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Comparison of a Novel Whole Blood Transglutaminase-based ELISA With a Whole Blood Rapid Antibody Test and Established Conventional Serological Celiac Disease Assays

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ABSTRACT

Objectives: Serum immunoglobulin A–class tissue transglutaminase (tTG-ab) and endomysial antibody (EMA) tests play a key role in the diagnostic evaluation of celiac disease. Recently, a novel whole blood rapid test based on self-tissue transglutaminase (tTG) was developed for celiac disease case finding. Based on the same principle, a whole blood self-tTG enzyme-linked immunosorbent assay (ELISA), especially applicable to large-scale screening of celiac disease, has been produced. We assessed the value of this new test in celiac disease antibody detection.

Patients and Methods: The new test uses endogenous tTG found in red blood cells of whole blood in IgA-class tTG-ab measurement by detecting tTG–tTG-ab complexes formed after hemolysis of the whole blood sample. Stored whole blood samples from 150 untreated celiac disease patients and 107 control individuals without celiac disease were evaluated, and the test results were compared with those of the whole blood rapid test, 2 conventional serum-based tTG-ab ELISA tests, and 2 EMA tests.

Results: A total of 15 whole blood samples were found to be clotted or dried after storage and were excluded from further evaluation. The whole blood ELISA test had a specificity (98%) comparable to that of the conventional serological tests, the sensitivity (91%) being slightly lower. The test was concordant with the whole blood rapid test in 92% of cases, with 2 serological ELISA tests in 91% and 94% of cases and with EMA tests in 94% and 93% of cases.

Conclusions: Whole blood self-tTG–based testing is accurate in celiac antibody detection, also when an ELISA method is applied. The testing requires no serum separation or external tTG. ***JPGN 47:562–567, 2008.*** **Key Words:** Celiac disease—Self tissue transglutaminase—Enzyme-linked immunosorbent assay—Endomysial antibodies. © 2008 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

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Celiac disease is a gluten-induced genetically determined lifelong autoimmune disorder presenting with a variety of symptoms ranging from gastrointestinal problems to extraintestinal manifestations, or with a total absence of symptoms (1–3). The condition affects approximately 1% of the European population, and high prevalences are widely reported around the world (4–9). However, as revealed by screening studies, in 85% to 90% of affected people, the condition still remains undiagnosed (3,10). Diagnosis is based on the histological finding of small bowel mucosal villous atrophy with crypt hyperplasia (11). Gluten-triggered tissue

autoantibodies, endomysial antibodies (EMA), and tissue transglutaminase antibodies (tTG-ab) can be used as a first-step noninvasive celiac disease screening method in people with symptoms indicative of the disease and in celiac disease risk groups such as first-degree family members or patients having other autoimmune diseases (5,6,12–14).

Immunoglobulin A (IgA)–class EMA, measured from serum samples by an indirect immunofluorescence (IF) method, are highly specific for celiac disease (15). However, the IF method is laborious and requires visual interpretation, which is subjective (16). Since the recognition of tissue transglutaminase (tTG) as the major celiac disease autoantigen, serum tTG-ab enzyme-linked immunosorbent assays (ELISAs) have been produced for easier and objective celiac disease antibody detection (17–21). Many of the ELISA tests today use human recombinant tTG for the detection of antibodies. Recognition that the enzyme tTG is also present in human red blood cells (22) led to the introduction of serological tTG ELISA tests with human native red blood cell–derived antigen (23,24). Recently, a whole blood–based rapid self-tTG method was developed to detect IgA-class tTG antibodies directed to the patient's own red blood cell tTG, self-tTG (25). In self-tTG testing, a whole blood sample is hemolyzed, resulting in liberation of the enzyme from the red blood cells and in complexing with autoantibodies if they are present in the serum of the sample (26). This novel means of detecting tTG-ab from whole blood (25,27–29) is also suitable for celiac antibody detection by use of a recently developed self-tTG–based ELISA method applicable to the large-scale screening of celiac disease. We have now assessed the value of the self-tTG–based tTG-ab ELISA in detecting patients with untreated celiac disease and control individuals without celiac disease, and compared the test results with those of the rapid test, 2 widely used serum tTG-ab ELISAs, and 2 EMA tests. Small bowel mucosal histology was used as reference.

PATIENTS AND METHODS

The study group consisted of 150 consecutive patients with untreated celiac disease (96 female, median age 9 years, range 1.4–40 years) and 107 control individuals without celiac disease (41 female, median age 12 years, range 1–55 years). The patients were examined between 2000 and 2005 at the Department of Gastroenterology-Nephrology, Heim Pál Children's Hospital, Budapest, Hungary, and at the Department of Paediatrics in Tampere University Hospital, Finland. The diagnosis of celiac disease was based on severe partial, subtotal, or total villous atrophy with crypt hyperplasia, also defined as Marsh IIIA–IIIC in the Marsh classification, in the small bowel and on the clinical and/or histological response to a gluten-free diet (11,30). Control individuals with celiac disease who had normal small-bowel mucosal morphology comprised individuals with dyspepsia (n = 44), autoimmune conditions such as Crohn disease (n = 20) or colitis ulcerosa (n = 7), gastroesophageal reflux disease (n = 12), intestinal polyposis (n = 8), noninfectious unspecific gastroenteritis or colitis (n = 2), retarded growth (n = 2), unspecific abdominal pain (n = 3), melena (n = 1), constipation (n = 1), vomiting (n = 1), abscess of the buttocks (n = 1), hematochezia (n = 1), cystic fibrosis (n = 1), congenital sucrase-isomaltase deficiency (n = 1), intestinal lymphangiectasia (n = 1), and Shwachman-Diamond syndrome (n = 1). Serum and ethylene diaminetetraacetic acid (EDTA) or sodium citrate whole blood samples were obtained at the time of biopsy and stored at −20°C until tested.

Whole Blood Self-tTG Antibody Testing

In self-tTG–based celiac antibody testing, the patient's own tTG found in red blood cells in whole blood is used as an antigen for IgA-class tTG-ab detection by hemolyzing a whole blood sample and liberating tTG from the red blood cells (26). If tTG-ab is present in the sample, it will complex with its liberated autoantigen. The complexes are captured from the hemolyzed sample by tTG binding protein to a solid support. IgA-class tTG-ab is further visualized by a solution conjugated with horseradish peroxidase–labeled antihuman IgA. This innovative test principle to detect tTG-ab without the need for external tTG and serum separation was first investigated in point-of-care fashion (25,29) and subsequently by a commercial rapid lateral flow immunochromatographic test (27,28). Inasmuch as the testing proved accurate in IgA-class tTG-ab detection, the principle was further developed into a commercial ELISA method suitable for large-scale celiac disease antibody screening.

In the self-tTG–based whole blood tTG-ab ELISA (Celiac IgA EIA, catalog number 6300100, Ani Labsystems Ltd Oy, Vantaa, Finland, abbreviated here as self-tTG ELISA), IgA-class tTG-ab is detected by binding the patient's own tTG immunocomplexed by its autoantibodies to a specific antigen attached to the polystyrene surface of a 96-well Microstip (Table 1). Autoantibodies, if present, are further discerned as a color reaction by the use of solutions containing horseradish peroxidase–labeled anti-human IgA and chromogen. In this study, the whole blood tTG-ab ELISA was carried out in the laboratory in blinded fashion according to the manufacturer's instructions (31). A value at or above 5.0 U/mL was considered positive, as suggested by the manufacturer.

Additionally, self-tTG testing was carried out with the whole blood self-tTG rapid test (Biocard Celiac Test, catalog number 3-027-000, Ani Biotech, Vantaa, Finland) according to the manufacturer's instructions (Table 1). The whole blood rapid test, using the same principle as the whole blood self-tTG ELISA, is a lateral flow immunochromatographic test measuring IgA-class tTG-ab within 5 minutes (27). In the test used in this study, the signal generator, gold-labeled mouse antibodies to human IgA, was bound to the filter tip of the tube containing the hemolyzing sample buffer. Otherwise, the test functioned in the same manner as previously described (27).

Conventional Serum-based Antibody Testing

The methods used here to measure celiac disease antibodies are presented in Table 1. IgA-class tTG-ab was determined by a commercial ELISA using native human tTG isolated from red

TABLE 1. *Methods used to measure celiac disease antibodies in this study*

Abbreviation	Commercial name and producer	Antigen	Test principle	Cutoff level
Self-tTG ELISA	Celiac IgA EIA, Ani Labsystems, Vantaa, Finland	Whole blood sample red blood cell self-tTG	Whole blood–based ELISA	5.0 U/mL
Self-tTG rapid test	Biocard Celiac Test, Ani Biotech, Vantaa, Finland	Whole blood sample red blood cell self-tTG	Whole blood–based immunochromatographic test	Visible color formation
nh-tTG ELISA	QUANTA Lite h-tTG IgA, INOVA Diagnostics, San Diego, CA	Native human red blood cell-derived tTG	Serum-based ELISA	20 U
hr-tTG ELISA	Celikey, Phadia GmbH, Freiburg, Germany	Human recombinant tTG	Serum-based ELISA	5.0 U/mL
In-house EMA	S-EMAbA, Service Laboratory, Celiac Disease Study Group, University of Tampere, Finland	Human umbilical cord	Serum-based indirect IF	1:5
Commercial EMA	ImmuGlo Anti-Endomysial Antibody Test System, IMMCO Diagnostics, Buffalo, NY	Primate smooth-muscle tissue	Serum-based indirect IF	1:2.5

blood cells as antigen (QUANTA Lite h-tTG IgA, INOVA Diagnostics, San Diego, CA, abbreviated here as nh-tTG ELISA), the suggested cutoff value being 20 U or more. The tTG-ab were also determined by another commercial ELISA using human recombinant tTG as antigen (Celikey, Phadia GmbH, Freiburg, Germany, abbreviated here as hr-tTG ELISA) with concentrations of 5.0 U/mL or higher being considered positive.

Serum IgA-class EMA was determined by an indirect IF method using human umbilical cord as antigen (abbreviated here as in-house EMA) (19,32). A titer of 1 to 5 or greater was considered positive. Additionally, EMA was also measured by a commercial indirect IF assay using primate smooth-muscle tissue as a substrate and IgA/IgG conjugate (ImmuGlo Anti-Endomysial Antibody Test System, IMMCO Diagnostics, Buffalo, NY, abbreviated here as commercial EMA), a titer of 1 to 2.5 or higher being considered positive.

Statistical Analysis

Sensitivities, specificities, negative and positive predictive values, and efficiencies of the tests were calculated for the 6 celiac disease antibody tests (33).

Ethical Considerations

The study protocol was approved by the local ethical committees in Hungary and Finland. All of the subjects gave informed consent.

RESULTS

The results obtained from 242 intact samples are shown in Table 2 for each antibody test; whole blood samples from 11 patients with untreated celiac disease and 4 control individuals without celiac disease were found to be clotted or dried after storage and were excluded from further evaluation. The whole blood self-tTG ELISA had a specificity (98%) similar to that of the serum tests, but the sensitivity (91%) was slightly lower compared with that of 99% of all serological tests. The corresponding figures for the rapid test were 94% and 93%, respectively. The positive predictive values of the self-tTG ELISA and of the rapid test were 98% and 96%, respectively. Table 3 shows the false negative and

TABLE 2. *Results of the different antibody tests in patients with untreated biopsy-proven celiac disease and control individuals without celiac disease*

	Whole blood testing				Serum testing							
	Self-tTG ELISA		Self-tTG rapid test		nh-tTG ELISA		hr-tTG ELISA		In-house EMA		Commercial EMA	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Celiac disease patients, n = 139*	126	13	129	10	138	1	137	2	138	1	138	1
Control individuals, n = 103*	2	101	6	97	7	96	1	102	0	103	0	103
Sensitivity, %	91		93		99		99		99		99	
Specificity, %	98		94		93		99		100		100	
PPV, %	98		96		95		99		100		100	
NPV, %	89		91		99		98		99		99	
ET, %	94		93		97		99		100		100	

* A total of 15 damaged samples were excluded from evaluation.

TABLE 3. *False negative and false positive results obtained with whole blood self-tTG antibody ELISA in patients with biopsy-proven untreated celiac disease and control individuals without celiac disease having normal mucosal morphology, respectively*

	Whole blood testing		Serum testing			
Patient	Self-tTG ELISA (cutoff 5.0 U/ml)	Self-tTG rapid test (test result negative or positive)	nh-tTG ELISA (cutoff 20 U)	hr-tTG ELISA (cutoff 5.0 U/mL)	In-house EMA (test result negative or positive)	Commercial EMA (test result negative or positive)
Patients with untreated celiac disease, false negative						
1*	0	−	4	0.0	−	+
2	1.0	−	103	82.3	+	+
3	1.5	+	80	20.3	+	+
4	2.0	+	30	2.6	+	+
5	2.0	+	145	68.8	+	+
6	2.0	+	190	118.5	+	+
7	3.0	+	94	19.7	+	+
8	3.0	−	53	6.5	+	+
9	3.5	−	148	80.5	+	+
10	3.5	+	152	97.5	+	+
11	4.5	+	208	109.6	+	+
12	4.5	+	250	118.3	+	+
13	4.5	+	47	12.0	+	+
Control individuals without celiac disease, false positive						
14	5.5	+	8	1.4	−	−
15	8.5	+	19	3.0	−	−

* Patient was subsequently revealed to have selective IgA deficiency and thus had positive results only in the commercial EMA test using IgA/IgG conjugate.

false positive self-tTG ELISA results compared with those of the other tests.

In patients with untreated celiac disease and control individuals without celiac disease, the concordance between the self-tTG ELISA and the whole blood self-tTG rapid test was 92%, the self-tTG ELISA and the serum nh-tTG ELISA 91%, the self-tTG ELISA and hr-tTG ELISA 94%, the self-tTG ELISA and the in-house EMA test 94%, and the self-tTG ELISA and the commercial EMA test 93%. Whole blood and serum celiac disease antibody test results for each of the 6 tests in patients with untreated celiac disease patients and control individuals without celiac disease, and the discordances between the different tests, are shown in Fig. 1. The 6 antibody test results were concordant throughout in 86% of all cases. The whole blood self-tTG ELISA agreed with at least 1 other celiac disease antibody test in 97% of cases.

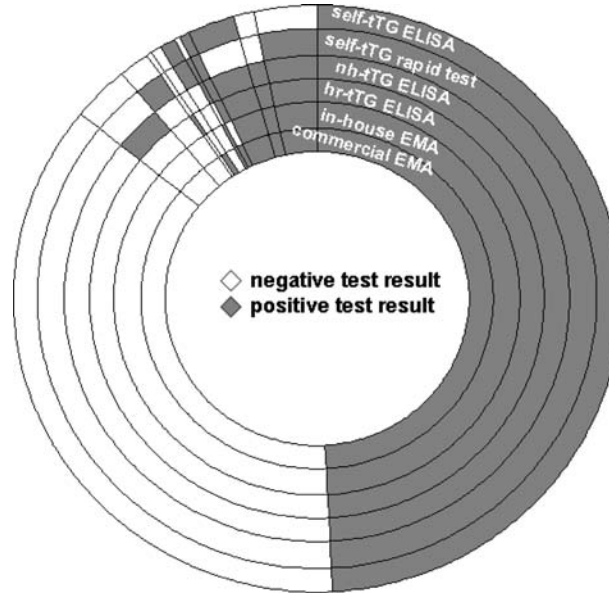

FIG. 1. Comparison between the IgA-class whole blood self tissue transglutaminase ELISA (self-tTG ELISA), the IgA-class whole blood self-tTG rapid test, IgA-class serum native human red blood cell–derived tTG ELISA (nh-tTG ELISA), serum human recombinant tTG ELISA (hr-tTG ELISA), serum in-house endomysial antibody (EMA), and commercial EMA test results obtained in 242 cases (139 untreated patients with celiac disease and 103 control individuals without celiac disease).

DISCUSSION

It has been shown that the use of the patients' own red blood cell tTG in self-tTG–based whole blood celiac disease antibody tests using either Nunc-Immunostick or the lateral flow immunochromatographic strip system is reliable in case findings and dietary monitoring of celiac disease (25,27–29). In the present study, we further show that the same principle may also be used in an ELISA

format suitable for large-scale screening purposes. In our series, the specificity of the IgA-class whole blood self-tTG ELISA was comparable to those of the IgA-class serological celiac disease antibody tests, whereas the sensitivity of the test was slightly lower. Additionally, self-tTG–based whole blood rapid testing was accurate in detecting celiac disease, as previously shown (27,28), and was extremely easy and quick to carry out compared with the other tests.

In this article we present a novel method, the whole blood self-tTG ELISA, for IgA-class tTG-ab detection and selection of patients to undergo diagnostic endoscopy without the need for serum separation or external antigen. Inasmuch as the sensitive antigen tTG is not included in the test kit, the shelf life of the test is long. The test may thus also preserve its functionality better in demanding conditions, such as exceptional storage temperatures and humidity, than serum tTG-ab ELISA using external tTG serum (22). Additionally, the whole blood self-tTG ELISA, like the serum tTG-ab ELISA tests, is objective in interpretation and easier and faster to carry out compared with serum indirect IF EMA tests (16). Moreover, theoretically the new whole blood self-tTG ELISA test could be more economical in the future than the conventional serum-based celiac disease antibody tests because labor costs can be reduced when serum separation is not needed and the external tTG antigen is not required. When the self-tTG–based whole blood rapid testing is applied, even more cost savings may be generated because the testing can be easily and quickly carried out without sample transportation, expert personnel, and laboratory facilities (34).

As previously shown, we also detected discordance between results obtained from different tests even though they measure the same antibodies (20,35–39) (Fig. 1). Some discrepant test results were also detected between the 2 self-tTG whole blood antibody tests. This may be partly explained by the use of different kinds of test formats and reagents, although the test principle is the same. Likewise, discordance was seen not only between the 2 whole blood self-tTG tests but also between 2 different well-established serum ELISA and EMA tests (Fig. 1). This has been the case in all of the serological tests so far developed (20,35–39), and no ideal test exists.

The present study shows, however, that in whole blood self-tTG testing the sample should be of good quality to guarantee effective exploitation of its red blood cell tTG, which is a sensitive protein (22) and, if damaged, may prove incompetent to form immunocomplexes with its serum autoantibodies. To ensure proper function of the antigen and the test, multiple freezings, thawings, or prolonged storage of samples should be avoided. Moreover, clotted whole blood samples should not be used, and where possible fresh whole blood samples should be preferred. In our series, 15 of the whole blood samples were discarded because they were clearly damaged after storage as a result of clotting or drying. When we carried out self-tTG ELISA with damaged whole blood samples, we often obtained negative test results. By contrast, when the counterpart serum samples were mixed with fresh celiac antibody-negative whole blood containing undamaged red blood cells, all but 1 false negative test results were again positive (data not shown). This finding suggests that the sensitivity of the whole blood self-tTG tests may be improved when whole blood samples with functional tTG able to form immunocomplexes are applied.

IgA-class serum tTG-ab and EMA, and the whole blood self-tTG rapid or ELISA tests, are not suitable for determination of celiac disease autoantibodies in patients with selective IgA deficiency, which is found more frequently in patients with celiac disease than in the general population (40). In such cases, celiac disease antibodies should be measured in IgG-class with the conventional serum tTG-ab and EMA tests (32,41,42), or untreated celiac disease should be excluded by intestinal biopsy. In this study, 1 patient with celiac disease had selective IgA deficiency and thus showed positive results in the commercial EMA test using IgA/IgG conjugate. Furthermore, it is known that seronegative celiac disease exists, but mainly among adults (43). It was shown earlier that in such patients, the self-tTG–based whole blood testing works as well as the conventional serological celiac disease antibody tests (29).

In conclusion, whole blood self-tTG–based testing is suitable for large-scale IgA-class celiac disease antibody screening using the ELISA method. Good whole blood sample quality is required for a reliable test result. The self-tTG–based ELISA and rapid antibody tests offer health care professionals an alternative to the known serological IgA-class EMA and tTG-ab tests, and when such tests are used, there is no need for serum separation or external recombinant or purified tTG antigen.

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