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Antibodies against Synthetic Deamidated Gliadin Peptides as Predictors of Celiac Disease: Prospective Assessment in an Adult Population with a High Pretest Probability of Disease

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Background: **Noninvasive serologic tests have shown high diagnostic accuracy for celiac disease (CD) in selected populations. Our aim was to determine prospectively the performance of CD-related serology in individuals undergoing intestinal biopsy because of clinical suspicion of small-bowel disorders.**

Methods: **We enrolled 141 unselected consecutive adult patients attending a small-bowel disease clinic. Patients underwent endoscopy and biopsy; serum samples were obtained at that time for measurements of anti–tissue transglutaminase (a-tTG), IgA and IgG anti–deamidated gliadin-related peptide (a-DGP), and IgA antiactin antibodies (AAAs). Characterization of patients was based on histological criteria (Marsh type II lesion or greater).**

Results: **The prevalence of CD was 42.5%. Sensitivity, specificity, and positive and negative predictive values were >90% for most assays. Diagnostic accuracy based on ROC curve analysis was similar for all assays [area under the curve (95% CI): 0.996 (0.967–0.998) for a-tTG, 0.995 (0.964–0.998) for IgA a-DGP, 0.989 (0.954–0.999) for IgG a-DGP, 0.996 (0.966–0.998) for blended conjugated of IgA + IgG a-DGP in a single assay, and 0.967 (0.922–0.990) for AAA]. The combinations of 2 tests, IgG a-DGP plus IgA a-tTG or the single blended conjugate detecting IgA + IgG a-DGP plus IgA a-tTG had 100% positive and negative predictive values if concentrations of both tests in either combination were above or below the cutoff.**

Conclusions: **In a population with high pretest probability, the newly developed a-DGP tests have diagnostic accuracy that is at least equivalent to that of established assays.**

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Intestinal biopsy is still considered the gold standard for diagnosis of celiac disease (CD)[3] *(1–4)*. For more than 30 years, CD-related serology has been used as a valuable marker for diagnosis, screening, and noninvasive follow-up of patients *(1, 5–9)*. Over the years, celiac serology has evolved, with the identification of newer and more specific antibodies and the improvement of technical approaches resulting in more reliable assays *(10)*.

Currently, the well-known endomysial (EmA) and anti–tissue transglutaminase (a-tTG) autoantibodies are considered to be among the most reliable noninvasive tools for detecting a disease in all of internal medicine *(5, 11–15)*. Although these exhibit very high sensitivity and specificity for CD *(16–21)*, recent investigations have shown that accuracy of tests remains controversial in 2 areas. First, sensitivity has been considered unacceptable both in patients with minor degrees of mucosal damage and in cases with a more indolent clinical course *(22, 23)*. Second, current antibodies seem to have variable accuracy in the follow-up of patients on a gluten-free diet *(24)*.

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[3] Nonstandard abbreviations: CD, celiac disease; EmA, endomysial; a-tTG, anti–tissue transglutaminase; DGP, deamidated gliadin-related peptide; a-DGP, anti-DGP; AAA, antiactin antibodies; IEL, intraepithelial lymphocyte.

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Recently, a series of new antibody tests have demonstrated very high sensitivity for diagnosis of CD. One of these new assays detecting IgA antibodies to the fibrillar form of the depolymerized actin protein of the cytoskeleton is promising but not completely defined *(25–27)*. Very recently, we have reported highly encouraging results with the use of ELISAs to detect antibodies binding synthetic deamidated gliadin-related peptides (DGPs). Both isotypes (IgA and IgG) of the peptide antibodies [anti-DGPs (a-DGPs)] have been shown to be highly sensitive and specific for active CD *(28–31)*.

The outstanding performance of the newer noninvasive CD serology opens the possibility that the tests can be used not only as a marker of the disease but, more importantly, as a substitute for intestinal biopsy in selected populations. Interestingly, although intestinal biopsy is still considered the gold standard for diagnosis of CD, the procedure has disadvantages—for instance, it is often refused by patients and has inherent difficulties that can lead to improper diagnosis *(1, 5)*. Thus, serology can be a very interesting adjunct or alternative to biopsy.

Most prior studies assessing the diagnostic performance of the available CD serologic tests have suffered from several potential design biases, including the use of preselected populations. Also important is the inherent interdependence between the assays' test results, the diagnostic criteria used to define CD, and the selection bias that results *(6, 7)*. Ideally, a marker for screening of a disorder must have high predictive values (both positive and negative). The predictive value of a test is determined by the sensitivity and specificity of the test and is also affected by the prevalence or pretest probability of the disorder in the assessed population. Considering all these aspects, our main objective has been to assess prospectively the performance of serologic tests in populations with different prevalence. In this study, our aim was to report on the performance of some established and newer tools for detecting CD in adult individuals with high pretest probability undergoing intestinal biopsy because of clinical suspicion of a small-bowel disorder.

Materials and Methods

PATIENTS

From December 2004 to December 2005, we studied a series of serum samples collected prospectively from a group of 141 consecutive adult patients with suspected intestinal disorders during their 1st clinic visit at the Small Bowel Diseases Clinic of the "Dr. Carlos Bonorino Udaondo" Gastroenterology Hospital. Patients considered for study inclusion underwent an upper gastrointestinal endoscopy and intestinal biopsy. Those with a previous diagnosis of CD (n = 176), prior treatment with a gluten-free diet (n = 32), CD-related serology tests performed before enrollment (n = 235), or diagnosis of dermatitis herpetiformis (n = 3) were excluded from the study. After giving informed consent, all study patients underwent intestinal biopsy irrespective of the clinical and/or endoscopic findings, and serum samples were obtained from all study patients at the time of the endoscopic procedure. Demographic and clinical data of all patients enrolled are reported in Table 1. We based the categorization of patients and controls on currently accepted histological criteria, the presence of a type II or more severe enteropathy (Marsh modified classification) *(1, 21)*. The final diagnosis of CD was supported by the additional presence of positive a-tTG antibodies or EmA and/or the histological response to a gluten-free diet. Patients were categorized according to the clinical status at the time of diagnosis as presenting with classic symptomatic disease (mainly gastrointestinal symptoms); an atypical form (subclinical CD) such as chronic anemia, hypertransaminasemia, or autoimmune diseases; or a silent clinical course (asymptomatic CD). The latter group consisted of patients detected during evaluation of 1st-degree relatives of index cases *(32)*.

CD-RELATED SEROLOGY

Serum samples were kept frozen at −30 °C until assays were performed. Tests were performed by personnel who did not have knowledge of the diagnoses. The following 5 assays were used. (*a*) IgA a-tTG (Quanta Lite™ h-tTG IgA, Inova Diagnostic) by ELISA (cutoff provided by the manufacturer, 20 kU/L) was used. (*b*) For patients with negative a-tTG test and controls with positive a-tTG antibodies, we further tested for IgA EmA by immunofluorescence on primate esophagus substrate (Inova Diagnostics) tested at a 1:5 dilution. (*c*) Newly developed ELISAs were used to separately detect IgA and IgG antibodies reacting with a fully synthetic, selectively glutamine-to-glutamate substituted gliadin-analogous peptide that incorporates several B-cell epitopes (IgA a-DGP and IgG a-DGP) by use of a reagent set provided by the manufacturer for research use only (Quanta Lite Gliadin IgA and IgG II, Inova Diagnostic; cutoff determined in a former study, 20 kU/L) *(31)*. (*d*) A single reagent set was used to assess simultaneously the presence of both antibody isotypes (IgA + IgG a-DGP; Quanta Lite Celiac DGP Screen; Inova Diagnostic; cutoff provided by the manufacturer, 20 kU/L). Assays (*c*) and (*d*) use as antigen the same gliadin-analogous peptide described. The peptide is constructed so that each epitope is pre-

Table 1. Demographic and clinical data of the overall population enrolled.

n	141
Median age (range), years	38 (16–80)
Female/male, n	114/27
Mean body mass index (SD), kg/cm^2	20.6 (3.2)
Symptom inducing consultation, n	
Chronic diarrhea	114
Weight loss	115
Chronic anemia	95
Distension	11

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sented in a proper conformational shape. The conjugate is a blend of both antihuman IgA and IgG, with most of the reactivity biased toward the IgG (approximate IgG vs IgA ratio, 70:30). (*e*) IgA-type antiactin antibodies (AAAs) determined by use of a modification of a commercial ELISA assay for IgG type AAAs (Quanta Lite Actin; Inova Diagnostics) and an antihuman IgA conjugate (Inova Diagnostic) were used. Serum samples were studied at 1:101 dilutions. According to our previous study, the cutoff for normal AAA concentration was 25 kU/L *(27)*.

All reagents were generously provided by the manufacturer.

ENDOSCOPIC PROCEDURE AND SMALL-BOWEL HISTOLOGY

We obtained biopsy samples from the distal duodenum by duodenoscopy, following a standard protocol *(31)*. All procedures were performed by the same operator (J.A.) blinded to the clinical and laboratory data. Endoscopic examination of the 2nd duodenal portion was reported with and without air insufflations. The endoscopic markers evaluated were scalloped duodenal folds, mosaic pattern, and reduction in the number of folds *(33)*. Their performance in the suspicion of CD will be reported separately. At least 3 samples were obtained using conventional endoscopic forceps (no enrichment, open cup: 8 mm). Samples were oriented carefully on paper, fixed in 10% formalin, embedded in paraffin wax, and conventionally stained with hematoxylin and eosin.

Morphology and quantitative assessments [intraepithelial lymphocyte (IEL) density] were performed by 1 of 2 experienced observers (A.C. and Z.K.) who were unaware of the clinical and laboratory findings of the patients. Morphology was categorized according to the modified Marsh classification *(1, 22)*. Briefly, type 0 is normal mucosa; type I is an infiltrative stage marked by a normal mucosal architecture in which the villous epithelium has intraepithelial lymphocytosis (>30 IELs/100 epithelial cells). Type II shows the addition of enlarged crypts (hyperplasic stage), and type III comprises a large spectrum of changes ranging from minor villous atrophy to complete villous atrophy (subcategorized as Marsh IIIa, IIIb, and IIIc). Qualitative and quantitative findings are listed here as reported by each observer.

ETHICS AND STATISTICS

The protocol was approved by the Research and Ethical Committees of the Gastroenterology Hospital. Data were analyzed using Statistix 7 for Windows Analytical Software (2000 Analytical Software). According to data distribution, results are reported as mean (SD) or median and range, and statistical analyses were used as appropriate. The diagnostic performance of single tests was determined by comparing sensitivity, specificity, 95% CIs, positive and negative predictive values, and likelihood ratios calculated using conventional formulas (Medcalc, version 9.2.1.0). The area under the curve for the ROC curves and their 95% CIs were determined by use of Medcalc. The performance of different combinations of assays reported (e.g., a-DGPs plus a-tTG) was assessed taking into account that a given combination of tests is considered positive if at least 1 of the 2 tests has concentration above the cutoff and negative if both tests are below the cutoff. Comparisons were performed using the Student *t*-test, Mann–Whitney *U*-test, χ^2 test, or Fisher exact test, as appropriate.

Results

DIAGNOSTIC CHARACTERIZATION OF PATIENTS AND CLINICAL FINDINGS

With histological criteria used as the gold standard, CD enteropathy was diagnosed in 60 patients. The remaining 81 patients had no histological evidence of active CD. As expected and according to the design of the protocol, we detected CD only in patients with symptomatic disease (patients with classic symptoms or atypical manifestations; Table 2). The very high prevalence of CD in the population enrolled was, as expected, very similar to that reported by our group in previous publications. Despite the fact that we avoided preselection bias by excluding patients in whom CD was previously suspected or diagnosed, the high prevalence could be due to the fact that our referral clinic receives only patients with small-bowel and diarrhea disorders. As reported in Table 2, the mean body mass index of CD patients was significantly lower than that of control individuals ($P < 0.002$). Table 2 also shows the histological characteristics of small-bowel biopsies according to the modified Marsh classification.

Table 2. Comparison of demographic data, clinical characteristics, and histological features of CD patients and controls.

	CD	Control
n	60	81
Female/male, n	53/7	62/19
Mean age (range), years	36 (19–72)	38 (16–80)
Mean body mass index (SD), kg/cm^2	19.3 (3.2)	21.5 (4.2)[a]
Clinical categorization of CD		
Classic	51	
Subclinical	9	
Asymptomatic	0	
Marsh classification, n		
Type 0	0	80
Type I	0	
Type II	0	
Type IIIa	2	1[b]
Type IIIb	9	
Type IIIc	49	
Mean IELs/100 epithelial cells (SD)	40.8 (15.5)	11.2 (4.4)[c]

[a] $P < 0.002$.
[b] Control case with a type IIIa histology had a gastrojejunal anastomosis with small-intestinal bacteria overgrowth.
[c] $P < 0.00001$ (Student *t*-test).

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Table 3. Patients with a positive test and concentrations (kU/L) of serology tests in CD patients and controls.

	CD	Control
n	60	81
Serology tests		
IgA a-DGP		
Positive, n	59	5
Mean concentration (SD)	277.4 (168.9)	6.5 (10.5)[a]
IgG a-DGP		
Positive, n	58	0
Mean concentration (SD)	121.1 (54.9)	2.0 (2.8)[a]
IgA + IgG a-DGP		
Positive, n	59	1
Mean concentration (SD)	176.8 (86.2)	2.0 (3.6)[a]
IgA a-tTG		
Positive, n	57	2
Mean concentration (SD)	142.8 (99.2)	6.9 (6.2)[a]
AAA		
Positive, n	52	4
Median concentration (range)	143.3 (12.0–550.0)	11.9 (1.0–64.0)[a]

[a] $P < 0.00001$ (Student *t*-test).

Only a minority of patients had mild histological changes (Marsh type II and IIIa); most patients showed severe villous atrophy (Marsh IIIb or IIIc). No CD patient or control had histological evidence of Marsh type I lesion, and the highest IEL density determined in controls was 23.3%. All these features confirm that the newly diagnosed CD population represents a more severe end of the clinical spectrum of the disorder. Three patients had negative a-tTG and EmA tests, and the diagnosis of CD was supported by improvement of the intestinal mucosa at rebiopsy.

ANTIBODY TESTING

Our serology assessment did not detect patients or controls with IgA deficiency. Table 3 shows the number of patients and controls with positive tests and the mean concentrations for all serology tests assessed. Compared with controls, CD patients had highly significantly greater absolute values for all assays assessed ($P < 0.00001$ for all comparisons). Tables 4 and 5 show results of individual tests expressed in the binary form (positive or negative). Interestingly, whereas 59 of 60 patients had >1 positive test, 1 case was positive only for IgA a-tTG antibody and had a Marsh IIIb lesion. In contrast, 10 controls had only 1 positive test, and 1 case had 2 positive tests (IgA a-DGP and IgA a-tTG). This individual was IgA EmA negative and presented with a nonatrophic histology with normal IEL density. Final diagnoses in non-CD patients with positive serology were undetermined in 3; irritable bowel syndrome in 4 (2 of these were 1st-degree relatives of CD patients); and colorectal cancer, choleretic diarrhea, and chagasic megacolon in 1 patient each. In these tables, we can also establish the frequency of positive and negative results for combinations of tests in both patients and controls to determine the best combinations and whether this approach is better than using a single determination of 1 assay.

Table 6 shows the statistical analysis of all serology tests assessed. Both IgA a-DGP and the dual conjugate a-DGP had a diagnostic sensitivity of 98.3%. Each test missed only 1 patient. The IgG a-DGP was negative in all controls. In addition, the dual-conjugate test (IgA plus IgG a-DGP) had only 1 false-positive result. Thus, the positive predictive value was 100% for IgG a-DGP and 98.3% for the IgA plus IgG a-DGP test. Furthermore, the highest point estimates of positive and negative likelihood ratios were produced by the same assays.

We analyzed the performance of different combinations of 2 tests, considering a result to be positive if at least 1 of the assays produced a concentration above the cutoff and negative if both were below the cutoff. Thus, the associations of (*a*) IgG a-DGP plus IgA a-tTG or (*b*) the dual-specificity conjugate (IgA plus IgG a-DGP) plus IgA a-tTG exhibited 100% sensitivity and negative predictive value (Tables 4–6). On the other hand, if we consider only cases with both assays positive (concentrations above the cutoff), the positive predictive value increases to 100% for both combinations, with sensitivities of 93.3% and 91.7%, respectively.

Discussion

Screening and diagnostic algorithms to detect CD are based on the sequential use of serologic tests followed by a confirmatory intestinal biopsy *(20, 21, 34)*. CD is a unique autoimmune disorder in which very specific serological testing has demonstrated high clinical utility. In

Table 4. Positive (+) and negative (−) results for the different tests in the CD subgroup.

n[a]	IgA a-DGP	IgG a-DGP	IgA + IgG a-DGP	IgA a-tTG	IgA AAA	Biopsy
50	+	+	+	+	+	+
5	+	+	+	+	−	+
2	+	+	+	−	−	+
1	+	+	+	−	+	+
1	+	−	+	+	+	+
1	−	−	−	+	−	+

[a] n, number of patients with the given combination.

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Table 5. Positive (+) and negative (−) results for different tests in individuals without CD.

n	IgA a-DGP	IgG a-DGP	IgA + IgG a-DGP	IgA a-tTG	IgA AAA	Biopsy
70	−	−	−	−	−	−
4	−	−	−	−	+	−
4	+	−	−	−	−	−
1	+	−	−	+	−	−
1	−	−	+	−	−	−
1	−	−	−	+	−	−

the last 30 years, a series of alimentary antibodies (antigliadin) and autoantibodies (antireticulin, a-tTG, and EmA) have been successfully used for detecting CD. Recently, newly developed assays such as AAA and a-DGP have increased interest in the use of these noninvasive serological tools *(25–31)*. In this prospective study, we aimed to determine the performance of individual tests or combinations of these tests that could result in cost-effective selection of cases referred to a specialized center. Furthermore, although the very high prevalence of the disease in our population imposes some limitations to the interpretation of results, these newer assays have high predictive values, and their use could minimize the often-refused option of endoscopy and intestinal biopsy.

Because EmA is a highly operator-dependent assay *(6, 7)* and has a history of a lack of sensitivity *(22–24)*, we used it only to resolve conflicting results such as a-tTG–negative patients or positive controls *(19)*. We also decided to not include standard AGA assays because of their well-known lack of sensitivity and specificity.

Considering the performance of individual tests, all newer serology tests evaluated exhibited results similar to those reported in studies of other populations. Although without statistically significant differences, the a-DGP tests had especially impressive results, such as those obtained with the widely used and reliable a-tTG. This study confirmed the high diagnostic accuracy for the IgG isotype reported in our earlier study *(30)* and contrasts with findings reported for other antibodies (including traditional gliadin antibodies, EmA, and a-tTG).

In the present study, we tested for the 1st time the performance of a newly developed assay to detect both isotypes (IgA and IgG) of the a-DGP simultaneously. This new test was designed on the basis of our prior observation that some patients are positive only for the IgG a-DGP antibody whereas others are positive only for the IgA antibodies against DGP *(31)*. On the basis of the high sensitivity and extremely high specificity of the IgG isotype of the a-DGP antibody *(30)*, the single conjugate set was blended in favor of IgG (70% vs 30% for the IgA). As expected, the blended conjugate was very useful, correctly identifying 98.3% of CD cases and 98.8% of individuals without CD.

Interestingly, only 11 individuals in the control group had any false-positive results. Whereas 1 had 2 positive assays (a-tTG and IgA a-DGP; the final diagnosis was irritable bowel syndrome), 10 presented only 1 positive test. In our critical analysis of these false-positive cases it seems likely that in 2 or more of these controls the positive serology might indicate the potential for developing CD.

Table 6. Statistical performance of individual CD serologic tests and some combinations.[a]

Test	Sensitivity, %	Specificity, %	PPV,[b] %	NPV, %	PLR	NLR	AUC/ROC
IgA a-DGP	98.3	93.8	92.2	98.7	15.9	0.02	0.995
95% CI	91.0–99.7	86.2–97.9					0.964–0.998
IgG a-DGP	96.7	100.0	100.0	97.6	NC	0.03	0.989
95% CI	88.4–99.5	95.5–100.0					0.954–0.999
IgA + IgG a-DGP	98.3	98.8	98.3	98.8	79.6	0.02	0.996
95% CI	91.0–99.7	93.3–99.8					0.966–0.998
IgA a-tTG	95.0	97.5	96.6	96.3	38.5	0.05	0.996
95% CI	86.1–98.9	91.3–99.6					0.967–0.998
IgA AAA	86.7	95.1	92.9	90.6	17.5	0.14	0.967
95% CI	75.4–95.2	87.8–98.6					0.922–0.990
IgA a-DGP + IgA a-tTG	100.0	92.6	90.9	100.0	13.5	0	
95% CI	94.0–100.0	84.6–97.2					
IgG a-DGP + IgA a-tTG	100.0	97.5	96.7	100.0	40.0	0	
95% CI	94.0–100.0	91.3–99.6					
IgA + IgG a-DGP + a-tTG	100.0	96.3	95.2	100.0	27.0	0	
95% CI	94.0–100.0	89.5–99.2					

[a] The statistical performance of the 3 combinations assessed is reported considering a result to be positive if at least 1 of the 2 tests has a concentration above the cutoff and negative if both are below the cutoff.

[b] PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; AUC, area under the curve.

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The evaluation of combinations of 2 different assays also shows very interesting results. If we take into account that a given combination of tests is considered positive if at least 1 of the 2 tests has a concentration above the cutoff and negative if both tests are below the cutoff, all 3 combinations assessed have absolute sensitivity (100%) and specificity comparable to single assays. However, if we consider as positive a finding that both assays were above the cutoff, the associations of either the blended conjugate of IgA plus IgG a-DGP plus IgA a-tTG or IgA a-tTG plus IgG a-DGP had a positive predictive value of 100%. This serological approach allowed identification of 93% and 92% of new cases for each option, respectively. In addition, no patients with negative results for both assays of the given combination were found to have CD. These final observations suggest that these combinations could obviate the need for intestinal biopsy in 95% of patients in this clinical scenario. Unfortunately, we could not explore the utility of single or combined tests in the identification of IgA-deficient CD cases because we did not encounter this situation in our study population.

In conclusion, this prospective study on a high pretest probability population showed that the newer noninvasive serology tests are highly accurate markers of CD. The newly developed a-DGP test, alone or in combination with the more commonly used a-tTG test, can potentially be used in many cases to avoid intestinal biopsy, thus having an impact on cost savings and better acceptance by patients.

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