

## Publication of the Month

November 11/11: Usefulness of deamidated gliadin peptides

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**Key messages:**

- Screening for anti-DGP IgA and IgG is a promising tool in detecting early stage celiac disease
  - Anti-DGP IgA and IgG is useful to monitor the compliance with GFD in childhood CD
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**Antibodies against deamidated gliadin peptides in early-stage celiac disease**

*J Clin Gastroenterol* 2011;45:673-8

**Background:** Early stage celiac disease (CD) is hardly detectable by biopsy and endomysial (EmA) or transglutaminase 2 (anti-tTG) antibodies may also remain negative. The determination of antibodies against deamidated gliadin peptides (anti-DGP) could be a useful tool for diagnosis in such cases and are also important for follow-up.

**Summary:** In this study the sensitivity of anti-DGP was superior to anti-tTG and comparable to EmA in patients having early-stage celiac disease with normal villous morphology. Testing for IgA and IgG-class anti-DGP offers important benefits like the detection of monospecific antibodies or IgG-class antibodies in IgA deficient patients. The results show that CD specific antibodies occur and can be detected before mucosal damage.

**Conclusions:** This study showed that the combined testing for IgA and IgG-class anti-DGP is a promising new method for case-finding and follow-up in early-stage celiac disease.

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Monzani A, Rapa A, Fonio P, et al

**Use of deamidated gliadin peptide antibodies to monitor diet compliance in childhood celiac disease**

*J Pediatr Gastroenterol Nutr* 2011;53:55-60

**Background:** Anti-tTG IgA levels seem to be less reliable than duodenal biopsy in monitoring the compliance with gluten-free diet (GFD) in children with celiac disease. However, the determination of anti-DGPs could be useful to follow-up the diet success.

**Summary:** During the first year of GFD anti-DGP IgA and anti-DGP IgA+G were found to be reliable tools for monitoring. Both showed a higher sensitivity than anti-tTG and anti-gliadin antibodies IgA in monitoring diet compliance.

**Conclusions:** Anti-DGP showed to be useful for monitoring the compliance with GFD. Combining anti-DGP IgA and IgG seems to perform better than anti-DGP IgA alone. Nevertheless, anti-DGP did not outperform anti-tTG IgA for CD screening.

**Comment:** These two articles demonstrate that deamidated gliadin peptide antibodies of the IgA and IgG isotypes are useful markers for diagnosis of early stage CD and for monitoring the compliance with GFD in childhood CD.



# Antibodies Against Deamidated Gliadin Peptides in Early-stage Celiac Disease

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**Background and Goals:** The widely used serum endomysial (EmA) and transglutaminase 2 (TG2-ab) antibodies predict forthcoming villous damage and celiac disease when the small-bowel mucosa structure is still normal. However, these autoantibodies may remain negative in this early stage of the disease. We hypothesized that the antibodies against deamidated gliadin peptides (DGP-AGA) might appear before the other antibodies and would thus be useful in the diagnosis and follow-up of patients with early-stage celiac disease.

**Study:** Serum DGP-AGA, TG2-ab, and EmA were measured at baseline and after 1 year on a gluten-free diet in 42 adults proven to have early-stage celiac disease despite normal small-bowel mucosal morphology (Marsh I-II), and in 20 celiac subjects evincing villous atrophy (Marsh III). Thirty-nine subjects with no signs of celiac disease served as nonceliac controls.

**Results:** Sensitivity to detect early-stage celiac disease was 79% for DGP-AGA, 64% for TG2-ab, and 81% for EmA. Specificities were 95%, 100%, and 100%, respectively. The corresponding efficiencies of the tests were 89% for DGP-AGA, 81% for TG2-ab, and 91% for EmA. All 3 antibodies were significantly decreased on a gluten-free diet.

**Conclusions:** This study showed that the sensitivity of DGP-AGA was superior to TG2-ab and comparable to EmA in celiac patients having early-stage celiac disease with normal villous morphology. On the basis of these results, DGP-AGA would seem to offer a promising new method for case-finding and follow-up in this entity.

**Key Words:** celiac disease, early-stage, antibodies to deamidated gliadin peptides, endomysial antibodies, transglutaminase 2 antibodies

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The current diagnostic criteria for celiac disease are based on the demonstration of small-bowel mucosal villous atrophy and crypt hyperplasia.<sup>1</sup> However, the mucosal damage develops gradually from inflammation (Marsh I) to crypt hyperplasia (Marsh II) and finally to villous atrophy (Marsh III)<sup>2</sup>; the latter representing only the end-point in the diverse clinical presentation of the disorder.<sup>3</sup> Highly specific serum endomysial (EmA) and transglutaminase 2 (TG2-ab) antibodies may appear while the villous morphology is still normal.<sup>4,5</sup> Although such cases are often considered false-positive, there is evidence that this antibody-positivity may actually predict forthcoming celiac disease.<sup>6,7</sup> Furthermore, patients with positive EmA but only Marsh I to II can suffer from a similar gluten-dependent disorder as those having Marsh III, indicating that early recognition of these gluten-sensitive cases would be important.<sup>8,9</sup> In contrast, it has been suggested that EmA and TG2-ab lack sensitivity at lesser degrees of mucosal damage<sup>10,11</sup> and might not thus be optimal for the detection of early-stage celiac disease.

In celiac disease, the immune system reacts against food-derived gluten peptides by producing antibodies against whole gliadin.<sup>12</sup> Earlier these antibodies were commonly applied in case-finding, but because of low specificity they are no longer in use.<sup>13</sup> A necessary step in the disease pathogenesis is a process where TG2 deamidates gliadin to form more specific epitopes to the immune system,<sup>14,15</sup> and antibodies against these deamidated gliadin peptides (DGP-AGA) have been shown to be accurate indicators in celiac disease with villous atrophy and crypt hyperplasia.<sup>16–18</sup> Interestingly, DGP-AGA might also be among the first antibodies to appear in the sera of subjects, who later develop celiac disease.<sup>19–22</sup> It has been speculated that the immune response against food-derived gliadin peptides occurs first, whereas the tissue-targeted TG2-ab and EmA would be produced only later through epitope spreading and molecular mimicry.<sup>23,24</sup> This hypothesis is supported by data showing that DGP-AGA from celiac patient sera are able to bind TG2 and thus share common epitopes.<sup>24</sup> These observations indicate that DGP-AGA might offer a promising new method of identifying early-stage celiac disease.

The aim of this study was to compare DGP-AGA to the widely used TG2-ab and EmA in the diagnosis and follow-up of subjects in an early stage of celiac disease with normal small-bowel mucosal morphology. The control groups comprised comparable patients with villous atrophy and crypt hyperplasia and subjects with no signs of celiac disease.

## MATERIALS AND METHODS

### Patients and Study Design

The study was conducted at the Department of Gastroenterology and Alimentary Tract Surgery in Tampere University Hospital. The early-stage celiac disease group ( $n = 42$ ) comprised 2 subgroups of participants with normal small-bowel mucosal villous structure while on a gluten-containing diet. The first subgroup comprised 16 cases who evinced normal small-bowel mucosal morphology (Marsh I to II) in the first biopsy specimens, but when the gluten consumption was continued, developed mucosal villous atrophy and crypt hyperplasia (Marsh III) diagnostic for celiac disease.<sup>8,25</sup> Ultimately, in these cases on a gluten-free diet, the mucosal damage was again abolished; this further confirming their diagnosis. The serum samples collected at the time of the first investigation, while the mucosal structure was normal (Marsh I to II), represented early-stage (latent) celiac disease. The second subgroup contained 26 early-stage celiac disease patients collected from our earlier studies.<sup>7,26,27</sup> These cases had been investigated because of suspicion of celiac disease, but had normal mucosal villous morphology (Marsh I to II). However, they had either human leukocyte antigen (HLA) DQ2 or DQ8 genotype and the increased density of CD3<sup>+</sup> ( $> 37$  cells/mm) and  $\gamma\delta$ + ( $> 4.3$  cells/mm) intraepithelial lymphocytes (IELs) characteristic for celiac disease,<sup>28–30</sup> and after clinical and histologic response to a gluten-free diet, the diagnosis of early-stage celiac disease was established. The differentiation between Marsh I and II is often difficult from histologic samples and the intraobserver variation is high.<sup>31</sup> Therefore, distinction was made between Marsh I-II and Marsh III.

Twenty adults diagnosed as suffering from classic celiac disease with small-bowel mucosal villous atrophy and crypt hyperplasia (Marsh III) served as disease controls (villous atrophy group). The nonceliac control group comprised 39 subjects who had been investigated because of gastrointestinal symptoms or signs of malabsorption, but had normal small-bowel mucosal structure while on a gluten-containing diet in a follow-up time up to 9 years.

The serologic samples were drawn both at baseline and at every follow-up visit from all study participants and stored at  $-70^{\circ}\text{C}$  until tested and used for the subsequent antibody measurements.

### Small-bowel Mucosal Morphology and IELs

Upper gastrointestinal endoscopy was performed in all participants and the mucosal morphology was determined from at least 3 separate biopsy specimens taken from the distal duodenum. The villous structure was further evaluated in detail by measuring the villous height-crypt depth ratio as a mean of at least 5 well-orientated villous-crypt pairs; a ratio less than 2.0 was regarded compatible with Marsh III and celiac disease.<sup>27</sup> Immunohistochemical stainings were performed using 5- $\mu\text{m}$ -thick frozen sections from small-bowel mucosal biopsy specimens. CD3<sup>+</sup> IELs were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA) and  $\gamma\delta$ + IELs with T-cell receptor- $\gamma$  antibody (Endogen, Woburn, MA). Positive IELs were counted with a 100 $\times$  flat-field light microscope objective throughout the surface epithelium and expressed as cells/mm of epithelium.<sup>30</sup>

### Serology and HLA-typing

An enzyme-linked immunosorbent assay was used for the serum IgA and IgG-class DGP-AGA (QuantaLite Celiac DGP Screen; Inova Diagnostics, San Diego, CA) and for the serum IgA-class TG2-ab (Celikey; Phadia, Freiburg, Germany) measurements. Unit (U) values of  $\geq 20$  U for DGP-AGA and  $\geq 5.0$  U for TG2-abs were considered positive according to the manufacturer's instructions. Serum IgA-class EmA titers were determined by an indirect immunofluorescence method using human umbilical cord as substrate; a dilution of 1: $\geq 5$  or more was considered positive.<sup>32,33</sup> Positive sera were further diluted 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000, and 1:4000. The celiac-type HLA was determined using either the DELFIA Celiac Disease Hybridization Assay (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) or the SSP DQB1 low resolution kit (Olerup SSP AB, Saltsjöbaden, Sweden) according to the manufacturer's instructions.

### Statistics

Quantitative data were expressed as means or as medians and ranges. The sensitivities and specificities of the antibodies were calculated using standard statistical methods.<sup>34</sup> The efficiencies of the tests were calculated from the ratio of the sum of true-negative and true-positive tests results and the sum of all untreated celiac disease and nonceliac disease patients.<sup>34</sup> Furthermore, cut-off values for the best possible efficiencies of the DGP-AGA and TG2-ab were calculated using receiver operating characteristics (ROC) analysis. A paired Student *t* test or Wilcoxon signed rank test was used to compare changes in the antibody values within the groups on treatment and a *P* value of less than 0.05 was considered statistically significant.

### Ethical Considerations

The study protocol was approved by the Ethics Committee of Tampere University Hospital. All subjects gave written informed consent.

## RESULTS

The study groups were comparable in respect of age, sex, primary reason for endoscopy, and family history of celiac disease (Table 1). All participants in the early-stage celiac disease and villous atrophy groups had the HLA DQ2 or DQ8 genotype, whereas only 21 (54%) of the 39 nonceliac control subjects were DQ2 or DQ8-positive. None of the participants had selective IgA deficiency.

The most sensitive single test for early-stage celiac disease was EmA, which found 35 (83%) of the 42 subjects (Fig. 1, Table 2). The sensitivity of DGP-AGA was 79% and that of TG2-ab was 64%. The best overall sensitivity (93%) could be achieved by a combination of DGP-AGA and EmA, missing only 3 of the 42 early-stage celiac disease patients. In the villous atrophy group, the sensitivity was 95% for DGP-AGA and 100% for both TG2-ab and EmA (Fig. 1, Table 2). There were 2 DGP-AGA-positive subjects in the nonceliac control group, giving a corresponding specificity of 95%. Both of these positive cases had the HLA-DQ2 genotype and an increased density of mucosal  $\gamma\delta$ + IELs, but the small-bowel mucosal morphology and density of CD3<sup>+</sup> IELs were normal. The specificities of both EmA and TG2-ab were 100% (Fig. 1, Table 2).

EmA showed the best efficiency of a test for early-stage celiac disease, whereas DGP-AGA had slightly and TG2-ab showed considerably lower efficiency (Table 2). The best

**TABLE 1.** Demographic Data, Primary Symptoms, Vh/CrD, and Presence of Celiac-type HLA in the Participants at Baseline

	Early-stage Celiac Disease (n = 42)	Villous Atrophy Celiac Disease (n = 20)	Nonceliac Controls (n = 39)
Female, n (%)	29 (69)	16 (80)	28 (72)
Age (y), median (range)	48 (13-70)	41 (20-71)	46 (17-63)
Primary reason for endoscopy, n (%)			
Abdominal symptoms	31 (72)	12 (60)	30 (77)
Malabsorption or anemia	4 (9)	5 (25)	6 (15)
Extraintestinal symptoms*	2 (5)	1 (5)	1 (3)
Risk group for celiac disease†	5 (12)	2 (10)	2 (5)
Family history of celiac disease, n (%)	14 (33)	5 (25)	6 (15)
Vh/CrD, mean (range)‡	2.8 (2.0-4.5)	0.3 (0.0-0.7)	3.3 (2.3-4.7)
Presence of HLA-DQ2 or DQ8, n (%)	42 (100)	20 (100)	21 (54)

\*Arthritis, delayed growth, gynecologic disorders, and osteoporosis.

†Type I diabetes mellitus, autoimmune thyroid disease, Sjögren syndrome, and family history of celiac disease.

‡Normal value  $\geq 2.0$ .<sup>27</sup>

HLA indicates human leukocyte antigen; Vh/CrD, villous height/crypt depth ratio.

overall efficiency could be obtained by a combination of either DGP-AGA and EmA or TG2-ab and EmA. Using ROC analysis, it was noted that by lowering the cut-off value of DGP-AGA to 13 U, an efficiency of 93% (sensitivity 90% and specificity 95%) could be achieved. Similarly, in the TG2-ab a cut-off value of 4.0 U would give an efficiency of 89% (sensitivity 81% and specificity 97%) for early-stage celiac disease.

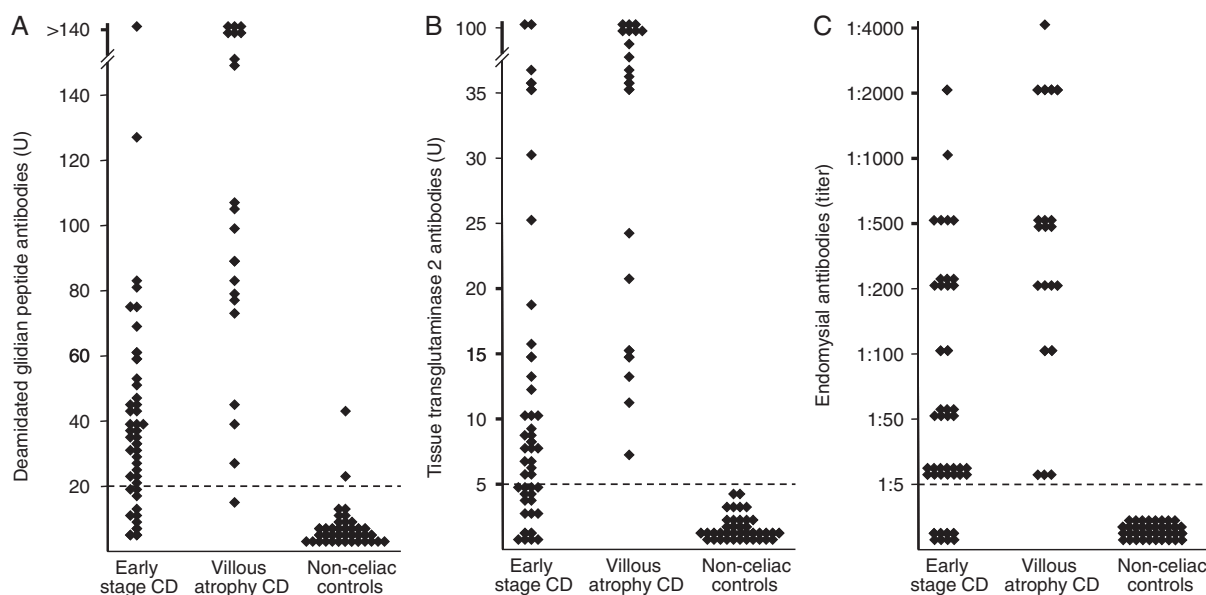
During 1 year on a gluten-free diet, the DGP-AGA, TG2-ab, and EmA values decreased significantly and equally in both the early-stage celiac disease and the villous atrophy groups (Fig. 2).

### DISCUSSION

The constantly increasing screening from at-risk groups discovers more and more subjects having a strong clinical suspicion of celiac disease but normal small-bowel

mucosal villous morphology (Marsh I to II). At the same time, evidence is mounting that at least some of these individuals may either benefit of an early introduction of a gluten-free diet, or will develop diagnostic mucosal lesion (Marsh III) if the gluten consumption is continued.<sup>6-9,25,26,35,36</sup> These results show that there is an urgent demand for new reliable methods to find such patients having an early-stage celiac disease.

In this study, EmA showed the best sensitivity and efficiency for early-stage celiac disease, but results with DGP-AGA were almost good. The rather poor sensitivity of TG2-ab when compared with EmA was somewhat surprising, as both of these autoantibodies are targeted against TG2 and have yielded comparable results in classic celiac disease with villous atrophy and crypt hyperplasia.<sup>24,37</sup> EmA were measured by human umbilical cord based in-house test, but we have earlier compared the method with commercial EmA using primate tissue as



**FIGURE 1.** Serum deamidated gliadin peptide (A), transglutaminase 2 (B), and endomysial antibodies (C) at baseline. The dotted horizontal line represents the cut-off value for the antibody in question as recommended by the manufacturer. CD indicates celiac disease.

**TABLE 2.** Sensitivities, Specificities, and Efficiencies of the Antibodies in Early-stage and Villous Atrophy CD

	DGP-AGA	TG2-ab	EmA	DGP-AGA + TG2-ab	DGP-AGA + EmA	TG2-ab + EmA
Sensitivity (%)						
Early-stage CD	79	64	83	88	93	88
Villous atrophy CD	95	100	100	100	100	100
Specificity (%)						
	95	100	100	95	95	100
Efficiency (%)						
Early-stage CD	89	81	91	91	94	94
Villous atrophy CD	95	100	100	97	97	100

CD indicates celiac disease; DGP-AGA, antibodies to deamidated gliadin peptides; EmA, endomysial antibodies; TG2-ab, transglutaminase 2 antibodies.

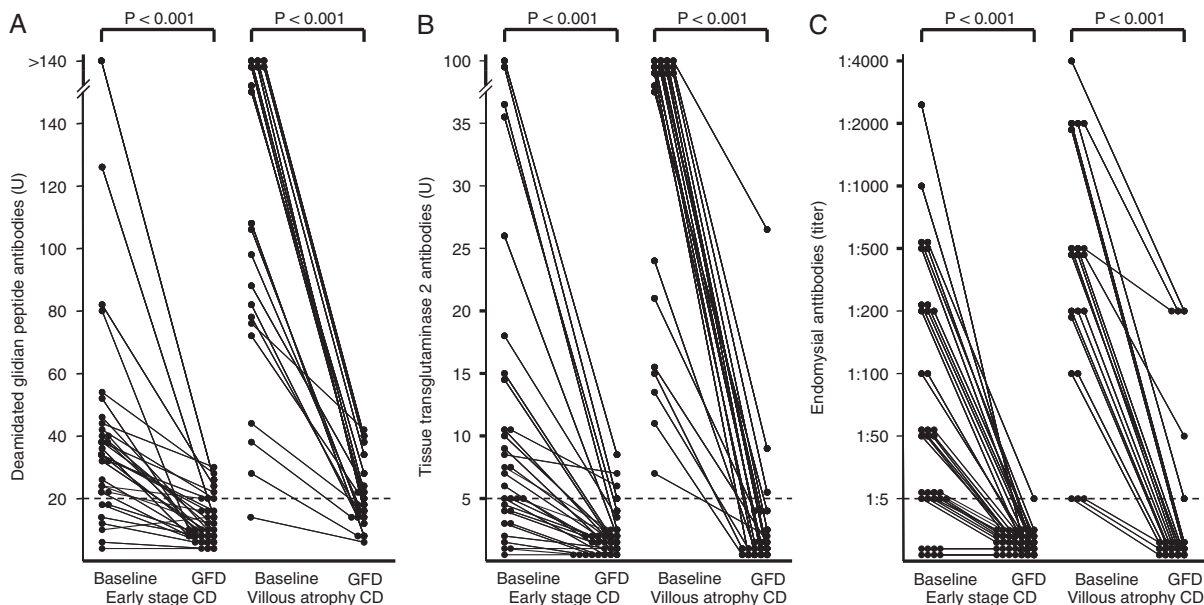
a substrate, and excellent correlation between the tests was observed.<sup>33</sup> We consider that the sensitivity of EmA is more dependent of laboratory personnels' experience than the method used. In any case, the immunofluorescence method required for EmA is rather subjective and laborious, particularly when compared with DGP-AGA and TG2-ab, which can be measured using a practical enzyme-linked immunosorbent assay. It was thus of significance that even after ROC analysis, the DGP-AGA had higher sensitivity and efficiency for early-stage celiac disease than TG2-ab.

Our results were in line with those of earlier studies showing the sensitivity of DGP-AGA and TG2-ab to be lower in subjects with lesser degrees of mucosal damage than in those with villous atrophy.<sup>38,39</sup> Nevertheless, there were also certain disparities. A study by Volta and associates<sup>18</sup> involved 7 individuals with potential celiac disease and both TG2-ab and EmA found these cases better than DGP-AGA. In addition, a group under Vermeersch et al<sup>39</sup> investigated 15 patients who had normal villous structure but serologic or histologic response to a gluten-free diet, and again TG2-ab detected some DGP-AGA-negative cases. These contradictory results might be at least partly explained by different study designs and the larger number of cases having early-stage celiac disease in our trial.

DGP-AGA had somewhat lower specificity for celiac disease than TG2-ab and EmA. However, this result was based on findings in the 2 DGP-AGA-positive subjects in the nonceliac group, both of whom had the HLA-DQ2 genotype and an increased density of  $\gamma\delta$ + IELs typical for celiac disease while consuming normal amount of dietary gluten.<sup>28,29</sup> It is thus possible that, in line with our original hypothesis on the early appearance of DGP-AGA, these 2 individuals are actually forthcoming celiac disease patients. It will be of great interest to see whether they develop further signs of gluten sensitivity in the future.

All tested antibodies showed parallel responses to the 1-year treatment with a gluten-free diet. The results are in accordance with those in earlier trials undertaken with patients having villous atrophy and crypt hyperplasia,<sup>22,38,39</sup> and show that DGP-AGA can be used as a reliable follow-up method in both early-stage and overt celiac disease.

The dual test for IgA and IgG-class DGP-ab was chosen for this study, as it offers some important benefits. Sometimes celiac disease patients are only either IgA or IgG-class antibody-positive, and measuring both types of antibodies may improve the test's overall sensitivity.<sup>40,41</sup> In addition, the use of IgG-class antibodies may reveal



**FIGURE 2.** Response of the serum deamidated gliadin peptide (A), transglutaminase 2 (B), and endomysial antibodies (C) to 1 year on a gluten-free diet (GFD). The dotted horizontal line represents the cut-off value for the antibody in question as recommended by the manufacturer. CD indicates celiac disease.

patients having selective IgA deficiency, which is particularly important in celiac disease where the prevalence of this condition is increased.<sup>42,43</sup> The use of IgG-class DGP-AGA in such cases is also supported by the notion that they seem to have higher sensitivity for celiac disease than the corresponding IgG-class TG2-ab.<sup>43,44</sup>

The results of this and earlier studies<sup>6-9,25,26,35,36</sup> have extended the concept of celiac disease beyond small-bowel mucosal villous atrophy. This leads to the inevitable question whether histologic evaluation is needed in all patients with positive celiac antibodies and beneficial response to a gluten-free diet. In this study, DGP-AGA showed better specificity than TG2-ab with a reasonable high specificity. However, the specificity was not 100%, suggesting that there is still need for small-bowel mucosal biopsy. Then again, the results of the study show that DGP-AGA positivity in subjects with normal mucosal villous morphology should not be considered immediately as false positive.

In conclusion, this study showed that the combined test for IgA and IgG-class DGP-AGA is a promising new method for case-finding and follow-up in early-stage celiac disease. In the future, more studies are needed to confirm our results in clinical settings.

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# Use of Deamidated Gliadin Peptide Antibodies to Monitor Diet Compliance in Childhood Celiac Disease

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## ABSTRACT

**Objective:** The aim of this study was to evaluate performance of serum antibodies against deamidated gliadin peptides (a-DGPs) in detecting compliance with gluten-free diet (GFD) in children with celiac disease (CD).

**Patients and Methods:** Serum samples were collected the same day of endoscopy in 95 children with CD and 106 controls. We preliminarily calculated the cutoff of a-DGP immunoglobulin A (IgA) and a-DGP IgA+G in our population by receiver operating characteristic (ROC) curves. Of 95 children with CD, 28 were studied during the first year after GFD introduction, with interview and serum collection every 3 months. In addition, serum samples were collected in 106 children with CD on GFD for more than 1 year (range 1–14). In both groups of children with CD on GFD, we compared a-DGP IgA and IgA+G performance in monitoring compliance with GFD with anti-tissue transglutaminase antibodies (anti-tTG) IgA and anti-gliadin antibody (AGA) IgA.

**Results:** The cutoff resulted in 13.1 arbitrary units (AU) for a-DGP IgA (sensitivity 87.4, 95% confidence interval [CI] 79%–92%, specificity 97.2, 95% CI 92%–99%) and 16.5 for a-DGP IgA+G (sensitivity 94.7, 95% CI 88%–98%, specificity 89.6, 95% CI 84%–95%). In the first year of GFD, at 6 to 8 months prevalence of positive a-DGPs was significantly higher in partially versus strictly compliant children, and at 9 to 12 months only prevalence of positive a-DGP IgA+G remained significantly higher. Moreover, at 9 to 12 months sensitivity to detect transgressions to GFD was 44% for a-DGP IgA and 100% for a-DGP IgA+G ( $P = 0.03$ ). In the 106 children on GFD for more than 1 year, sensitivity to detect transgressions to GFD was 60% for a-DGP IgA and 76% for a-DGP IgA+G. Anti-tTG IgA and AGA IgA sensitivity was much lower (24% and 4%, respectively). The 4 tests showed comparable high specificity.

**Conclusions:** Both a-DGPs showed higher sensitivity than anti-tTG IgA and AGA IgA in monitoring compliance with GFD, but a-DGP IgA+G seemed to perform better. a-DGPs did not outperform anti-tTG IgA for CD screening.

**Key Words:** anti-gliadin, anti-tissue transglutaminase, celiac disease, gluten-free diet

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Celiac disease (CD) is an autoimmune-mediated enteropathy triggered by the ingestion of wheat, rye, or barley gluten. The criterion standard for the diagnosis of CD is the histological evaluation of the duodenal mucosal tissue obtained by biopsy, and serologic tests are useful to identify individuals who require a duodenal biopsy. Classic serologic tests include anti-gliadin antibodies (AGAs), anti-endomysial antibodies (EMA), and anti-tissue transglutaminase antibodies (anti-tTG) of the immunoglobulin A (IgA) class. AGA testing has little diagnostic value because of its low sensitivity and specificity, especially in children aged 18 months or older (1). EMA and anti-tTG exhibit better sensitivity and specificity, close to 95% to 100%, and have replaced conventional AGA as the best serologic tools for screening CD (1–3). Because the dominant antigen of endomysial antibodies is transglutaminase, both assays measure the same antibodies (4). Therefore, determination of anti-tTG IgA alone may be used as a screening test because it is accurate and not operator dependent (5). In addition, serologic tests may be used to monitor compliance with gluten-free diet (GFD), but this is still a controversial issue, because anti-tTG IgA levels were reported to be scarcely reliable in detecting compliance when compared with duodenal biopsy (6,7); however, they were reported to be significantly lower in a group of adults with CD strictly adhering to the diet (8).

In the last decade, deeper understanding of the immunopathology of the small bowel mucosal damage in CD increased interest in deamidated gliadin peptides (a-DGPs). Dietary gluten is partially digested into gliadin peptides that pass through the small bowel epithelium and are bound to the human leukocyte antigen HLA-DQ2 or DQ8 on the surface of antigen-presenting cells. This binding is strongly enhanced by deamidation, catalyzed by anti-tTG, thus resulting in a greatly enhanced T-cell (9,10) and antibody response (11). Recent studies have shown that deamidation increases binding of antibodies to gliadin in the sera of patients with CD but not of controls (11–14). The reliability of serum antibodies against a-DGP in detecting adherence to GFD was studied in 4 small groups of adults with CD (15–18) and in only a few children with CD (19,20).

The aim of this study was to assess the usefulness of a-DGP antibodies for the evaluation of compliance with GFD in children with CD both in the first year after the diagnosis of CD and the introduction of GFD and after 1 or more years of GFD. To do so we preliminarily calculated the cutoff of both a-DGP IgA and a-DGP IgA+G (a combined detection of IgA and IgG isotypes of a-DGP) with a receiver operating characteristic (ROC) curve in a group of children with active CD and gastroenterological controls. We also compared a-DGPs diagnostic accuracy with that of anti-tTG IgA and classical AGA IgA.

## PATIENTS AND METHODS

### Patients

For the preliminary analysis of the cutoff of a-DGPs in our pediatric population consecutive serum samples were collected in

children the same day of endoscopy performed for failure to thrive or abdominal pain or other gastrointestinal symptoms. Upper gastrointestinal endoscopy with duodenal biopsy confirmed active CD in 95 children (male/female [M/F] = 39/56, median age 6.6, range 0.8–16.8 years) and normal duodenal histology in 106 (M/F = 43/63, median age 7.2, range 0.4–17.1 years), which were considered gastrointestinal controls. Histologic duodenal damage was graded according to the Marsh classification (21). In controls histology showed esophagitis in 49, gastritis in 19, esophagogastritis in 18, and normal mucosa in 20 children.

We then analyzed serum samples of 134 children with CD on GFD: 28 of them (M/F = 11/17, median age 8.1, range 1–16.8 years) were newly diagnosed and studied for the first year of GFD with serum samples to be collected every 3 months, and 106 (M/F = 43/63, median age at endoscopy 6.4, range 0.8–16.2 years) were on GFD for 1 year or longer (range 1–14 years) and their median age at serum collection was 10.6 years (range 2.3–22.1). The 28 children with CD studied in the first year of GFD were part of the 95 with active CD.

Compliance with GFD was ascertained through a structured interview always performed by the same investigator (G.O.). Parents and children were instructed to follow specific dietary restrictions and received additional counseling to reinforce the instructions at each follow-up visit. The same operator always performed a physical examination and assessed growth parameters. Dietary compliance assessment at follow-up visits was based on self-reported dietary habits by the child and the parents, and was categorized as strict or partial compliance with GFD. When a discrepancy between parents and child reports was demonstrated by the structured interview, the final categorization (ie, partially or strictly compliant) was assigned on the basis of the investigator's opinion. The investigator was completely blind to the serologic test results because the interview was done on the same day of the serum samples collection.

Written informed consent for the anonymous use of their data was obtained from the parents of all of the children included in the study, and the study protocol was approved by local ethical committee.

## Laboratory Assays for CD-specific Serology

After centrifugation at 3000 rpm for 10 minutes, sera were stored at  $-20^{\circ}\text{C}$  until the enzyme-linked immunosorbent assays (ELISAs) were performed. AGA and anti-tTG—both of the IgA class—determinations were performed by EliA Gliadin IgA and EliA Celikey IgA assays, respectively (Phadia AB, Uppsala, Sweden), with a manufacturer's suggested cutoff of 10 U/mL. a-DGP IgA and a-DGP IgA+G (a combined detection of IgA and IgG isotypes of a-DGP) determinations were performed by Quanta Lite Gliadin IgA II and Quanta Lite Celiac DGP Screen, respectively (INOVA Diagnostics, San Diego, CA), with a manufacturer's suggested cutoff of 20 arbitrary units (AU). All of the tests were conducted in accordance with the manufacturer's instructions. Total IgA assay was conducted by an immunonephelometric assay (BN ProSpec; Siemens Healthcare Diagnostics, Marburg, Germany).

## Statistical Analysis

To determine in our population the cutoff value of a-DGP IgA and a-DGP IgA+G, we performed ROC curve analysis. Using ROC curves, we identified the cutoff value associated with the highest sensitivity and specificity by plotting for each cutoff value sensitivity versus  $(1 - \text{specificity})$ . The area under the ROC curve and the 95% confidence interval (95% CI) were assessed. We then

compared the sensitivity and specificity of a-DGP IgA, a-DGP IgA+G, anti-tTG IgA, and AGA IgA to detect compliance with GFD in the first year of GFD and after 1 year or longer of GFD by 2-tailed Fisher exact test or  $\chi^2$  test, when appropriate. For all of the serologic assays sensitivity, specificity, positive likelihood ratio (LR+), and negative likelihood ratio (LR-) were calculated.

Levels of CD-specific antibodies were expressed as median, interquartile range (IQR), and range values. Comparisons among groups were performed by the Mann-Whitney *U* test and the Kruskal-Wallis ANOVA test, as appropriate. Post-hoc comparisons were performed with Bonferroni correction. The *k* statistic was used to analyze agreement between a-DGPs and anti-tTG or AGA assays. The level of agreement was classified as  $k < 0.20$ : poor; 0.21 to 0.40: fair; 0.41 to 0.60: moderate; 0.61 to 0.80: good; and 0.81 to 1.00: excellent.

Statistical analysis was performed with STATISTICA software version 6.1 (Statsoft Inc, Tulsa, OK) and ROC curve analysis with MedCalc software version 11.0.1 (MedCalc Software, Mariakerke, Belgium).

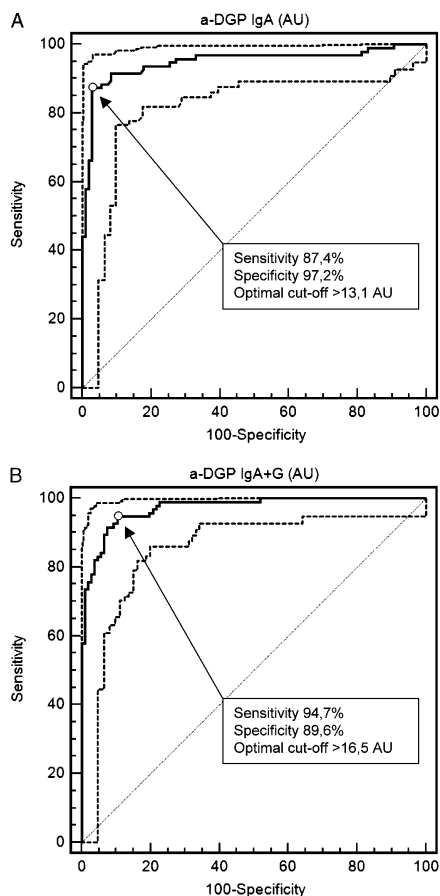
## RESULTS

### Cutoff Determination of A-DGP IgA and IgA+G Antibodies by ROC Curves

The ROC curve analysis performed on the a-DGP IgA and a-DGP IgA+G levels measured in 95 children with active CD at diagnosis and 106 gastroenterological controls showed that the cutoff value for a-DGP IgA was 13.1 AU (ie, all children with an a-DGP IgA level 13.1 AU were considered negative). The cutoff value for a-DGP IgA+G was 16.5 AU (ie, all children with an a-DGP IgA+G level 16.5 AU were considered negative). Figure 1 shows the ROC curves for a-DGP IgA and a-DGP IgA+G. Area under the curve was 0.948 (95% CI 0.908–0.975,  $P = 0.0001$ ) for a-DGP IgA and 0.972 (95% CI 0.939–0.990,  $P = 0.0001$ ) for a-DGP IgA+G.

According to these cutoffs, a-DGP IgA were positive in 83 of 95 children with CD and in 3 of 106 controls; their median value was 95 AU (IQR 24.3–127.8 AU) in children with CD and 3.8 AU (IQR 2.6–5.7 AU) in controls. a-DGP IgA+G were positive in 90 of 95 children with CD and in 11 of 106 controls; their median value was 98.3 AU (IQR 46.1–114.5 AU) in children with CD and 6.6 AU (IQR 4.3–9.3 AU) in controls. Table 1 shows sensitivity, specificity, positive, and negative likelihood ratios for a-DGP IgA, a-DGP IgA+G, anti-tTG IgA, and AGA IgA. Anti-tTG IgA were positive in 94 of 95 children with CD and in 1 of 106 controls. AGA IgA were positive in 68 of 95 children with CD and in 6 of 106 controls.

When comparing sensitivity of the 4 serologic assays, we found both a-DGP IgA and a-DGP IgA+G significantly more sensitive than AGA IgA ( $P = 0.01$  and  $P < 0.0001$ , respectively) and no difference between a-DGP IgA+G and anti-tTG IgA, whereas we found a-DGP IgA to be significantly less sensitive than anti-tTG IgA ( $P = 0.002$ ), but no difference was seen between a-DGP IgA and a-DGP IgA+G. When comparing specificity of the 4 assays, we found a-DGP IgA+G to be significantly less specific than anti-tTG IgA ( $P = 0.005$ ) but found no difference between a-DGP IgA and anti-tTG IgA. Neither a-DGP IgA nor a-DGP IgA+G specificity was different from that of AGA IgA. Specificity of a-DGP IgA was slightly higher than that of a-DGP IgA+G ( $P = 0.049$ ). Agreement between a-DGPs and anti-tTG assays was excellent ( $k = 0.85$  for a-DGP IgA and  $k = 0.84$  for a-DGP IgA+G) and between a-DGPs and AGA assays was good ( $k = 0.73$  for a-DGP IgA and  $k = 0.63$  for a-DGP IgA+G). In our series we did not detect children with CD or controls with IgA deficiency.



**FIGURE 1.** Receiver operating characteristic curves with 95% confidence bounds for a-DGP IgA (A) and a-DGP IgA+G (B).

We further analyzed the diagnostic performance of all of the serologic assays according to age. For this purpose, we divided children with CD in 2 subgroups:  $\leq 3$ -year-olds ( $n=24$ ) and  $>3$ -year-olds ( $n=71$ ). Serum a-DGP IgA were positive in all 24  $\leq 3$ -year-old children with CD and in 59 of 71 (83.1%, 95% CI 72–91)  $>3$ -year-old children with CD, showing a significantly higher sensitivity in younger children ( $P=0.03$ ). a-DGP IgA+G were positive in all of the  $\leq 3$ -year-old children with CD and in 66 of 71 (93%, 95% CI 84–98)  $>3$ -year-old children with CD, with a similar sensitivity in both younger and older children. AGA IgA were positive in all 24  $\leq 3$ -year-old children with active CD but only in 44 of 71 (61.9%, 95% CI 49–73)  $>3$ -year-old children with CD, showing a significantly higher sensitivity in younger children

( $P=0.0001$ ). Anti-tTG IgA were positive in all 24  $\leq 3$ -year-old children with active CD and in 70 of 71 (98.6%, 95% CI 92–100)  $>3$ -year-old children with CD. No difference was found as regards the specificity in any of the 4 serologic assays in relation to age of the control children.

### Serum Levels in the First Year After CD Diagnosis and Introduction of GFD

Of the 95 newly diagnosed children with CD, 28 were studied in the first year after introduction of GFD with a structured interview, physical examination, evaluation of growth parameters, and serology that were to be performed every 3 months. Not all of the children strictly followed the instructions, and timekeeping was not always correct. At 2 to 4 months, data and serum samples were available for 21 children (13 strictly compliant, 8 partially compliant), at 6 to 8 months for 13 children (8 strictly, 5 partially compliant), and at 9 to 12 months for 20 children (11 strictly, 9 partially compliant). Table 2 shows changes in prevalence of positive/negative samples over time. At 2 to 4 months of GFD, the prevalence of positive anti-tTG IgA was significantly higher in partially compliant children, but the difference was no longer significant in later samples. At 6 to 8 months, the prevalence of positive a-DGP IgA and a-DGP IgA+G was significantly higher in partially compliant children, but at 9 to 12 months only the prevalence of positive a-DGP IgA+G was still significantly higher in partially versus strictly compliant children. In particular, none of the partially compliant children was negative for a-DGP IgA+G at 6 months or later (sensitivity 100%). Moreover, at 9 to 12 months, sensitivity to detect transgressions to GFD was significantly higher for a-DGP IgA+G than for a-DGP IgA (100% versus 44%,  $P=0.03$ ).

Figure 2 shows the trends of all of the serologic tests. At 2 to 4 and 6 to 8 months, median anti-tTG IgA levels decreased in strictly compliant children only, whereas at 9 to 12 months, the levels decreased also in partially compliant children and were not significantly different in strictly versus partially compliant children. Median levels of a-DGP IgA and a-DGP IgA+G were decreased in all of the children already at 2 to 4 months; however, the difference between strictly and partially compliant children became significant only after 6 months or longer ( $P=0.008$  for a-DGP IgA and  $P=0.04$  for a-DGP IgA+G) and 9 to 12 months ( $P=0.03$  for a-DGP IgA and  $P=0.02$  for a-DGP IgA+G).

### Serology According to Compliance With Diet After $\geq 1$ Year of GFD

According to interview, physical examination, and evaluation of growth parameters, strict compliance was found in 81 (76.4%, strictly compliant) of the 106 children with CD whose serum samples were collected  $\geq 1$  year (range 1–14 years) after the

**TABLE 1.** Diagnostic performance of a-DGP IgA, a-DGP IgA+G, anti-tTG IgA, and AGA IgA assays as screening test for CD

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	LR+ (95% CI)	LR– (95% CI)
a-DGP IgA*	87.4 (79.2–92.6)	97.2 (92–99)	30.9 (10.1–94.4)	0.13 (0.08–0.22)
a-DGP IgA+G*	94.7 (88.3–97.7)	89.6 (83.8–95.5)	9.1 (5.2–16)	0.06 (0.03–0.14)
Anti-tTG IgA†	98.9 (94.3–99.8)	99.1 (94.8–99.8)	104.9 (14.9–737.8)	0.01 (0–0.08)
AGA IgA†	71.6 (61.8–79.7)	94.3 (88.2–97.4)	12.6 (5.7–27.8)	0.30 (0.22–0.42)

a-DGP = antibodies against deamidated gliadin peptides; AGA = anti-gliadin antibodies; anti-tTG = anti-tissue transglutaminase antibodies; CD = celiac disease; LR = likelihood ratio.

\* According to the calculated cutoff of 13.1 arbitrary units for a-DGP IgA and of 16.5 arbitrary units for a-DGP IgA+G.

† According to the manufacturer's cutoff.

TABLE 2. Prevalence of positive/negative samples for a-DGP IgA, a-DGP IgA+G, anti-tTG IgA, and AGA IgA in 28 children with CD at diagnosis and throughout the first year after GFD introduction

	a-DGP IgA	a-DGP IgA+G	Anti-tTG IgA	AGA IgA	a-DGP IgA	a-DGP IgA+G	Anti-tTG IgA	AGA IgA
At diagnosis								
Pos/neg	27/1	27/1	28/0	24/4				
Time after GFD introduction	Strictly compliant				Partially compliant			
2–4 mo (n=21)								
Pos/neg	8/5	9/4	7/6*	1/12	7/1	7/1	8/0*	3/5
6–8 mo (n=13)								
Pos/neg	1/7 <sup>†</sup>	2/6 <sup>‡</sup>	5/3	0/8	4/1 <sup>†</sup>	5/0 <sup>‡</sup>	4/1	1/4
9–12 mo (n=20)								
Pos/neg	1/10	3/8 <sup>#</sup>	5/6	1/10	4/5	9/0 <sup>#</sup>	5/4	0/9

Strictly compliant children were 13 at 2–4 months, 8 at 6–8 months, and 11 at 9–12 months. Partially compliant children were 8 at 2–4 months, 5 at 6–8 months, and 9 at 9–12 months. a-DGP = antibodies against deamidated gliadin peptides; AGA = anti-gliadin antibodies; anti-tTG = anti-tissue transglutaminase antibodies; CD = celiac disease; GFD = gluten-free diet. \**P* = 0.04. <sup>†</sup>*P* = 0.03. <sup>‡</sup>*P* = 0.02. <sup>#</sup>*P* = 0.001 by Fisher exact test.

introduction of GFD, whereas transgressions were found in 25 (23.6%, partially compliant). None of the children were fully non-compliant. Of the 25 partially compliant children, a-DGP IgA was positive in 15 (60%) and negative in 10, a-DGP IgA+G was positive in 19 (76%) and negative in 6, anti-tTG IgA was positive in 6 (24%) and negative in 19, and AGA IgA was positive in 1 (4%) and negative in 24. Of the 81 strictly compliant children, a-DGP IgA was positive in 11 and negative in 70 (86.4%), a-DGP IgA+G was

positive in 7 and negative in 74 (91.4%), anti-tTG IgA was positive in 3 and negative in 78 (96.3%), and AGA IgA was positive in 1 and negative in 80 (98.8%).

When comparing performance of the 4 serologic tests to detect partial compliance (Table 3), sensitivity of a-DGP IgA was significantly higher than that of both anti-tTG IgA (*P* < 0.0001) and AGA IgA (*P* < 0.0001) but not significantly different from that of a-DGP IgA+G. Similarly, sensitivity of a-DGP IgA+G was

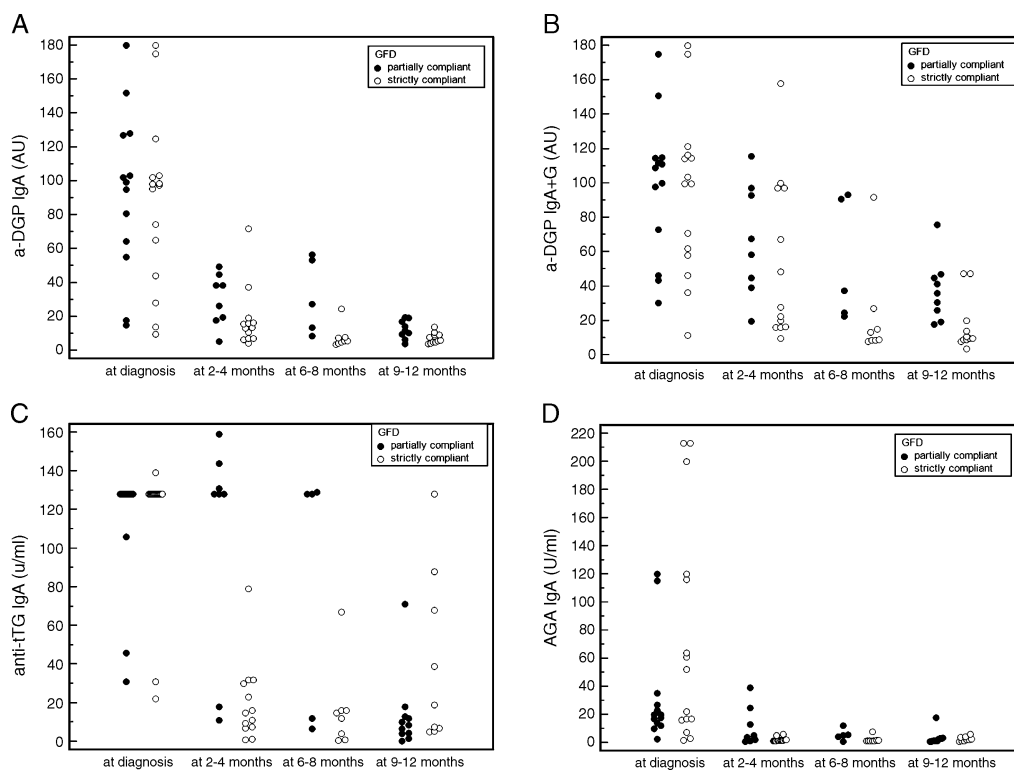


FIGURE 2. Distribution of a-DGP IgA (A), a-DGP IgA+G (B), anti-tTG IgA (C), and AGA IgA (D) levels in 28 children with CD at diagnosis and in the first year after gluten-free diet (GFD) introduction, according to compliance with GFD.

TABLE 3. Diagnostic performance of a-DGP IgA, a-DGP IgA+G, anti-tTG IgA, and AGA IgA assays in monitoring compliance with diet after  $\geq 1$  year of GFD

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	LR+ (95% CI)	LR- (95% CI)
a-DGP IgA*	60 (40.7-76.6)	86.4 (77.3-92.2)	4.42 (2.34-8.34)	0.46 (0.28-0.75)
a-DGP IgA+G*	76 (57.6-88.5)	91.4 (83.2-95.8)	8.79 (4.19-18.46)	0.26 (0.13-0.53)
Anti-tTG IgA†	24 (11.5-43.4)	96.3 (89.7-98.7)	6.48 (1.75-24.05)	0.79 (0.63-0.99)
AGA IgA†	4 (0.7-19.5)	98.8 (93.3-99.8)	3.24 (0.21-49.94)	0.97 (0.89-1.06)

a-DGP = antibodies against deamidated gliadin peptides; AGA = anti-gliadin antibodies; anti-tTG = anti-tissue transglutaminase antibodies; GFD = gluten-free diet; LR = likelihood ratio.

\* According to the calculated cutoff of 13.1 arbitrary units for a-DGP IgA and of 16.5 arbitrary units for a-DGP IgA+G.

† According to the manufacturer's cutoff.

significantly higher than that of both anti-tTG IgA ( $P < 0.0001$ ) and AGA IgA ( $P < 0.0001$ ). Specificity of a-DGP IgA in detecting compliance with GFD was lower than that of both anti-tTG IgA ( $P = 0.047$ ) and AGA IgA ( $P = 0.005$ ) but similar to that of a-DGP IgA+G, whereas specificity of a-DGP IgA+G was not significantly different from that of anti-tTG IgA and AGA IgA. We also found the 11 strictly compliant children with positive a-DGP IgA (false-positive, median 13.9, range 13.3–17.3 AU) had significantly lower levels than the 25 partially compliant (true-positive, median 20.5, range 7.5–28.5 AU,  $P = 0.0003$ ).

No other differences were found between strictly compliant and partially compliant children as regards age at diagnosis, sex, duration of GFD, or age at sample collection for serology determination.

## DISCUSSION

In this study we ascertained whether the new serological tests measuring a-DGPs were sensitive enough to detect transgression to GFD in children with CD. To do this we had to preliminarily determine the cutoff of a-DGP IgA and a-DGP IgA+G in our population of children with active CD and gastroenterological controls by a ROC curve analysis.

The cutoff values we calculated (ie, 13.1 AU for a-DGP IgA and 16.5 AU for a-DGP IgA+G) were lower than the manufacturer's suggested 20 AU. This is in accordance with some previous studies that suggested lowering a-DGP cutoff. In a group of children, the best cutoff was 9.7 for IgA and 9.8 for IgG (22). In other studies including both children and adults, the best cutoffs were 14.8 for IgA and 9.6 for IgG (16) or 11.4 for IgA and 9.6 for IgA+G (18) or 17.1 for IgG (23). Other studies, however, confirmed the manufacturer's cutoff of 20 as the optimal one in children both for screening and for follow-up after GFD (20), even in those with total IgA deficiency (24). On the contrary, some authors suggested raising the cutoff to 30 for a-DGP IgA, IgG, and IgA+G (25) or even to 45 for a-DGP IgA+G when used for CD mass screening (26). According to our cutoff, both a-DGP IgA and a-DGP IgA+G showed good sensitivity as screening tests for CD, but sensitivity was still lower than that of anti-tTG IgA. Therefore, neither of them could replace anti-tTG to screen for CD before endoscopy, thus confirming conclusions of a recent meta-analysis, according to which anti-tTG perform better than a-DGP IgA as a serologic test for CD screening (27). Both a-DGP IgA and a-DGP IgA+G showed higher sensitivity and specificity than that of AGA IgA. Moreover, in children younger than 3 years of age, the sensitivity of a-DGP was 100% with behavior similar to that of AGA. Indeed, in younger children AGA testing is still recommended because of the high prevalence of children with false-negative anti-tTG reported in many series (1); thus, a-DGP could replace AGA completely even in very young children.

The main aim of our study was to ascertain whether a-DGPs were accurate in monitoring compliance with GFD. To assess their changes in time and evaluate when the difference between strictly and partially compliant became significant, we studied a group of 28 newly diagnosed children with CD during the first year of GFD. It was not possible to longitudinally follow every child, because any of them could be strictly or partially compliant at different time points, so prevalence of negative and positive samples—and not children—at each time point was assessed. Moreover, not all of the samples of all 28 children were available at each time point because not all of the children strictly followed visit timekeeping and some missed  $\geq 1$  appointments. Despite these limits, we showed that all of the serologic levels decreased over time in the first year after the introduction of GFD, even in partially compliant children, as already reported in 20 children with CD in whom compliance was not controlled for (25). The general decrease we have seen in all of the serologic tests was probably the result of all of the children being on GFD, and the partially compliant having only occasional dietary transgressions. The most interesting finding in our series was that the prevalence of positive a-DGP levels (both IgA and IgA+G) was significantly higher in partially compliant than in strictly compliant children with CD only at 6 to 8 months, and remained so at 9 to 12 months only for a-DGP IgA+G. In particular, none of the partially compliant children was negative for a-DGP IgA+G at 6 months or longer, showing 100% sensitivity. This finding confirms the improved performance of serology testing reported in adults on GFD for 12 months or longer (28), possibly because it takes time for subjects with CD to reach a thorough comprehension of the intricacy of GFD and a complete adherence to it.

Neither anti-tTG IgA nor AGA IgA seems to be reliable in monitoring compliance with GFD in children because of their low sensitivity. The nonreliability of anti-tTG is still controversial in adults. Their levels were reported to be negative even in subjects with persistent duodenal atrophy (6,7) but were reported to be significantly lower in adults with CD strictly compliant with GFD when compared with those reporting dietary transgressions (8). When comparing anti-tTG and a-DGP accuracy in detecting GFD compliance it was found to be similar in predicting the status of duodenal mucosa in 53 adults with CD after 1 year of GFD (17), whereas a-DGPs were reported to be more reliable than anti-tTG in a small group of 9 adults with CD after 1 year of GFD (15). In children treated with GFD both anti-tTG and a-DGP decreased significantly; however, because of more rapid resolution of a-DGP (19) and a higher prevalence of negative values in children compliant with GFD (20), this antibody measurement was suggested as more useful than anti-tTG in monitoring dietary compliance. Our children studied in the first year after the introduction of GFD a-DGP IgA and a-DGP IgA+G were found to be reliable for monitoring compliance with GFD, when the diet had been

consumed for at least 6 months or longer, and this finding was confirmed in our series of 106 children with CD on GFD for  $\geq 1$  year, where both a-DGP IgA and a-DGP IgA+G showed higher sensitivity than anti-tTG IgA and AGA IgA. This is at variance from data reported in adults (29), in which the prevalence of positive levels of a-DGP was still high in strictly compliant subjects after 1 year of GFD and not significantly different from partially compliant ones. The more rapid decrease of a-DGP seen in our population of children should be attributed to the younger age at CD diagnosis and GFD introduction. Moreover, in strictly compliant children with positive a-DGP IgA (false-positive) median a-DGP IgA levels were significantly lower than they were in partially compliant children (true-positive) and levels were all below the manufacturer's cutoff of 20 AU (range 13.3–17.3), so they could be considered weakly positive. We did not have any fully noncompliant child, probably because they were clinically supervised and repeatedly given dietary counseling.

Specificity of all of the tests was satisfactory, but we believe that the most clinically relevant characteristic of such tests is sensitivity, because it is better to strengthen dietary instruction in a strictly compliant child than to miss a partially compliant one. Therefore, high sensitivity is essential in detecting dietary transgressions that were reported to enhance the risk of long-term complications (30) and worsen quality of life (31–33).

A limit of our study could be that we did not calculate the optimal cutoff for anti-tTG IgA and AGA IgA because this was not the objective of our study. Manufacturers' cutoffs for AGA and anti-tTG are routinely used as such in most laboratories; however, this could be a source of bias.

In conclusion, both a-DGP IgA and a-DGP IgA+G showed good sensitivity in monitoring compliance with GFD, higher than that of anti-tTG IgA and of AGA IgA, at comparable high specificity, but a-DGP IgA+G seemed to perform better than a-DGP IgA in children on GFD for 9 to 12 months or  $>1$  year. Both a-DGP IgA and a-DGP IgA+G showed good sensitivity and specificity as screening tests for CD, but specificity was higher for a-DGP IgA; however, they did not outperform anti-tTG IgA, which remains the best screening test for CD. In very young children sensitivity of both a-DGPs was high and therefore traditional AGA IgA could be substituted in this age group for their comparable performance.

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