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ORIGINAL ARTICLE

Celiac disease serology in patients with different pretest probabilities: Is biopsy avoidable?

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Abstract

AIM: To establish the diagnostic performance of several serological tests, individually and in combination, for diagnosing celiac disease (CD) in patients with different pretest probabilities, and to explore potential serological algorithms to reduce the necessity for biopsy.

METHODS: We prospectively performed duodenal biopsy and serology in 679 adults who had either high risk (n = 161) or low risk (n = 518) for CD. Blood samples were tested using six assays (enzyme-linked immunosorbent assay) that detected antibodies to tissue transglutaminase (tTG) and deamidated gliadin peptide (DGP).

RESULTS: CD prevalence was 39.1% in the high-risk population and 3.3% in the low-risk group. In high-risk patients, all individual assays had a high diagnostic efficacy [area under receiving operator characteristic curves (AU ROC): 0.968 to 0.999]. In contrast, assays had a lower diagnostic efficacy (AU ROC: 0.835 to 0.972) in the low-risk group. Using assay combinations, it would be possible to reach or rule out diagnosis of CD without biopsy in 92% of cases in both pretest populations. We observed that the new DGP/tTG Screen assay resulted in a surplus compared to more conventional assays in any clinical situation.

CONCLUSION: The DGP/tTG Screen assay could be considered as the best initial test for CD. Combinations of two tests, including a DGP/tTG Screen, might be able to diagnose CD accurately in different clinical scenarios making biopsy avoidable in a high proportion of subjects.

Key words: Celiac disease; Serology; Gliadin peptide antibodies; Tissue transglutaminase; Antigliadin antibodies; Small bowel biopsy; Diagnostic accuracy

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INTRODUCTION

The current criterion for diagnosing celiac disease (CD) is mainly based on the presence of a characteristic enteropathy in an intestinal biopsy and evidence that these changes are gluten-triggered[1,2]. While intestinal biopsy is still considered necessary for diagnosing CD, the presence of positive CD-specific serology tests associated with enteropathy are the most commonly used surrogate markers for gluten dependency of damage[3,4]. The assessment of intestinal biopsies requires a certain level of expertise and skill, and the diagnostic accuracy can be affected by variability in sample quality and subjective interpretation[5]. Thus, while pathologists specialized in gastroenterology might have difficulties interpreting mild forms of damage; general pathologists might be susceptible to misdiagnosis, especially if a severe villous atrophy is not present[6,7].

Serological tests have been used for more than 20 years as valuable markers for screening candidates for the need of a duodenal biopsy. Clinical research has demonstrated very high sensitivity and specificity in the detection of IgA antibodies to human tissue transglutaminase [anti-tissue transglutaminase (a-tTG) and anti-endomysial antibodies (EmA)], which are now widely used for identifying patients who might require biopsy, or as a non-invasive confirmation of the CD enteropathy[6-10]. More recently, a new generation of promising assays detecting the presence of deamidated synthetic peptides of gliadin (a-DGP) have shown very high diagnostic performance equivalent to conventional tests[11,12].

As the difficulties in histological diagnosis of CD in clinical practice have been well documented[4,13,14], the appropriate use of simpler and more accurate tools would add reliability to the diagnosis of a condition with high comorbidity and mortality[15]. A new diagnostic standard based on serology alone, which could accurately detect patients in any clinical setting, has been previously suggested[16]. Thus, based on the very high positive predictive values (PPVs) of reliable serological tests, some authors have proposed that intestinal biopsy could no longer be mandatory for the diagnosis of CD in some patients[16-19].

The utility of serological tests finding the search for a new reliable and accurate diagnostic standard for CD requires additional analysis[20]. To obtain the best serological algorithm, studies need to evaluate as many tests and combinations of tests as possible. Furthermore, they should assess a diversity of patient populations, taking in consideration different relevant aspects, such as the disease prevalence and the magnitude of intestinal mucosal damage[10,21-23]. Our aims in this prospective study were threefold: (1) to determine the diagnostic effectiveness of a complete panel of CD-specific serological tests as compared against CD enteropathy in two groups of patients with different risk characteristics; (2) to analyze the performance of individual tests and two-test combinations; and (3) to explore the performance of serology-based algorithms that can potentially obviate the need for intestinal biopsies for diagnosis. The gold standard used in the study to confirm the CD diagnosis was the presence of enteropathy in duodenal biopsies, as assessed by expert gastrointestinal (GI) pathologist.

MATERIALS AND METHODS

Study design

This is a prospective, cross-sectional study on the predictive performance of a set of CD-related antibodies in the diagnosis of CD in two patient populations with different pretest probabilities for having the disorder. All study patients underwent an intestinal biopsy, regardless of the clinical, laboratory and endoscopic findings. For the serological tests, all serum samples were obtained from study subjects before the endoscopy. Endoscopy and biopsies were performed at the endoscopy units of two tertiary care institutions: the "Dr. Carlos Bonorino Udaondo" Gastroenterology Hospital in Buenos Aires and the Endoscopy Service at the HIGA "San Martín" Hospital of La Plata, Buenos Aires Province, Argentina.

Subjects

Between December 2004 and December 2006, we enrolled and performed intestinal biopsies in 679 adult subjects who underwent upper GI endoscopy. Based on their pretest probability of having CD, subjects were categorized as having either high or low risk for the disorder.

High-risk group: During the study period, 161 consecutive adults with suspected but undiagnosed intestinal disorders were enrolled in the study upon their first visit to the Small Bowel Diseases clinic at "Dr. Carlos Bonorino Udaondo" Gastroenterology Hospital. Inclusion criteria required that patients (1) were referred to the clinic because of a suspected small bowel disorder (diarrhea, weight loss, chronic iron-deficiency anemia, malabsorption signs, *etc.*), (2) had no previously known diagnosis of a GI disorder and (3) signed the informed consent. Patients with CD serology performed before the endoscopy, a previous diagnosis of CD, prior treatment with a gluten-free diet, or a former diagnosis of dermatitis herpetiformis, were

excluded from the study. Some data from this population was reported in a prior study[12]. Here, we report the results of a new test in the same population and the analysis of a combination of tests addressing our aims.

Low-risk group: We randomly selected 518 subjects from patients who had been referred for routine upper GI endoscopy because of nonspecific symptoms not primarily related to CD (heartburn, regurgitation, epigastric pain, non-ulcer dyspepsia, *etc*.). Exclusion criteria were the same as those for the high risk group. The first two patients appointed daily for the Endoscopy Unit who fulfilled the inclusion/exclusion criteria were invited to enroll in the study.

Study endpoints

The CD enteropathy was diagnosed based on currently accepted histological criterion: the presence of a type Ⅲ a or more severe enteropathy according to the Marsh's modified classification[24,25]. The final diagnosis of CD was supported by additional presence of positive anti-tTG antibodies or EmA. In addition, the clinical and/or histological response to a gluten-free diet was assessed when possible, and the typical CD-related HLA DQ was tested in some seronegative patients with enteropathy.

Endoscopic procedure and small bowel histology

All endoscopic procedures were performed by experienced endoscopists who were blinded to the clinical and laboratory data. At least three biopsy samples were obtained from a subject's descendent duodenum at different levels distal to the papilla. Morphological and quantitative assessments (intraepithelial lymphocyte -IEL-density) were performed by one experienced pathologist from one center (A.C.). Morphology was categorized according to the modified Marsh classification[25].

Celiac disease-specific serology

Serum samples were kept frozen at -20℃ until the assay was performed in only one center. The CD-related tests and cut-offs were: (1) a-tTG IgA by enzyme-linked immunosorbent assay (QUANTA Lite™, h-tTG IgA, Inova Diagnostic Inc., San Diego, CA, USA); cut-off at 20 units (U)/mL; (2) IgA and (3) IgG antibodies reacting with deamidated gliadin-derived peptides (IgA and IgG a-DGP), (QUANTA Lite™ DGP IgA or IgA. Inova Diagnostic Inc., San Diego, CA); cut-off: 20 U/mL; (4) IgA + IgG isotypes of a-DGP in a single assay (DGP Dual) (cut-off at 20 U/mL); (5) detection of IgA + IgG isotypes of both a-DGP and a-tTG in a single assay (DGP/tTG Screen) (QUANTA Lite™, h-DGP/tTG Screen. Inova Diagnostic. Cut-off: 20 U/mL); (6) IgA antiactin antibodies (AAA) (QUANTA Lite™ F-Actin IgA. Inova Diagnostic Inc., San Diego, CA; cut-off at 25 U/mL); and (7) total serum IgA by radial immunodiffusion (Diffu-Plate, Biocientífica S.A., BA, Argentina) only for cases with enteropathy but negative IgA serology tests. IgA endomysial antibody (IgA EmA) by immunofluorescence on primate esophagus substrates (INOVA Diagnostics Inc., San Diego, CA) was used only in cases with discrepancies between histology and serology. The characteristics of the tests have been reported in previous studies[11,12]. Positive tests were checked in duplicate assays.

Statistical analysis

Based on data distribution, descriptive data are reported as either mean and standard deviation (SD) or median and range. The diagnostic performance of individual serological tests was determined by calculating the sensitivity, specificity, 95% confidence intervals (95% CI), positive and negative predictive values (PPVs and NPVs), and likelihood ratios using cut-offs provided by the manufacturer and at cut-off values that would give a PPV of 100%. Data were analyzed using MedCalc® version 9.3.8.0 (MedCalc Software; Broekstraat, Mariakerke, Belgium). The area under the receiving operator characteristic curve (AU ROC) and corresponding 95% CIs were determined using MedCalc. When assessing the performance of different assay combinations, a particular combination was considered positive if both tests produced concentrations above the cut-off. Comparisons were performed using the Student's *t* test, Mann-Whitney *U* test, χ^2 test, or Fisher's exact test, as appropriate.

To explore the effectiveness of serology for predicting CD in a theoretical context of reducing the necessity of intestinal biopsy, we compared different algorithms for individual assays and two-assay combinations, in both the high-risk and low-risk groups. For individual assays, we devised an algorithm in which only patients with positive serology results would receive biopsy. For combinations of two tests, we explored an algorithm where a patient would receive a biopsy if only one of the assays was positive while the other was negative. For each algorithm, we estimated the number of true positives, false positives, false negatives, and the proportion (%) of biopsies correctly avoided.

RESULTS

Subject characteristics and CD diagnosis

The demographics and some clinical and histological features of the subjects in both groups are presented in Table 1. Compared to those with a high probability of having CD, subjects with low-risk for the disease had a significantly higher mean body mass index (BMI) ($P < 0.0001$). The prevalence of CD correlated with the pretest probability of the disease. Sixty-three (39.1%) of the 161 patients in the high-risk group were diagnosed with CD. In contrast, 17 (3.3%) of the 518 subjects undergoing routine upper GI endoscopy at the two endoscopic units (low-risk group) had a diagnosis of CD. As expected, newly diagnosed CD patients in the high-risk group had a significantly more severe clinical picture and greater degree of histological damage (*P* values between < 0.001 to < 0.0001) compared to those diagnosed in the low-risk group (Table 1).

Table 1 Demographic, clinical, and histological characteristics of subjects categorized by their pretest probability of having CD and from newly diagnosed CD patients from each subgroup

Characteristic	High pretest	Low pretest	*P* value
No. of subjects enrolled (F/M)	161 (131/30)	518 (351/167)	
Mean age (range), yr	40 (16-80)	46 (16-87)	
No. of CD patients (%)	63 (39.1)	17 (3.3)	
Body mass index			
mean ± SE (kg/m^2)	20.6 ± 3.9	25.2 ± 5.0	< 0.0001
Histological characterization of duodenal biopsy samples (Marsh's modified)[25]			
Type 0 (No. of patients)	97	495	
Type Ⅰ	0	5	
Type Ⅱ	1	1	
Type Ⅲa	4	6	
Type Ⅲb	12	4	
Type Ⅲc	47	7	< 0.0001
Newly diagnosed CD patients			
No. of patients	63	17	
Mean age (range), yr	37 (24-74)	37 (19-72)	
Body mass index			
mean ± SE (kg/m^2)	19.6 ± 3.1	23.6 ± 5.0	< 0.0001
Clinical categorization at diagnosis			
No. of patients (%)			
Classical CD	52	1	< 0.0001
Atypical CD	11	10	< 0.0010
Silent CD	0	6	< 0.0001

CD: Celiac disease.

Performance of serological tests

Individual assays in high-risk group: Some of the data collected from patients in the high-risk group in this study were reported in a previous publication[12]. Data reported here, however, includes results of the newly developed assay (DGP/tTG Screen), explores the value of combinations of two assays, and analyzes the performance of tests using theoretical cut-offs with an absolute (100%) PPV. All newly diagnosed CD patients in the high-risk group had at least one positive serology result.

Table 2 shows that sensitivities for the different assays ranged from 95.2% (for IgA a-tTG) to 100% (for the DGP/tTG Screen), except for the AAA assay (87.3%), which had the worst performance. The IgG a-DGP test had an optimal specificity and PPV (100%). Very high values of AU ROC curves were seen for all individual assays (0.968 to 0.999). Table 2 also shows the performance of assays if the cut-off values had been set to values that would give a PPV of 100%. In most assays, a slight deterioration of the sensitivity was associated with almost absolute specificity. Two assays require further consideration: the IgG a-DGP tests, which had a 100% PPV at the manufacturer's cut-off level (20 U/mL); and the AAA assay, which reached the optimal PPV at a 64 U/mL cut-off, resulting in poor sensitivity (52.4%).

Individual assays in the low-risk group: Table 2 shows the statistical performance of individual assays in the low-risk population. Using the cut-offs provided by the manufacturer, all assays had lower diagnostic efficacy compared with their performance in the high-risk group. (The AU ROC ranged from 0.835 for AAA to 0.972 for the DGP/tTG Screen). The IgA a-DGP and DGP/tTG Screen tests had high sensitivity (82.3%), and all assays had very high specificity (ranging from 88.2% to 99.0%). The PPVs were overall quite low, ranging from 17.6% for AAA to 70.6% for IgG a-DGP. The PPVs for both, the widely used a-tTG (at a cut-off value of 20 U/mL) (50.0%) and the sensitive DGP/tTG Screen (19.2%) were frustratingly low. Once again, the performance of the assays was also determined using cut-off values that would produce a 100% PPV (Table 2). The sensitivity at a 100% PPV cut-off was also disappointing for all assays. For example, the cut-off for the IgA a-tTG would be 139 U/mL, consistent with a sensitivity of 35.3%. The DGP/tTG Screen was the most sensitive test (64.7%) at this cut-off value.

Two-assay combinations in both groups

Table 3 shows the performance of some of the assay combinations in the high-risk group. As expected, the combination tests were more specific, but less sensitive, than individual tests. Furthermore, combinations had excellent AU ROC curves (0.962 to 0.984). Most of these combinations had PPVs of 100% when both assays were positive. Considering the association of two assays in the low-risk group (Table 3), the best sensitivity (82.3%) was achieved by the a-tTG plus the DGP/tTG Screen with an almost absolute specificity (99.0%) and the best NPV and AU ROC curve.

Seronegative patients with mild enteropathy and type Ⅰ damage

In the low-risk group, three patients had mild enteropathy (both with type Ⅲa villous atrophy) but a negative CD-specific serology (one case was positive for AAA and negative for the haplotype DQ2 or DQ8). She had a positive clinical response to the gluten-free diet. The remaining two cases died during the follow-up period: one from an acute myocardial infarction and the other as result of an esophageal malignancy discovered at the time of the endoscopy.

Five other patients had histological features characterized as type I of Marsh's modified classification (IELs count > 30%) and, based on the inclusion criteria, were not categorized as having a CD diagnosis. Two of these patients had a positive serology (IgA a-DGP, IgA a-tTG).

Exploring the value of tests aiming to reduce the necessity of intestinal biopsy

Based on the serology findings, we explored the diagnostic algorithms that might reduce the number of patients required to undergo the invasive diagnostic duodenal biopsy. The algorithm for the use of individual assays would avoid biopsies for patients with negative serology and require biopsy only for those with positive serology. For the theoretical algorithm using combination assays, patients

Table 2 Statistical performance of individual CD serologic tests for high- and low-risk populations at cut-offs provided by the manufacturer and when the cut-off is set for a 100% PPV

Test	% (95% CI)			(%)	
	Sensitivity	Specificity	AU ROC	PPV	NPV
High-risk population					
IgA a-tTG					
(cut-off 20 U/mL)	95.2 (86.7-99.0)	97.9 (92.8-99.7)	0.997 (0.971-0.998)	96.9	96.8
(cut-off 34 U/mL)	93.6 (84.5-98.2)	100.0 (96.3-100.0)	0.968 (0.928-0.989)	100.0	96.0
IgA a-DGP					
(cut-off 20 U/mL)	98.4 (91.4-99.7)	92.7 (85.5-97.1)	0.995 (0.968-0.999)	90.0	98.9
(cut-off 77 U/mL)	87.3 (76.5-94.3)	100.0 (96.3-100.0)	0.995 (0.968-0.999)	100.0	92.5
IgG a-DGP					
(cut-off 20 U/mL)	95.2 (86.7-99.0)	100.0 (96.2-100.0)	0.989 (0.958-0.998)	100.0	97.0
(cut-off 20 U/mL)					
DGP Dual					
(cut-off 20 U/mL)	96.8 (89.0-99.5)	99.0 (94.4-99.8)	0.995 (0.967-0.999)	98.4	97.9
(cut-off 22 U/mL)	96.8 (89.0-99.5)	100.0 (96.3-100.0)	0.984 (0.951-0.997)	100.0	98.0
DGP/tTG Screen					
(cut-off 20 U/mL)	100.0 (94.3-100.0)	92.8 (85.8-97.1)	0.999 (0.976-1.000)	90.3	100.0
(cut-off 54 U/mL)	98.4 (91.4-99.7)	100.0 (96.3-100)	0.992 (0.963-0.999)	100.0	99.0
IgA AAA					
(cut-off 25 U/mL)	87.3 (76.5-94.3)	94.9 (88.5-98.3)	0.968 (0.927-0.989)	91.9	91.8
(cut-off 64 U/mL)	52.4 (39.4-65.0)	100.0 (96.3-100.0)	0.770 (0.697-0.832)	100.0	76.6
Low-risk population					
IgA a-tTG					
(cut-off 20 U/mL)	76.5 (50.1-93.0)	97.4 (95.6-98.6)	0.921 (0.894-0.942)	50.0	99.2
(cut-off 139 U/mL)	35.3 (14.3-61.6)	100.0 (98.9-100.0)	0.706 (0.665-0.745)	100.0	97.8
IgA a-DGP					
(cut-off 20 U/mL)	82.3 (56.6-96.0)	96.2 (94.1-97.7)	0.932 (0.907-0.952)	42.4	99.4
(cut-off 313 U/mL)	35.3 (14.3-61.6)	100.0 (99.3-100.0)	0.706 (0.655-0.745)	100.0	97.9
IgG a-DGP					
(cut-off 20 U/mL)	70.6 (44.1-89.6)	99.0 (97.7-99.7)	0.926 (0.900-0.947)	70.6	99.0
(cut-off 109 U/mL)	29.4 (10.4-55.9)	100.0 (99.3-100.0)	0.676 (0.634-0.717)	100.0	97.7
DGP Dual					
(cut-off 20 U/ml)	76.5 (50.1-93.0)	95.8 (93.7-97.4)	0.963 (0.943-0.978)	38.2	99.2
(cut-off 77 U/mL)	47.1 (23.0-72.1)	100.0 (99.3-100.0)	0.735 (0.695-0.773)	100.0	98.2
DGP/tTG Screen					
(cut-off 20 U/mL)	82.3 (56.6-96.0)	88.2 (85.1-90.9)	0.972 (0.954-0.984)	19.2	99.3
(cut-off 128 U/mL)	64.7 (38.4-85.7)	100.0 (98.9-100.0)	0.824 (0.788-0.855)	100.0	97.8
IgA AAA					
(cut-off 25 U/mL)	52.9 (27.9-77.0)	91.6 (88.8-93.9)	0.835 (0.800-0.866)	17.6	98.3
(cut-off 106 U/mL)	29.4 (10.4-55.9)	100.0 (99.3-100.0)	0.647 (0.604-0.688)	100.0	97.7

The statistical performance of tests if concentrations are above the cutoff. PPV: Positive predictive value; NPV: Negative predictive value; tTG: Tissue transglutaminase; DGP: Deamidated gliadin peptide; AAA: IgA antiactin antibodies; AU ROC: Area under the ROC curve.

would not have a biopsy if both assays were congruent (either both positive or both negatives), but would have a biopsy only if the two serology results disagree (one positive and the other negative).

Table 4 shows the performance of the algorithm for single assays. In the high-risk group, the percentage of cases that would not require biopsy ranged from 56.5% to 62.7%, and the single use of DGP/tTG Screen would not miss any CD case. In the low-risk group, the use of single assays would avoid biopsy in most cases (90.3% to 96.7%). However, a substantial number of CD diagnoses (three to eight cases) would be missed, mainly because three CD patients were negative for all serology tests.

The simultaneous combination of two assays for the high-risk group would allow significant reduction in the percentage of intestinal biopsies (92.0% to 98.7%) with no case missed for any of the following four pairs of tests (a-tTG + IgG a-DGP, a-tTG + IgA a-DGP, a-tTG + DGP Dual and a-tTG + DGP/tTG Screen) (Table 5). The use of assay combinations in the low-risk group would result in a similarly high proportion of biopsies avoided (92.1% to 99.0%), but three to five CD cases would be missed.

DISCUSSION

Small bowel histology is still considered the gold standard for diagnosing CD, notwithstanding the fact that the morphological features are not specific, and that other conditions can produce similar findings[1,2]. The possibility of a noninvasive diagnostic algorithm for CD has been explored before[16-19], but no definitive standard has been established yet. Our first aim was to assess the diagnostic performance of serological tests in two patient groups

Table 3 Statistical performance of combinations of two tests in the high- and low-risk populations at the cut-off provided by the manufacturer

Test	% (95% CI)			(%)	
	Sensitivity	Specificity	AU ROC	PPV	NPV
High-risk					
IgA a-DGP + IgA a-tTG	93.6 (84.5-98.2)	99.0 (94.4-99.8)	0.963 (0.921-0.986)	98.4	95.9
IgG a-DGP + IgA a-tTG	90.5 (80.4-96.4)	100.0 (96.3-100)	0.952 (0.907-0.980)	100.0	94.0
DGP Dual + IgA a-tTG	92.0 (82.4-97.3)	100.0 (96.3-100)	0.960 (0.917-0.985)	100.0	95.0
DGP/tTG Screen + IgA a-tTG	95.2 (86.7-99.0)	100.0 (96.3-100.0)	0.976 (0.939-0.994)	100.0	96.9
IgA a-DGP + DGP/tTG Screen	98.4 (91.4-99.7)	96.9 (91.3-99.3)	0.977 (0.940-0.994)	95.4	99.0
IgG a-DGP + IgA a-DGP	95.2 (86.7-99.0)	100.0 (96.3-100.0)	0.976 (0.939-0.994)	100.0	97.0
IgA a-DGP + DGP Dual	96.8 (89.0-99.5)	100.0 (96.3-100.0)	0.984 (0.951-0.997)	100.0	98.0
IgG a-DGP + DGP Dual	95.2 (86.7-99.0)	100.0 (96.3-100.0)	0.976 (0.939-0.994)	100.0	97.0
IgG a-DGP + DGP/tTG Screen	95.2 (86.7-99.0)	100.0 (96.3-100.0)	0.976 (0.939-0.994)	100.0	97.0
DGP Dual + DGP/tTG Screen	96.8 (89.0-99.5)	100.0 (96.3-100.0)	0.984 (0.951-0.997)	100.0	98.0
Low-risk					
IgA a-DGP + IgA a-tTG	72.2 (46.5-90.2)	99.8 (98.9-100.0)	0.860 (0.827-0.889)	92.9	99.0
IgG a-DGP + IgA a-tTG	66.7 (41.0-86.6)	100.0 (99.3-100.0)	0.833 (0.798-0.864)	100.0	98.8
DGP Dual + IgA a-tTG	72.2 (46.5-90.2)	99.8 (99.3-100.0)	0.861 (0.828-0.890)	92.8	99.0
DGP/tTG Screen + IgA a-tTG	72.2 (46.5-90.2)	98.8 (97.4-99.6)	0.855 (0.822-0.884)	68.4	99.0
IgA a-DGP + DGP/tTG Screen	82.3 (56.6-96.0)	99.0 (97.7-99.7)	0.907 (0.878-0.930)	73.6	99.4
IgG a-DGP + IgA a-DGP	70.6 (44.1-89.6)	100.0 (99.3-100.0)	0.853 (0.819-0.882)	100.0	99.0
IgA a-DGP + DGP Dual	76.5 (50.1-93.0)	99.6 (98.6-99.9)	0.880 (0.849-0.907)	86.6	99.2
IgG a-DGP + DGP Dual	70.6 (44.1-89.6)	99.0 (97.7-99.7)	0.848 (0.814-0.878)	70.5	99.0
IgG a-DGP + DGP/tTG Screen	70.6 (44.1-89.6)	99.4 (98.3-99.9)	0.850 (0.816-0.880)	80.0	99.0
DGP Dual + DGP/tTG Screen	76.5 (89.0-99.5)	99.0 (96.3-100.0)	0.877 (0.846-0.904)	72.2	99.2

The statistical performance of the 10 combinations assessed is reported, considering a result to be positive if both tests of the combination have concentrations above the cutoff.

Table 4 Performance of individual assays in both risk populations in the theoretical analysis aiming to avoid duodenal biopsy when serology is negative

Individual serology tests	High-risk		Low-risk	
	Biopsy avoided (%)	Missed CD cases (*n*)	Biopsy avoided (%)	Missed CD cases (*n*)
IgA a-tTG	61.5	3	95.4	4
IgA a-DGP	57.1	1	93.6	3
IgG a-DGP	62.7	3	96.7	5
DGP Dual	61.5	2	96.0	4
DGP/tTG Screen	56.5	0	91.3	3
AAA	62.7	8	90.3	8

with different risk levels of having CD. In this context, we postulated that different assays might perform differently in populations with low pre-test risk for the disease compared to those with high-risk. We hypothesized that this could change the selection of the best serological algorithm to be used in case finding among disorders with increased prevalence of CD (e.g. chronic anemia, osteoporosis, irritable bowel syndrome, *etc.*) or for screening the general population. Notably, the use of serological tests for the selection process of cases in clinical situations with low pretest probabilities has been mostly based on the performance of assays assessed in cohort studies with post-test probabilities greater than 95%.

Considering the high-risk group, we confirmed that all the individual serological assays had very high diagnostic efficacy. Interestingly, our present study shows the DGP/tTG Screen test is the only assay with optimal sensitivity, and only the IgG a-DGP test had 100% specificity and PPV at the cut-off provided by the manufacturer. If the cut-off values were set to obtain 100% PPV, the sensitivity would be minimally reduced for the IgA a-DGP and the DGP/tTG Screen, but more profoundly affected for IgA a-tTG, IgG a-DGP and DGP Dual. The sensitivity for AAA would be reduced from 87.3% to 52.4% with the 100% PPV cut-off, making its use non recommendable in the diagnostic work-up. Therefore, our results highlight the value of the new DGP/tTG Screen, which was the most sensitive assay in detecting CD among subjects with high-risk for the disorder at both cut-offs: the value set by the manufacturers and at a 100% PPV. As far as we know, this is the second study showing the efficacy of the newly developed DGP/tTG Screen test for CD[26].

As we expected, the serological tests did not perform as well in the low-risk group. The sensitivity of individual tests varied between 52.9% and 82.3%, and the highest were again the DGP/tTG Screen and the IgA a-DGP assays. The specificity was high, ranging from 88.2% to 99.0%. As expected, the NPVs were excellent (98.3% to 99.4%) and the PPVs were disappointingly low for all the assays, ranging from 17.6% for the IgA AAA test to 70.6% for the IgG a-DGP. The commonly used IgA a-tTG assay had an unacceptable PPV of 50.0%. Once again, we assessed the performance of individual assays at cut-off values that would result in a 100% PPV. Sensitivity dropped

Table 5 Performance of combinations of two assays in both risk populations exploring the potential avoidance of duodenal biopsy if the procedure is only performed in +/- cases

Combination of two tests	High risk		Low risk	
	Biopsy avoided (%)	Missed CD cases (*n*)	Biopsy avoided (%)	Missed CD cases (*n*)
IgA a-tTG + IgA a-DGP	93.2	0	96.7	4
IgA a-tTG + IgG a-DGP	95.6	0	94.4	3
IgA a-tTG + DGP Dual	95.0	0	96.1	4
IgA-tTG + DGP/tTG Screen	92.0	0	94.4	3
IgA aDGP + DGP/tTG Screen	95.0	2	92.1	3
IgG a-DGP + IgA a-DGP	94.4	1	95.0	3
IgA a-DGP + DGP Dual	94.4	1	95.2	3
IgG a-DGP + DGP Dual	98.7	2	99.0	4
IgG a-DGP + DGP/tTG Screen	93.8	0	93.6	3
DGP Dual + DGP/tTG Screen	93.8	0	93.8	5

significantly to unacceptable values, with the highest being the DGP/tTG Screen at 64.7%. Notably, the sensitivity of the commonly used IgA a-tTG was 35.3%. Overall, these observations for patients with a low-risk for CD (with a prevalence that is intermediate between those disorders at risk and that of the general population) suggest the possibility of underdiagnosis using the most commonly employed serological algorithms. Interestingly, the sensitivity of the assays in the low-risk group was affected by the fact that three of the 17 new patients with villous atrophy (17%) had a minor damage (type IIIa) and were negative for all tests. These cases were considered as having a CD-like enteropathy. We confirmed former observations that a minor degree of damage is frequently detected in patients diagnosed from populations with low pretest probability[27]. Furthermore, our observations on the behavior of serological tests in this group are consistent with former findings showing that CD patients with a minor histological damage might have seronegative results[21,22,25]. However, confirmation of a definitive diagnosis of CD in seronegative cases (and even more in cases with lesser degree of histological damage) requires additional features indicative of gluten dependency that often are difficult to meet. This was the case with the small group identified in this study. Interestingly, one patient had a positive clinical response to the GFD, but was negative for the HLA-DQ2 and DQ8 investigated in the β1 chain. The other two cases died some time after the biopsy without having performed a GFD, one due to a myocardial infarct, and the other due to an esophageal malignancy diagnosed at the time of endoscopy. We estimate that the lack of the specific HLA alleles in the first case minimized the possibility of the patient having a gluten-triggered enteropathy[28]. However, although the inclusion of these three not well defined cases has a negative impact on the performance of serology in the low-risk population, the fact of all patients in our study have had a biopsy evaluation, makes our study a reflection of real clinical practice. We consider that although the diagnosis of these cases is uncertain, they should be included in the analysis, unlike those cases with mild inflammation (Marsh's type I) with a positive serology, which were excluded on the bases of our strict protocol.

To determine if a combination of assays could improve diagnostic accuracy, we explored the performance of all possible combinations of two serological tests, with the condition that a given combination was considered positive or negative if both assays were concordantly above or below the cut-off values, respectively. We observed that the performance of all combinations for the high-risk group was slightly lower than that of single assays, as evidenced by the AU ROC (> 0.960). However, the specificity and PPVs increased to 100% with acceptable sensitivity (above 90.5%) for all possible combinations excluding from this analysis AAA. Furthermore, we observed that combinations of the DGP/tTG Screen with either IgA a-tTG or IgA a-DGP add accuracy to the diagnosis or exclusion of CD.

In the low-risk group, all combinations had poorer performances than the single assays due to a lower sensitivity with minimally increased specificity. However, PPVs improved significantly (with most combinations approaching 100.0%) as expected. Once again, as it was for the high-risk population, the DGP/tTG Screen assay used in combination with the IgA a-DGP exhibited the best performance with acceptable sensitivity (82.3%), optimal specificity, and predictive values. Our observations are in agreement with a recent study from our group that clearly showed that the use of the DGP/tTG Screen assay enhances the sensitivity of detecting gluten sensitivity among a-tTG seronegative patients with CD-like enteropathy (including cases with dermatitis herpetiformis)[29]. Interestingly, it is well-established in clinical practice to use the IgA a-tTG test to select patients for biopsy, in both case-finding processes and in population screening studies. The performance of this popular assay in the low pretest population suggests that its use alone does not seem to be a wise strategy, because it would miss up to 23% of potential new cases.

Based on the present findings, we finally analyzed the number of cases missed or biopsy procedures avoided in the theoretical situation where serology could be used as a single non-invasive tool for diagnosing CD. With this aim, we assessed the effectiveness of two algorithms for the use of a single assay or two-assay combinations. The algorithm for single assays was devised such that biopsy should only be performed for patients with a positive test, as the pretest risk was below 50% in most clinical situations and the number of biopsies avoided would be greater. The second algorithm was designed for combinations of two serological assays, in which biopsy would be omitted if a patients had two positive or negative assays. Biopsy would be reserved for patients with conflicting results.

For the high-risk group, the DGP/tTG Screen assay was the only single test that did not miss any CD cases, and would avoid duodenal biopsy in 56.5% of subjects. In contrast, the algorithm exploring combinations of two assays was highly effective, and could avoid intestinal biopsy in more than 92% of subjects. The higher performance was seen in the combinations using DGP/tTG Screen with other assays. As expected, the serological algorithms

did less well in the low-risk group, as three cases were negatives in all assays. In this population, the two diagnostic algorithms did not differ significantly in terms of false negatives or biopsies avoided. The use of the DGP/tTG Screen and the IgA anti-DGP assays alone, in combination with each other, or in combination with other assays, would miss the three mentioned cases and avoid biopsy in 91.3% to 95.4% of subjects.

In conclusion, we suggest that appropriate use of CD serology might accurately identify the vast majority of CD patients in populations with different pretest probabilities. Furthermore, a negative serology might still miss underlying CD, but the clinical importance of the disease in such patients is probably minimal. The combination of two assays makes diagnostic accuracy higher. However, a proportion of patients (17%) in the low-risk group would be missed by all serology tests or their combinations. Definitive confirmation of CD in these seronegative cases is often difficult and doubtful, sometimes requiring long-term observation. We also confirm the additional value of the new DGP/tTG Screen assay, which resulted in a surplus to more conventional assays and should be considered as the best initial test in investigation for CD. Our study also suggests that this assay in combination with IgA a-tTG or IgA a-DGP could be used to obviate the need for duodenal biopsy in more than 92% of individuals in the high- and low-risk populations. Future validation of the algorithms is required to confirm our findings before new diagnostic guidelines are proposed.

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COMMENTS

Background

Diagnosis of celiac disease is based on a characteristic enteropathy in an intestinal biopsy and evidence that these changes are gluten-triggered. The appropriate use of simpler and more accurate tools would add reliability to the diagnosis of celiac disease. Thus, the celiac disease-related serology might have a key role in defining new diagnostic standards for celiac disease.

Research frontiers

A new diagnostic standard based on serology alone could make intestinal biopsy no longer mandatory for diagnosis of celiac disease. With this purpose in mind, the authors aimed to establish the diagnostic performance of several serological tests, individually and in combination, for diagnosing celiac disease in patients with different pretest probabilities and to explore potential serological algorithms to reduce the necessity for biopsy.

Innovations and breakthroughs

This study demonstrates that the deamidated gliadin peptide (DGP)/tissue transglutaminase (tTG) Screen assay could be considered as the best initial test for suspected CD. The authors also show that combinations of two serology tests, including the DGP/tTG Screen and IgA a-tTG or IgA a-DGP, might be able to diagnose celiac disease accurately in different clinical scenarios, and that they could diagnose or rule out the disorder, avoiding intestinal biopsy in almost 92% of subjects under study.

Applications

This study confirms the diagnostic value of the DGP/tTG Screen assay and proposes that it should be considered as the first line test in the screening algorithm for celiac disease. The combination of two tests, which include the DGP/tTG Screen and either IgA a-tTG or IgA a-DGP, strengthen the serological diagnosis of celiac disease, rendering biopsy unnecessary if both tests are congruent.

Terminology

The DGP/tTG Screen assay is a single kit that assess simultaneously the presence of both antibody isotypes (IgA and IgG), the fully synthetic selectively deamidated gliadin peptide, and the human recombinant tTG.

Peer review

Although the study seems to be an "add-on" to their original work, the methodology is adequate and it merits publication for discussion amongst the wider scientific community and for debate into the merits of saving unnecessary biopsies or in patients where gastroscopies are contraindicated/declined.

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