Effectiveness of antigliadin antibodies as a screening test for celiac disease in children

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Abstract

Objective: To test the effectiveness of serologic antigliadin antibody (AGA) testing in predicting celiac disease in children.

Design: Prospective clinical assessment.

Setting: Hôpital Sainte-Justine, Montreal.

Patients: A total of 176 children with possible celiac disease who were referred for duodenal biopsy between January 1992 and June 1995.

Outcome measures: IgA and IgG AGA titres, as determined by enzyme-linked immunosorbent assay (ELISA); duodenal biopsy; clinical outcome on a gluten-free diet.

Results: Of the 176 children 30 were found to have celiac disease according to the criteria of the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN). The sensitivity and specificity of the IgA AGA titre, as well as its positive and negative predictive values, were 80%, 92%, 67% and 96% respectively; the corresponding values for the IgG AGA titre were 83%, 79%, 45% and 96%. The respective values for IgA and IgG AGA titres combined were 93%, 71%, 43% and 98%. Only 2 of the 30 patients with celiac disease had false-negative results for both IgA and IgG AGA titres. The IgA and IgG AGA titres decreased significantly \( p < 0.005 \) in all 11 patients after being on a gluten-free diet for at least 10 months and reached normal values in 8.

Conclusion: AGA screening for celiac disease permits better selection of patients for duodenal biopsy and adds specificity to the histologic diagnosis. Such screening cannot replace intestinal biopsy, which remains the gold standard for diagnosis.

Résumé

Objectif : Éprouver l'efficacité du dosage sérologique des anticorps antigliadine (AGA) dans la prédiction de la maladie coeliaque chez l'enfant.

Conception : Évaluation clinique prospective.

Contexte : Hôpital Sainte-Justine, Montréal.


Mesures de résultats : Titres de l’IgA et de l’IgG AGA, tels que déterminés par une épreuve immuno-enzymatique (ELISA); biopsie duodénale; résultat clinique après un régime sans gluten.

Résultats : On a dépisté la maladie coeliaque, selon les critères de l’ESPGAN (European Society of Pediatric Gastroenterology and Nutrition), chez 30 des 176 enfants. La sensibilité et la spécificité du titrage IgA AGA, ainsi que ses valeurs prédictives positive et négative, ont été respectivement de 80 %, 92 %, 67 % et 96 %; les valeurs correspondantes du titrage IgG AGA ont été de 83 %, 79 %, 45 % et 96 %. Les valeurs correspondantes des titrages IgA et IgG AGA combinés ont été de 93 %, 71 %, 43 % et 98 %. Seulement 2 des 30 patients atteints d’une maladie coeliaque ont eu des résultats faussement négatifs aux 2
Celiac disease, or gluten enteropathy, is induced by a permanent sensitivity to the gliadin fraction of gluten, a protein found primarily in wheat, rye, barley, triticale and, to a lesser extent, oats. The diagnosis is supported by histologic evidence of flattened villi and hyperplastic crypts on a biopsy specimen of the small intestine while the patient is ingesting gluten. It is confirmed by an unequivocal clinical remission after withdrawal of dietary gluten.

The clinical presentation of celiac disease varies greatly. In fact, many patients have no gastrointestinal symptoms or signs of malabsorption. Much effort has therefore been expended in attempting to develop reliable, noninvasive screening methods. Small-bowel barium contrast studies may indicate a nonspecific malabsorptive disorder, although in some cases the films appear normal. Various screening tests, including the D-xylose absorption test, the measurement of fecal fat and the hydrogen breath test to determine lactose intolerance, have unsatisfactory sensitivities and specificities as well as poor negative predictive values.

In recent years, assays for circulating antibodies have been the main focus of studies on screening for celiac disease. The detection of high titres of antigliadin antibody (AGA), antireticulin antibody (ARA) or antien domysium (EMA) antibody at the time of diagnosis, and the subsequent decrease in titres after removal of dietary gluten, add specificity to the histologic findings. Although assays for ARA and, in particular, EMA are reported to be more specific than those for AGA in screening for celiac disease, they are technically more demanding and far more expensive. Moreover, EMAs are uniquely of IgA isotype and thus will inevitably lead to false-negative results in celiac patients with selective IgA deficiency, a frequent association. In addition to its low cost, the AGA assay can be performed easily in any serology laboratory, and the quantitative results provided allow for the comparison of serial measurements.

We performed this study (a) to determine the sensitivity and specificity as well as the positive and negative predictive values of the AGA assay when used as a screening tool in a well-described cohort of children suspected of having celiac disease and (b) to assess prospectively changes in the AGA titres in identified celiac patients after removal of gluten from their diet.

**Methods**

**Patients**

From January 1992 to June 1995 we prospectively recruited children with suspected celiac disease who were referred to the Division of Pediatric Gastroenterology at the Hôpital Sainte-Justine, Montreal. Presenting symptoms generally included failure to thrive, diarrhea, abdominal distention, vomiting, weight loss or abdominal pain. Patients were included if the consulting pediatric gastroenterologist felt that an intestinal biopsy was indicated and if the patient or his or her legal guardian consented to endoscopy and blood testing. Patients were excluded if they had previously undergone tests for celiac disease or had been on a gluten-free diet.

**Investigations**

An intestinal biopsy specimen was obtained from all patients with a pediatric video endoscope. At least 2 distal duodenal biopsy specimens, adequate in size and orientation, were examined by an experienced pathologist (P.R.), blinded to the AGA titre results. Celiac disease was diagnosed on the basis of histologic findings of a flat intestinal mucosa on a gluten diet and a subsequent clinical remission on a gluten-free diet, as per the revised criteria of the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN).

A serum sample of 1–2 mL was taken at the time of biopsy and stored at −70°C until testing for AGA, as described later. Serum samples from patients with abnormal histologic findings and negative AGA results were assayed to exclude selective IgA deficiency.

Serologic testing and histologic examinations were performed independently by investigators who were blind to each other’s findings. A subgroup of patients found to have celiac disease underwent additional AGA determination at follow-up after eating a gluten-free diet for a mean of 18.3 (range 10–32) months.

**Antigliadin antibody assay**

IgA and IgG AGA titres were determined by means of enzyme-linked immunosorbent assay (ELISA). Gliadin
was prepared from wheat gluten (product no. G-3375; Sigma Chemical Company, St. Louis, Mo.) in 70% ethanol (1 mg/mL). A 200-μL aliquot of gliadin (5 μg/mL) in carbonate buffer [0.015M sodium carbonate + 0.03M sodium bicarbonate adjusted to pH 9.6 in water] was added to each well of a microtitre ELISA plate (Falcon 3915; Becton Dickinson Labware, Lincoln Park, NJ) and kept overnight at 4°C. Subsequently, 200 μL of PBS–BSA 1% Tween (phosphate-buffered saline [PBS; Gibco, Grand Island, NY], bovine serum albumin [BSA; Sigma] and Tween 20 [Sigma]) were added to each well, and the plate was left at room temperature for 1½ hours. Each serum sample (100 μL) to be tested was added to the wells in quadruplicate at a 1:100 dilution in PBS–BSA 1% Tween. Subsequently, 100 μL of either peroxidase-conjugated goat antihuman IgA or IgG (Sigma) was added at a concentration of 1:500 or 1:20 000, respectively, in PBS–BSA 1% Tween, each in duplicate wells. After a 1-hour incubation at 37°C, 100 μL of OPD (o-phenylenediameine dihydrochloride) substrate solution (10 mL of buffer [0.2M sodium hydrogen phosphate + 0.1M citric acid] adjusted to pH 5.0), 4 μL of hydrogen peroxide and 4 mg of OPD (Sigma) were added to each well. The plates were then incubated at room temperature, without exposure to light, for 30 minutes. Washing steps (× 3) with PBS–Tween were incorporated after each of the above interaction stages to remove any nonimmobilized species.

To stop the reaction, 25 μL of 4N sulfuric acid was added to each well. Optical density was read at 492 nm using an automated ELISA detector. The optimal cut-off points for the predictive ability of the IgA and IgG AGA titres were individually determined by varying the cut-off point from the smallest to the largest possible value in order to achieve maximum sensitivity. Our primary objective was to achieve the highest sensitivity, with an acceptable false-positive rate. The optimal discriminative ability of IgA AGA and IgG AGA using our method was at an optical density of 0.25 and 0.30, respectively. The same celiac patient’s serum was used as a positive control with each batch, to confirm the reproducibility of the AGA assays on different days. Furthermore, appropriate negative controls were routinely carried out, using the anti-human immunoglobulin antibodies without serum. The background optical densities from these negative control wells were subtracted from results with each patient’s serum.

### Statistical analysis

Because all patients in this study underwent concurrent duodenal biopsy and serologic testing, estimates of the sensitivity and specificity of AGA assays are unbiased. The positive predictive value of each test is defined as the proportion of patients with a true-positive result among the total number of patients with a positive test result. The negative predictive value is defined as the proportion of patients with a true-negative result among the total number of patients with a negative test result. In the subgroup analysis, AGA titres were compared before and after the gluten-free diet using the paired t-test, with a p value below 0.05 considered significant.

### Results

A total of 176 children met the inclusion criteria. Their mean age at the time of biopsy and serologic testing was 5.2 (range 0.5–18.1) years. Celiac disease was diagnosed in 30 (17%) of the patients (13 boys, 17 girls). Their mean age at diagnosis was 3.7 (0.6–11.2) years. The proportion of children found to have celiac disease was highest in the group aged less than 2 years (22% [16/72]); it was 16% among those 2–8 years old (9/55) and 10% among those 9 and older (5/49). One patient had diabetes mellitus, and another had an intestinal malrotation. Only 2 (7%) of the celiac patients had a family history of the disease.

The symptoms and signs at presentation of the patients found to have celiac disease are summarized in Table 1. A classic presentation (diarrhea and failure to thrive, with or without other symptoms) was noted in 62% (10/16) of those less than 2 years of age, 56% (5/9) of those 2–8 years of age (9/55) and 10% among those 9 and older (5/49).

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. (and %) of patients</th>
<th>Median age (and range), yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical presentation</td>
<td>Chronic diarrhea and failure to thrive</td>
<td>17 (57)</td>
</tr>
<tr>
<td>Atypical presentation</td>
<td>Diarrhea without failure to thrive</td>
<td>9 (30)</td>
</tr>
<tr>
<td>Unusual presentation</td>
<td>Abdominal distension with or without abdominal pain</td>
<td>4 (13)</td>
</tr>
<tr>
<td></td>
<td>Recurrent abdominal pain with nausea or reflux</td>
<td>2 (7)</td>
</tr>
</tbody>
</table>

Table 1: Presenting symptoms in 30 pediatric patients with celiac disease

Because all patients in this study underwent concurrent duodenal biopsy and serologic testing, estimates of the sensitivity and specificity of AGA assays are unbiased. The positive predictive value of each test is defined as the proportion of patients with a true-positive result among the total number of patients with a positive test result. The negative predictive value is defined as the proportion of patients with a true-negative result among the total number of patients with a negative test result. In the subgroup analysis, AGA titres were compared before and after the gluten-free diet using the paired t-test, with a p value below 0.05 considered significant.
IgA deficiency. Therefore, only 2 of the 30 celiac patients (1.2 and 6.5 years of age, respectively) had a false-negative result for both the IgA and the IgG AGA assays. As expected, IgG AGA sensitivity was somewhat higher than that for IgA AGA, but its specificity was considerably lower (Table 2). The negative predictive value if both IgA and IgG AGA titres were below threshold was 98%.

When the IgA and IgG AGA data for each patient were expressed as percent of a standard positive control serum, the sensitivity, specificity and predictive values were unchanged (data not shown).

Eleven of the 30 celiac patients had AGA measured both at the time of biopsy and after a mean of 18.3 (range 10–32) months on a gluten-free diet (Fig. 2). Both the IgA and the IgG AGA titres decreased significantly \((p < 0.005)\) after the diet therapy. The mean IgA AGA titre decreased from 1.5 to 0.10 and the mean IgG AGA titre from 0.81 to 0.21. These reductions were consistent with the complete resolution of symptoms and satisfactory weight gain noted in all patients. Despite this uniform decrease in titres on a gluten-free diet, 3 (27%) of the 11 patients still had IgA and IgG AGA titres above the threshold value after a follow-up of at least 10 months. However, because repeat biopsies were not clinically indicated, histologic data are unavailable for comparison with the follow-up serologic results.

![Fig. 1: IgA (left) and IgG (right) antigliadin antibody (AGA) titres, as determined by enzyme-linked immunosorbent assay, at time of duodenal biopsy in 176 pediatric patients suspected of having celiac disease. Titres are expressed as optical density (OD).](image)

![Table 2: Reported effectiveness of IgA and IgG antigliadin antibody (AGA) testing in screening for celiac disease](image)
Arguably, the most important characteristic of a screening test for celiac disease is a high negative predictive value. A clinician would then be able to estimate whether celiac disease is improbable for an individual patient, without resorting to a biopsy. In a pediatric population similar to ours, the negative predictive values of the D-xylose test, fecal-fat measurement and the lactose breath test were only 86%, 85% and 72%, respectively, substantially lower than that for AGA testing (Table 2). The highest predictive value reported to date was obtained by performing both AGA and EMA assays. In our study, the 98% negative predictive value observed compares favourably with that previously reported for AGA testing.

Variation in the results of AGA testing from one study to another is likely due to a number of reasons, as recently discussed by Stern and associates. First, the ratio of celiac:control patients differed between studies (Table 2). It is well established that both positive and negative predictive values are related to the prevalence of the disease in the population studied. Screening studies in healthy populations in Sweden have shown AGA titres to be generally of low predictive value, as compared with reports in populations of symptomatic pediatric patients, in which the disease prevalence will be higher. Second, the techniques used to measure AGA differed between reports and included radioimmunoassay, immunofluorescence and ELISA. The advantage of easily analysing large series of serum samples at relatively low cost has rendered ELISA the most widely used technique. Even among studies using ELISA, the results varied widely because of differences in methodology and in the interpretation of results. Third, different cut-off points were used to determine positivity of IgA and IgG AGA assays in the studies, which allows the test to achieve optimal specificity, but only at the cost of reduced sensitivity. This general approach is important when screening healthy populations, in which an unwarranted biopsy in people with a false-positive antibody titre is undesirable. Because we studied a pediatric group of referred patients, in which celiac disease is far more prevalent than in the general population, we adjusted the test to achieve optimal sensitivity at the cost of specificity in order not to miss anybody with celiac disease. Hence, the false-positive rate in our study was relatively high, in part because of our choosing a low cut-off point and in part because AGA can be found in patients with other inflammatory bowel diseases.

Another potential limitation is the method used to describe and evaluate AGA titres obtained by ELISA. The results of the test samples are most often presented as optical density units against a pool of negative serum samples, as reported herein. However, they may also be expressed as a percent of the optical density obtained using a standard positive serum or a pool of highly positive serum samples as a reference value. When our data were reanalysed as a percent of the standard positive serum, no difference was observed in sensitivity, specificity or predictive value. The importance of defining intra-assay and interassay variations has been pointed out recently. We chose to develop our own ELISA for AGA determination because of the unsatisfactory results reported using the first generation of commercially available kits. Moreover, the cost of materials for our ELISA method is less than 10% of the cost of a commercial kit. The cost of labour does not differ, because the time involved for both assays is similar.

Although IgG AGA is far less specific than IgA
AGA, it is generally measured simultaneously because of the relatively high incidence of IgA deficiency among celiac patients. Patients with selective IgA deficiency are at least 10 times more likely to have celiac disease than the general population. Thus, IgA deficiency may serologically mask celiac disease. Serum IgA levels should be routinely measured if the IgG AGA test result is positive, despite absent or very low IgA AGA levels. In our study of 6 IgA-negative celiac patients had positive IgG AGA titres, including 2 who had IgA deficiency.

Obtaining a repeat biopsy subsequent to a gluten challenge after a patient has been on a gluten-free diet is no longer considered necessary to confirm the diagnosis of celiac disease, except in dubious cases. However, it has been suggested that serologic testing may verify compliance with dietary treatment and reflect the mucosal aspect of the intestine. In our study, all 11 celiac patients who underwent repeat AGA assay had a significantly decreased AGA titre after 10–32 months on a gluten-free diet. It has been reported that up to 6 months may be required before AGA levels return to normal on a gluten-free diet. Two of our patients who had lower but still positive AGA levels after 5 months on a gluten-free diet had negative levels after 13 months. On the other hand, 3 asymptomatic patients had persistently high or borderline positive levels for IgA or IgG AGA, or both, after being on a gluten-free diet for 16, 25 and 32 months respectively. Because repeat biopsies were not performed, we can only speculate that these results were due to slight dietary indiscretions or simply reflected the normal rate of decrease in antibody levels for these children.

Although some investigators have claimed that AGA testing is useful in monitoring gluten challenge, in our study 2 of 9 children who underwent a gluten challenge had no symptoms or rise in AGA levels after 4 and 13 months on gluten, respectively. Repeat biopsies in both cases showed characteristic histologic evidence of active celiac disease despite normal AGA levels, which confirmed the possibility of false-negative AGA values in patients with positive histologic findings, as reported by others. Thus, in our limited experience, AGA testing seems to be far more accurate for screening celiac disease than for evaluating relapse upon gluten challenge. Other investigators have shown that the AGA titre may not increase for years on a gluten challenge, but that an eventual increase in titres may assist the clinician in timing repeat biopsies. Therefore, during a gluten challenge, a negative AGA titre does not necessarily indicate normal histology, and a positive AGA titre usually, even after years on gluten, indicates a histological relapse.

The diagnosis of celiac disease can easily be overlooked because the classic clinical presentation of weight loss and diarrhea is not always present, as observed in our study.

Serologic screening has shown that clinically symptomatic cases represent only a small proportion of the total population with celiac disease, the “tip of the iceberg.” Whereas a biopsy is not recommended as a first step for patients presenting with isolated anemia, osteopenia or recurrent abdominal pain, serologic screening for celiac disease is desirable in such circumstances, because dietary treatment can change the natural history of the disease. Positive results should thus prompt an intestinal biopsy. Indeed, an early diagnosis may help to prevent complications in untreated patients, including growth retardation, cancer, osteopenia, complications during pregnancy, and cerebral calcifications associated with epilepsy.

Serologic AGA testing is a simple, noninvasive tool that is more efficient than other tests, provided that selective IgA deficiency is ruled out. Its negative predictive value can be very useful in screening for celiac disease. However, serologic AGA testing is not infallible and cannot replace biopsy as the definitive diagnostic tool. Although AGA testing can also be useful for the follow-up of celiac patients, our data are similar to results from recent studies in which AGA and EMA test results may occasionally be negative in the presence of slight or intermittent dietary indiscretions, despite the presence of mucosal damage. Moreover, our study involved a pediatric population. Further testing of the reliability of AGA testing among adults is necessary to establish the appropriate cut-off values.

Considerations about cost savings for our health care system are becoming more and more important in Canada. AGA testing should replace the other numerous, out-of-date, nonspecific screening methods because of its greater efficiency and lower cost and because it would lead to fewer unnecessary biopsies and additional costs. North America has lagged far behind Europe in the establishment of serological testing to aid in the diagnosis of celiac disease. Furthermore, early diagnosis of celiac disease with the help of serologic screening could prevent complications, which would represent additional savings.

**Clinical implications:** AGA testing is an effective, low-cost method for screening celiac disease, even in asymptomatic patients.

**Study limitations:** False-negative results are possible, especially in patients with IgA deficiency. Although repeat AGA testing may be useful for following up celiac patients, it is not accurate in determining slight or intermittent dietary indiscretions.
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References


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