

Serodiagnostic Assays for Celiac Disease Based on the Open or Closed Conformation of the Autoantigen, Transglutaminase 2

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Abstract The autoantigen of celiac disease, transglutaminase 2 (TG2), adopts an open conformation during enzymatic activation. We studied diagnostic accuracy of serodiagnostic assays using TG2 in its open and closed conformation as antigens in patients with diagnostic difficulties. The open TG2 antibody (TG2ab) test identified 93% of untreated celiac patients in contrast to 44%, 27%, and 68% detected by closed and conventional TG2ab and endomysial antibody (EmA) tests, respectively. The assay was able to detect 60% of non-responding celiac patients seronegative for conventional TG2ab and EmA. The titers of the openTG2abs were higher than those of the closed TG2abs. The serological test utilizing TG2 in an open conformation was more accurate than the

other assays in finding active celiac disease even in patients having negative or borderline conventional celiac autoantibodies and in revealing poor dietary response non-invasively. It thus offers a promising tool in the diagnostics and follow-up of celiac disease.

Keywords Celiac disease · gluten · transglutaminase 2 · open conformation · diagnostic accuracy

Introduction

In a subgroup of genetically predisposed individuals carrying the human leukocyte antigen (HLA) DQ2 and DQ8 molecules, the ingestion of wheat-, rye-, and barley-derived gluten prolamins causes an abnormal immune reaction called celiac disease. This disorder is characterized by small-bowel mucosal villous atrophy, crypt hyperplasia, and the presence of serum immunoglobulin class A endomysial autoantibodies targeted against a multifunctional protein, transglutaminase 2 (TG2) [1]. It is widely accepted that TG2 is the major, if not sole, autoantigen in celiac disease and plays a central role in the disease pathogenesis [2].

The celiac disease autoantigen TG2 is a ubiquitously expressed protein found in different cellular compartments and with a multitude of functions. In the cytosol, TG2 functions mainly as a G-protein and in the cell surface as a co-receptor involved in adhesion [3]. In the extracellular environment, it acts as an enzyme promoting the cross-linking of extracellular matrix proteins by catalyzing the transamidation of glutamine residues to lysine, resulting in proteolytically resistant ϵ -(γ -glutamyl)lysine isopeptide bonds [3]. Extracellular TG2 is catalytically inactive under normal physiological conditions [4] but upon enzymatic activation, for instance in response to tissue injury, TG2 is

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known to adopt an extended open conformation where the active site of the enzyme is exposed [5]. Interestingly, previous results have suggested that the enzymatic activity of TG2, and thereby the conformation, might affect the binding of celiac autoantibodies [6, 7].

Enzyme-linked immunosorbent assay (ELISA) tests with TG2 as the antigen are widely employed in the diagnostic work-up of celiac disease, although conspicuous problems exist [8–10]. Approximately 10–20% of patients with untreated celiac disease remain negative for serum celiac autoantibodies [11]. Evidence suggests that seronegativity is common particularly in subjects having mild small-bowel histological lesions [12] although such cases may already evince symptoms and signs typical for celiac disease [13]. On the other hand, increased serum autoantibody levels normalize fairly rapidly after commencement of a gluten-free diet and disappear completely after strict long-term treatment despite persistent small-bowel mucosal damage [14–16]. There is thus clearly a need for a reliable non-invasive test which would firstly identify seronegative celiac disease patients and secondly provide a suitable means to monitor the activity of the disease during a gluten-free diet.

Since enzymatically active TG2 has an extended open conformation, it has been hypothesized that an ELISA test using this open conformation as antigen would be superior to closed TG2-based assay in detecting serum celiac autoantibodies. We tested this hypothesis in a selected population of consecutive untreated and treated celiac disease patients known to have borderline or low autoantibody levels despite active condition and in non-celiac controls suspected but excluded for celiac disease.

Materials and Methods

Patients

The first study group comprised 44 adults with untreated celiac disease fulfilling the current diagnostic criteria [17] (Table 1). Of these, 30 had Marsh III and 14 had Marsh I–II lesion at diagnosis. They all had either HLA DQ2 or DQ8 genotype and increased density of $\gamma\delta$ +intraepithelial lymphocytes characteristic for celiac disease [11, 13]. Out of the 44 untreated celiac patients, serum samples and follow-up histology were available for altogether 32. All of these individuals evincing good response to a gluten-free diet constitute the second study group. The third group comprised 15 non-responsive celiac disease patients who had persistent villous atrophy despite a strict gluten-free diet. Regardless of ongoing small-bowel mucosal damage, 13 out of this 15 were asymptomatic. Two had refractory celiac disease (one type I and the other type II with aberrant T-cell clonality) [18]. Finally, 25 subjects with normal villous morphology, earlier

suspected but excluded for celiac disease, served as controls. None of the participants had selective IgA deficiency.

The participants underwent upper gastrointestinal endoscopy with small-intestinal mucosal biopsies at the Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital between 2005 and 2010. Blood samples were drawn at the time of endoscopy, frozen, and stored in -20°C until studied in 2010. No adverse events occurred in performing the tests. The study protocol was approved by the Ethical Committee of Tampere University Hospital. All patients gave their written informed consent.

Serological Testing

Serum IgA-class autoantibodies against the open (open TG2ab) and closed (closed TG2ab) conformation of TG2 were measured by ELISA using kits provided by the manufacturer according to instructions (Open tTG and closed, standard tTG, Zedira, Darmstadt, Germany). For both tests, the cut-off level was set at 3.0 U/ml as instructed by the manufacturer. For comparison, conventional serum IgA-class antibodies against TG2 were detected by ELISA using human recombinant TG2 as antigen (Celikey, Phadia, GmbH, Freiburg, Germany). Concentrations over 5 U/ml were considered positive in accordance with previous studies [11, 13, 19, 20]. Serum endomysial antibodies (EmA) were determined by an indirect immunofluorescence method, a serum dilution of $1:\geq 5$ being considered positive [6]. In these conventional TG2 assays, the conformation of the antigen has not been defined. In addition to manufacturers' recommended reference values also cut-off values determined by receiver operating characteristic (ROC) plots, maximizing the sum of specificity and sensitivity, were used to calculate the diagnostic performance of each test. All samples were analyzed blindly by an experienced technician. Analysis of the diagnostic accuracy were carried out according to the STARD guidelines [21].

HLA Typing

Patients were genotyped based on polymerase chain reaction with allele-specific primers identifying celiac-type HLA DQ2 and DQ8 and carried out on a Dynal DQ low-resolution SSP kit (Dynal AS, Oslo, Norway).

Small-Bowel Mucosal Morphology

Small-bowel mucosal biopsy specimens were obtained during upper gastrointestinal endoscopy. For morphometrical analysis, paraffin-embedded specimens were processed and stained with hematoxylin–eosin. Villous height crypt depth ratio was counted in well-oriented biopsy samples as previously described and a ratio <2.0 was

Table I Demographic and background data on study groups

	Celiac disease patients			Controls <i>n</i> =25
	Untreated <i>n</i> =44	Treated responsive <i>n</i> =32	Treated non-responsive <i>n</i> =15	
Female, <i>n</i>	32 (73%)	22 (69%)	13 (87%)	19 (76%)
Age; median (range), years	45 (12–70)	46 (17–69)	48 (39–76)	47 (24–81)
Indication for endoscopy, <i>n</i>				
Abdominal symptoms	28 (63%)	–	–	17 (68%)
Anemia or malabsorption	3 (7%)	–	–	4 (16%)
Extraintestinal manifestation ^a	3 (7%)	–	–	3 (12%)
Screening of celiac disease	10 (23%)	–	–	1 (4%)
Monitoring gluten-free diet	0	32	15	0
Duration of gluten-free diet; median (range), years	0	1 (1–1)	1 (1–24)	0
Family history of celiac disease	27 (61%)	17 (53%)	5 (33%)	1 (4%)
Celiac-type HLA DQ2 or DQ8+, <i>n</i>	44 (100%)	32 (100%)	14 ^b (100%)	6 (24%)
Small-bowel histology, <i>n</i>				
Marsh 0	0	20 (63%)	0	20 (80%)
Marsh I–II	14 (32%)	12 (37%)	0	5 (20%)
Marsh III	30 (68%)	0	15 (100%)	0

^a Infertility, dermatitis herpetiformis, aphthous stomatitis^b DNA samples missing for one individual

considered compatible with Marsh III [11]. This ratio was ≥ 2.0 in patients with Marsh I and II.

Statistical Analysis

Quantitative data were expressed as medians and ranges or means and 95% confidence intervals (CI). The efficiency of a test was determined as the ratio of the sum of true-negative and true-positive test results and the sum of all untreated celiac disease patients and non-celiac controls. Likelihood ratio of a positive test result was calculated by sensitivity divided by 1-specificity. ROC plot analysis was performed on each ELISA-based method to determine the

area under curve (AUC). Statistical differences were evaluated using two-sided Mann–Whitney *U* test and Wilcoxon's signed-rank test as appropriate. *P* values < 0.05 were considered statistically significant. All calculations were made using Statistical Package for the Social Sciences version 17 (SPSS Inc. Chicago, IL, USA).

Results

When manufacturers' provided cut-off values were applied the serum open TG2ab test showed better performance, detecting 93% of untreated celiac disease patients in

Table II Serum autoantibody test positivity in study groups according to manufacturers' provided cut-off values

	Open TG2ab+ (cut-off 3.0 U/ml)	Closed TG2ab+ (cut-off 3.0 U/ml)	Conventional TG2ab+ (cut-off 5.0 U/ml)	EmA+ (cut-off 1:≥5)
Untreated CD, <i>n</i> =44	41 (93%)	18 (44%)	12 (27%)	30 (68%)
Treated responsive CD, <i>n</i> =32	5 (15%)	0	0	1 (3%)
Treated non-responsive CD, <i>n</i> =15	9 (60%)	3 (20%)	0	0
Controls, <i>n</i> =25	4 (16%)	1 (4%)	0	0
Sensitivity	93%	44%	27%	68%
Specificity	84%	96%	100%	100%
Efficiency of the test	90%	61%	54%	80%
Likelihood ratio of a positive test result	5.8	11.0	∞	∞

TG2ab transglutaminase-2 antibody; EmA endomysial antibody; CD celiac disease

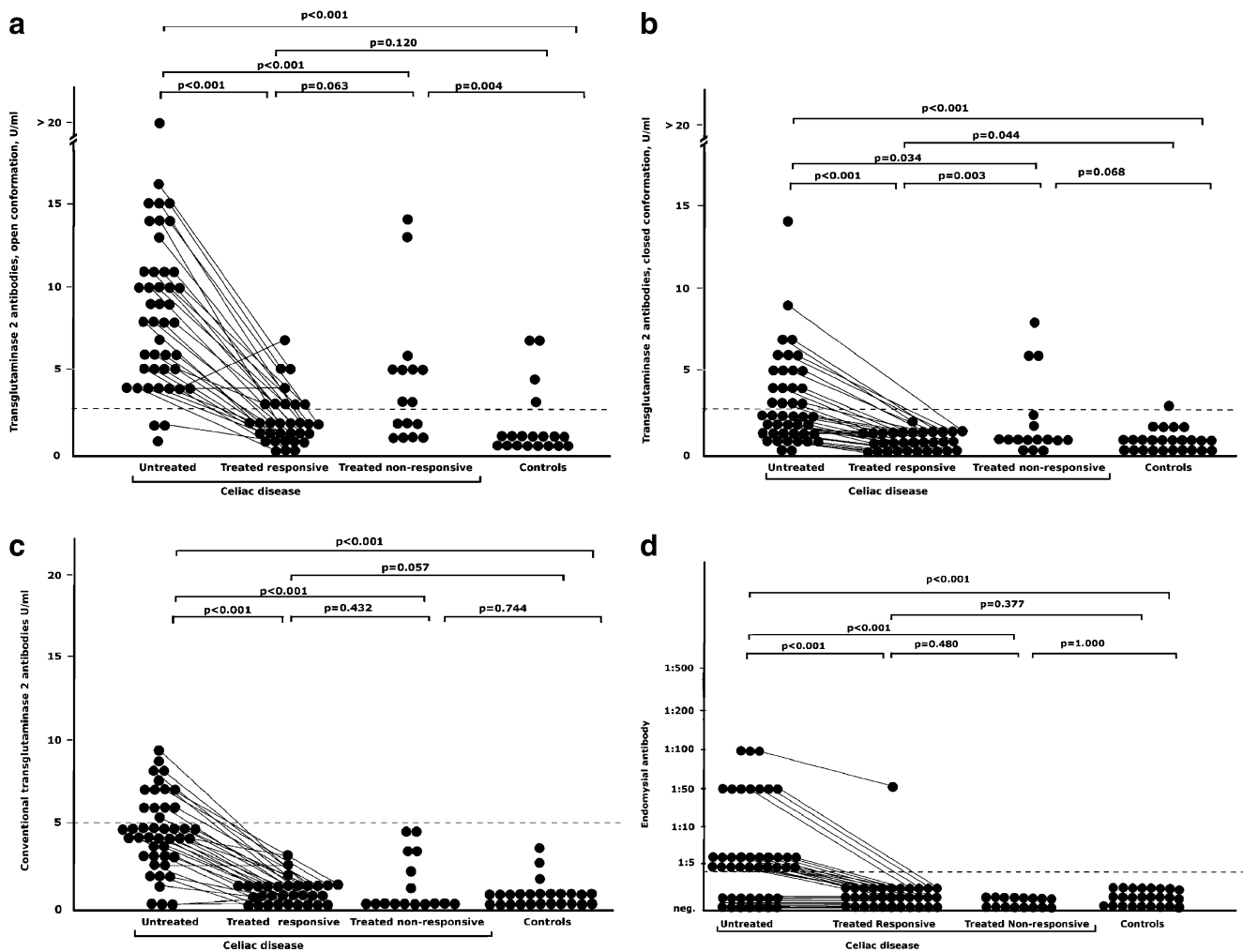


Fig. 1 Serum IgA-class autoantibodies against the open (a) and closed (b) conformation of transglutaminase 2, conventional transglutaminase 2 (c), and endomysial antibody (d) titers in study groups.

Dashed lines indicate the upper limit of normal level based on manufacturers' provided cut-off values. *P*-values below 0.05 are considered statistically significant

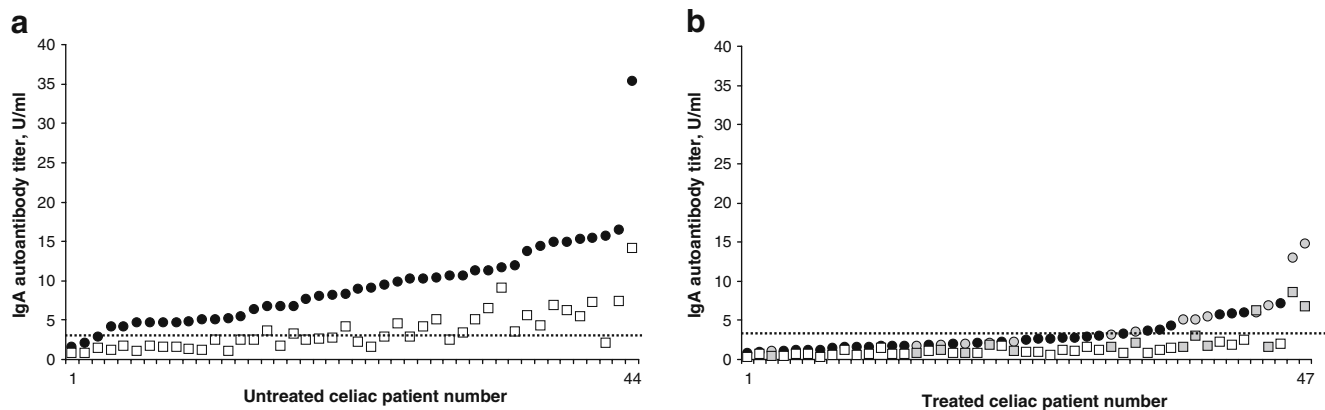


Fig. 2 Titers of open (closed circles) and closed (open squares) TG2abs in altogether 44 untreated (a) or 47 treated celiac disease patient (b). Non-responders to gluten-free diet are indicated in gray.

Dashed line indicates the upper limit of normal level based on manufacturers' provided cut-off values

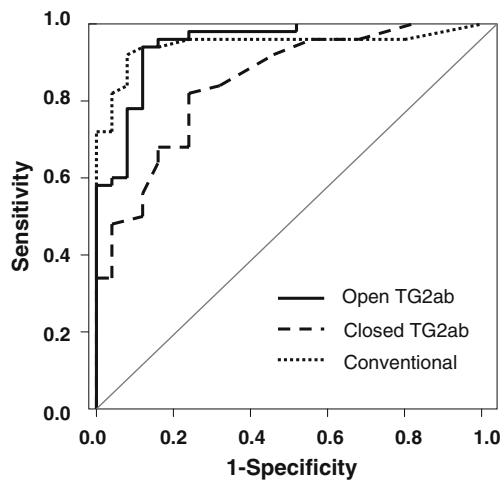


Fig. 3 Receiver operating characteristic curves for IgA-class open transglutaminase 2 antibody (TG2ab), closed TG2 ab, and conventional TG2ab tests based on 44 untreated celiac patients and 25 controls

contrast to 44% detected by closed TG2ab assay, 27% by conventional TG2ab kit and 68% by EmA test (Table II and Fig. 1). Three untreated patients with Marsh III celiac disease were missed by the open TG2ab test, but these cases were also negative for all other tested celiac autoantibody tests. Interestingly, one of these three patients also had dermatitis herpetiformis.

The serum levels of open TG2abs decreased significantly during a gluten-free diet in the histologically responsive celiac patients (Fig. 1a). This was likewise evident in all the other antibody tests, although the autoantibody titers were relatively low before dietary treatment (Fig. 1b–d).

In non-responsive celiac disease when using manufacturers' provided cut-off values the open TG2ab test was able to identify 60% and the closed TG2ab test 20% cases having persistent small-bowel atrophy on a gluten-free diet, while the conventional TG2abs and EmA assays missed all these patients.

When we compared the titers for the open and closed TG2ab assays in all celiac patient sera we noticed that there was a tendency toward higher titers for the open TG2abs (Fig. 2). The difference was more obvious in positive sera.

Based on manufacturers' recommended cut-off values none of the controls had serum celiac autoantibodies detected by conventional TG2 or EmA autoantibody tests, whereas four (16%) of them were positive for open TG2abs and one subject for closed TG2ab. Three out of the four positive for open TG2abs showed signs of early developing celiac disease (Marsh 0 but small-bowel mucosal IgA deposits and increased densities of $\gamma\delta$ +intraepithelial lymphocytes) and two had celiac-type HLA. Nevertheless, none of these four subjects has developed celiac disease during an 11-year follow-up on a gluten-containing diet.

Analysis of the ROC curves showed comparable AUCs for open and conventional TG2ab test (0.945 [95% CI 0.890–0.999] and 0.941 [95% CI 0.879–1.000], respectively) but 0.845 (95% CI 0.752–0.938) for the closed TG2ab test (Fig. 3). When cut-offs achieving a balance between sensitivity and specificity were applied the sensitivity of the closed TG2 ab test increased from 44% to 68% and that of the conventional TG2ab test from 27% to 91% (Table III). Simultaneously the specificity of the two above mentioned tests leveled that of the open TG2ab test.

Discussion

Our result showing celiac patients with active condition to have increased levels of autoantibodies against TG2 in the open conformation was logical, since it is reported that TG2 adopts an open conformation in the catalytically active form [5]. It is important that the new open TG2ab assay was also able to identify such untreated and non-responsive patients who were seronegative for other TG2-based tests, utilizing the antigen in an undefined or closed conforma-

Table III Serum autoantibody test positivity in study groups with modified cut-off levels based on receiver operating characteristic curves

	Open TG2ab+ (cut-off 3.7 U/ml)	Closed TG2ab+ (cut-off 2.1 U/ml)	Conventional TG2ab+ (cut-off 1.9 U/ml)
Untreated CD, <i>n</i> =44	41 (93%)	30 (68%)	40 (91%)
Treated responsive CD, <i>n</i> =32	6 (19%)	2 (6%)	2 (6%)
Treated non-responsive CD, <i>n</i> =15	7 (47%)	4 (27%)	5 (33%)
Controls, <i>n</i> =25	3 (12%)	4 (16%)	2 (8%)
Sensitivity	93%	68%	91%
Specificity	88%	84%	92%
Efficiency of the test	91%	74%	91%
Likelihood ratio of a positive test result	7.8	4.3	11.4

TG2ab transglutaminase-2 antibody; CD celiac disease

tion. Notably, in our study, the untreated celiac patients were either seronegative or had extremely low titers of conventional TG2abs (range 0–9.4 U/ml) or EmA (0–1:100) compared to what is often reported in the literature (often >100 U/ml and 1:>1,000, respectively) [22, 23]. Interestingly, the titers of the open TG2ab test were considerably higher than those of the closed TG2ab test (Fig. 2) and therefore borderline line cases could be easily interpreted as negative if the one uses a test where TG2 is not in its open conformation. Our finding on the performance of the open TG2ab assay is significant for clinical practice in that attempts to replace control biopsy with non-invasive serological tests have so far not been successful [14–16, 24, 25]; in this respect, the open TG2ab test is thus promising although in need of further verification. It is noteworthy that the levels of the open TG2abs significantly declined (Fig. 1a) during a 1 year gluten-free diet although the autoantibody levels did not completely normalize in all cases. However, this is in line with previous reports suggesting that negative seroconversion sometimes takes more than 1 year [11, 14].

As the open TG2ab test also identified four non-celiac subjects who were negative for conventional celiac autoantibodies, this would suggest that the test is not entirely specific. This issue, however, is of no major importance, as the positive test result can be verified with small-intestinal mucosal biopsy. Since three of the four control subjects with positive openTG2abs showed signs of early developing celiac disease which have previously been reported to predict the onset of the disease [13, 20], we cannot rule out the possibility that these open TG2ab-seropositive individuals will develop or manifest celiac disease later in life. Although these patients were followed up for 11 years, it is known that the development of villous atrophy and celiac disease may take even several decades [26].

According to current literature it is well known that different celiac autoantibody tests may give slightly discordant results, although they measure the same antibodies [27, 28]. This might partly be explained by the use of different kinds of test formats and reagents. Interestingly, in our study, many of the EmA positive but conventional TG2ab-negative individuals had conventional TG2ab values that were rather close to the cut-off value (3.0–4.9 U/ml) even though they were in the normal range (Fig. 1b). The catalytic activity of TG2 and therefore also its open conformation requires calcium [3], which is present in the conventional TG2ab assay used in the current study suggesting that also possible in this assay TG2 is in its open conformation. This conception is also supported by the fact that AUC were comparable between the open and conventional TG2ab tests. Changing the cut-off levels of the conventional TG2ab test from the manufacturer's recommendation of 5.0 to 1.9 U/ml increased its sensitivity but on the other hand specificity

was compromised. However, as our study population was highly selected we do not know whether the lower cut-off of the conventional TG2ab test would work more widely for instance in population screening. It is of note however that EmA test which is based on a distinct staining pattern in immunofluorescence is more like an on/off test but still the open TG2ab test operated better than EmA. Furthermore, the open TG2ab test might be especially helpful in detecting non-responsive celiac disease (Table III).

As the patients were investigated in a tertiary clinic and the patient material was highly selected in terms of certain diagnostic problems, we would discourage any generalization of the finding to all celiac patients. Also, we wanted to select a control group that is clinically relevant in differential diagnostics of patients having borderline small-bowel mucosal changes. Such diagnostic problems are common in clinical practice and the new open-TG2ab test might offer help in such difficult cases. For the moment, we conclude that the celiac autoantibody test utilizing the antigen TG2 in an open conformation constitutes a promising new non-invasive tool for the diagnostics and follow-up of celiac disease, especially in patient with diagnostic difficulties. In clinical practice, this patient group (i.e., those with negative or indeterminate conventional TG2 ab levels) would benefit the most from the new test.

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Conflicts of Interest The authors declare no conflicts of interest.

References

1. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med*. 1997;3:797–801.
2. Schuppan D, Junker Y, Barisani D. Celiac disease: from pathogenesis to novel therapies. *Gastroenterology*. 2009;137:1912–33.
3. Griffin M, Casadio R, Bergamini CM. Transglutaminases: nature's biological glues. *Biochem J*. 2002;368:377–96.
4. Siegel M, Strnad P, Watts RE, Choi K, Jabri B, Omary MB, et al. Extracellular transglutaminase 2 is catalytically inactive, but is transiently activated upon tissue injury. *PLoS ONE*. 2008;3:e1861.
5. Pinkas DM, Strop P, Brunger AT, Khosla C. Transglutaminase 2 undergoes a large conformational change upon activation. *PLoS Biol*. 2007;5:e327.
6. Sulkanen S, Halttunen T, Laurila K, Kolho KL, Korponay-Szabó IR, Sarnesto A, et al. Tissue transglutaminase autoantibody

- enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology*. 1998;115:1322–8.
7. Roth EB, Sjöberg K, Stenberg P. Biochemical and immunopathological aspects of tissue transglutaminase in coeliac disease. *Autoimmunity*. 2003;36:221–6.
 8. Carroccio A, Vitale G, Di Prima L, Chifari N, Napoli S, La Russa C, et al. Comparison of anti-transglutaminase ELISAs and an anti-endomysial antibody assay in the diagnosis of celiac disease: a prospective study. *Clin Chem*. 2002;48:1546–50.
 9. Parizade M, Bujanover Y, Weiss B, Nachmias V, Shainberg B. Performance of serology assays for diagnosing celiac disease in a clinical setting. *Clin Vaccine Immunol*. 2009;16:1576–82.
 10. van der Windt DA, Jellema P, Mulder CJ, Kneepkens CM, van der Horst HE. Diagnostic testing for celiac disease among patients with abdominal symptoms: a systematic review. *JAMA*. 2010;303:1738–46.
 11. Salmi TT, Collin P, Korponay-Szabo IR, Laurila K, Partanen J, Huhtala H, et al. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. *Gut*. 2006;55:1746–53.
 12. Rostami K, Kerckhaert J, Tiemessen R, von Blomberg BM, Meijer JW, Mulder CJ. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. *Am J Gastroenterol*. 1999;94:888–94.
 13. Kurppa K, Collin P, Viljamaa M, Haimila K, Saavalainen P, Partanen J, et al. Diagnosing mild enteropathy celiac disease: a randomized, controlled clinical study. *Gastroenterology*. 2009;136:816–23.
 14. Kaukinen K, Sulkanen S, Mäki M, Collin P. IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease. *Eur J Gastroenterol Hepatol*. 2002;14:311–5.
 15. Dipper C, Maitra S, Thomas R, Lamb CA, McLean-Tooke AP, Ward R, et al. Anti-tissue transglutaminase antibodies in the follow-up of adult coeliac disease. *Aliment Pharmacol Ther*. 2009;30:236–44.
 16. Vecsei AK, Graf UB, Vogelsang H. Follow-up of adult celiac patients: which noninvasive test reflects mucosal status most reliably? *Endoscopy*. 2009;41:123–8.
 17. When is a coeliac a coeliac. Report of a working group of the United European Gastroenterology Week in Amsterdam 2001. *Eur J Gastroenterol Hepatol*. 2001;13:1123–8.
 18. Cellier C, Delabesse E, Helmer C, Patey N, Matuchansky C, Jabri B, et al. Refractory sprue, coeliac disease, and enteropathy-associated T-cell lymphoma. French Coeliac Disease Study Group. *Lancet*. 2000;356:203–8.
 19. Collin P, Kaukinen K, Vogelsang H, Korponay-Szabó I, Sommer R, Schreier E, et al. Antiendomysial and antihuman recombinant tissue transglutaminase antibodies in the diagnosis of coeliac disease: a biopsy-proven European multicentre study. *Eur J Gastroenterol Hepatol*. 2005;17:85–91.
 20. Salmi TT, Collin P, Järvinen O, Haimila K, Partanen J, Laurila K, et al. Immunoglobulin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease. *Aliment Pharmacol Ther*. 2006;24:541–52.
 21. Bruns DE, Huth EJ, Magid E, Young DS. Toward a checklist for reporting of studies of diagnostic accuracy of medical tests. *Clin Chem*. 2000;46:893–5.
 22. Volta U, Granito A, Parisi C, Fabbri A, Fiorini E, Piscaglia M, et al. Deamidated gliadin peptide antibodies as a routine test for celiac disease: a prospective analysis. *J Clin Gastroenterol*. 2010;44:186–90.
 23. Donaldson MR, Firth SD, Wimpee H, Leiferman KM, Zone JJ, Horsley W, et al. Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. *Clin Gastroenterol Hepatol*. 2007;5:567–73.
 24. Plebani M, Basso D. Diagnostic testing for celiac disease. *JAMA*. 2010;304:639.
 25. Van Meensel B, Hiele M, Hoffman I, Vermeire S, Rutgeerts P, Geboes K, et al. Diagnostic accuracy of ten second-generation (human) tissue transglutaminase antibody assays in celiac disease. *Clin Chem*. 2004;50:2125–35.
 26. Matysiak-Budnik T, Malamut G, de Serre NP, Grosdidier E, Segulier S, Brousse N, et al. Long-term follow-up of 61 coeliac patients diagnosed in childhood: evolution toward latency is possible on a normal diet. *Gut*. 2007;56:1379–86.
 27. Green PH, Barry M, Matsutani M. Serologic tests for celiac disease. *Gastroenterology*. 2003;124:585–6.
 28. Raivio T, Korponay-Szabó IR, Paajanen T, Ashorn M, Iltaanen S, Collin P, et al. Comparison of a novel whole blood transglutaminase-based ELISA with a whole blood rapid antibody test and established conventional serological celiac disease assays. *J Pediatr Gastroenterol Nutr*. 2008;47:562–7.