Prediction of celiac disease at endoscopy

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Background and study aims: Celiac disease is increasingly recognized worldwide, but guidelines on how to detect the condition and diagnose patients are unclear. In this study the prevalence and predictors of celiac disease were prospectively determined in a cross-sectional sample of Lebanese patients undergoing esophagogastroduodenoscopy (EGD).

Patients and methods: Consecutive consenting patients (n = 999) undergoing EGD answered a questionnaire and had blood taken for serologic testing. Endoscopic markers for celiac disease were documented and duodenal biopsies were obtained. The diagnosis of celiac disease was based on abnormal duodenal histology and positive serology. Risk factors were used to classify patients to either high or low risk for celiac disease. Independent predictors of celiac disease were derived via multivariate logistic regression.

Results: Villous atrophy (Marsh 3) and celiac disease were present in 1.8 % and 1.5 % of patients, respectively. Most were missed on clinical and endoscopic grounds. The sensitivity of tissue transglutaminase (tTG) testing for the diagnosis of villous atrophy and celiac disease was 72.2 % and 86.7 %, respectively. The positive predictive value of the deamidated gliadin peptide (DGP) test was 34.2 % and that of a strongly positive tTG was 80 %. While the strongest predictor of celiac disease was a positive tTG (odds ratio [OR] 131.7, 95 % confidence interval [CI] 29.0 – 598.6), endoscopic features of villous atrophy (OR 64.8, 95 %CI 10.7 – 391.3), history of eczema (OR 4.6, 95 %CI 0.8 – 28.8), anemia (OR 6.7, 95 %CI 1.2 – 38.4), and being Shiite (OR 5.4, 95 %CI 1.1 – 26.6) significantly predicted celiac disease. A strategy of biopsying the duodenum based on independent predictors had a sensitivity of 93 % – 100 % for the diagnosis of celiac disease, with an acceptable (22 % – 26 %) rate of performing unnecessary biopsies. A strategy that excluded pre-EGD serology produced a sensitivity of 93 % – 94 % and an unnecessary biopsy rate of 52 %.

Conclusion: An approach based solely on standard clinical suspicion and endoscopic findings is associated with a significant miss rate for celiac disease. A strategy to biopsy based on the derived celiac disease prediction models using easily obtained information prior to or during endoscopy, maximized the diagnosis while minimizing unnecessary biopsies.

Introduction

Celiac disease is a common disorder that affects genetically predisposed individuals on ingestion of gluten [1,2]. It is underdiagnosed due to lack of specificity of clinical symptoms, and the diagnosis is often made after considerable delay [3 – 5].

Dyspepsia, abdominal pain, bloating, and gastroesophageal reflux symptoms are more common in patients with celiac disease than in the general population [6 – 11]. These symptoms are common indications for upper gastrointestinal endoscopy (esophagogastroduodenoscopy [EGD]) and celiac disease is common in patients undergoing duodenal biopsy for various indications, with a prevalence of 1.0 % – 5.2 % [10, 12 – 15]. Thus, a protocol for detecting celiac disease in patients presenting for EGD would be of value because the best screening strategy in this population is not known. Treatment of those with celiac disease may improve symptoms and outcome [16]. Hopper et al. suggested duodenal biopsy for all high risk patients with the symptoms of diarrhea, weight loss, or anemia, and for all patients with positive tissue transglutaminase (tTG) antibody test [15].

The diagnosis of celiac disease is challenging. Most screening algorithms begin by serologic tests for antiendomysial (EMA), tTG, or deamida-

ted gliadin peptide (DGP) antibodies. The value of sequential testing or performing multiple serologic tests is not clear [17]. Most patients undergoing EGD for dyspepsia, however, do not undergo serologic testing for celiac disease prior to the procedure. The gold standard for celiac disease diagnosis remains duodenal biopsy [1,18,19], but how do endoscopists decide which patients to biopsy?

Several endoscopic markers are associated with the presence of villous atrophy [20,21], but their sensitivity and specificity for celiac disease is variable [21]. Methods to increase the visualization of villi are being utilized [22], but are time consuming, require expertise and are not always available.

Despite improvement in its detection, celiac disease remains underdiagnosed. Some patients have undergone EGD in the 5 years preceding their diagnosis without having duodenal biopsies [23]. Hence, the number of patients who undergo biopsies is still insufficient, leading to missed cases and a delay in the diagnosis [24].

The current study used parallel biopsy, serology, and prospective collection of potential risk factors in a cross-sectional sample of patients presenting for EGD to determine 1) the reliability of standard clinical judgement, 2) endoscopic findings, and 3) serologic testing to diagnose celiac disease (confirmed by positive histological evidence). The study also aimed to derive an accurate and reliable clinical decision tool for celiac diagnosis as a means to minimize the likelihood of missed cases while also reducing the number of unnecessary biopsies in patients undergoing EGD.

Methods

Patients

This was a prospective cross-sectional study of 1000 Lebanese patients (18 – 70 years) who were scheduled to undergo EGD at the American University of Beirut – Medical Center (26 February 2007 to 22 April 2009). The study was approved by the Institutional Review Board (protocol number: IM.KB.06).

Patients were excluded if they had active bleeding, coagulopathy, known celiac disease, gastric outlet obstruction, or if they were undergoing EGD prior to bariatric surgery or had undergone serologic testing for celiac disease before the endoscopy, regardless of results. Informed consent was obtained from all patients. Enrolled patients were interviewed about their chief complaint, and medical and family history using a questionnaire. Serum samples were obtained and stored for serologic testing and duodenal biopsies were performed in all patients. The interview, serum sampling, and duodenal biopsies were performed during the same appointment/setting as the EGD examination.

Esophagogastroduodenoscopy

EGD was performed by five experienced endoscopists. For each patient, endoscopists were asked whether they had any clinical suspicion of celiac disease. At endoscopy, 4 – 6 biopsies were taken from the duodenum, including the duodenal bulb in 70 % of the patients. Endoscopists were asked to state with a "yes" or "no" whether there were any endoscopic features of celiac disease in the duodenum, such as mucosal atrophy, scalloping, nodularity, loss of mucosal folds or mosaic pattern. A patient was considered to have endoscopic features of celiac disease if one or more features were present.

Before initiating the study, endoscopists were shown endoscopy photographs of mucosal atrophy, scalloping, nodularity, loss of mucosal folds, and mosaic pattern. The intraobserver and interobserver agreement among the endoscopists was determined by showing them 50 endoscopic images of the duodenum for 50 patients in the study. This was done in a blinded fashion with no information available on the patient's name, endoscopist's name, and the diagnosis. Nine of the 50 images contained villous atrophy.

Celiac serology

Serum samples were tested at the University of Maryland Center for Celiac Disease research laboratory for the presence of IgA anti-tTG antibody alone and for the presence of IgG or IgA anti-tTG/DGP antibodies combined. For quantification of IgA anti-tTG alone, the Celikey htTG-IgA ELISA kit (Phadia GmbH, Frieburg, Germany) or the QUANTA Lite h-tTG (INOVA Diagnostics Inc., San Diego, California, USA) were used. For quantification of IgA/IgG anti-tTG/DGP combined, the QUANTA Lite h-tTG/DGP Screen ELISA kit (INOVA Diagnostics Inc.) was used.

Cases with a positive histology or positive serology for IgA anti-tTG or positive DGP were then tested for the presence of EMA using the IgA anti-EMA indirect immune-fluorescence antibody assay (Scimedx Corp., Denville, New Jersey, USA). Serologic tests were conducted by investigators who were blinded to the clinical, endoscopy, and histology status of patients.

Histopathology

Duodenal biopsies were examined by two histopathologists without knowledge of the clinical history or the serology. The two histopathologists independently reviewed each case. When discrepancy in their diagnoses occurred, a consensus was reached through discussion without knowledge of any of the clinical or serologic data relating to the patient. The following parameters were assessed: villous atrophy, intraepithelial lymphocytes (IEL), villous height to crypt depth ratio, and the Marsh – Oberhuber grade (0 – 4). Quantification of IEL was done by counting the number of lymphocytes present at the villous tips per 20 epithelial cells. Five villi were selected and the number of IELs was expressed per 100 epithelial cells [4,5]. Atrophy and acute inflammation were each graded as absent, mild, moderate, or severe.

The Marsh grading system was used to classify patients as having Marsh 0, 1, 2, 3a, 3b, 3c, or 4 changes. Marsh type 0 (pre-infiltrative) is assigned for any biopsy with < 30 IELs/100 epithelial cells. For determination of the Marsh score, only fragments that had proper orientation were included in the analysis.

Questionnaire

The following contact and demographic details were obtained and documented: self-assessment of ethnicity and religious background, chief complaint, detailed gastrointestinal symptom history, history of conditions that may be associated with celiac disease such as diabetes and skin diseases such as dermatitis herpetiformis and eczema (as indicated by the patient and/or a medical record evaluation by a dermatologist whenever available), as well as family history of celiac disease.

Cost analysis and frequency of missed celiac disease and unnecessary duodenal biopsy

To determine the optimal strategy for the diagnosis of celiac disease at endoscopy, an analysis was conducted to estimate the following: the frequency of missed celiac disease or villous atrophy, the frequency of unnecessary duodenal biopsy, and the cost per

patient for the following five strategies: 1) a strategy involving doing an IgA tTG test on all patients referred for EGD and performing duodenal biopsy on those who are tTG antibody positive; 2) a strategy involving performing duodenal biopsy on all patients referred for EGD, and doing tTG testing on those with Marsh 1–4 changes; 3) the strategy of Hopper et al. [15] (performing biopsy on patients at high risk for celiac disease as well as those with positive tTG test results); 4) our strategy which is based on performing duodenal biopsies on patients with independent predictors of celiac disease, with and without testing the rest for IgA anti-tTG and performing duodenal biopsy on those who are IgA anti-tTG positive.

In conducting the cost analysis, it was assumed that a positive tTG test would require confirmation with an EMA test.

Data analysis and statistical methods

For the analysis of data, two definitions were used to classify patients as having celiac disease: the first was based on Marsh 3 histologic findings (villous atrophy), irrespective of serologic findings; the second definition was based on abnormal duodenal histology and positive serology, namely villous atrophy and any positive serology or lymphocytic duodenosis in addition to positive EMA serology (celiac disease) [25]. A primary analysis compared the villous atrophy group with all patients who did not have celiac disease. The latter group comprised three subgroups: 1) normal histology and negative serology (normal), 2) positive serology and normal histology, and 3) lymphocytic duodenosis with evidence of duodenal inflammation (no atrophy) and irrespective of serologic findings. For all groups, another analysis was performed in which patients with celiac disease were compared with patients in the normal group.

Patients were subclassified to high risk and low risk for celiac disease based on having one or more of the following: chronic diarrhea, anemia, weight loss, or family history of celiac disease.

For the diagnosis of villous atrophy, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each of the following was determined: clinical impression of the gastroenterologist, endoscopic markers of celiac disease, the three serologic tests for antibodies to tTG, DGP, and EMA, as well as the IEL count. The same measures were calculated for the diagnosis of celiac disease.

Continuous variables are expressed as means ± SD and were compared by using 2-sample *t* tests for independent samples. Differences in proportions of categorical variables were compared using a chi-squared or Fisher's exact test, as appropriate. Multivariate modeling to identify independent predictors of celiac disease was done using backward logistic regression. Factors that were associated univariately with a significance of $P < 0.2$ in addition to age, sex, and body mass index (BMI) were considered in the multivariate analyses. Forward selection logistic regression and 1000 times bootstrapping (with replacement) were also used to confirm the prediction models. The discrimination of these logistic regression models was assessed using the area under the receiver operating characteristic curves, and the Hosmer–Lemeshow goodness-of-fit statistics were used to assess the performance and calibration of the model. In all instances, predictors were retained if found to be significant at the $P < 0.05$ level.

All statistical analyses were done with SPSS 20 software (IBM Corp., Armonk, New York, USA).

Results

The final analysis was conducted on 999 patients, as one patient inadvertently underwent gastric rather than duodenal biopsies. Overall, 1863 patients were excluded from the analysis either because they met the exclusion criteria or because they did not consent to the study.

Celiac disease

Of the 999 patients who underwent EGD, 18 were diagnosed as having villous atrophy (1.8%) based on the presence of Marsh 3 changes (two Marsh 3a, seven Marsh 3b, and nine Marsh 3c). Villous atrophy was significantly greater among patients at high risk for celiac disease (12/409; 3.0%) compared with those at low risk (1.02%, odds ratio [OR] 0.34, 95% confidence interval [CI] 0.11–0.98; $P = 0.029$). The chief symptoms leading to EGD in the 999 patients are shown in **Table 1**. Demographics, clinical history, celiac disease-associated manifestations, endoscopy, and serology data for patients with villous atrophy (n = 18; seven males (38.9%); mean age 41.2 ± 11.9 years) vs. those without (n = 981; 442 males (45.1%); mean age 44.1 ± 14.2 years) are summarized and compared in **Table 1**. A total of 388 patients classified themselves as Shiite. Anemia, weight loss, and chronic diarrhea were more prevalent in the patients with villous atrophy ($P = 0.035$, 0.006, and 0.043, respectively). Prevalence of villous atrophy was significantly higher among Shiite patients (14/388 [3.6%] vs. 4/590 [0.68%]; OR 5.48, 95%CI 1.67–19.8; $P = 0.001$).

A total of 15 patients (14 with villous atrophy and any positive serology and one with Marsh 1 changes and positive EMA) were categorized as having celiac disease, giving a prevalence of 1.5%. Weight loss ($P = 0.022$) and anemia ($P = 0.007$) were similarly more prevalent in patients with celiac disease compared with patients in the normal group, whereas the increased prevalence of diarrhea did not reach statistical significance ($P = 0.078$; **Table 1**). Here too, prevalence of celiac disease was higher among Shiite patients ($P = 0.013$; **Table 1**).

Diagnostic accuracy of clinical suspicion/endoscopic features of celiac disease and/serology to detect celiac disease

Clinical suspicion

A total of 51 patients were clinically suspected of having celiac disease prior to EGD, most of whom fit the high risk category for celiac disease (48/51; 94.1%). For patients with villous atrophy, this clinical suspicion was born out in only five cases (all high risk), and the remaining 13 villous atrophy cases were not suspected. Pre-EGD clinical suspicion had a very low sensitivity (27.8%; 95%CI 10.9–52.4) and PPV (9.8%; 95%CI 3.8–18.5) of celiac disease (**Table 2**). Clinical suspicion had a similarly poor performance in identifying patients with actual celiac disease, with a sensitivity and PPV of 26.7 and 8%, respectively.

Endoscopic features of celiac disease

During EGD, 18 patients (15 high risk) were considered to have endoscopic markers of celiac disease, but only nine had villous atrophy leading to a 50.0% (95%CI 29–69) sensitivity and 99.1% (95%CI 98.7–99.4) specificity (**Table 2**). Similar findings were obtained when applied to actual celiac disease cases. Combining pre- and post-EGD suspicion of celiac disease did not improve the diagnostic value, with rather low sensitivity (50.0%) and PPV (15.3%).

Table 1 Demographic, clinical, and serologic data of patients with and without villous atrophy and celiac disease.

Patient factor	No villous atrophy/ no celiac disease (n = 980)	Villous atrophy (n = 18)	*P* value (2 sided)	Celiac disease (n = 15)	*P* value (2-sided)
Continuous data, mean ± SD					
Age, years	44.1 ± 14.2	41.2 ± 11.9	0.378	43.1 ± 13.3	0.775
Height, cm	168 ± 9	168 ± 9	0.847	166.4 ± 8.1	0.517
Weight, kg	74 ± 16	69 ± 15	0.242	68.1 ± 13.4	0.178
BMI, kg/m^2	26 ± 4.7	24.6 ± 4.9	0.211	24.6 ± 4.6	0.238
Categorical data, n (%)					
Shiite	374 (38.2)	14 (77.8)	0.001	11 (73.3)	0.013
Male	442 (45.1)	7 (38.9)	0.642	5 (33.3)	0.439
Family history of celiac disease	25 (2.6)	1 (5.6)	0.38	1 (6.7)	0.38
Clinical symptoms, n (%)					
Chronic diarrhea	108 (11.0)	5 (27.8)	0.043	4 (26.7)	0.078
Abdominal pain	389 (39.7)	10 (55.6)	0.131	7 (46.7)	0.603
Weight loss	201 (20.5)	9 (50.0)	0.006	7 (46.7)	0.022
Dyspepsia	499 (50.9)	10 (55.6)	0.813	7 (46.7)	0.8
GERD	596 (60.8)	13 (72.2)	0.465	10 (66.7)	0.792
Eczema	65 (6.6)	4 (22.2)	0.03	2 (13.3)	0.268
Low risk for celiac	585 (59.7)	6 (33.3)	0.03	4 (26.7)	0.061
Anemia	193 (19.7)	8 (44.4)	0.035	8 (53.3)	0.007
Clinical suspicion	46 (4.7)	5 (27.8)	0.001	4 (26.7)	0.005
Endoscopic markers	9 (0.9)	9 (50.0)	< 0.001	8 (53.3)	< 0.001
Clinical or endoscopic suspicion	50 (5.1)	9 (50.0)	< 0.001	8 (53.3)	< 0.001
Serology results, n (%)					
EMA positive*	3 (0.3)	13 (72.2)	< 0.001	14 (93.3)	< 0.001
tTG positive	16 (1.6)	13 (72.2)	< 0.001	13 (86.7)	< 0.001
tTG/DGP positive	25 (2.6)	13 (72.2)	< 0.001	13 (86.7)	< 0.001
tTG/DGP and EMA positive	2 (0.2)	13 (72.2)	< 0.001	13 (86.7)	< 0.001
tTG and EMA positive	2 (0.2)	12 (66.7)	< 0.001	12 (80.0)	< 0.001
Clin/Endo/tTg (any positive)	64 (6.5)	15 (83.3)	< 0.001	14 (93.3)	< 0.001
Clin/Endo/tTg/EMA (any positive)	65 (6.6)	15 (83.3)	< 0.001	15 (100)	< 0.001
Clin/Endo/DPG (any positive)	74 (7.6)	14 (77.8)	< 0.001	13 (86.7)	< 0.001

BMI, body mass index; GERD, gastroesophageal reflux disease; EMA, endomysium antibody.
* EMA test done on 96 patients only (positive tTG, DGP or histology).

Table 2 Sensitivity, specificity, positive predictive value, and negative predictive value of various serologic tests, as well as clinical and endoscopic suspicion of villous atrophy.

Diagnostic test	Patients, n	Positive, n	True positive	False positive	False negative	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Clinical suspicion	998	51	5	46	13	27.8	95.3	9.8	98.6
High risk	407	48	5	43	7	41.7	89.1	10.4	98.1
Low risk	591	3	0	3	6	0	99.5	0	99
Endoscopic suspicion	997	18	9	9	9	50.0	99.1	50	99.1
High risk	407	15	7	8	5	58.3	98	46.7	98.7
Low risk	590	3	2	1	4	33.3	99.8	66.7	99.3
tTG	999	29	13	16	5	72.2	98.4	44.8	99.5
High risk	408	15	9	6	3	75	98.5	60	99.2
Low risk	591	14	4	10	2	66.7	98.3	28.6	99.7
tTG/DGP	998	38	13	25	5	72.2	97.4	34.2	99.5
High risk	408	21	9	12	3	75	97	42.9	99.2
Low risk	590	17	4	13	2	66.7	97.8	23.5	99.7
EMA	999	16	13	3	5	72.2	99.7	81.3	99.5
High risk	408	10	9	1	3	75	99.7	90	99.2
Low risk	591	6	4	2	2	66.7	99.7	66.7	99.7

tTG, tissue transglutaminase; DGP, deamidated gliadin peptide; EMA, endomysium antibody; PPV, positive predictive value; NPV, negative predictive value.
All three tests had the same sensitivity (72.2%) and NPV (99.5%) for the diagnosis of villous atrophy. However, the specificities of tTG (98.4%) and EMA (99.7%) were higher than that of tTG/DGP (97.4%).

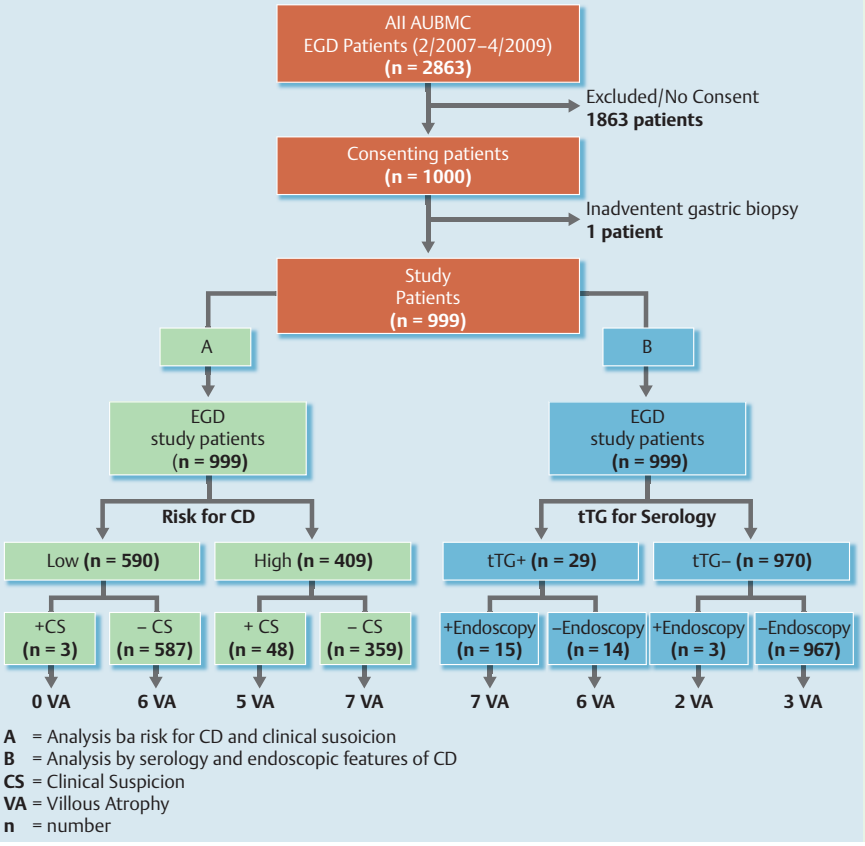


Fig. 1 Absolute prevalence of villous atrophy in groups of patients presenting for esophagogastroduodenoscopy based on (A) risk for celiac disease and clinical suspicion, or (B) on results of tTG-IgA test result and endoscopic features of celiac disease. Information on clinical suspicion was not available for two patients. AUBMC, American University of Beirut–Medical Center; EGD, esophagogastroduodenoscopy.

When tested on still photographs of the duodenum, the intraobserver agreement for the endoscopists was 93%. However, the interobserver agreement was marginal with an alpha of 0.38 when taking all five endoscopists together.

Serology

The sensitivity, specificity, PPV, and NPV of all serologic tests are shown in **Table 2**. A total of 29, 38, and 16 patients were positive for IgA anti-tTG, tTG/DGP, and EMA, respectively. EMA was performed only in patients who were positive for any serologic test or who had any histopathology abnormality and was otherwise assumed to be negative. A total of 14 patients with villous atrophy tested positive for one or more antibodies; 12 were positive to all 3; 1 was positive for tTG only; and 1 was positive for tTG/DGP and EMA only. Four patients were negative for all 3 tests, two of whom were in the high risk group. In two of these four patients, there was resolution of symptoms on a gluten-free diet, and one of these two had resolution of villous atrophy on follow-up endoscopy and duodenal biopsy. One patient did not adhere to a gluten-free diet, and one was lost to follow-up.

All three tests had the same sensitivity (72.2%; 95%CI 48.0–88.9) and NPV (99.5%; 95%CI 99.0–99.8) for the diagnosis of villous atrophy. However, the specificities of tTG (98.4%; 95%CI 97.9–98.7) and EMA (99.7%; 95%CI 99.3–99.9) were higher than that of tTG/DGP (97.4%; 95%CI 97.0–97.8). In addition, all three tests performed better in the high risk group compared with the low risk group in terms of their sensitivity and PPV (**Table 2**). In addition, PPV was highest for EMA and lowest for tTG/DGP. Finally, a strongly positive tTG (>30 U/mL with INOVA or >8 U/mL with Celikely) had a PPV of only 80.0%.

For actual celiac disease cases, the sensitivities of tTG, combined tTG/DGP, and EMA were 86.7%, 86.7%, and 93.3%, and the NPVs were 99.8%, 99.8%, and 99.9%, respectively.

A flow chart summarizing the overall results is shown in **Fig. 1**.

Diagnostic accuracy of IEL count to detect celiac disease

All patients with villous atrophy and all patients with celiac disease had an IEL count >30/100 epithelial cells. The sensitivity, specificity, NPV, and PPV of this count for the diagnosis of celiac disease were 100% (95%CI 76–100), 96.2% (95%CI 95.9–96.2), 100% (95%CI 99.6–100), and 28.8% (95%CI 21.8–28.8), and for the diagnosis of villous atrophy were 100% (95%CI 78.1–100), 96.2% (95%CI 95.9–96.2), 100% (95%CI 99.6–100), and 31.5% (95%CI 24.6–31.5). The number of IEL/100 epithelial cells (mean ±SD) increased significantly and substantially with increasing Marsh changes: 0 (13.9±5.5), Marsh 1 (33.1±2.8), Marsh 2 (41.1±6.8), and Marsh 3 (52.2±13.8) ($P<0.001$). Finally, there were 47 patients with an IEL count of 25–29/100 epithelial cells; none of these patients had celiac disease, villous atrophy, or crypt hyperplasia and none of them was tTG or EMA positive, strongly suggesting that these patients do not have celiac disease or potential celiac disease.

Among patients with no villous atrophy, there were 41 cases of lymphocytic duodenosis (29 patients with Marsh 1 and 12 with Marsh 2 changes). Compared with patients in the normal group, patients with lymphocytic duodenosis were more likely to have abdominal pain ($P=0.021$); other demographic and clinical characteristics were similar (**Table 3**). *Helicobacter pylori* status was available for only 17 patients with lymphocytic duodenosis, and seven were positive. Positive serology was present in three of the 41 patients with lymphocytic duodenosis (7.3%), with two tTG/DGP positive and one EMA positive. Their chief symptoms were

Table 3 Demographic, clinical, and serologic characteristics of patients with lymphocytic duodenosis or patients with positive serology and normal histology compared with "normal" (normal histology and negative serology).

	Normal (n = 906)	Lymphocytic duodenosis (n = 41)	Positive serology + normal histology (n = 37)	Normal vs. lymphocytic duodenosis *P* value[1]	Normal vs. positive serology + normal histology *P* value*
Continuous data, mean ± SD					
Age, years	44.2 ± 14.2	41.1 ± 14.6	47.2 ± 15.3	0.169	n.s.
BMI, kg/m^2	26.1 ± 4.8	25 ± 4.1	25.5 ± 4.5	0.149	n.s.
Categorical data, n (%)					
Male	414 (45.7)	12 (29.3)	16 (43.2)	0.053	n.s.
Clinical symptoms, n (%)					
Chronic diarrhea	101 (11.2)	4 (9.8)	3 (8.1)	n.s.	n.s.
Abdominal pain	355 (39.2)	24 (58.5)	11 (29.7)	0.021	n.s.
Weight loss	180 (19.9)	9 (22)	12 (32.4)	n.s.	0.092
Dyspepsia	465 (51.3)	21 (51.2)	16 (43.2)	n.s.	n.s.
GERD	550 (60.7)	26 (63.4)	23 (62.2)	n.s.	n.s.
Low risk for celiac	543 (59.9)	22 (53.7)	22 (59.5)	n.s.	n.s.
Associated manifestations, n (%)					
Diabetes	17 (1.9)	0 (0.0)	0 (0.0)	n.s.	n.s.
Aphthous ulcers	89 (9.8)	3 (7.3)	5 (13.5)	n.s.	n.s.
Eczema	58 (6.4)	4 (9.8)	4 (10.8)	n.s.	n.s.
Anemia	175 (19.3)	12 (29.2)	7 (18.9)	0.172	n.s.
Clinical suspicion, n (%)					
Before endoscopy	43 (4.8)	1 (2.4)	2 (5.4)	n.s.	n.s.
After endoscopy	9 (1.0)	0 (0.0)	0 (0.0)	n.s.	n.s.
Before/after endoscopy	47 (5.2)	1 (2.4)	2 (5.4)	n.s.	n.s.
Serology results, n (%)					
EMA positive*	0 (0.0)	1 (2.4)	3 (8.1)	0.043	< 0.001
tTG/DGP positive	0 (0.0)	2 (4.9)	25 (67.6)	0.002	< 0.001
tTG positive	0 (0.0)	0 (0.0)	16 (43.2)	N/A	< 0.001

BMI, body mass index; n.s., not significant ($P > 0.2$); N/A, not applicable; GERD, gastroesophageal reflux disease; EMA, endomysium antibody; tTG, tissue transglutaminase; DGP, deamidated gliadin peptide.

* EMA test done in 96 patients only (positive tTG, DGP or histology).

epigastric pain (n = 2) and gastroesophageal reflux disease (n = 1); one patient met the high risk criteria for celiac disease. The patient with lymphocytic duodenosis and positive EMA was included in the celiac disease group.

Patients with positive serology and normal duodenal histology

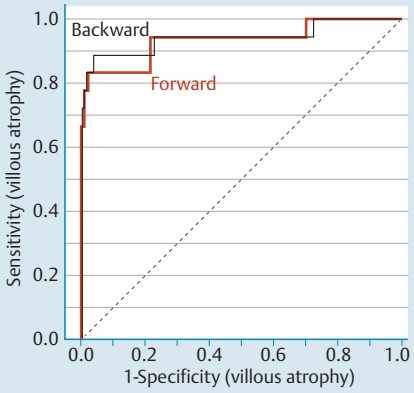
A total of 37 patients (3.7 %) with Marsh 0 had one or more positive serologic tests for celiac disease: 16 positive for tTG (13 moderate, 3 strong), 3 positive for EMA, and 25 positive for tTG/DGP. These patients had similar demographic and clinical characteristics to patients with normal histology and negative serology (**Table 3**). The three patients with strongly positive tTG were negative for both EMA and tTG/DGP. Five patients that were moderately positive for tTG were also positive for tTG/DGP. Two patients (one high risk) were positive for all three tests and were considered to have potential celiac disease. They were excluded from the analysis of predictors and disease characteristics.

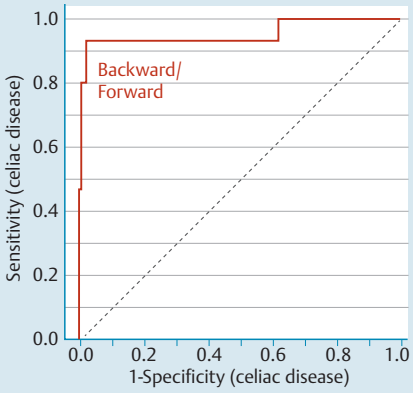
Independent predictors of celiac disease

Logistic regression modeling (backward stepwise) identified being Shiite (OR 5.4, 95 %CI 1.1 – 26.6; $P = 0.036$) and presence of eczema (OR 4.6, 95 %CI 0.8 – 28.8; $P = 0.099$ [confirmed by bootstrapping]) as the only pre-EGD factors to independently predict villous atrophy. This modeling also identified endoscopic markers (OR 64.8, 95 %CI 10.7 – 391.3; $P < 0.001$) and a positive tTG (OR 131.7, 95 %CI 29.0 – 598.6; $P < 0.001$) as other independent predictors. Positive tTG test, endoscopic markers, and being Shiite were confirmed as independent predictors of villous atrophy by forward selection, and all three as well as pre-EGD eczema were identified as predictors by the 1000 times bootstrapping analysis. The prediction model was found to have excellent discrimination (**Fig. 2**) and was well calibrated (Hosmer – Lemeshow $P = 0.589$). When the Shiite variable was removed, positive tTG ($P < 0.001$), endoscopic markers ($P < 0.001$), anemia ($P = 0.029$), and eczema ($P = 0.08$) remained as independent predictors of villous atrophy. For celiac disease, three independent predictors were found: positive tTG (OR 510.1, 95 %CI 54.3 – 4796.0; $P < 0.001$], endoscopic markers (OR 111.6, 95 %CI 8.9 – 1392.6; $P < 0.001$], and anemia (OR 6.7, 95 %CI 1.2 – 38.4; $P = 0.032$) (**Table 4**; **Fig. 2**).

Strategies for screening for celiac disease in patients referred for EGD

Various strategies of screening for celiac disease in patients referred for EGD were investigated. Some strategies were based on performing duodenal biopsies on patients who belong to groups at high risk for celiac disease or who have independent predictor (s) of celiac disease, as well as on patients who test positive for tTG (if the test were available to the endoscopist prior to the endoscopy and were ordered for all individuals undergoing EGD) regardless of other parameters. The results are shown in **Table 5**. In the EGD population in the current study, an approach based on performing biopsies on patients with indepen-





ROC analysis

Model (villous atrophy	AUC	SE*	P value
Univariate			
tTG	0.853	0.064	0.000
Endoscopic suspicion	0.746	0.076	0.000
Shiite	0.697	0.058	0.004
Eczema	0.577	0.075	0.266
Multivariate			
Forward model	0.935	0.041	0.000
Backward model	0.943	0.041	0.000

AUC, area under the curve
*Under the nonparametric assumption.

ROC analysis

Model	AUC	SE*	P value
Univariate			
Anemia	0.662	0.077	0.032
Endoscopic suspicion	0.762	0.082	0.000
tTG	0.925	0.052	0.000
Multivariate			
Forward/backward model	0.957	0.042	0.000

AUC, area under the curve (C statistic)
*Under the nonparametric assumption.

Fig. 2 Receiver operating characteristic (ROC) curves derived from multivariate logistic regression models for villous atrophy vs. nonvillous atrophy (left) and celiac disease vs. nonceliac disease (right). The tables summarize the area under the curve data for each of the ROC curves shown. *Eczema data are shown as this was confirmed as a predictor by bootstrapping analysis (see *Methods* and **Table** 3).

dent predictors of celiac disease as well as on patients who test positive for tTG among the remaining patients seems to have the highest detection rate for celiac disease and the lowest rate of unnecessary biopsies. Thus, performing duodenal biopsies on high risk patients who are Shiite, those with eczema, and those who test positive for tTG among all others would miss none of the celiac disease cases and would result in unnecessary biopsies in 22.2% of patients. Alternatively, performing duodenal biopsies on patients who have anemia, eczema, or endoscopic features of celiac disease as well as on those who are tTG positive would result in missing 11% of celiac disease cases and doing unnecessary biopsies in 25.8% of patients. If serology is not available to the endoscopist, a strategy based on performing biopsies on patients with independent predictors of celiac disease has a sensitivity of 93%–94% for detecting celiac disease, but is associated with a 52 % rate of unnecessary biopsies (**Table 5**).

Cost analysis

At our institution, the total cost of a duodenal biopsy is US$100, that of serologic tests for tTG is US$45, and for EMA the cost is US $46. The estimated cost per patient for the various strategies discussed above is shown in **Table 5**. The strategy of performing biopsies on all those undergoing EGD followed by tTG serology on those with Marsh 1–4 changes will have the lowest miss rate for villous atrophy and a low miss rate for celiac disease, but it is the most expensive and involves doing a large number of unnecessary biopsies. Alternatively, a strategy that involves doing tTG on all those undergoing EGD, and doing biopsies on those with positive tTG will be the cheapest and will involve doing the lowest number of unnecessary duodenal biopsies, but would have an unacceptably high miss rate for celiac disease. The strategies proposed in the current study (based on doing duodenal biopsies on patients with independent predictors of celiac disease, and on those who are tTG positive among the remaining patients) have comparable costs to the strategy of Hopper et al. [15], have sim-

Patient factor	B	SE	*P* value*	OR [95%CI]
Backward model – villous atrophy				
tTG	4.88	0.77	<0.001	131.7 [29.0–598.6]
Endoscopic suspicion	4.17	0.92	<0.001	64.8 [10.7–391.3]
Shiite	1.69	0.81	0.036	5.4 [1.1–26.6]
Eczema	1.54	0.93	0.099	4.6 [0.8–28.8]
Constant	–6.97	0.89	<0.001	
Backward model – celiac disease				
tTG	6.235	1.143	<0.001	510.1 [54.3–4796.0]
Endoscopic suspicion	4.715	1.288	<0.001	111.6 [8.9–1392.6]
Anemia	1.905	0.89	0.032	6.7 [1.2–38.4]
Constant	–7.777	1.197	<0.001	

B, model coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.
Variables considered were: endoscopic suspicion, tTG, eczema, Shiite, clinical suspicion, age, sex, body mass index, weight loss, anemia, chronic diarrhea, risk of celiac disease.
* Two-tailed significance.

Table 4 Independent predictors of villous atrophy and celiac disease derived by multivariate logistic regression analysis.

Table 5 Comparison of various strategies to diagnose celiac disease in patients referred for esophagogastroduodenoscopy.

	tTG-IgA on all patients*	Duodenal biopsy on all patients†	Strategy of Hopper et al.‡	Our strategy (1)§	Our strategy (2)¶	Our strategy excluding serology**
Missed villous atrophy, %	27	0	11	0	11	5.5
Missed celiac disease, %	13.3	6.6	6.6	0	6.6	6.6
Unnecessary duodenal biopsy, %	1.6	93	42	22	26	52
Cost per patient in US$	49	105	71	60	74	62

* Duodenal biopsy performed in patients who are tTG positive.
† tTG serology done on patients with Marsh 1–4 changes.
‡ Duodenal biopsy performed in patients at high risk for celiac disease and in low risk patients who are positive for tTG antibody.
§ Duodenal biopsy performed in high risk Shiite patients, patients with eczema, and those who are tTG antibody positive among the remaining patients.
¶ Duodenal biopsy performed in patients with anemia, eczema, or endoscopic features of celiac disease, and on patients who are tTG positive among the remaining patients.
** Duodenal biopsy performed in any patient with independent predictor of celiac disease.

ilar or lower miss rate for celiac disease (at least for the EGD population in the current study), and involve performing fewer unnecessary biopsies.

Discussion

The current study suggests that villous atrophy (Marsh 3 changes regardless of serology) and celiac disease (villous atrophy and positive serology or lymphocytic duodenosis and positive EMA) are common in patients referred for EGD. The study also prospectively identified specific independent predictors of celiac disease, including anemia, history of eczema, being Shiite, and endoscopic features of villous atrophy. These predictors are easily discernible by the endoscopist prior to or during the endoscopy. A decision to perform duodenal biopsies based on these factors increases the sensitivity of celiac disease diagnosis while having an acceptable rate of unnecessary biopsies. Elucidating these few pieces of clinical information would have enabled the identification of most cases of celiac disease that would otherwise have been missed. Although a positive tTG is a powerful independent predictor of celiac disease, performing the test on patients prior to the EGD seems to result in reducing the number of unnecessary biopsies without significantly improving the rate of celiac disease diagnosis (**Table 5**).

Patients with villous atrophy/celiac disease constituted 1.8%/1.5% of the whole group. Having anemia, chronic diarrhea, weight loss, or being in a "high risk" category were associated with celiac disease, and anemia was an independent predictor. The study also shows for the first time that a history of eczema is an independent predictor of villous atrophy via multivariate analysis (**Table 4**). Four of 69 patients with eczema were found to have villous atrophy. Three of these four patients met the low risk definition and two were negative for all three serology tests. An association between celiac disease and atopic dermatitis in adults has been suggested but its pathogenesis is not well understood [26]. Further research is needed to validate this association.

The data from the current study suggest that endoscopic markers of celiac disease are independent predictors of the disease, and that they have a low sensitivity and high specificity, in line with what is known [27]. While intraobserver agreement at our endoscopy unit was acceptable at 93%, interobserver agreement among our endoscopists was marginal. This is in part due to having tested concordance among the endoscopists using still photographs of the duodenum, which may not reflect the total picture of the duodenum. Emerging techniques of endoscopic imaging might improve the sensitivity of endoscopic markers [22], but these are still restricted to the research setting. Strategies to enhance our ability to both see and recognize features of villous atrophy, such as the use of high magnification endoscopy and water immersion examination of the small intestine are needed. However, awareness and specific training of endoscopists to detect these features would likely further enhance or increase sensitivity of celiac disease detection.

All three serologic tests in this study had a lower sensitivity and PPV than previous reports [17], particularly if the gold standard is presence of villous atrophy regardless of serology results. The disappointing performance of serologic tests in clinical practice has been reported previously [13,28,29]. Unlike other investigators [30], we found that the DGP test had the lowest specificity and PPV, making it the least suitable in this setting. Furthermore, no correlation was found between the presence of a moderately or strongly positive IgA anti-tTG and villous atrophy, which has been suggested previously [31,32]. Thus, 16 patients with strongly or moderately positive IgA anti-tTG tests had normal duodenal histology. The reason for the difference may be the inclusion of patients with high IgA anti-tTG levels who had duodenal biopsies prior to initiating the studies [31,32], suggesting a high pretest probability of celiac disease.

The histopathologic analysis in the current study was conducted by pathologists who were blinded to the status of the patients. Well established criteria based on the Marsh classification were used to establish the presence of villous atrophy and lymphocytic duodenosis. A relatively higher threshold was used to establish the presence of lymphocytic duodenosis (>30 IEL/100 epithelial cells) than the one used by others [25]. This count seems to have a high sensitivity and specificity for the diagnosis of celiac disease and villous atrophy. Furthermore, none of the patients with an IEL count of 25–29 IEL/100 epithelial cells had celiac disease or potential celiac disease. Overall, the findings, if confirmed by other studies, suggest that that an IEL count of >30/100 epithelial cells has better accuracy than a count of >25/100 epithelial cells in the diagnosis of celiac disease.

In the current study, 39% of patients identified themselves as Shiite; this is similar to the population as a whole. The reasons for the high prevalence of celiac disease in Shiite patients in the current study are unclear. Celiac disease is highly prevalent in people belonging to certain ethnicities, such as the Saharawi people [33], and is "heritable" and tends to cluster in families [34], with first-degree relatives of index cases having a 4%–12% risk of having the disease [35]. A recent study suggested that the prevalence of celiac disease was 0.3% in Germany and 2.4% in Finland [36]. Together, these studies suggest a role for ethnic

background, genetic factors, and possibly consanguinity in celiac disease development.

Shiite patients were as likely to belong to the high risk category (44.3% vs. 38.3%) as other patients. They were significantly more likely to be younger, to have weight loss, to have endoscopic markers of celiac disease, and to be positive for tTG and EMA. However, the multivariate analysis identified being Shiite as an independent predictor of celiac disease. Bias from self-referral is unlikely to account for this finding as patients were recruited from all those referred to the Endoscopy Unit for EGD. Finally, prevalence of celiac disease in high risk Shiite patients was significantly greater than in high risk non-Shiite patients (6.4 vs. 1.3%; $P = 0.014$).

Intermarriage among people belonging to the same sect is known in Lebanon, but in the Shiite patients in the current study, consanguinity and its degree were not determined. Further research on the prevalence of certain human leukocyte antigen genes and other genetic and environmental factors is needed to explain the current findings.

Celiac disease is common in patients referred for EGD, but the best strategy for detection in patients is debatable. Performing serologic tests and duodenal biopsies on all patients is costly and may expose patients to unnecessary risks. The strategy suggested by Hopper et al. [15] has some limitations in our patients. More than 90% of patients at high risk for celiac disease do not actually have celiac disease, and too many patients would undergo unnecessary biopsies. Hence, strategies to improve the PPV of being at high risk are needed. Such strategies would include patients who belong to groups with very high prevalence of celiac disease. The current study suggests that such a strategy might reduce the number of unnecessary biopsies without compromising its sensitivity for diagnosing celiac disease. Furthermore, a serologic test that is very sensitive in low risk patients is needed. None of the three serologic tests in the current study fulfill these criteria. The current study suggests that "clinical decision tools" or algorithms need to be developed for each country or region to optimize the diagnosis of celiac disease in given populations. In patients referred for EGD, a strategy based on performing duodenal biopsies in patients with independent predictors of celiac disease, as well as on those who are tTG positive among the others may have the lowest miss rate for celiac disease as well as the lowest rate of unnecessary duodenal biopsies. The cost analysis carried out in the current study also lends support to this strategy. Even if serology is not available to the endoscopist prior to EGD, the sensitivity of this strategy for the diagnosis of celiac disease remains high.

The current study has several strengths. It was prospective, and a comprehensive analysis was conducted on a large number of patients. All investigators were blinded to the diagnosis, which was declared only after all pertinent data became available. Independent predictors of celiac disease were determined, which will help to identify patients that may otherwise be missed. In addition, two separate analyses were conducted based on different criteria for diagnosing celiac disease.

The study also has some limitations. EMA was not tested for all patients, and the cause of lymphocytic duodenosis was not determined in all patients. In addition, celiac disease in patients with IgA deficiency might have been missed, as IgA level was not measured. Finally, it was not possible to ascertain the diagnosis of celiac disease in two patients with villous atrophy.

In conclusion, this study has shown that celiac disease is common in patients referred for EGD and that the majority of patients would be missed if only clinical and endoscopic markers were used. Independent predictors of celiac disease have been identified. A strategy to identify candidates for duodenal biopsies, derived from those predictors, maximized celiac disease diagnosis at a low rate of unnecessary biopsies.

Competing interests: None.

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