018 Oct;67(4): 494-500 (PMID: 29901554).

22. Gidrewicz D, Potter K, Trevenen CL, Lyon M, Butzner JD. Evaluation of the ESPGHAN Celiac Guidelines in a North American Pediatric Population. Am J Gastroenterol 2015;110:760-7 (PMID: 25823767).