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*** Point by Point Response**

- A paragraph was added at the top of p.11 in the Discussion section addressing the possibility that other conditions (sp. self-limited enteritis) could mimic the histological findings of celiac disease. The appropriate reference was also added as recommended by the reviewer.

UTILITY IN CLINICAL PRACTICE OF IGA ANTI-TISSUE TRANSGLUTAMINASE ANTIBODY FOR THE DIAGNOSIS OF CELIAC DISEASE

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ABSTRACT

BACKGROUND & AIMS

The diagnosis of celiac disease often relies on the use of the anti-tissue transglutaminase antibody test. The aim of this study is to evaluate the sensitivity and specificity of this blood test in clinical practice with the use of commercial laboratories, where the test characteristics may differ from research laboratories.

METHODS

We identified 122 patients with suspected celiac disease who had anti-tissue transglutaminase antibody serologies as well as upper endoscopy with duodenal biopsies. Those patients with celiac disease were classified as either classical (with diarrhea or other symptoms of malabsorption) or silent (asymptomatic). Biopsies from celiac disease patients were classified as either partial (Marsh IIIA) or total (Marsh IIIB or IIIC) villous atrophy.

RESULTS

The overall sensitivity, specificity, positive predictive value, and negative predictive value of the anti-tissue transglutaminase antibody test were 70.6%, 65.0%, 91.1%, and 30.2%, respectively. The sensitivity was 90.0% for patients with total villous atrophy and 42.3% for patients with partial villous atrophy ($p<0.0001$). There were statistically significant differences in both sensitivity and specificity between the two most commonly used commercial laboratories. The sensitivity for Lab #1 was 40.0% vs. 86.4% for Lab

#2 ($p<0.0001$). The specificity for Lab #1 was 100.0% and was 41.7% for Lab #2 ($p=0.02$).

CONCLUSIONS

The sensitivity of the anti-tissue transglutaminase antibody in clinical practice, with the use of commercial laboratories is not as high as previously reported in research laboratories. The sensitivity is significantly lower in patients with partial villous atrophy. There is also significant variability in test characteristics between major commercial laboratories in the United States. These results need to be confirmed in prospective studies.

Introduction

Celiac disease is a genetically determined autoimmune-like disorder induced by gluten, the storage protein of wheat and similar proteins found in barley and rye[1]. The autoimmune component of the disease is evidenced by the development of auto-antibodies to the endomysium, reticulin and tissue transglutaminase [2]. Originally antibodies to the gliadin component of gluten were used for diagnostic purposes, however these tests are considered to lack sensitivity and specificity [3]. The endomysial antibody (EMA) has a very high specificity for celiac disease and has became the "gold standard" serological test [4].

The recognition that the enzyme tissue transglutaminase 2 (tTG) is the main autoantigen for the EMA [5] allowed development of automated enzyme linked immunoassays (ELISA). Initially guinea pig tTG was used as the antigen; subsequently human tTG, either recombinant or derived from red blood cells (H-tTG), was used in the assays. Systematic review of the available studies revealed that the IgA tTG antibody test has greater than 90% sensitivity and specificity for celiac disease [3].

Celiac serologic tests have a history of not performing as well in the clinical setting as the original research studies suggest they should [6-9]. Within the United States serologic tests are performed by a variety of commercial laboratories that use different test kits from different manufacturers. Because there is no data as to the sensitivity of the IgA anti-tTG test as used in the clinical practice setting in the United States, we determined the sensitivity of this test in a large group of patients who were undergoing

biopsy for the diagnosis of celiac disease and who had serologic testing performed at commercial referral laboratories.

Methods

We identified 122 consecutive patients seen between January 2000 and December 2003 at the Celiac Disease Center at Columbia University (New York, NY) with suspicion for celiac disease. Patients were selected for this study if they had immunoglobulin A anti-tissue transglutaminase (human) antibody determination performed prior to upper endoscopy for duodenal biopsies. Serologic testing was performed at various commercial laboratories around the country. Patients were not included in the study if they were <16 years old at time of diagnosis, had selective immunoglobulin A deficiency (defined as a total serum immunoglobulin A level <0.05 g/L), were already on a gluten-free diet, were taking immunosuppressants at the time of initial evaluation, or had initial serologic testing performed at more than one laboratory. All patients were included in the study if the above criteria were met, regardless of whether celiac disease was proven. To receive a diagnosis of celiac disease, patients had to demonstrate either histological or serological improvement after six months on a gluten-free diet. No patients, at the time of the initial biopsy, were on a reduced gluten diet. During the study period, patient information was prospectively entered into a database (Access, Microsoft Office).

All duodenal biopsies were reviewed by a single pathologist, who was unaware of the antibody status, in a blinded fashion. A pathologic diagnosis of celiac disease required intraepithelial lymphocytosis and either partial villous atrophy (Marsh IIIA) or

total villous atrophy (Marsh IIIB and IIIC). The control group consisted of those in whom the biopsy was normal.

The clinical presentation was classified as either classical, in which diarrhea with or without a malabsorption syndrome was present, or silent, asymptomatic. Silent or asymptomatic celiac disease included patients presenting with iron deficiency, osteoporosis, dermatitis herpetiformis, or neurological symptoms, or patients identified by screening or with incidental findings on endoscopy of atrophic or scalloped duodenal folds.

Statistical analysis was performed using chi-square or Fisher's exact tests for categorical variables and two-sided *t*-tests for continuous variables. We calculated 95% confidence intervals for the sensitivities of the anti-tTG antibody. STATA Release 8 was used for all data analysis.

Results

There were a total of 122 patients who met the inclusion criteria for the study (**Table 1**). All the patients were Caucasian, 12% had at least one first degree relative already diagnosed with celiac disease. During the screening process, six patients were excluded from the study due to selective IgA deficiency. Celiac disease was diagnosed in 102 of the patients. Among celiac disease patients, 70.6% (72/102) tested positive for anti-tissue transglutaminase, whereas 65.0% (13/20) of the non-celiac disease group was negative for the antibody. The positive and negative predictive values for the anti-tissue transglutaminase antibodies were 91.1% and 30.2%, respectively. Within the celiac disease group, 90.0% (54/60) of patients with total villous atrophy on initial biopsy and

42.3% (18/42) of patients with partial villous atrophy had positive anti-tissue transglutaminase antibodies ($p<0.0001$) (**Table 2**). The anti-tissue transglutaminase antibody was positive in 65.9% (29/44) of patients with classical celiac disease, compared to 74.1% (43/58) patients with silent celiac disease ($p=0.37$). There were no significant differences in sensitivity when comparing the various modes of presentation. There was a trend towards significance seen in patients diagnosed with celiac disease who initially presented with anemia, of which 88.9% (16/18) tested positive for anti-tissue transglutaminase antibody compared to 66.7% (56/84) among patients with other modes of presentation ($p=0.06$).

Of the various commercial laboratories used to test for anti-tissue transglutaminase, 108/122 (88.5%) of the patients' samples were tested at one of two laboratories (Lab #1 and Lab #2). The overall sensitivity was 40.0% for Lab #1 and 86.4% for Lab #2 ($p<0.0001$). The specificity for Lab #1 was 100.0% and was 41.7% for Lab #2 ($p=0.02$) (**Table 3**). Significantly more celiac disease patients had total villous atrophy in samples sent to Lab #2 (67.8%, 40/59) compared to Lab #1 (43.3%, 13/30) ($p=0.03$). However, there was still a significant difference between the sensitivities of these two labs in patients with both total and partial villous atrophy. Within each of the two primary laboratories, the difference in sensitivity between patients with total and partial villous atrophy remained significant (**Table 4**).

Discussion

Our study of patients undergoing biopsy for the diagnosis of celiac disease revealed that the sensitivity of the serum IgA tTG was 70%. This is considerably lower

than the value of >90% reported by Rostom et al in their review of the literature [3, 10]. The lower sensitivity is due mainly to the inclusion of patients with lesser degrees of villous atrophy. The low overall sensitivity may also be due the comparison of the results of commercial laboratory tests (as opposed to those of a research laboratory) against the gold standard duodenal biopsies, which were interpreted by a single expert histopathologist. However, this does not account for the marked difference in sensitivity between patients with total and partial villous atrophy, as the pathologist was blinded to the test results. The test had a sensitivity of 90% when total villous atrophy was present compared to 43% in the presence of partial villous atrophy. This has been noted previously for tTG [11-13] as well as the EMA [7, 8, 13].

The disappointing sensitivity of serologic tests in the diagnosis of celiac disease in the clinical practice setting has been noted previously [6, 9, 14], and could be explained by selection bias in the initial studies from research laboratories for the tTG test, in which patients with total villous atrophy and positive EMA are included [15-17]. These studies often used anti-tTG assays developed in the research laboratory, adding another bias. Our patients were representative of those seen with celiac disease in that women predominated, less than 50% had diarrhea as their primary presentation and 60% had total villous atrophy [18, 19]. However the main bias of our study is the referral nature of the population as well as the high rate of celiac disease diagnosis. However, this selection bias does not account for the difference in sensitivity between patients with total villous atrophy and partial villous atrophy, as both groups should be equally affected by this bias.

In our study, 84% of the patients were ultimately diagnosed with celiac disease. This high percentage is due to the fact that we are a referral center for celiac disease, and

our patient population may not be reflective of that seen by community gastroenterologists. There also was a greater than usual number of patients who were relatives of patients with the disease. As such, interpretation of the positive and negative predictive values should be done with caution, as these values are heavily influenced by disease prevalence in the population being studied. However, the fact that this study was carried out at a referral center should have no impact on the sensitivity and specificity, as these values are inherent to the test, independent of disease prevalence.

We found no difference in the sensitivity of the tissue transglutaminase antibody in patients with classical, diarrhea predominant, celiac disease as compared to patients presenting with silent or asymptomatic celiac disease. The serologic tests likely have equal utility in the diagnostic evaluation of both groups of patients. The degree of villous atrophy is the major determinant of whether the test is positive [12]. It is interesting to note that the patients who presented with anemia tested positive for the tissue transglutaminase antibody more than the patients with other presentations, although this finding did not reach statistical significance.

The IgA tTG assay has replaced the EMA test in many laboratories, yet the anti-tTG test is not as specific for celiac disease as the EMA. There are many reports of the IgA tTG test positive in the absence of celiac disease [20-22], and in a diverse spectrum of diseases including arthritis [23], heart failure [24], chronic liver disease [25-27], diabetes [28] and inflammatory bowel disease [25, 27, 29], even when human tTG is used as the antigen in the tests. During the period of this study only human tTG were used by the testing laboratories. The EMA and anti-tTG are not always concordant [6, 14], indicating the value of performing both the EMA and tTG in patients at risk for celiac disease.

A source of variability in all of these studies is the sensitivity and specificity of the different test kits. Several studies have demonstrated that different tests kits have different characteristics [30-32]. This is born out in our study in which the two principal laboratories in this study used different kits for the tTG test. One laboratory had high specificity and low sensitivity, while the other laboratory had high sensitivity and low specificity. There were significantly more patients with total villous atrophy tested at Lab #1 compared to Lab #2. This finding alone could explain the difference in overall sensitivity between the two laboratories. However, after analyzing the sensitivities by degree of histological damage, there was still a difference in the tissue transglutaminase sensitivity between the labs. The sensitivity of the tissue transglutaminase antibody was lower in patients with partial villous atrophy compared to total villous atrophy in both Lab #1 and Lab #2. We are not aware how each laboratory determines cut off values or whether they in fact use the manufacturers recommended values. In turn, we do not know how the manufacturer determines the normal range for their individual kits. It is not realistic for physicians in clinical practice to be aware of these subtle yet important details. Perhaps a standard assay and cutoff should be agreed upon and observed by all commercial reference laboratories as has been attempted in Europe [33]. For a more valid evaluation of interlaboratory variability, future studies should compare patients' serum that is tested using different kits commonly used in the United States. We have in fact seen patients that have had tTG tests performed at both laboratories and noted conflicting results. These patients were not included in this study. We are not aware of the applicability of our results to other laboratories or the different manufacturers' kits that are used in the United States.

It is also possible, though remote, that not all the patients had celiac disease. Patients with biopsy findings similar to that found in celiac disease have been reported to spontaneously remit, suggesting the presence of an acute non-specific enteritis that is not gluten-related [34]

The test sensitivity for the tissue transglutaminase antibody is significantly lower in patients with lesser degrees of histological damage. The cutoffs for a positive test and the assay used to test for the antibody are not uniform across different commercial reference laboratories. The result is a marked inter-laboratory variability in test sensitivity and specificity observed in clinical practice. The clinician should therefore have a very low threshold for obtaining biopsies in any patient with a negative tissue transglutaminase and a moderate clinical suspicion for a celiac disease diagnosis. A significant proportion of patients with celiac disease and a negative antibody will be missed if there is no further work-up. Relying on the antibody tests as the sole means of identifying patients with celiac disease will result in many missed diagnoses, in particular in patients with partial villous atrophy.

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Table 1. Celiac Disease Patient Characteristics

	No. of Patients	%
Total	102	100
Sex		
Male	33	32.4
Female	69	67.6
Race		
Caucasian	102	100
Other	0	0
Type of Celiac Disease		
Classical	44	43.1
Silent	58	56.9
Presentation*		
Diarrhea	44	43.1
Iron deficiency	18	17.6
Osteoporosis	14	13.7
Screening	10	9.8
Other	40	39.2
Histology		
Total villous atrophy	60	58.8
Partial villous atrophy	42	41.2
Age at Diagnosis (yr)	44.5 (SD=15.4)	

*Some patients had multiple presentations.

Table 2. Tissue transglutaminase (tTG) sensitivities by degree of villous atrophy*.

	Sensitivity (%)	(95% CI)	
Overall	70.1	(60.8-79.2)	
TVA	90.0	(79.5-96.2)	
PVA	42.9	(27.7-59.0)	p<0.0001†

*TVA = total villous atrophy, PVA = partial villous atrophy

†p-value calculated for the difference in sensitivity between TVA and PVA

Table 4. Comparison of tTG sensitivity in each laboratory by histology*.

	Lab 1 (%tTG+)	(95% CI)	Lab 2 (%tTG+)	(95% CI)	p-value
TVA	10/13 (76.9%)	(46.2-95.0)	39/40 (97.5%)	(86.8-100)	0.04
PVA	2/17 (11.8%)	(1.5-36.4)	12/19 (63.2%)	(38.4-83.7)	0.002
p-value†	0.0003		0.0003		

*TVA = total villous atrophy, PVA = partial villous atrophy

†p-value for comparison of TVA vs. PVA sensitivities

Table 3. Anti-tissue transglutaminase test characteristics at two major commercial laboratories.

	Lab #1	(95% CI)	Lab #2	(95% CI)	p-value
Sensitivity	12/30 (40.0%)	(22.7-59.4)	51/59 (86.4%)	(75.0-94.0)	<0.0001
Specificity	7/7 (100.0 %)	(65.2-100)	5/12 (41.7%)	(15.2-72.3)	0.02
PPV*	12/12 (100.0%)	(77.9-100)	51/58 (87.9%)	(76.7-95.0)	0.34
NPV*	7/25 (28.0%)	(12.1-49.4)	5/13 (38.5%)	(13.9-68.4)	0.71

*PPV = positive predictive value, NPV = negative predictive value

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