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Accuracy of Serologic Tests and HLA-DQ Typing for Diagnosing Celiac Disease

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Background: Estimates of the diagnostic performance of serologic testing and HLA-DQ typing for detecting celiac disease have mainly come from case–control studies.

Objective: To define the performance of serologic testing and HLA-DQ typing prospectively.

Design: Prospective cohort study.

Setting: University hospital.

Patients: Patients referred for small-bowel biopsy for the diagnosis of celiac disease.

Interventions: Celiac serologic testing (antigliadin antibodies [AGA], antitransglutaminase antibodies [TGA], and antiendomysium antibodies [EMA]) and HLA-DQ typing.

Measurements: Diagnostic performance of serologic testing and HLA-DQ typing compared with a reference standard of abnormal histologic findings and clinical resolution after a gluten-free diet.

Results: Sixteen of 463 participants had celiac disease (prevalence, 3.46% [95% CI, 1.99% to 5.55%]). A positive result on both TGA and EMA testing had a sensitivity of 81% (CI, 54% to 95.9%), specificity of 99.3% (CI, 98.0% to 99.9%), and negative predictive value of 99.3% (CI, 98.0% to 99.9%). Testing positive for either HLA-DQ type maximized sensitivity (100% [CI, 79% to 100%]) and negative predictive value (100% [CI, 98.6% to 100%]), whereas testing negative for both minimized the negative likelihood ratio (0.00 [CI, 0.00 to 0.40]) and posttest probability (0% [CI, 0% to 1.4%]). The addition of HLA-DQ typing to TGA and EMA testing, and the addition of serologic testing to HLA-DQ typing, did not change test performance compared with either testing strategy alone.

Limitation: Few cases of celiac disease precluded meaningful comparisons of testing strategies.

Conclusions: In a patient population referred for symptoms and signs of celiac disease with a prevalence of celiac disease of 3.46%, TGA and EMA testing were the most sensitive serum antibody tests and a negative HLA-DQ type excluded the diagnosis. However, the addition of HLA-DQ typing to TGA and EMA testing, and the addition of serologic testing to HLA-DQ typing, provided the same measures of test performance as either testing strategy alone.

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The high prevalence and clinical heterogeneity of celiac disease necessitate noninvasive tests for diagnosis. Specifically, tests are needed to select which patients should undergo small-bowel biopsy. Although celiac disease serologic tests, especially IgA tissue antitransglutaminase antibodies (TGA) and IgA antiendomysium antibodies (EMA), are often used for this purpose because of their reported high sensitivity (1), they may perform less well in the clinical setting (2). Most studies have not defined the usefulness of serologic tests prospectively (3–7), and in addition, some authors doubt the high sensitivity of these tests (8, 9).

Susceptibility to celiac disease is related to the presence of distinct HLA-DQ heterodimers—the DQ2 heterodimer encoded by the alleles HLA-DQA1*05 and HLA-DQB1*02, and the DQ8 heterodimer encoded by the alleles HLA-DQA1*03 and HLA-DQB1*0302 (10–14). One way to improve the selection of patients to undergo small-bowel biopsy may be to combine serologic tests with HLA-DQ typing (15, 16).

We designed a prospective study to define the value of specific serologic tests, HLA-DQ typing, or both in diagnosing celiac disease.

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METHODS

The institutional review board of the VU University Medical Center, Amsterdam, the Netherlands, approved the study protocol. All participants received oral and written information according to the usual recommendations for medical research and the Declaration of Helsinki (17) and gave written informed consent.

Patients

The study was performed in an academic, mixed secondary and tertiary referral center that serves a population of about 200 000 people. In the design phase of the study (1999–2000), the staff of departments of internal medicine and gastroenterology reviewed the literature and agreed that serologic tests could not substitute for small-bowel biopsy in the diagnostic work-up of celiac disease.

Therefore, the policy was to perform small-bowel biopsy when celiac disease was suspected. Adults suspected of having celiac disease who were attending the endoscopy department for small-bowel biopsy were requested to give blood samples for serum antibody testing and HLA-DQ typing. We excluded patients younger than 18 years of age, those with known celiac disease, and patients who declined to undergo endoscopy.

Endoscopy

We performed upper gastrointestinal endoscopy with Olympus video endoscopes (GIF-NT140/160, Olympus Nederland, Zoeterwoude, the Netherlands) and obtained 4 oriented biopsy specimens from the distal duodenum (18).

Serum Antibody Tests

We performed serologic tests after obtaining small-bowel biopsy specimens in all patients to avoid referral bias. All serologic tests were determined anonymously without knowledge of the clinical status or histologic result. We determined IgA and IgG antigliadin antibodies (AGA-IgA and AGA-IgG, respectively) by using enzyme-linked immunosorbent assay (ELISA). We tested for EMA according to the method of Lerner and colleagues (19) by indirect immunofluorescence assay using monkey esophagus (16). Finally, TGA was determined by ELISA, essentially as described by Dieterich and colleagues (20), with guinea pig TGA (gp-TGA) (Sigma-Aldrich, Poole, United Kingdom; coating 10 g/mL in Tris hydrochloride [pH, 7.5] with 5-mmol/L of $CaCl_2$) as the substrate (20). Sera were diluted and preincubated (30 minutes at room temperature) with 1% bovine serum albumin to avoid nonspecific binding (16, 21). The cutoff values for the titers of AGA and gp-TGA tests are based on measurements in control groups (blood donors, patients without celiac disease, and the general population age 2 to 4 years), and optimization was done by a receiver-operating characteristic curve analysis in well-defined patient groups.

Because the recombinant human TGA (rh-TGA) assay became available when the study was already ongoing, we retrospectively reevaluated all samples from patients with an abnormal result on serologic testing or histologic examination by using rh-TGA as substrate (Roboscreen, Leipzig, Germany; coating 5 g/mL and same conditions as those for gp-TGA). When serologic test results did not match histologic findings, we measured total serum IgA and repeated the serologic tests. In cases of IgA deficiency, we evaluated TGA-IgG antibodies. We defined seropositivity as 1 or more positive measured antibody test results and seronegativity as negative results on all 4 tests.

HLA-DQ Typing

Whole blood was obtained for HLA-DQA1 and HLA-DQB1 genotyping. Polymerase chain reaction–amplified exon 2 amplicons were generated for low- to medium-resolution typing in a combined, single-stranded conformation polymorphism–heteroduplex assay by a semiautomated electrophoresis and gel-staining method on the PhastSystem (Amersham Pharmacia Biotech, Uppsala, Sweden). Alleles DQA1*05 and DQB1*02 (encoding the HLA-DQ2 heterodimer) and alleles DQA1*03 and DQB1*0302 (encoding the HLA-DQ8 heterodimer) could be reliably characterized in homozygous and heterozygous states. This method has been validated by using a panel of reference DNA against the Dynall Allset sequence-specific primers high-resolution typing kits (Dynal A.S., Oslo, Norway) (16, 22).

Context

The value of adding HLA genetic typing to serologic testing for celiac disease is not well defined.

Contribution

In this prospective study of patients referred for evaluation of celiac disease, the test performance of combinations of genetic typing and serologic testing was similar to that of either strategy alone.

Caution

The small number of cases of celiac disease precluded meaningful comparisons of testing strategies.

Implications

The combination of genetic typing and serologic testing is about as accurate as either strategy alone. Neither is a substitute for small-bowel biopsy in the diagnosis of celiac disease.

—The Editors

Histologic Studies

A gastrointestinal pathologist who was masked to clinical data evaluated the biopsy material, and an independent pathologist reviewed the samples when histologic examination was abnormal. Consensus was reached on the final diagnosis. Villous (crypt) anatomy and density of intraepithelial lymphocytes were assessed uniformly by using hematoxylin–eosin and immunohistologic anti-CD3 staining, respectively. **Appendix Figures 1** and **2** (available at www.annals.org) show the histologic grading of abnormalities, based on the most severe change found according to the modified Marsh classification (23, 24).

Diagnosis and Follow-up of Celiac Disease

The diagnosis of celiac disease was based on the European Society for Paediatric Gastroenterology, Hepatology and Nutrition criteria, revised in 1989 and published in 1990, by identifying characteristic histologic findings (Marsh III) on small-bowel biopsy and unequivocal clinical resolution after a gluten-free diet was initiated (25). Thus, by this definition and for this study, the diagnosis of celiac disease did not require follow-up biopsy. However, we assessed histologic response in most patients and serologic response in all patients who were found to have celiac disease. We defined a serologic response as the disappearance

of initially positive celiac disease antibody test results and histologic response as the regression of villi to Marsh 0 to II on a repeated biopsy at least 12 months after a gluten-free diet was initiated (24).

Statistical Analysis

We compared results of serologic tests and HLA-DQ typing with the diagnosis of celiac disease as previously defined. We performed statistical analysis by using SPSS software, version 11.0 (SPSS, Chicago, Illinois). To calculate exact binomial CIs, we used StatXact software, version 7.0.0 (Cytel Software, Cambridge, Massachusetts). We used 2×2 tables (Bayes theorem) to calculate sensitivities and specificities, predictive values, and likelihood ratios. We used the *t* test and Fisher exact test to compare continuous data and categorical data, respectively. We calculated posttest probabilities (and CIs) of celiac disease for different diagnostic tests or a combination thereof by using the method recommended by Altman (26).

Role of the Funding Source

The study received no funding.

RESULTS

Patients

Between January 2001 and January 2004, 502 consecutive patients (originally from community practices in the Amsterdam area) were referred from outpatient internal medicine and gastroenterology clinics for endoscopy and small-bowel biopsy for the diagnosis of celiac disease. Another 16 inpatients were referred from the internal medicine and gastroenterology inpatient wards. No referred patient was under the care of the investigators. We excluded 55 (10.6%) patients because they declined to participate in the study after the small-bowel biopsy. Age, sex, body mass index, ethnicity, and indications for referral did not statistically significantly differ between those included in and those excluded from the study (data not shown). The available serologic data ($n = 20$), HLA-DQ data ($n = 4$), and histologic examinations ($n = 55$) suggested the absence of celiac disease in excluded patients.

Therefore, 463 patients were included in the study: 346 (75%) were unrelated Dutch Caucasian persons and 117 (25%) were not Caucasian (Indian, Chinese, North African [Arab], and Central African [black], in descending order of frequency). All patients were on a normal diet at the time of inclusion. **Table 1** summarizes the general characteristics and indications for small-bowel biopsy of patients without and with celiac disease.

Patients with Celiac Disease

Of the 463 patients enrolled, 16 (3.46% [95% CI, 1.99% to 5.55%]) fulfilled the diagnostic criteria for celiac disease (**Figure**) (25) within a median follow-up interval of 22 months (range, 11 to 44 months). Biopsy readings of the 2 pathologists were concordant in all but 3 cases (classified as Marsh II and Marsh I by each of the 2 investigators). This disagreement did not influence the study results, because only Marsh III was considered to be diagnostic of celiac disease. **Table 2** shows the clinical features, diagnostic findings, and follow-up data of patients proven to have celiac disease. All patients reported symptomatic relief on a gluten-free diet for a median interval of 3.5 weeks (range, 2 to 8 weeks), and 13 initially seropositive patients had a serologic response by the end of follow-up.

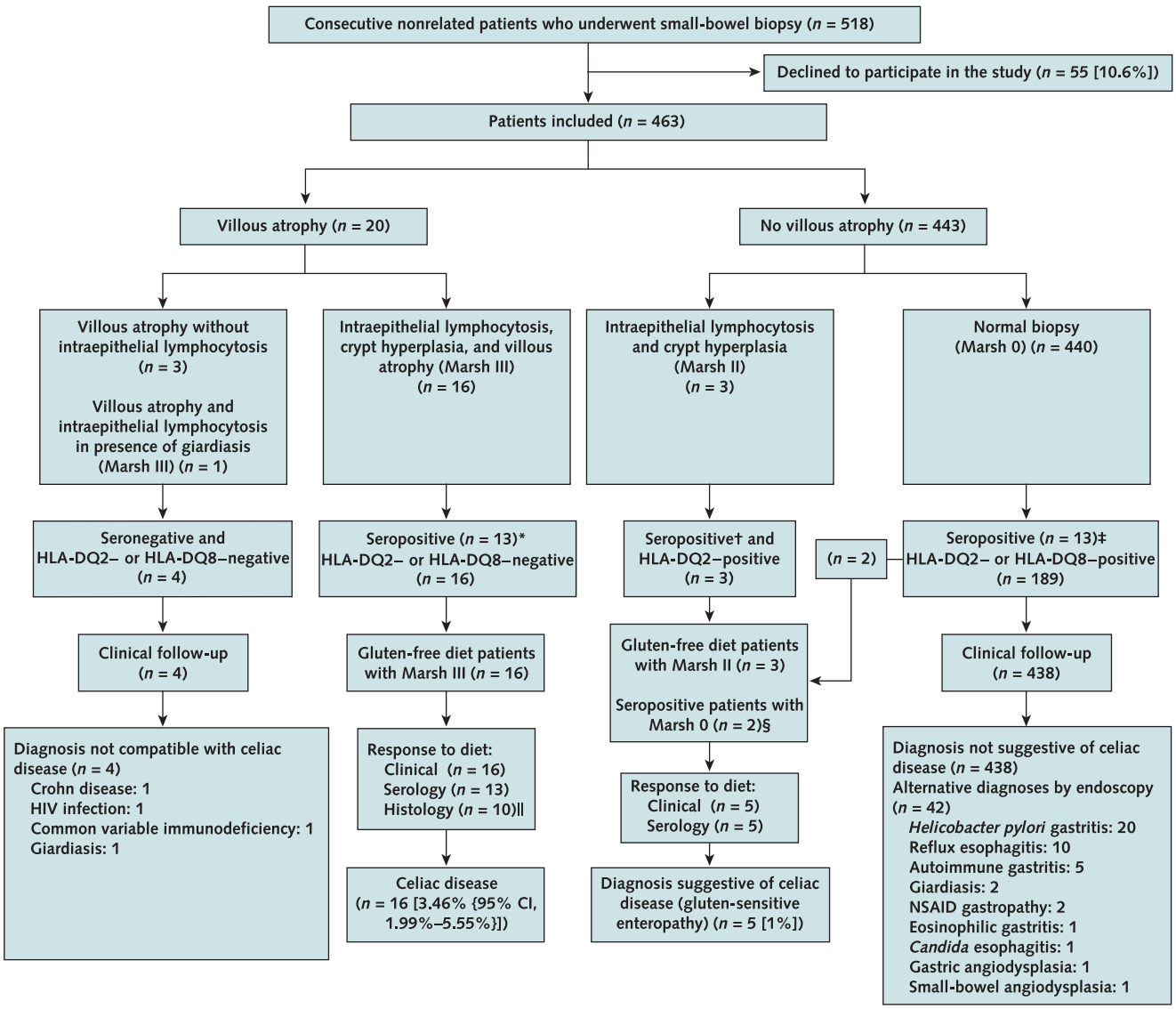
Three (19% [CI, 4% to 46%]) patients with celiac disease remained seronegative on repeated measurement.

Table 1. **Characteristics of and Indications for Referral in Patients without and with Celiac Disease**

Characteristic	All Patients ($n = 463$)	Patients without Celiac Disease ($n = 447$)	Patients with Celiac Disease ($n = 16$)	*P* Value*
Mean age (SD), *y*	46.1 (16.0)	46.4 (15.9)	39.0 (16.1)	0.072
Men, *n* (%)	140 (30)	134 (30)	6 (37)	0.58
Mean body weight (SD), *kg*	68.3 (14.6)	68.4 (14.7)	64.2 (9.7)	0.24
Mean height (SD), *cm*	169.0 (9.4)	168.9 (9.3)	172.8 (9.2)	0.106
Mean body mass index (SD), kg/m^2	23.9 (4.8)	24.0 (4.9)	21.7 (3.6)	0.025
Caucasian ethnicity, *n (%)*	346 (75)	333 (74)	13 (81)	0.77
Family history of celiac disease, *n (%)*	40 (9)	36 (8)	4 (25)	0.041
Associated disorders, *n (%)*†	46 (10)	39 (9)	7 (43)	<0.001
Indications for referral, *n (%)*				
Abdominal pain, dyspepsia	167 (36)	164 (37)	3 (19)	–
Diarrhea	129 (28)	121 (27)	8 (50)	–
Anemia	101 (22)	98 (22)	3 (19)	–
Weight loss	47 (10)	46 (10)	1 (6)	–
Family screening	19 (4)	18 (4)	1 (6)	–

* Continuous data were compared by using the *t* test (2-sided), and categorical data were compared by using the Fisher exact test (2-sided).
† Type 1 diabetes mellitus, autoimmune thyroid disease, the Sjögren syndrome, lymphocytic colitis, collagenous colitis, dermatitis herpetiformis, and pernicious anemia.

Figure. **Study flow diagram.**



NSAID = nonsteroidal anti-inflammatory drug. *Tests were positive for IgG antigliadin antibodies (AGA) in 4 patients, AGA-IgA in 8 patients, and guinea pig–IgA tissue antitransglutaminase (TGA) and antiendomysium antibodies (EMA) in 13 patients. †Tests were positive for AGA-IgG in 1 patient, tissue TGA in 1 patient, and EMA in 2 patients. ‡Tests were positive for AGA-IgG in 5 patients, AGA-IgA in 8 patients, tissue TGA in 3 patients, and EMA in 2 patients. §A gluten-free diet was prescribed to 2 seropositive patients at their request despite normal small-bowel histologic findings. ||One patient could not be reached for a follow-up small-bowel biopsy.

All had villous atrophy classified as Marsh IIIa ($n = 2$) or IIIb ($n = 1$). One of these seronegative patients had IgA deficiency and was clearly positive for TGA-IgG. Only 1 seronegative patient had a first-degree family history of celiac disease. All 3 patients reported diarrhea when using a normal diet that resolved after initiating a gluten-free diet. Repeated small-bowel biopsy revealed recovery of villi from Marsh IIIa and IIIb to Marsh I in 2 patients; the third patient could not be reached again for a follow-up small-bowel biopsy.

Patient Subsets

The **Figure** shows the clinically relevant patient subsets. Five seropositive patients (AGA-IgG [$n = 2$], TGA [$n = 2$], and EMA [$n = 3$]) who also tested positive for HLA-DQ2 had Marsh II changes ($n = 3$) or normal histologic findings ($n = 2$) on small-bowel biopsy. All experienced symptomatic relief and seroconversion after a gluten-free diet. These 5 patients did not meet study criteria for the diagnosis of celiac disease but were considered to have gluten-sensitive enteropathy (27).

Table 2. **Clinical and Diagnostic Work-up of Patients with Proven Celiac Disease***

Patient	Age, y	Sex	Ethnicity	Associated Disorders	Indications for Small-Bowel Biopsy
1	29	F	North Africa (Arab)	NA	Abdominal pain
2	46	M	Caucasian	NA	Diarrhea
3	22	F	Caucasian	NA	Weight loss
4	24	F	Indochinese (Indonesia)	Dermatitis herpetiformis	Dermatitis herpetiformis
5	37	F	Caucasian	Type 1 diabetes mellitus	Diarrhea
6	66	F	Caucasian	NA	Diarrhea
7	41	M	Caucasian	NA	Family screening
8	42	F	Caucasian	NA	Iron deficiency anemia
9	49	M	North Africa (Arab)	Autoimmune thyroid disease	Abdominal pain
10	26	F	Caucasian	NA	Iron deficiency anemia
11	42	F	Caucasian	NA	Diarrhea
12	55	M	Caucasian	Autoimmune thyroid disease	Abdominal pain
13	68	M	Caucasian	Collagenous colitis	Diarrhea
14	19	M	Caucasian	Sjögren syndrome and IgA deficiency	Diarrhea
15	54	F	Caucasian	Collagenous colitis	Diarrhea
16	25	F	Caucasian	NA	Diarrhea

* AGA = antigliadin antibody; EMA = antiendomysium antibody; F = female; GFD = gluten-free diet; gp = guinea pig; M = male; NA = not applicable; ND = not done; rh = recombinant human; TGA = antitransglutaminase antibody.
† Normal serum IgA level, 0.55–4.14 g/L.
‡ At presentation, the patient reported having no symptoms. However, the general condition improved and the previously ignored dyspeptic symptoms disappeared after the patient began a gluten-free diet.

Another 4 patients had villous atrophy but were seronegative and HLA-DQ2– and HLA-DQ8–negative. Diagnoses in these patients were Crohn disease ($n = 1$), HIV infection ($n = 1$), common variable immunodeficiency ($n = 1$), and giardiasis ($n = 1$). In the patient with giardiasis, intraepithelial lymphocytosis observed on histologic examination resolved completely on repeated biopsy after treatment with metronidazole.

Other diagnoses made by endoscopy included *Helicobacter pylori*–associated gastritis ($n = 20$), reflux esophagitis ($n = 10$), autoimmune gastritis ($n = 5$), giardiasis ($n = 2$), nonsteroidal anti-inflammatory drug gastropathy ($n = 2$), eosinophilic gastritis ($n = 1$), *Candida* esophagitis ($n = 1$), gastric angiodysplasia ($n = 1$), and small-bowel angiodysplasia ($n = 1$).

Serum Antibody Tests

Table 3 and the **Appendix Table** (available at www.annals.org) provide serologic test performance results in the study sample. A positive result on TGA testing alone or EMA testing alone maximized sensitivity to 81% (CI, 54% to 96%), specificity to 99.1% (CI, 97.7% to 99.7%), and negative predictive value to 99.1% (CI, 98% to 99.7%); results were the same when both were positive, except for a trivial increase in specificity. A negative result on TGA testing, EMA testing, or both in combination minimized the negative likelihood ratio (0.19 [CI, 0.07 to 0.47]) and posttest probability (0.67% [CI, 0.14% to 1.9%], based on study prevalence estimate of 3.46%). When all 4 serologic test results (AGA-IgG, AGA-IgA, TGA, and EMA) were positive, specificity (100% [CI, 99.2% to 100%]), positive predictive value (100% [CI, 29% to 100%]), and posttest probability (100% [CI, 29% to 100%]) were maximal but sensitivity was minimized to 19% (CI, 4% to 46%).

Sixteen (3.46%) patients without villous atrophy were seropositive (AGA-IgG in 4, AGA-IgA in 8, TGA in 4, and EMA in 4). Nine of these 16 patients were also positive for HLA-DQ2 or HLA-DQ8. At the end of follow-up, 5 seropositive patients received a diagnosis of gluten-sensitive enteropathy and alternative diagnoses were made in 11 patients. The rh-TGA assays confirmed seropositivity in all sera that were initially positive on gp-TGA ELISA and confirmed seronegativity in all sera from patients with histologic abnormalities and negative results on gp-TGA ELISA.

HLA-DQ Typing

Table 4 summarizes the distribution of HLA-DQ2 and HLA-DQ8 heterodimers in patients without and with celiac disease. Of 463 patients, 208 (45%) had HLA-DQ2 heterodimers, HLA-DQ8 heterodimers, or both, including 2 of 4 patients with dermatitis herpetiformis and 27 (67.5%) of 40 patients with a positive family history for celiac disease. Of all patients with celiac disease, all Dutch Caucasian patients, 2 patients from Morocco (DQ2 heterozygote and DQ2 homozygote), and 1 patient from Indonesia (DQ2 heterozygote) tested positive for celiac disease–specific heterodimers.

Table 3 describes the HLA-DQ typing test performance. Testing positive for either HLA-DQ2 or HLA-DQ8 maximized sensitivity (100% [CI, 79% to 100%]) and negative predictive value (100% [CI, 98.6% to 100%]). Testing negative for either type minimized the

Table 2—Continued

Serum Antibody Tests						Serum IgA Level, *g/L*†	HLA-DQ Type	Histologic Findings	Treatment	Response		
AGA		EMA	TGA							Clinical	Serologic	Histologic
IgA	IgG	IgA	IgA (gp)	rh	IgG (gp)							
−	−	+	+	+		NA	DQ2	Marsh IIIa	GFD	+	+	ND
+	−	+	+	+		NA	DQ2	Marsh IIIb	GFD	+	+	Marsh 0
−	+	+	+	+		NA	DQ2	Marsh IIIb	GFD	+	+	Marsh 0
+	−	+	+	+		NA	DQ2	Marsh IIIa	GFD	+	+	ND
−	−	+	+	+		NA	DQ2 and DQ8	Marsh IIIa	GFD	+	+	Marsh 0
−	−	−	−	−		2.4	DQ2	Marsh IIIa	GFD	+	ND	ND
−	−	+	+	+		NA	DQ2	Marsh IIIa	GFD	+‡	+	Marsh 0
+	+	+	+	+		NA	DQ2	Marsh IIIb	GFD	+	+	Marsh I
−	−	+	+	+		NA	DQ2	Marsh IIIc	GFD	+	+	Marsh IIIa
+	+	+	+	+		NA	DQ2	Marsh IIIc	GFD	+	+	Marsh IIIa
+	−	+	+	+		NA	DQ8	Marsh IIIa	GFD	+	+	Marsh 0
+	+	+	+	+		NA	DQ2	Marsh IIIc	GFD	+	+	Marsh I
+	−	+	+	+		NA	DQ2	Marsh IIIb	GFD	+	+	ND
−	−	−	−	−	+	0.07	DQ2	Marsh IIIa	GFD	+	ND	Marsh I
−	−	−	−	−		3.1	DQ2	Marsh IIIb	GFD	+	ND	Marsh I
+	–	+	+	+		NA	DQ2	Marsh IIIa	GFD	+	+	Marsh 0

negative likelihood ratio (0.00 [CI, 0.00 to 0.40]) and posttest probability (0% [CI, 0% to 1.4%], based on study prevalence estimate of 3.46%).

Combined Serologic and HLA-DQ Testing

Sensitivity (100% [CI, 79% to 100%]) and negative predictive value (100% [CI, 98.5% to 100%]) were maximized when test results for either TGA or EMA or either of the 2 HLA-DQ heterodimers were positive. Specificity (99.3% [CI, 98.0% to 99.9%]), positive predictive value (81% [CI, 54% to 95.9%]), positive likelihood ratio (121 [CI, 39 to 409]), and posttest probability (81% [CI, 54% to 96%]) were maximized when TGA and EMA results and HLA-DQ typing were all positive (**Table 3**). When all 4 serologic test results and HLA-DQ typing were negative, the likelihood ratio (0.00 [CI, 0.00 to 0.43]) and posttest probability (0% [CI, 0% to 1.5%]) were minimized. The addition of HLA-DQ typing to TGA and EMA testing provided the same performance as TGA and EMA testing alone. The addition of serologic testing to HLA-DQ typing provided the same performance as HLA-DQ typing alone.

Discussion

Our prospective study in a well-defined clinical setting of a sample of patients attending a university hospital suggests that TGA and EMA tests alone or in combination were specific and were the most sensitive of the 4 commonly used serum antibody tests to diagnose celiac disease. Testing for HLA-DQ2 or HLA-DQ8 was 100% sensitive for the diagnosis and yielded a negative likelihood ratio and posttest probability of 0. However the addition of HLA-DQ typing to TGA and EMA testing provided the same performance as TGA and EMA testing alone, as did the addition of TGA and EMA testing to HLA-DQ typing alone.

Our data on the sensitivity of serologic tests are consistent with those of other studies (28–32), although Collin and colleagues (6) observed higher sensitivities (94% for TGA and 89% for EMA). This difference may be explained by patient selection, because these authors compared serologic test results in patients with known celiac disease with those in control patients. A recent prospective report from an Australian celiac disease study group investigating the diagnostic accuracy of celiac disease serologic tests and using a similar approach to ours showed similar results in the sensitivity and specificity of AGA and specificity of EMA (33). However, the group reported lower sensitivity for EMA (68% vs. 81%) and higher sensitivity and lower specificity for TGA (88% vs. 81% and 84% vs. 99.1%, respectively) compared with our findings (33). Both studies are alike in design; therefore, selection bias might be a plausible explanation for these differences in outcome. However, we show, as have the Australian study (33) and others (1), that sensitivity and specificity of AGA are inferior to those of TGA and EMA. In our study, TGA-IgG provided additional value when the test result was clearly positive in 1 patient with celiac disease and IgA deficiency (34), and therefore the test may be useful in seronegative patients with a high pretest probability of disease when serologic results are negative and HLA typing is

Table 3. **Frequencies and Likelihood Ratios of Serologic Tests and HLA-DQ Typing***

Test Result	Small-Bowel Biopsy Result and Clinical Resolution with Gluten-Free Diet, *n*		Sensitivity (95% CI), %†	Specificity (95% CI), %†	Predictive Value (95% CI), %†	Likelihood Ratio (95% CI), %†	Posttest Probability, (95% CI), %‡
	Positive (*n* = 16)	Negative (*n* = 447)					
Serologic testing							
AGA			56 (30–80)	97.3 (95.4–98.6)			
Positive	9	12			43 (22–66)	21 (9.9–41)	43 (22–66)
Negative	7	435			98.4 (96.7–99.4)	0.45 (0.23–0.72)	1.6 (0.64–3.2)
AGA-IgA			50 (25–75)	98.2 (96.5–99.2)			
Positive	8	8			50 (25–75)	28 (11–65)	50 (25–75)
Negative	8	439			98.2 (96.5–99.2)	0.51 (0.27–0.77)	1.8 (0.77–3.5)
AGA-IgG			25 (7.3–52)	98.7 (97.1–99.5)			
Positive	4	6			40 (12–74)	19 (5.1–55)	40 (12–74)
Negative	12	441			97.3 (95.4–98.6)	0.76 (0.50–0.93)	2.6 (1.4–4.6)
gp-TGA			81 (54–95.9)	99.1 (97.7–99.7)			
Positive	13	4			76 (50–93.2)	91 (33–271)	76 (50–93)
Negative	3	443			99.3 (98.0–99.9)	0.19 (0.072–0.47)	0.67 (0.14–1.9)
EMA			81 (54–95.9)	99.1 (97.7–99.7)			
Positive	13	4			76 (50–93.2)	91 (33–271)	76 (50–93)
Negative	3	443			99.3 (98.0–99.9)	0.19 (0.072–0.47)	0.67 (0.14–1.9)
AGA and TGA			56 (30–80)	99.8 (98.8–99.99)			
Positive	9	1			90 (55–99.8)	251 (42–2160)	90 (55–99)
Negative	7	446			98.4 (96.8–99.4)	0.44 (0.25–0.71)	1.5 (0.62–3.2)
AGA and EMA			56 (30–80)	99.8 (98.8–99.99)			
Positive	9	1			90 (55–99.8)	251 (42–2160)	90 (55–99)
Negative	7	446			98.4 (96.8–99.4)	0.44 (0.25–0.71)	1.5 (0.62–3.2)
TGA and EMA			81 (54–95.9)	99.3 (98.0–99.9)			
Positive	13	3			81 (54–95.9)	121 (39–409)	81 (54–96)
Negative	3	444			99.3 (98.0–99.9)	0.19 (0.072–0.47)	0.67 (0.14–1.9)
All 4 serologic tests			19 (4–46)	100 (99.2–100)			
Positive	3	0			100 (29–100)	∞ (22–∞)	100 (29–100)
Negative	13	447			97.2 (95.2–98.5)	0.81 (0.56–0.96)	2.8 (1.5–4.8)
Any serologic test			81 (54–95.9)	96.4 (94.2–97.9)			
Positive	13	16			45 (26–64)	23 (13–39)	45 (26–64)
Negative	3	431			99.3 (97.9–99.9)	0.19 (0.073–0.49)	0.69 (0.14–20)
HLA-DQ typing							
HLA-DQ2			93.7 (70–99.8)	73 (69–77)			
Positive	15	121			11 (6.3–18)	3.5 (2.6–4.2)	11 (6.3–17)
Negative	1	326			99.7 (98.3–99.99)	0.085 (0.018–0.44)	0.30 (0.077–1.7)
HLA-DQ8			12 (1.6–38)	81 (77–85)			
Positive	2	85			2.3 (0.28–8.1)	0.66 (0.14–2.1)	2.2 (0.28–8.1)
Negative	14	362			96.3 (93.8–97.9)	1.1 (0.79–1.22)	3.7 (2.0–6.2)
HLA-DQ2 and -DQ8			6.2 (16–30)	96.9 (94.8–98.3)			
Positive	1	14			6.7 (0.17–32)	1.9 (0.18–11)	6.6 (0.17–32)
Negative	15	433			96.6 (94.5–98.1)	0.97 (0.74–1.0)	3.3 (0.054–1.6)
HLA-DQ2 or -DQ8			100 (79–100)	57 (52–62)			
Positive	16	192			7.7 (4.5–12)	2.3 (1.9–2.6)	7.7 (4.5–12)
Negative	0	255			100 (98.6–100)	0 (0–0.40)	0 (0–1.4)
Both serologic testing and HLA-DQ typing							
AGA and HLA-DQ			56 (30–80)	98.7 (97.1–99.5)			
Positive	9	6			60 (32–84)	42 (16–100)	60 (32–84)
Negative	7	441			98.4 (96.8–99.4)	0.44 (0.24–0.71)	1.5 (0.63–3.2)
TGA and HLA-DQ			81 (54–95.9)	99.3 (98.0–99.9)			
Positive	13	3			81 (54–95.9)	121 (39–409)	81 (54–96)
Negative	3	444			99.3 (98.0–99.9)	0.19 (0.072–0.47)	0.67 (0.14–1.9)
EMA and HLA-DQ			81 (54–95.9)	99.1 (97.7–99.8)			
Positive	13	4			76 (50–93)	91 (33–271)	76 (50–93)
Negative	3	443			99.3 (98.0–99.9)	0.19 (0.072–0.47)	0.67 (0.14–1.9)
TGA, EMA, and HLA-DQ			100 (79–100)	99.3 (98.0–99.9)			
Positive	13	3			81 (54–95.9)	121 (39–409)	81 (54–96)
Negative	3	444			99.3 (98.0–99.9)	0.19 (0.072–0.47)	0.67 (0.14–1.9)
Any serologic test or any HLA-DQ			100 (79–100)	55 (51–60)			
Positive	16	199			7.4 (4.3–12)	2.2 (1.8–2.5)	7.4 (4.3–19)
Negative	0	248			100 (98.5–100)	0 (0–0.43)	0 (0–1.5)
Any serologic test and any HLA-DQ			81 (54–95.9)	97.9 (96.2–99.1)			
Positive	13	9			59 (36–79)	40 (19–85)	59 (36–79)
Negative	3	438			99.3 (98.0–99.9)	0.19 (0.073–0.48)	0.68 (0.14–1.9)

* AGA = antigliadin antibody; EMA = antiendomysium antibody; gp = guinea pig; TGA = antitransglutaminase antibody.
† Exact binomial CIs.
‡ Based on study prevalence of 3.46%.

unavailable or is otherwise not performed. Although studies have reported that rh-TGA tests (35, 36) have generally higher values than gp-TGA tests, the 2 tests provided the same results in our study.

Our data confirm that the absence of HLA-DQ2, HLA-DQ8, or both virtually excludes the diagnosis of celiac disease (6, 16, 37), although the modest specificity of the test means that a positive result is not sufficient to diagnose the disease (having a low positive predictive value and positive likelihood ratio and yielding a low posttest probability). Even the presence of HLA-DQ2 or HLA-DQ8 in patients with positive serologic test results is strongly suggestive but not pathognomonic for celiac disease. Our findings also suggest that the HLA-DQ2 or HLA-DQ8 genotype, although originally studied in Caucasian populations, is a prerequisite for celiac disease in people of heterogeneous ethnic backgrounds (38, 39).

Nine patients without villous atrophy had positive serologic test and HLA-DQ typing results. Gluten-sensitive enteropathy (27) was diagnosed in 5 of these patients on the basis of improvement in symptoms and serologies, whereas a diagnosis other than celiac disease was established at the end of the follow-up in the other 4 patients. On the basis of this experience, gluten challenge might be useful for identifying patients with gluten sensitivity if initial biopsies reveal no or only minor abnormalities (40). Conversely, villous atrophy does not necessarily imply celiac disease, as 4 patients with villous atrophy had other disorders. The absence of intraepithelial lymphocytosis in 3 of the 4 cases suggested a diagnosis other than celiac disease, and HLA-DQ typing definitively excluded the diagnosis.

Our study has several limitations. The potential for referral bias exists in studies like ours that are conducted in academic medical centers, and the sample was small. With only 16 cases of celiac disease, we could not meaningfully compare testing strategies. Fifty-five (10.6%) patients declined to participate in the study, and although available data suggest that none of these patients had celiac disease, the study's estimates exclude these cases. Finally, the immediate clinical relevance of our findings is modest given that HLA-DQ typing is cumbersome and is not readily available (41). However, new tests that are less cumbersome, quicker, and cheaper are under development (42).

In summary, our study found a prevalence of celiac disease of 3.46% in an unselected population referred for symptoms and signs of celiac disease or for screening over 3 years. Estimates of diagnostic test accuracy in this population suggest the following: TGA and EMA alone or in combination were specific and were the most sensitive serum antibody tests; HLA-DQ typinig was 100% sensitive for the diagnosis and yielded a negative likelihood ratio and posttest probability of zero; and the addition of HLA-DQ typing to TGA and EMA testing and the addition of serologic testing to HLA-DG typing had the same performance as either testing strategy alone.

Table 4. **Distribution of HLA-DQ2 and HLA-DQ8 Heterodimers in Patients without and with Celiac Disease**

HLA-DQ2 and HLA-DQ8	Patients without Celiac Disease (n = 447), n (%)	Patients with Celiac Disease (n = 16), n (%)
HLA-DQ2/X*	99 (22)	9 (56)
HLA-DQ2 homozygous	8 (2)	5 (31)
HLA-DQ8/X*	65 (15)	–
HLA-DQ8 homozygous	6 (1)	1 (6)
HLA-DQ2 and -DQ8	14 (3)	1 (6)
HLA-DQ2 and/or -DQ8	192 (43)	16 (100)
Noncarrier of HLA-DQ2 and/or -DQ8	255 (57)	–

* X = any HLA-DQ except HLA-DQ2 or HLA-DQ8.

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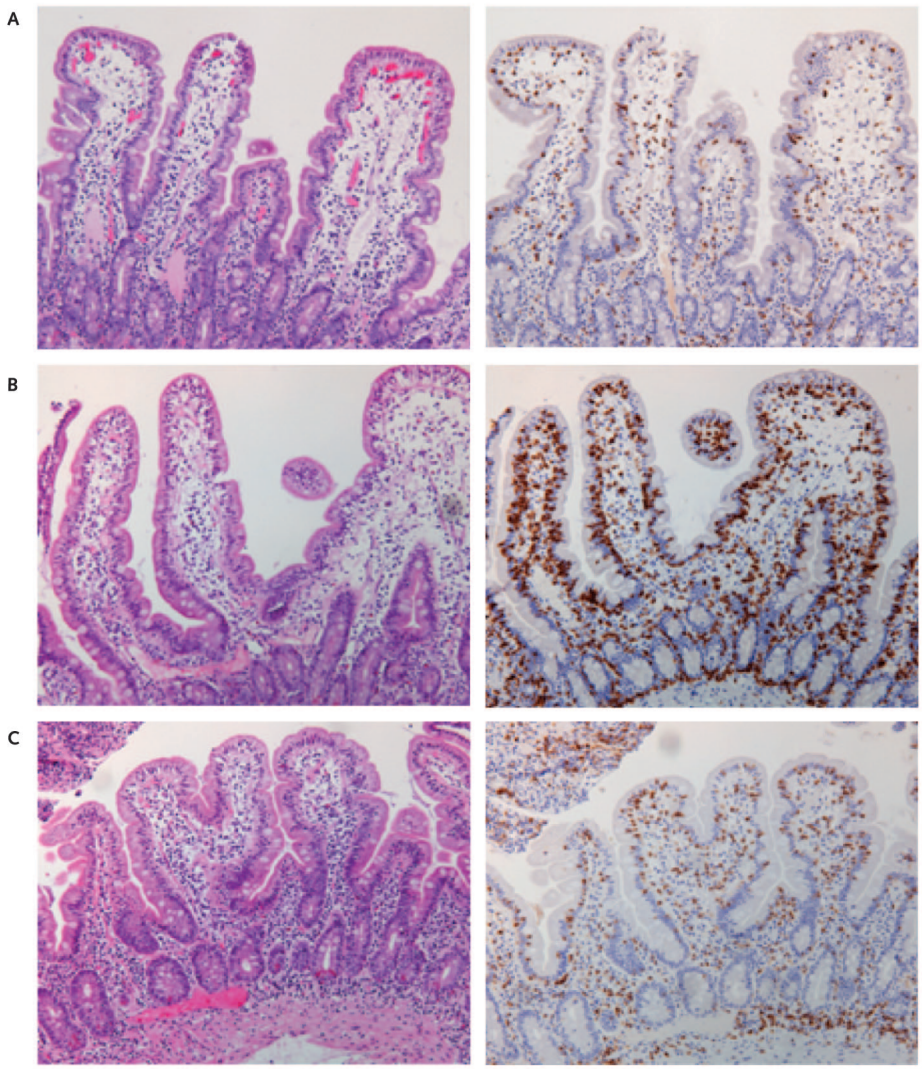
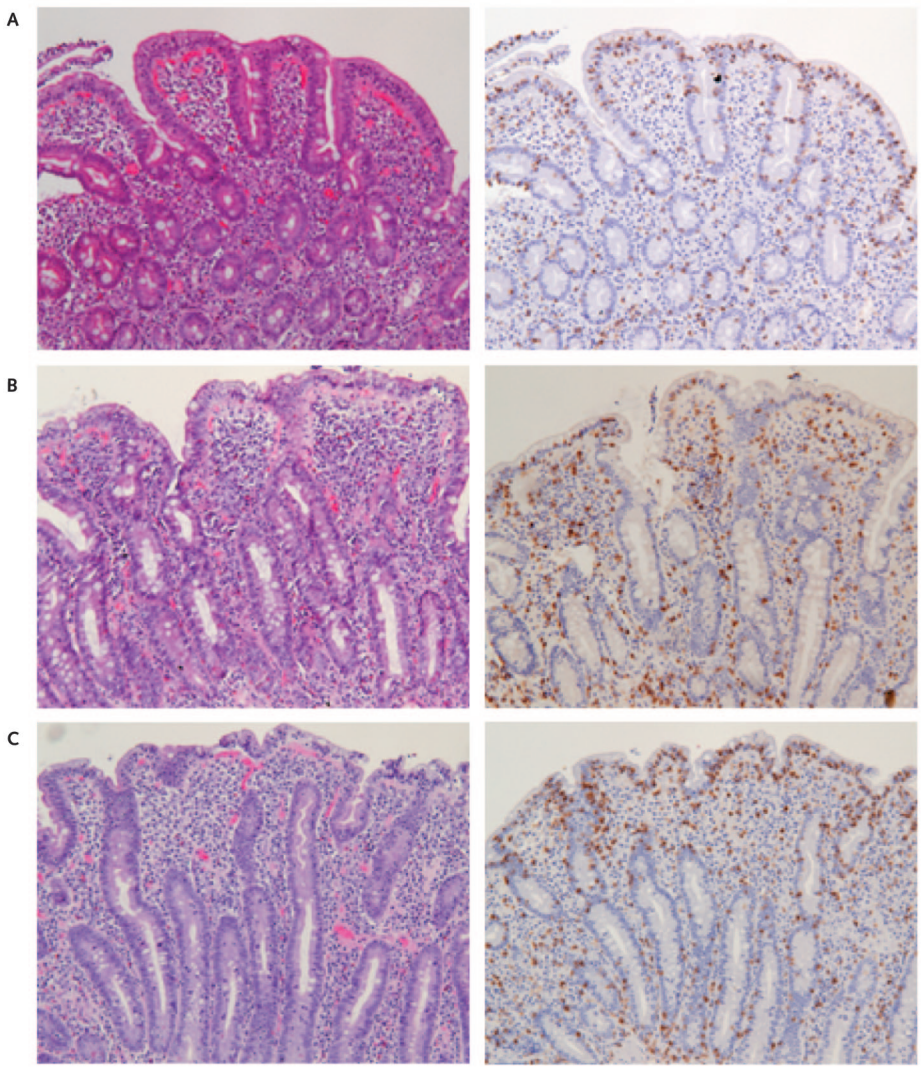
Appendix Figure 1. **Small-bowel histologic findings (Marsh 0 to II).**



Slides with hematoxylin–eosin staining (for assessment of villous [crypt] anatomy) appear on the left; those with immunofluorescence-labeled, monoclonal anti-CD3 immunohistologic staining (for assessment of density of intraepithelial lymphocytes) appear on the right. **A.** Normal small-bowel mucosa is characterized by normal villous architecture, villous–crypt ratio of 4:1, and intraepithelial lymphocyte count <30 cells per 100 enterocytes. **B.** A Marsh I lesion is characterized by intraepithelial lymphocytosis, defined as intraepithelial lymphocyte count >30 cells per 100 enterocytes in mucosa whose architecture appears normal. **C.** A Marsh II lesion is characterized by intraepithelial lymphocytosis accompanied by crypt hyperplasia (elongation and branching of crypts).

Appendix Figure 2. **Small-bowel histologic findings (Marsh IIIa to IIIc).**



Slides with hematoxylin–eosin staining (for assessment of villous [crypt] anatomy) appear on the left; those with immunofluorescence-labeled, monoclonal anti-CD3 immunohistologic staining (for assessment of density of intraepithelial lymphocytes) appear on the right. **A.** Marsh IIIa (partial villous atrophy) is a more severe stage that is characterized by intraepithelial lymphocytosis (defined as intraepithelial lymphocyte count >30 cells per 100 enterocytes in mucosa whose architecture appears normal), crypt hyperplasia, and a villous–crypt ratio <1:1. **B.** A Marsh IIIb lesion (subtotal villous atrophy) is characterized by atrophic but still recognizable villi. **C.** When villi are absent or rudimentary, the lesion is described as Marsh IIIc, or total villous atrophy.

Appendix Table. **Diagnostic Performance of Serum Antibody Tests and HLA-DQ Typing Alone or in Combination***

Positive Diagnostic Test Result	Frequency of No Celiac Disease (n = 447), n (%)	Frequency of Celiac Disease (n = 16), n (%)	Sensitivity (95% CI), %	Specificity (95% CI), %	Positive Predictive Value (95% CI), %	Negative Predictive Value (95% CI), %	Positive Likelihood Ratio (95% CI), %	Negative Likelihood Ratio (95% CI), %
Serologic testing								
AGA								
IgA or IgG†	12 (2.6)	9 (56)	56 (30–80)	97.3 (95.4–98.6)	43 (22–66)	98.4 (96.7–99.4)	21 (9.9–41)	0.45 (0.23–0.72)
IgA	8 (1.7)	8 (50)	50 (25–75)	98.2 (96.5–99.2)	50 (25–75)	98.2 (96.5–99.2)	28 (11–65)	0.51 (0.27–0.77)
IgG	6 (1.3)	4 (25)	25 (7.3–52)	98.7 (97.1–99.5)	40 (12–74)	97.3 (95.4–98.6)	19 (5.1–55)	0.76 (0.50–0.93)
TGA‡	4 (0.89)	13 (81)	81 (54–95.9)	99.1 (97.7–99.7)	76 (50–93.2)	99.3 (98.0–99.9)	90.8 (33–271)	0.19 (0.072–0.47)
EMA§	4 (0.89)	13 (81)	81 (54–95.9)	99.1 (97.7–99.7)	76 (50–93.2)	99.3 (98.0–99.9)	90.8 (33–271)	0.19 (0.072–0.47)
AGA or TGA	16 (3.6)	13 (81)	81 (54–95.9)	96.4 (94.2–97.9)	45 (26–64)	99.3 (97.9–99.9)	23 (13–39)	0.19 (0.073–0.49)
AGA or EMA	15 (3.4)	13 (81)	81 (54–95.9)	96.6 (94.5–98.1)	46 (28–66)	99.3 (98.0–99.9)	24 (13–43)	0.19 (0.073–0.49)
TGA or EMA	5 (1.1)	13 (81)	81 (54–95.9)	98.9 (97.4–99.6)	72 (47–90)	99.3 (98.0–99.0)	73 (29–204)	0.19 (0.072–0.48)
AGA and TGA	1 (0.22)	9 (56)	56 (30–80)	99.8 (98.8–99.99)	90 (55–99.8)	98.4 (96.8–99.4)	251 (42–2160)	0.44 (0.25–0.71)
AGA and EMA	1 (0.22)	9 (56)	56 (30–80)	99.8 (98.8–99.99)	90 (55–99.8)	98.4 (96.8–99.4)	251 (42–2160)	0.44 (0.25–0.71)
TGA and EMA	3 (0.67)	13 (81)	81 (54–95.9)	99.3 (98.0–99.86)	81 (54–95.9)	99.3 (98.0–99.9)	121 (39–409)	0.19 (0.072–0.47)
AGA-IgA, TGA, and EMA	0	8 (50)	50 (25–75)	100 (99.2–100)	100 (63–100)	98.2 (96.6–99.3)	∞ (57–∞)	0.50 (0.29–0.76)
All serologic tests positive	0	3 (19)	19 (4–46)	100 (99.18–100)	100 (29–100)	97.2 (95.2–98.5)	∞ (22–∞)	0.81 (0.56–0.96)
Serology	16 (3.6)	13 (81)	81 (54–95.9)	96.4 (94.2–97.9)	45 (26–64)	99.3 (97.9–99.9)	23 (13–39)	0.19 (0.073–0.49)
HLA-DQ typing								
HLA-DQ2	121 (27)	15 (94)	93.7 (70–99.8)	73 (69–77)	11 (6.3–18)	99.7 (98.3–99.9)	3.5 (2.6–4.2)	0.085 (0.018–0.44)
HLA-DQ8	85 (19)	2 (12)	12 (1.6–38)	81 (77–85)	2.3 (0.28–8.1)	96.3 (93.8–97.9)	0.66 (0.14–2.1)	1.1 (0.79–1.22)
HLA-DQ2 and DQ8	14 (3.1)	1 (6.2)	6.2 (16–30)	96.9 (94.8–98.3)	6.7 (0.17–32)	96.6 (94.5–98.1)	1.9 (0.18–11)	0.97 (0.74–1.0)
HLA-DQ	192 (43)	16 (100)	100 (79–100)	57 (52–62)	7.7 (4.5–12)	100 (98.6–100)	2.3 (1.9–2.6)	0 (0–0.40)
Both serologic testing and HLA-DQ typing								
AGA and HLA-DQ	6 (1.3)	9 (56)	56 (30–80)	98.7 (97.1–99.5)	60 (32–84)	98.4 (96.8–99.4)	42 (16–100)	0.44 (0.24–0.71)
AGA-IgG and HLA-DQ	3 (0.67)	4 (25)	25 (7.3–52)	99.3 (98.0–99.9)	57 (18–90)	97.4 (95.4–98.6)	37 (8.7–149)	0.75 (0.49–0.93)
AGA-IgA and HLA-DQ	4 (0.89)	8 (50)	50 (25–75)	99.1 (97.7–99.8)	67 (35–90)	98.2 (96.5–99.2)	56 (18–177)	0.50 (0.28–0.76)
TGA and HLA-DQ	3 (0.67)	13 (81)	81 (54–95.9)	99.3 (98.0–99.9)	81 (54–95.9)	99.3 (98.0–99.9)	121 (39–409)	0.19 (0.072–0.47)
EMA and HLA-DQ	4 (0.89)	13 (81)	81 (54–95.9)	99.1 (97.7–99.8)	76 (50–93)	99.3 (98.0–99.9)	90.8 (33–271)	0.19 (0.072–0.47)
EMA, TGA, or HLA-DQ	192 (43)	16 (100)	100 (79–100)	57 (52–62)	7.7 (4.5–12)	100 (98.6–100)	2.2 (1.8–2.5)	0 (0–0.43)
EMA, TGA, and HLA-DQ	3 (0.67)	13 (81)	81 (54–95.9)	99.3 (98.0–99.9)	81 (54–95.9)	99.3 (98.0–99.9)	121 (39–409)	0.19 (0.072–0.47)
Any serologic test or any HLA-DQ	199 (45)	16 (100)	100 (79–100)	55 (51–60)	7.4 (4.3–12)	100 (98.5–100)	2.2 (1.8–2.5)	0 (0–0.43)
Any serologic test and any HLA-DQ	9 (2.0)	13 (81)	81 (54–95.9)	97.9 (96.2–99.1)	59 (36–79)	99.3 (98.0–99.9)	40 (19–85)	0.19 (0.073–0.48)

* AGA = antigliadin antibody; EMA = antiendomysium antibody; TGA = antitransglutaminase antibody.
† The test result is positive when either AGA-IgA or AGA-IgG test result is positive.
‡ Guinea pig–IgA TGA.
§ EMA-IgG.