ARTICLE

Diagnosing Celiac Disease

A Comparison of Human Tissue Transglutaminase Antibodies With Antigliadin and Antiendomysium Antibodies

Jean-Jacques Baudon, MD; Catherine Johanet, PhD; Yvan Boniface Absalon, PhD; Georges Morgant, PhD; Sylvie Cabrol, MD; Jean-François Mougenot, MD

Objective: To evaluate and compare the sensitivity and specificity of the new serologic marker human antitissue transglutaminase antibodies (IgA anti-tTG) with those of antiendomysium (IgA EMA) and antigliadin antibodies (IgA and IgG AGA) for the diagnosis of celiac disease (CD).

Methods: The level of IgA antibodies to tTG in serum was determined by an enzyme-linked immunosorbent assay (ELISA) test using recombinant human tTG as the antigen; IgA EMA, by indirect immunofluorescence; and IgA and IgG AGA, by ELISA. Sixty-eight serum samples from 59 patients with CD were studied—30 patients had untreated CD, 22 were on gluten-free diets, and 16 had been reintroduced to gluten—and compared with serum samples from 116 children examined for failure to thrive, short stature, various digestive diseases, or other non-CD conditions.

Results: Twenty-eight of 30 patients with CD had anti-tTG (the 2 patients whose results were negative were 1 patient with IgA deficiency and 1 infant); 27 of 30 patients had IgA EMA (1 child was IgA anti-tTG positive and IgA EMA negative); 18 of 30 had IgA AGA; and 28 of 30 had IgG AGA. On gluten-free diets, 4 of 22 patients had anti-tTG but none had IgA EMA or IgA AGA. On normal diets, 15 of 15 children who had relapsed had anti-tTG; 9, IgA EMA; 4, IgA AGA; and 8, IgG AGA (1 child did not relapse). In subjects without CD, 3 of 116 had anti-tTG; 12, IgG AGA; and 1, IgA AGA, but none had IgA EMA. In the 3 children who had anti-tTG, CD could be excluded. The positive predictive value of IgA anti-tTG was 90% and the negative predictive value, 98%. In comparison, results for IgA EMA were 100% and 97%, IgA AGA 94% and 90%, and IgG AGA 70% and 98%, respectively.

Conclusion: The presence of human anti-tTG is a reliable indicator for the diagnosis and follow-up of CD.

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From the Hôpital d'Enfants Armand Trousseau (Drs Baudon, Morgant, and Cabrol), the Laboratoire d'Immunologie Hôpital Saint-Antoine (Drs Johanet and Absalon), and the Hôpital Robert Debré (Dr Mougenot); Assistance Publique-Hopitaux de Paris, Paris, France.

DESPITE THE INCREASING importance of serological methods, the diagnosis of celiac disease (CD) is still based on histological criteria.[1,2] Laboratory testing for antigliadin antibodies (AGA) by enzyme-linked immunosorbent assay (ELISA) and for antiendomysium antibodies (EMA) by immunofluorescence constitutes a valuable screening tool in the decision for intestinal biopsy.[1-4] These tests have also proven their value for the follow-up of CD and have revealed the high prevalence of undiagnosed CD.[5-7] Whereas the sensitivity and specificity of AGA are insufficient, the specificity of EMA is near 100%; however, the sensitivity of EMA is lower, the technique can be performed only in specialized laboratories, and the results depend to a certain extent on interpretation. Moreover, there are ethical concerns about the use of monkey esophagus as a substrate. Tissue transglutaminase (tTG) has been identified as the main antigen recognized by endomysial antibodies.[8] Therefore, the detection of IgA autoantibodies against tTG by the immunoenzymatic method appears to constitute a decisive step in the diagnosis.[9-18] Commercially available enzymes based on human recombinant tTG seem to be more accurate than those from guinea pigs.[19-26] The aim of our study was to compare IgA antibodies to antitissue transglutaminase (IgA anti-tTG) with IgA EMA and with IgA AGA and IgG AGA for the diagnosis and follow-up of CD.

METHODS

SUBJECTS

Sixty-eight serum samples from 59 patients with biopsy-confirmed CD were studied at

Table 1. Prevalence of Antibodies in Patients With Confirmed Celiac Disease and Control Subjects*

Antibody	Patients With Untreated Celiac Disease (n = 30)	Patients With Celiac Disease on GFD (n = 22)	Patients With Celiac Disease on Normal Diet (n = 16)	Control Subjects (n = 116)
IgA anti-tTG	28† (93.3 ± 4.5)	4 (18.2 ± 8.2)	15‡ (93.8 ± 6.1)	3 (2.6 ± 1.5)
IgA EMA	27 (90.0 ± 5.5)	0 (0)	9 (56.3 ± 12.4)	0 (0)
IgA AGA	18 (60.0 ± 8.9)	0 (0)	4 (25.0 ± 10.8)	1 (0.8 ± 0.8)
IgG AGA	28 (93.3 ± 4.5)	2 (9.1 ± 6.1)	8 (50 ± 12.5)	12 (10.4 ± 2.8)

Abbreviations: AGA, antigliadin; anti-ITG, antitissue transglutaminase; EMA, antiendomysium; GFD, gluten-free diet.
*Values are expressed as number (percentage ± SD) of patients with positive antibody results.
†One patient had an IgA deficiency.
‡One patient had no histological relapse after reintroduction of gluten into diet.

different stages of the disease. Thirty children were studied before excluding gluten from their diets and were classified as patients with untreated CD. The majority were infants or young children (median age, 2 years; range, 8 months-12 years); in our practice, symptoms of the disease appear early. Twenty-two patients had been on gluten-free diets for at least a year (median age, 9 years; range, 26 months-19 years). Their median age was older than that of the first group because the gluten-free diet was usually adhered to for several years. Sixteen patients had abandoned their gluten-free diets for a period of 3 months to 5 years (median, 9 months). They were primarily adolescents (median age, 12 years; range, 6-22 years). Intestinal biopsy results showed subtotal or partial villous atrophy in 15 of these 16 patients.

One hundred sixteen children (61 boys, 55 girls) with disorders other than CD constituted the control group. They were screened for CD at various ages because of the wide range of nonspecific symptoms that can reveal the disease in a pediatric population.[2] Forty-two of these children (median age, 23 months; range, 5.0 months-13.5 years) were studied for failure to thrive; 30 (median age,7 years; range, 7 months-16 years), for short stature (<2 SDs); 29 (median age, 17 months; range, 5 months-14.5 years), for various digestive diseases (chronic nonspecific diarrhea [n=10], cow's milk protein allergy [n=6], gastroesophageal reflux and gastritis [n=7], postinfectious diarrhea [n=3], AIDS and diarrhea [n=1], Crohn disease [n=1], encopresis [n=1]); 5 (median age, 20 months; range, 11 months-15 years), for iron deficiency anemia; 3, for mucoviscidosis; 6, for miscellaneous diseases (atopic dermatitis [n=3], asthma [n=2], thrombopenia [n=1]); and 1 infant belonging to a family with CD. Twenty-four subjects in the control group underwent an intestinal biopsy; results showed normal mucosa in 17, minor histological changes in 6, and partial villous atrophy in 1. In this infant, the partial villous atrophy was attributed to an allergy to cow's milk after its elimination from the diet and subsequent reintroduction. All had normal intraepithelial lymphocyte counts.

All but 10 of the serum samples were prospectively collected between January 2001 and February 2003; the remaining 10 were frozen serum samples from children with untreated CD, which were retrospectively studied. The study was performed according to the principles of the Helsinki Declaration, and oral informed consent was obtained from participants' parents.

METHODS

Before analysis, serum samples were stored at –80°C. All patients were screened for IgA deficiency by measuring immunoglobulin levels.

Specific IgA antibodies against tTG were detected with commercial ELISA using recombinant human tTG as the antigen (Eu-tTG umana IgA; Eurospital, Trieste, Italy). Briefly, serum samples diluted to 1:26 were incubated for 1 hour at room temperature, then washed 3 times, and subsequently incubated for another hour at room temperature with horseradish peroxidase–labelled sheep antihuman IgA. After washing, a chromogenic substrate was added. Optical density was read at 450 nm. Results were expressed in arbitrary units (AU) according to the reference calibrator. The cutoff value for positive outcome was 7 AU.[26]

Antigliadin antibodies were determined by ELISA.[27] Wells of flat-bottomed polystyrene plates (Microwell; Nunc Dutscher, Brumath, France) were coated with 100 µL of crude gliadin (Sigma, St Louis, Mo) at a concentration of 1 mg/mL in phosphate-buffered saline (PBS). The plates were incubated at 4°C overnight, then washed 3 times with PBS-tween. One hundred microliters of 1:200 PBS-diluted serum samples was added to each well, incubated for 45 minutes at room temperature, and then washed 3 times with PBS-tween. Alkaline phosphatase–conjugated goat antihuman IgG (Biosys; Compiègne, France) and IgA (Biosys) were diluted to 1:3000 for IgG and to 1:1000 for IgA, and 100 µL was added to each well. After 45 minutes of incubation, wells were washed twice in PBS-tween and once in 0.9% NaCl. One hundred microliters of a 1-mg/mL paranitrophenyl phosphate substrate (Sigma) in glycine-HCl (pH 10,4) was added to each well and incubated for 20 minutes at 37°C. Optical density was read at 405 nm. The antibody levels were expressed using a standard titration curve by testing control serum samples at 1:10 to 1:1000. The cutoff value for positive outcome for IgA and IgG AGA was 20 AU, corresponding to the 95th percentile in a blood-donor group.

Antiendomysium antibodies were determined by the standard immunofluorescence method using commercial slides of monkey esophagus sections (Eurospital).[28] Patients' serum samples were screened at a dilution of 1:10 and in case of positive samples, serum samples were titered to 1:640. A second antibody, fluorescein isothiocyanate–conjugated goat antibody to human IgA (Bio Rad, Marne la Coquette, France), was diluted to 1:50. Staining of the endomysium around the smooth muscle fibers in monkey esophagus was considered positive for EMA.

The sensitivity and specificity of anti-tTG, EMA, and AGA were calculated according to standard procedures with Statistical Package for the Social Sciences (SPSS Inc, Chicago, Ill), and precision of percentage was expressed in standard deviations.

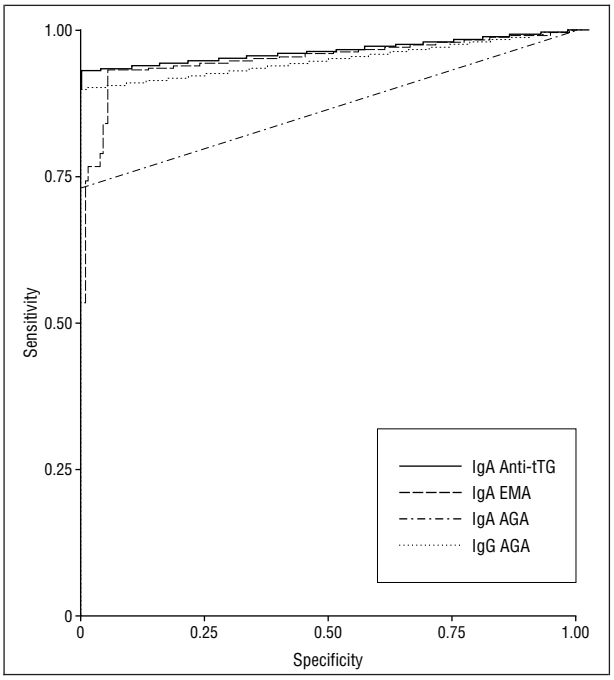
RESULTS

Results are summarized in **Table 1**, **Table 2**, and the **Figure**. Twenty-eight of 30 patients with untreated CD

Table 2. Sensitivity, Specificity, and Positive and Negative Predictive Values for IgA Antitissue Transglutaminase Antibodies (Anti-tTG), IgA Antiendomysium Antibodies (EMA), and IgA and IgG Antigliadin Antibodies (AGA) in Our Study Population*

	IgA Anti-tTg		IgA EMA		IgA AGA		IgG AGA	
	A	B	A	B	A	B	A	B
Sensitivity	93.3	93.8	90	56.3	60	25	93.3	50
Specificity	97.4		100		99.1		90	
Positive predictive value	90.3	83.3	100		94.7	80	70	40
Negative predictive value	98.2	100	97.4	94.3	90.5	90.5	98.1	92.8

*Values are expressed as percentage of patients. Sensitivity was based on (A) 30 patients with untreated celiac disease and (B) 16 patients with celiac disease after reintroduction of a normal diet. One patient had no histological relapse after reintroduction of gluten in the diet, so actual sensitivity is 100%, 60%, 26.6%, and 53.3%, respectively. Specificity was based on the 116 members of the control group.



Receiver operating characteristic curve. Data are shown for IgA antitissue transglutaminase (anti-tTG) (area under the curve [AUC], 0.97±0.03); IgA antiendomysium (EMA) (AUC, 0.95±0.03); IgA antigliadin (AGA) (AUC, 0.86±0.05); and IgG AGA (AUC, 0.95±0.03).

had IgA antibodies to tTG higher than the cutoff point (median, 21 AU; range, 13-33 AU). The 2 patients with negative results were a 2-year-old child in whom an IgA deficiency was discovered and an 8-month-old infant with IgA sufficiency. Four patients of the 22 on gluten-free diets had positive levels of antibodies (levels 7, 7, 9, and 11 AU). After reintroduction of gluten into the diet, 15 of 16 patients had anti-tTG (median, 20 AU; range, 10-32 AU). The remaining patient, who had negative anti-tTG results, did not relapse even though her HLA antigen group was DR7 DR13 DQ2; her intestinal mucosa remained normal after 5 years on a diet containing gluten.

Only 3 subjects had anti-tTG in the control group. The first, aged 28 months, had mental retardation and was investigated for failure to thrive. His anti-tTG level was slightly positive (10 AU); his EMA, IgA AGA, and IgG AAG levels were negative. Two months later, his results were negative for anti-tTG; his HLA antigen group was not DQ2 DQ8. No intestinal biopsy was performed. The second patient, 6 years of age, had erythemato-vesiculous lesions of the legs and buttocks that were evocative of dermatitis herpetiformis. However, cutaneous biopsy results revealed no granular IgA antibodies, and intestinal biopsy results showed no lesions. His anti-tTG level was 10 AU. The third patient, who was 3 years of age and had AIDS, had protracted diarrhea and failed to thrive. Her anti-tTG level was 9 AU; intestinal biopsy results showed only minor histological changes.

In untreated CD, the concordance rate between IgA anti-tTG and IgA EMA was 96.4% (27/28), including the patient with IgA deficiency. Only one of the 29 patients with IgA sufficiency had IgA anti-tTG and no IgA EMA; another had neither anti-tTG nor IgA EMA but did have IgA and IgG AGA. In the group of 30 patients with untreated CD, IgG AGA were present in 28 patients and IgA AGA in only 18. No patient on a gluten-free diet had either IgA EMA or IgA AGA, but 4 had IgA anti-tTG. After reintroduction of gluten (16 patients) and relapse of intestinal lesions (15/16), the 15 patients who relapsed had IgA anti-tTG; 9 had IgA EMA (concordance rate, 60%); 4, IgA AGA (concordance rate, 26.6%); and 8, IgG AGA (concordance rate, 53.3%). In the control group, no subject, not even the 3 subjects with IgA anti-tTG, had IgA EMA; 1 had IgA AGA, and 12 had IgG AGA. Of the latter, 6 had IgG AGA levels higher than 30 AU; intestinal biopsy results showed normal mucosa or minimal histological changes in 5 of these and partial villous atrophy in the sixth, who was allergic to cow's milk.

Finally, the positive predictive value of IgA anti-tTG was 90% and the negative predictive value, 98%. In comparison, results for IgA EMA were 100% and 97%; IgA AGA, 94% and 90%; and IgG AGA, 70% and 98%, respectively. All the patients with untreated CD had at least 1 of these antibodies. The receiver operating characteristic curve (Figure 1) showed the best results for IgA anti-tTG.

COMMENT

These results show that IgA antibodies to human tTG are reliable as a test for both the diagnosis and follow-up of childhood CD. In a series consisting exclusively of children, our results in untreated CD are similar to those of previous studies[21,22] using human tTG with an ELISA test that showed both high sensitivity (96%-100%) and specificity (96%-100%). In series comprising both children and adults,[19,21,22] sensitivity was 91.5% to 98.0% and specificity, 98% to 99%. In series solely of adults,[25,26] the sensitivity was the same but the specificity was lower (82%-97%). Except for the 1 case with IgA deficiency, our only negative result was observed in an 8-month-old infant who had only IgA and IgG AGA but no EMA. The sensitivity and specificity were higher than those obtained

What This Study Adds

Celiac disease is a permanent gluten-sensitive enteropathy characterized by villous atrophy in individuals with HLA antigen DQ2 or DQ8. Because of the wide range of symptoms and their lack of specificity, it is essential to develop simple, reliable tests that identify CD in various clinical conditions. Sensitivity and specificity of IgA and IgG AGA are insufficient. Those of IgA EMA exceed 90% but the immunofluorescence technique requires a specialized laboratory.

Faced with a number of clinical conditions, we compared the search for human anti-tTG by ELISA with the tests mentioned previously in screening for CD. The positive predictive value was 90% and the negative predictive value was 98%, making it a better ELISA test than AGA. However, none of the antibodies was 100% sensitive and specific. Two explanations may be advanced: (1) a small proportion of patients with CD is IgA deficient and (2) sensitivity of antibodies is lower in infants than in older children.

by using guinea pig transglutaminase as a substrate for the ELISA test.[11-14,17-19]

In previous studies comparing human anti-tTG and EMA for the diagnosis of untreated CD in both adults and children,[19,21,22,25] differences between IgA anti-tTG and IgA EMA were minute. The first are slightly more sensitive and the second, slightly more specific.[25] The absence of IgA EMA is more frequent in patients younger than 2 years than in older children.[3] The same holds true for IgA anti-tTG.[29] By comparison, IgA AGA are less sensitive (81%-83%)[3,14,22] and less specific (82%- 89%).[14,21,22] The sensitivity of IgG antibodies to gliadin is variable (82%- 99%),[3,14,21,22] and the specificity is relatively low (76%- 92%).[14,21,22] However, 1 infant and 1 child with IgA deficiency in our series would have been missed had we not also looked for IgA and IgG AGA.

After reintroduction of gluten to the diet, the search for IgA anti-tTG antibodies showed higher sensitivity than that for IgA EMA, and the results agreed with those of intestinal biopsy results because 15 of the patients had both IgA anti-tTG and histological relapse. We considered IgA EMA to be unreliable markers for slight dietary transgressions.[30] On the contrary, Hansson et al[21] found that after only 12 weeks of gluten challenge, 41 of 48 children had IgA anti-tTG.

In our control group, only 3 subjects of 116 had positive anti-tTG results. Celiac disease was ruled out by HLA antigen–DQ determination[2] for the first subject and by intestinal biopsy data for the other two. However, the high specificity of IgA anti-tTG in this series may not be entirely reliable because we performed intestinal biopsies in only 24 of the 116 control subjects. One might question the ethics of performing an intestinal biopsy in all children with short stature or other disorders. The combination of the 3 tests makes the probability of an incorrect diagnosis extremely slight. In cases with positive anti-tTG results, determination of HLA antigen DQ2 or DQ8 might be a valuable procedure before considering the intestinal biopsy.[2] Because no test is satisfactory to diagnose IgA deficiency associated with CD and even the search for IgG tTG gave conflicting results,[21,29] the test for IgG AGA remains useful.[14] Nevertheless, the cost of the tests in combination is relatively high, €76. Separately, each test costs €19.

In conclusion, although additional studies are required to establish a strategy for the detection of all cases of CD in infants and patients with IgA deficiency, the detection by ELISA of IgA anti-tTG is highly sensitive and specific. In both the diagnosis and follow-up of children with CD, respectively, 28 (93%) of 30 and 15 (100%) of 15 of our relapsing patients tested positive for these antibodies.

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Corresponding author: Jean-Jacques Baudon, MD, Hôpital d'Enfants Armand-Trousseau, 26 avenue du Dr Arnold-Netter, 75571 Paris, France (e-mail: jean-jacques.baudon@trs.ap-hop-paris.fr).

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