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A Brazilian experience of the self transglutaminase-based test for celiac disease case finding and diet monitoring

Lorete Maria da Silva Kotze, Ana Paula Brambila Rodrigues, Luiz Roberto Kotze, Renato Mitsunori Nisihara

Lorete Maria da Silva Kotze, Ana Paula Brambila Rodrigues, Luiz Roberto Kotze, Gastroenterology and Endoscopy Services, Cajuru Hospital, Pontifical Catholic University of Paraná, CEP 80240-220, Curitiba, Paraná, Brazil
Renato Mitsunori Nisihara, Evangelical University and Laboratory of Immunopathology, Clinical Hospital, Federal University of Paraná, CEP 80240-220, Curitiba, Paraná, Brazil
Author contributions: Kotze LM performed clinical trials and the majority of experiments; Brambila Rodrigues AP performed tests and endoscopic procedures; Kotze LR performed histological evaluations; Nisihara RM performed laboratory antibody determinations; All the authors participated in the trial, designed the study, wrote and revised the manuscript.
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Correspondence to: Lorete Maria da Silva Kotze, MD, PhD, Professor, FACG, Rua Bruno Filgueira, 369 - Cj 1205, CEP 80240-220, Curitiba, Paraná, Brazil. loretekotze@hotmail.com
Telephone: +55-41-33361088 Fax: +55-41-33361088
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Abstract

AIM: To evaluate the effectiveness of a rapid and easy fingertip whole blood point-of-care test for celiac disease (CD) case finding and diet monitoring.

METHODS: Three hundred individuals, 206 females (68.7%) and 94 males (31.3%), were submitted to a rapid and easy immunoglobulin-A-class fingertip whole blood point-of-care test in the doctor's office in order to make immediate clinical decisions: 13 healthy controls, 6 with CD suspicion, 46 treated celiacs, 84 relatives of the celiac patients, 69 patients with dyspepsia, 64 with irritable bowel syndrome (IBS), 8 with Crohn's disease and 9 with other causes of diarrhea.

RESULTS: Upper gastrointestinal endoscopy with duodenal biopsies was performed in patients with CD suspicion and in individuals with positive test outcome: in 83.3% (5/6) of the patients with CD suspicion, in 100% of the patients that admitted gluten-free diet transgressions (6/6), in 3.8% of first-degree relatives (3/79) and in 2.9% of patients with dyspepsia (2/69). In all these individuals duodenal biopsies confirmed CD (Marsh's histological classification). The studied test showed good correlation with serologic antibodies, endoscopic and histological findings.

CONCLUSION: The point-of-care test was as reliable as conventional serological tests in detecting CD cases and in CD diet monitoring.

Key words: Celiac disease; Gluten; Relatives; Dyspepsia; Diarrhea

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INTRODUCTION

Celiac disease (CD) is a multifactorial disorder that occurs in genetically susceptible individuals. CD is considered an immune-mediated enteropathy caused by a permanent intolerance of the small bowel mucosa to gluten, and affects approximately 1% of the world population[1]. In our geographic area the estimated prevalence is 1/475[2]. Individuals with CD can be asymptomatic or can present a variety of gastrointestinal symptoms or extra-intestinal manifestations and often the diagnoses of dyspepsia or irritable bowel syndrome (IBS) are suggested[3]. The diagnosis of CD is based on serologic and histopathologic findings. Antiendomysial (EMA) and anti-tissue transglutaminase antibodies, class IgA, are considered highly specific serological markers for CD. Biopsies of the proximal small bowel in positive individuals for these antibodies confirm the diagnosis of CD[1].

As CD is a disorder that can be ruled out in the differential diagnosis of several gastrointestinal diseases, non-invasive tests are recommended as a first-step to screening patients before small intestinal biopsy to confirm CD[1,4]. Also, gluten-triggered tissue autoantibodies can be determined to aid in monitoring the diet and for case findings of CD in at-risk groups such as relatives[5] and in patients with other autoimmune

Table 1 Demographic data of all the studied individuals[1]

Group	Number	Female	Male	Median age	Range	Positivity
Healthy control	13	6	7	40.0	7-75	0
Celiac at diagnosis	6	4	2	41.2	28-61	**5 (83.3%)[1]**
GFD adherence	40	29	11	39.6	6-76	0
GFD transgression	6	4	2	24.0	10-38	**6 (100.0%)[1]**
First-degree relatives	79	56	23	30.7	5-79	**3 (3.8%)[1]**
Second-degree relatives	6	3	3	33.5	9-60	0
Dyspepsia	69	46	23	40.6	12-78	**2 (2.9%)[1]**
Irritable bowel syndrome	64	46	18	46.8	12-81	0
Crohn's disease	8	5	3	35.6	27-45	0
Diarrhea	9	7	1	46.6	14-63	0
Total	300	206	94			

[1]In bold the number of individuals with a positive test and the respective percentages. GFD: Gluten-free diet.

diseases[6-8].

The conventional tests, EMA determined by indirect immunofluorescence and anti-tTG performed by ELISA, require sera samples, are laborious and time-consuming, need special laboratorial centers, are expensive and the results are available only after a time lag. So, a rapid test, with a result which can be available immediately, will help the physicians to make decisions at their own offices[9].

The aim of this study was to evaluate the effectiveness of a rapid and easy immunoglobulin-A-class whole blood point-of-care test for celiac autoantibody detection in patients routinely seen in the clinic, to enable immediate clinical decisions.

MATERIALS AND METHODS

Patients

The patients were tested at the Gastroenterology and Endoscopy Services, Pontifical Catholic University of Paraná, and also tested on site in the author's office, Curitiba city, State of Paraná, South of Brazil. The study was approved by the Research Ethic Committee of the institution. All the participants gave informed consent.

A total of 300 consecutive Caucasoid individuals born in southern Brazil were studied, 206 females (68.7%) and 94 males (31.3%) divided by groups: 13 controls not relatives of the celiac patients; 6 with CD suspicion; 46 treated celiacs, 40 with and 6 without compliance to a gluten-free diet (GFD); 85 relatives of the celiac patients, 79 first- and 6 second-degree relatives; 69 patients with clinical diagnosis of dyspepsia; 64 with irritable bowel syndrome; 8 patients with Crohn's disease; 9 with other causes of diarrhea (4 lactose intolerance, 2 collagenous colitis, 1 diarrhea/possible gastroenteritis, 1 severe psoriasis, 1 AIDS). Table 1 shows the demographic data of the studied groups.

Methods

The diagnosis of CD was based on serologic tests and on the findings of a small intestinal mucosal biopsy. Because some tests are operator-dependent, the same professional (RMN) performed all the serologic tests: Antiendomysial antibodies (IgA EmA) by indirect immunofluorescence assay using human umbilical cord as substrate, according to Volta *et al*[10], sera considered positive if fluorescence was observed at a dilution of 1/2.5 or higher; serum IgA anti-tissue transglutaminase antibodies (anti-tTG) titres measured by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (INOVA Diagnostics Inc., USA), based on the method described by Dieterich *et al*[11], results higher than 20 U were considered positive.

Upper gastrointestinal endoscopy was done by the same physician (APBR) with attention to the classical endoscopic markers of CD: erosions, scalloping of folds, decrease and atrophy of duodenal folds, mosaic patterns[12,13]. The histological analysis was performed by the same pathologist (LRK), familiar with the spectrum of mucosal changes in CD, using Marsh's classification[14] in Hematoxylin-Eosin stained fragments. These were, briefly, type Ⅰ (infiltrative) with increased number of intraepithelial lymphocytes; type Ⅱ (hyperplastic) with increased number of these cells and crypt hyperplasia; type Ⅲ (destructive) with villous atrophy, A: partial, B: subtotal and C: total; type Ⅳ (hypoplastic) with flat atrophic mucosa. The number of intraepithelial lymphocytes (IEL) was counted according to Ferguson and Murray[15]; 24% was considered normal for the Brazilian population, according to previous study[16].

All the individuals (patients and relatives) were submitted to a rapid test for celiac-specific immunoglobulin A class tissue transglutaminase antibody detection performed from fresh fingertip whole blood sample (*Biocard Celiac-Test*™, ANI Biotech, Vantaa, Finland). The basic concept is to liberate the individual's own tTG from the red blood cells by hemolysing an anticoagulated whole blood sample. When tTG-specific antibodies are present in the sera they recognize and form complexes with the liberated self-tTG. The complexes can be detected by binding tTG to a solid surface coated with tTG-capturing proteins. The bound antigen-antibody complexes can be seen in a color reaction with the help of labeled anti-human IgA solution[9]. According to the manufacturer, 5 min (not longer than 10 min) is the time till the interpretation of the results. However, a positive test result may appear within 1 to 2 min. The test result is positive when both

Table 2 Demographic data, correlation with antiendomysium antibodies, endoscopic and histological findings of duodenal biopsies for the positive individuals

	Age	Gender	IgA EmA	Biopsy	IEL	Endoscopy
Celiac at diagnosis						
CPA	28	F	Positive	Ⅲ-C	> 40	Scalloped folds, nodularity
CBO	38	F	Positive	Ⅲ-C	40	Mosaic pattern
NHF	41	F	Positive	Ⅲ-C	> 40	Nodularity, atrophic areas
AHR	42	M	Positive	Ⅲ-C	45	Erosions, edema
HB	61	M	Negative	Ⅲ-C	> 40	Decreased folds
GFD transgression						
GSCS	10	M	Positive	Ⅲ-C	55	Decreased folds, atrophic areas
ALM	19	F	Positive	Ⅲ-A	NC	Atrophic areas
VC	23	F	Positive	Ⅲ-C	> 40	Atrophic areas
FA	23	M	Positive	Ⅲ-C	35	Decreased folds, atrophic areas
LGH	31	F	Positive	Ⅲ-A	35	Decreased folds
RK	38	F	Positive	Ⅲ-C	> 50	Atrophic areas
First-degree relatives						
LGS	21	F	Positive	I	38	Normal
RG	48	F	Positive	Ⅲ-C	48	Atrophy of folds
ERA	52	F	Negative	R	R	R
Dyspepsia						
EJMS	21	F	Positive	Ⅲ-A	50	Atrophy
LA	26	F	Positive	Ⅲ-C	> 40	Scalloped folds, areas of atrophy

EmA: Antiendomysium; IEL: Percent of intraepithelial lymphocytes; NC: Not counted; R: Recommended.

the control line and the line in the test field can be seen; in negative cases only the control line forms[9]. The result was interpreted immediately on site at the Endoscopy Room or in the doctor's office by the same physicians (APBR and LMSK).

The patients previously diagnosed as having CD were invited to an interview with their same physician to monitor diet, answering a questionnaire concerning the adherence to a GFD[17]. The first-degree relatives of the patients were invited to participate in the study by phone call[5].

Patients with clinical complaints suggestive of CD were submitted to the point-of-care test and to intestinal biopsy plus IgA EmA determination for comparison[18].

The patients with dyspepsia, defined as persistent or recurrent pain or discomfort in the upper abdomen, were submitted to upper gastrointestinal endoscopy and biopsies by routine procedures. The mucosa of the bulb and the second portion of the duodenum was carefully studied[12,13] and, if the previous test was positive, or if there were any changes, 5 to 7 fragments were obtained for histologic evaluation and Marsh's classification[14]. In these cases IgA EmA test was also performed for correlation.

The diagnosis of IBS was based in Roma Ⅲ criteria[19]. Crohn's disease was diagnosed by clinical, endoscopic, imaging and histologic findings[20]. The final diagnosis of the other cases were based on recommended tests[3,8].

RESULTS

Table 1 shows the positivity of the Biocard Celiac-Test™ with the corresponding percentages.

The test was negative in the controls, in CD patients with strict adherence to a GFD, in the second-degree relatives, in IBS, in Crohn's disease and diarrhea of different etiologies. Positive tests were detected in 83.3% of patients with suspicion of CD, in 100% of the patients that admitted diet transgressions, in 3.8% of the first-degree relatives and in 2.9% of patients with dyspepsia.

Considering only the female gender, the positivity of the studied test was 75.0% (3/4) for patients with CD suspicion, 66.7% (4/6) for patients with GFD transgressions, 5.3% (3/56) for first-degree relatives, and 4.3% (2/46) for patients with dyspepsia.

One female (HK) was negative for the rapid test, but positive for IgA EmA. Biopsy confirmed CD. IgA anti-tTG was determined in the sera of the patient (HB) with Biocard test positive and IgA EmA negative, with a resulting antibody level of 193 U, and CD confirmed by duodenal biopsy. IgA EmA was also determined in all the individuals with Biocard test positive (n = 16); 2 were negative (12.5%) for these antibodies (Table 2).

Duodenal biopsy was performed in 15 of the 16 individuals with positive Biocard test and recommended for one first-degree relative. Lesions characterized as Marsh Ⅲ-C were seen in 11 cases or 73.3% (5 CD suspicion, 4 with GFD transgressions, 1 first-degree relative and 1 with and 1 without dyspepsia); Marsh Ⅲ-A were detected in 2 patients with GFD transgressions and in 1 patient with dyspepsia (20.0%); Marsh type I was seen in one young first-degree relative (6.7%) (mother and aunt with CD). The number of IEL was increased in all the positive individuals (100%). Table 2 shows the demographic data of these individuals in relation to the positivity of IgA EmA and the endoscopic and histological evaluations.

DISCUSSION

CD has become more common than in the past, and can

be diagnosed at any age[1]. However, it frequently remains undetected for long periods of time, because of failure by health care professionals to recognize the disorder, probably due to the variable clinical presentation or failure to perform appropriate diagnostic tests[21].

In the present study, there was a young adult preponderance in all groups, indicating that it is important to exclude CD in the differential diagnosis of gastrointestinal complaints in this age group. However, we must remember that this affliction occurs also in patients of more than 60 years (as is shown here in the case of a male, 62 years, with recent-onset diarrhea)[22].

The female preponderance, also reported by several authors in different countries, alerted us to investigate carefully women with digestive or systemic symptoms[8,23]. When we considered only female gender, we observed that the percentage of CD case findings was higher than the total number of positive individuals in first-degree relatives and in patients with dyspepsia.

In this investigation, in the CD-suspicion group, the point-of-care test was positive in 83.3% (5/6) patients, similar to the report of Korponay-Szabó *et al*[24]. It is possible that a patient can have only one CD-specific test positive, as was the case of the female patient with Biocard test negative and IgA EmA positive, or the male 62-year-old with this test positive, anti-tTG increased (193 U) and IgA EmA negative (Table 2). These cases demonstrate that more than one test is recommended if CD is suspected[4].

Patients with CD should be evaluated at regular intervals by a health care team, including a physician and a dietician, but there are no clear guidelines as to the optimal means to monitor adherence to a GFD. Dietary compliance as assessed by interview is the best marker of CD due to low cost, non-invasiveness and strong correlation with serological tests and intestinal damage[17]. Repeat serologic testing after 6 mo or more on a GFD can be helpful. The disappearance or decline of CD-specific serum antibodies during a diet is a further indication of dietary adherence and antibody testing is therefore recommended in dietary monitoring of CD (sensitivity varies from 29% to 100%)[24]. However, antibody tests might not reveal slight dietary transgressions[25]. In the present experience, the Biocard test recognized the disappearance of anti-tTG antibodies in all the patients with a GFD compliance and was positive in 14% (6/43) of the patients who admitted transgressions: they were young patients (median age 24 years), confirming the reports that adolescents had difficulties adhering strictly to the diet[26-28]. The positivity of the test presumes major dietary transgressions[25]. Notably, repeat biopsies are no longer required, but in the patients with positive tests the biopsy was performed for correlation (Table 2).

Dietary compliance varies a great deal in CD around the world. The compliance is related to the physician's recommendations but also to the collaboration of the families. It is important to know about the adherence to a GFD due to the probability of nutritional imbalances in children and adolescents[28]. So, an easy test at the office or at home can be very useful to detect transgressions because positivity rate correlates with dietary lapses[24].

The prevalence of CD in first-degree relatives of patients with CD undergoing intestinal biopsy varies from 5.5%[29] to 22.5%[30]. In a previous study with 115 Brazilian relatives, we reported 15.6% CD prevalence[5], with female preponderance, and in the present study there was 3.8% of test positivity (Table 2)[31]. All were female: 1 sister and 1 daughter of the same family; 1 mother. This mother currently presents severe dyspeptic complaints. Her positivity for the point-of-care test occurred 7 years after the first IgA EmA negative determination, showing that the relatives need to be re-evaluated periodically[32]. In some individuals, only more subtle changes of crypt lengthening with an increase in IEL, or simply an increase in IEL, are present. So, it is important that the slides be viewed by an experienced pathologist familiar with the spectrum of mucosal changes in CD. The example is the patient LGS (Table 2) with positive serologic tests, normal endoscopy and duodenal mucosa with preserved architecture, but with an increased number of IEL[15,16].

In this investigation, there was no positivity in second-degree relatives. However, this was a small group. In a previous study with more Brazilian relatives we reported 5.9% of positivity[32].

Although dyspepsia may be part of the clinical spectrum in CD patients, there are scarce data about its prevalence in silent CD[33]. Duodenal biopsy undertaken during routine upper GI endoscopy in adults has been gradually incorporated into clinical practice, and is a useful tool for the diagnosis of CD in high-risk groups such as those with anemia and/or chronic diarrhea.

In this study there was 2.9% of test positivity in dyspepsia patients, both young female patients without diarrhea (Table 1). The correlations with IgA EmA and intestinal biopsy were 100% (Table 2). This result is similar to the findings of Riestra *et al*[33] in Spain (2.2% also in women); Ozaslan *et al*[21] in Turkey (1.5% in women). In another Brazilian region, Lima *et al*[34] reported 1.4% positivity in women. In summary, CD should be kept in mind as a cause of dyspepsia during clinical assessments[35]. Serological screening can be recommended for patients with refractory dyspepsia, especially females[35]. A GFD may still bring symptomatic relief for dyspeptic symptoms in CD[36].

Diarrhea is part of the clinical spectrum of IBS; the habitual tests are normal and the nutritional state is not compromised. There is a female prevalence probably related to hormones[37-39]. In the present study 46 of 64 diarrhea patients were female (71.8%), median age 46.8 years, similar to previous reports[40]. Even when CD was suspected in IBS, none of our patients were positive for anti-tTG point-of-care test (Table 1).

Patients with Crohn's disease can present chronic diarrhea as the only manifestation. At the beginning of the evaluation, differential diagnosis with CD can be pertinent, mainly in children and adolescents. In this study 62.5% (5/8) were female, mean age 35.6 years,

almost the same characteristics of the women with CD[20,40]. None of the patients with Crohn's disease were positive for the point-of-care test (Table 1).

In the differential diagnosis of chronic diarrhea, a serological test to suggest or rule out CD is pertinent, especially in the doctor's office, to guide subsequent investigations. In 9 patients of this investigation, who were point-of-care test negative, the final diagnosis confirmed that the patients were not celiac patients[3,41,42].

In a cost-effectiveness analysis in our geographic area, south of Brazil, the cost of IgA EmA or anti-tTG is 8 times the cost of the studied test, another reason to use this screening. The same conclusions were reported by Crovella *et al*[42] in another Brazilian area (Recife, Northeast).

To conclude, in this study the results of the point-of-care test showed good correlation with positivity of IgA EmA antibodies, endoscopic and histological findings (Table 2). As the test is quick, economical, easy to perform and as reliable as the conventional serological tests in CD case finding and in diet monitoring, it can be performed on site in the physician's office and in primary care[24,43].

COMMENTS

Background

A rapid test, with a result which can be available immediately, will help the physicians to make decisions at their own offices for celiac disease.

Research frontiers

The literature only shows data in Finland populations and the authors emphasize the importance of the tissue transglutaminase antibody detection test in other geographic areas.

Applications

Due to low cost, the point-of-care test is important for celiac disease screening in developing countries.

Peer review

Overall this was a very good paper that demonstrate the value of TTG assay for diagnosis of celiac disease.

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