

Diagnostic value of duodenal antitissue transglutaminase antibodies in gluten-sensitive enteropathy

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SUMMARY

Background

In gluten-sensitive enteropathy, antitissue transglutaminase antibodies are synthesized in the duodenum.

Aim

To compare the diagnostic yield of these autoantibodies in cultured duodenal biopsies, duodenal aspirate and serum.

Methods

Patients ($n = 315$, 135 female, 180 male; age: 37.3 ± 1.1 years) referred for duodenal biopsies, were recruited and HLA-DQ2/DQ8 haplotyped. Histological measurements were made from duodenal biopsies and cultured duodenal biopsies were used for antitissue transglutaminase antibodies analysis by enzyme-linked immunosorbent assay. Duodenal aspirate was collected in a subgroup of 81 patients. Patients were classified, according to their histology, response to a gluten-free diet and DQ2/DQ8 status, as definite, likely or nongluten-sensitive enteropathy.

Results

Histology was normal in 59% of patients; 28% had lymphocytic enteritis, 1% had crypt hyperplasia and 13% showed atrophy. In Marsh III patients, there was complete agreement between duodenal and serological antitissue transglutaminase antibodies measurements. Marsh I patients showed a slight antitissue transglutaminase antibodies sensitivity improvement in cultured duodenal biopsy compared to serum in definite (22% vs. 19%) and likely gluten-sensitive enteropathy (20% vs. 14%) patients. Combined serum and cultured duodenal biopsy antitissue transglutaminase antibodies assessment increased serological sensitivity from 19% to 30% in Marsh I patients.

Conclusion

Duodenal antitissue transglutaminase antibodies detection improves serological determination sensitivity in Marsh I patients, providing diagnostic value and therapeutic impact.

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INTRODUCTION

Coeliac disease (CD) remains an underdiagnosed disorder. Serum antiendomysial antibodies (EmA) and anti-tissue transglutaminase antibodies (tTGA) have demonstrated a high sensitivity and specificity for CD diagnosis in patients with complete atrophy.^{1, 2} However, the sensitivity of these tests is lower in milder forms of the disease, such as in patients with lymphocytic enteritis (LE; Marsh I type lesion) where sensitivity ranges from 15% to 30%.²⁻⁴

Diagnosing the whole spectrum of gluten-sensitive enteropathy (GSE) has recently been emphasized, as patients with LE may show symptoms identical to those of patients with villous atrophy,⁴ as well as showing a good response to a gluten-free diet (GFD).⁵ A diagnostic strategy based on HLA-DQ2 genotyping followed by duodenal biopsy sampling in DQ2-positive relatives of CD patients is useful for identifying individuals with milder forms of the disease.⁴ This strategy can be successfully applied in genetically predisposed groups, such as first-degree relatives and patients with type I diabetes. However, when this diagnostic strategy is applied to individuals with clinically suspected CD, patients with LE that is unrelated to gluten may be identified. In fact, it is well established that LE,⁶ as well as villous atrophy,⁷ may have causes distinct from the immunological reaction to gluten.

As there is evidence showing that intestinal mucosa is the specific site of autoantibody production (EmA and tTGA) in CD,^{4, 8-10} the aims of the present study were to: (i) compare the diagnostic yield of two methods based on local tTGA detection produced in the duodenum in either duodenal aspirate (DA) and/or cultured duodenal biopsy (CDB) specimens with the tTGA level in serum and (ii) assess the accuracy of tTGA determination in CDB for GSE diagnosis.

MATERIALS AND METHODS

Patients and study design

Between the years 2003 and 2005, 315 individuals (135 female, 180 male; 37.3 ± 1.1 years) were recruited in the out-patient clinic of a tertiary referral hospital, to test for GSE. Of the 315 patients, 48 were children (age range: 1–16 years) and 267 were adults (age range: 17–82 years). Patients with IgA deficiency were excluded from the study. All

patients had either gastrointestinal or extraintestinal symptoms, or belonged to a CD risk group. The procedures followed by our department to diagnose patients with gastrointestinal complaints were previously described.¹¹⁻¹⁶ Genetic testing for CD is routinely performed, as a negative HLA-DQ2/DQ8 haplotype has a high predictive value of a negative CD diagnosis.¹⁷ Thus, most patients, who were referred for endoscopy to rule out GSE, tested positive for HLA-DQ2/DQ8.^{4, 18}

After obtaining a written informed consent from all cases (or their parents, in paediatric cases), all subjects underwent a duodenoscopy to collect duodenal biopsies, for histological study and tTGA detection in CDB. At the same time, a blood sample was drawn for serum tTGA and EmA detection. In a subgroup of 81 individuals, the tTGA were also measured in the DA. A GFD was prescribed for all patients who possibly had GSE. These patients had a follow-up after 12 months. Then another clinical, serological and/or histological assessment was performed. According to the obtained results, patients were classified into one of the five following groups (Figure 1).

1 Definite GSE: 70 DQ2- or DQ8-positive individuals who fulfilled the diagnostic criteria and had a clear clinical, histological and/or serological response to GFD. The CD diagnosis was based on the American Gastroenterological Association criteria.¹⁹

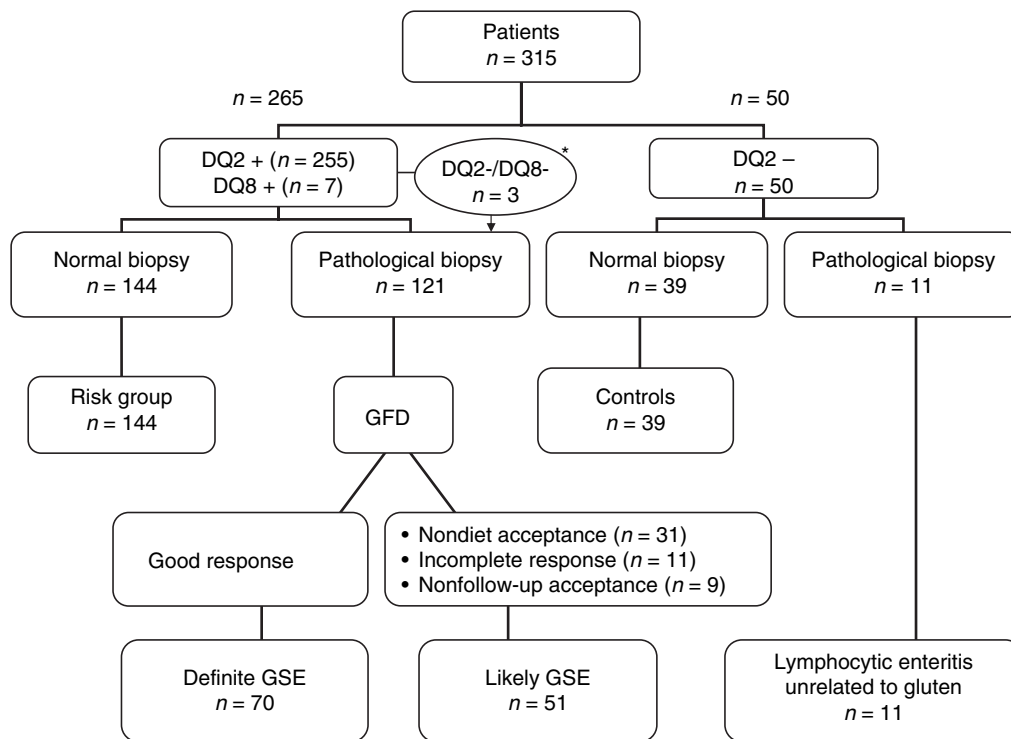
2 Likely GSE: 51 DQ2- or DQ8-positive patients who had some diagnostic criteria, but who did not accept the GFD or control biopsy after the GFD, or who showed an incomplete response.

3 Risk group with normal histology: 144 DQ2 or DQ8 positive, who were first-degree relatives of GSE patients, or who had type 1 diabetes mellitus or autoimmune thyroiditis.

4 Control group: 39 DQ2-negative (five DQ8 positive) patients, who had normal duodenal biopsies.

5 Group with LE unrelated to gluten: 11 DQ2/DQ8-negative patients (six with *Helicobacter pylori* infection and LE disappearance after eradication treatment, three with nonsteroidal anti-inflammatory drugs ingestion and two more patients with no response to a GFD making the diagnosis of GSE highly improbable).

Patients who had normal biopsies, but tested positive for tTGA in their serum, CDB and/or DA samples were encouraged to undergo retest and an intestinal biopsy at the end of the study.



* Patients with villous atrophy.

Figure 1. Flow diagram showing the diagnostic criteria of all the recruited patients.

Genetic markers

Standard techniques for DNA extraction, PCR amplification and product detection were used. To purify genomic DNA from whole blood, a commercial reagent was used (Generation Capture Column Kit; Gentra Systems Inc., Minneapolis, MN, USA). HLA-DQ2 (DQA1*0501 and DQB1*0201 alleles) and HLA-DQ8 (DQA1*0301 and DQB1*0302 alleles) genotyping was performed by PCR amplification using sequence-specific primers (PCR-SSP)²⁰ on a GeneAmp PCR 2400 System (Perkin-Elmer, Waltham, MA, USA). PCR products were detected by electrophoresis on 2% agarose gels and were visualized under UV light. Analysis of the HLA-DQ8 haplotype was performed only in patients who tested negative for DQ2.

Duodenal biopsy and aspirate collection

Four endoscopic biopsies from the second and third duodenal portions were obtained, fixed in 4% formaldehyde and embedded in paraffin. Specimens were stained with haematoxylin and eosin for morphologi-

cal assessment, and CD3 immunophenotyping was performed.²¹ The slides were blindly evaluated by an expert gastrointestinal pathologist. Histopathological findings were staged according to the Marsh criteria,²² as revised by Rostami *et al.*⁴ We made the diagnosis of intraepithelial lymphocytosis when more than 25 intraepithelial lymphocytes per 100 epithelial cells were observed.²³

The tTGA assay was performed in CDB supernatant by incubating two biopsy specimens from the same area at 37 °C for 48 h in culture medium [Bio-MPM-1 Multipurpose SFM for adherent cells (Biological Industries Ltd., Kibbutz Beit Haemek, Israel) with penicillin, streptomycin and L-glutamine], as previously described.^{9, 24} DA samples were collected through the suction channel of the endoscope into a disposable sterile trap. After DA collection, a protease inhibitor was added. Both CDB and DA samples were stored at -70 °C until analysis.

Antibody detection assays

Serum and CDB. Serum IgA-EmA levels were measured using the indirect immunofluorescence assay in

serum samples in a 1:5 dilution, as previously described.²⁵

IgA-tTGA levels were measured in serum and CDB samples (diluted at 1:100 and 1:20 respectively), using a quantitative automated enzyme-linked immunosorbent assay (ELISA) method and a commercially available detection kit (Varelisa Celikey, Phadia AB, Freiburg, Germany). Serum values of tTGA that were higher than 2 U/mL were considered positive (manufacturer's recommended cut-off = 8 U/mL), according to results of an epidemiological study performed in our regional health area (Gut 2007; 56: A110).

The commercial ELISA to detect IgA-tTGA on CDB was performed following a previously described protocol.⁴ The intra-assay coefficient of variation (CV) was 5% and the interassay CV was 8% ($n = 10$). The chosen cut-off of 0.17 U/mL was established using ROC curves, which allowed for a 97% specific and 60% sensitive GSE diagnosis.

Duodenal aspirate. For the detection of IgA-tTGA in DA samples, a noncommercial ELISA was performed, which was based on previously described methods.²⁶⁻²⁸ Ninety-six well ELISA plates were coated with guinea-pig liver tissue-transglutaminase (Sigma-Aldrich, St. Louis, MO, USA), diluted in phosphate-buffered saline. DA samples were diluted at 1:100, and the plates were incubated with peroxidase-conjugated goat antihuman IgA, using 3,3',5,5'-tetramethylbenzidine as the substrate (Sigma-Aldrich). The cut-off was established at 1.1 U/mL as determined by ROC curves (95% specificity and 55% sensitivity). The intra-assay CV for the IgA-tTGA ELISA on the DA samples was 3%, and the interassay CV was 20% ($n = 20$). The DA samples were also analysed using the commercially available ELISA kit used in the CDB experiments.

Total IgA detection assay. To ensure reliability of tTGA detection, we measured total IgA in all samples. In serological samples, total IgA was analysed by nephelometry. In the CDB and DA samples, an ELISA technique was used, as previously described.²⁷

Statistical analysis

The number and percentage of positive tests in all groups were provided according to their histopathological grade. Quantitative values were expressed as the mean and standard error of the mean. The sensi-

tivity, specificity, positive and negative predictive values, and accuracy of the assessed methods, were calculated.²⁹ True positive cases were patients with definite GSE, and true negative cases included all individuals from the control group and from the LE group, who had LE unrelated to gluten. The agreement rates between CDB, DA and serum samples were analysed by kappa statistics. The percentages of positive values obtained using different techniques and samples were compared using the McNemar's test. The tTGA-positive groups were compared using the Fisher's exact test.

RESULTS

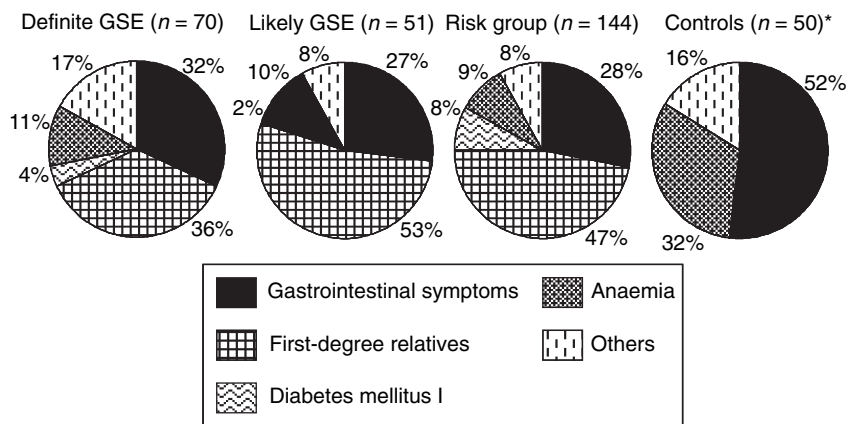
Histological findings, clinical manifestations and serology

One of the 315 recruited patients was excluded because of IgA deficiency. Duodenal histology was normal in 59% of the 314 included patients, while 28% had LE, 1% had crypt hyperplasia and 13% had villous atrophy. More Marsh III type lesions were observed in paediatric patients [19 of 48 (40%)] than in adults [23 of 267 (9%)].

The reasons for clinical referral for definite and likely GSE patients, and the risk and control groups are illustrated in Figure 2. In terms of the serological analyses, all of the EmA-positive patients ($n = 39$) had GSE spectrum histological lesions (four Marsh I, one Marsh II and 34 Marsh III), and all of these patients had positive tTGA levels, which were higher than 8 U/mL. All but one of the tTGA-positive patients ($n = 53$) had abnormal histological findings (one Marsh 0, 12 Marsh I, one Marsh II and 39 Marsh III). Patients with serological tTGA ranging from 2 to 8 U/mL ($n = 14$) were negative for EmA (one Marsh 0, eight Marsh I and five Marsh III). In 60% of the individuals with pathological mucosa, the serology analyses were negative for both EmA and tTGA (76 LE, one crypt hyperplasia and three villous atrophies).

Comparison of the diagnostic yield of tTGA detection in CDB and DA

In 32 of 81 DA samples (40%), the total concentration of IgA was undetectable. For this reason, these samples were not subjected to IgA-tTGA analysis. Thus, the DA and CDB samples of 49 patients were analysed (Table 1). Data were categorized according to the established groups and degree of histological damage.



* Control group with normal biopsy and disease control group with lymphocytic enteritis unrelated to gluten.

Figure 2. Reason for clinical referral.

Table 1. Comparison of tTGA detection in serum, CDB and DA samples ($n = 49$)

Groups	Histology	tTGA-positive cases in serum	tTGA-positive cases in CDB	tTGA-positive cases in DA	Total tTGA-positive cases*
Definite GSE ($n = 5$)	2 Marsh I	0	0	1	4
	3 Marsh III	3	3	3	
Likely GSE ($n = 7$)	7 Marsh I	1	2	3	4
Risk group ($n = 30$)	30 Marsh 0	0	1	2	2
Control group ($n = 7$)	6 normal and 1 LE unrelated to gluten	0	0	0	0

Results expressed as the number of positive samples.

Serum cut-off = 2 U/mL, CDB cut-off = 0.17 U/mL, DA cut-off = 1.1 U/mL.

Results equal or greater than the cut-off were considered positive.

* Total number of positive cases detected when analysing tTGA in all three samples (serum, CDB and DA). Combined assessment of tTGA detection in serum, CDB and DA increases the serological sensitivity from 11% (1/9) to 56% (5/9) in Marsh I patients with definite and likely GSE ($n = 9$).

GSE, gluten-sensitive enteropathy; DA, duodenal aspirate; tTGA, antitissue transglutaminase antibodies; CDB, cultured duodenal biopsy; LE, lymphocytic enteritis.

The kappa coefficient obtained by comparing the tTGA results in DA and CDB specimens was 0.56 (95% CI: 0.38–0.73; $P < 0.0001$). There were no significant differences in the percentage of positive tTGA between DA and CDB samples [DA: 9/49 (18%); CDB: 6/49 (12%); McNemar's test: $P = 0.375$]. We observed a complete concordance between DA and CDB samples in patients with atrophy, although the sample size was too small to make a solid conclusion ($n = 3$).

Combined assessment of tTGA in serum, along with measurements from CDB and DA samples increased serological detection in Marsh I patients with definite and likely GSE from 11% (1/9) to 56% (5/9) (Table 1). However, as previously mentioned, it should be taken into account that the frequency of positive tTGA in DA decreases when assessed by intention to test (24% positive tTGA in Marsh I patients), probably because of IgA degradation. Subjects of the control group were

negative for tTGA. In addition to detecting tTGA in DA samples using a noncommercial assay, the samples were assayed using a commercially available ELISA kit (Varelisa Celikey, Phadia AB). There were no significant differences in the percentages of positive tTGA samples in comparing the two methods (Varelisa Celikey: 18%, noncommercial ELISA: 18%; McNemar's t -test, $P = 1$).

Diagnostic value of tTGA in CDB compared to tTGA in serum

The levels of tTGA were analysed in both CDB and serum in 314 subjects, as shown in Table 2. In two of 314 samples (1%), the total IgA concentration was undetectable in the CDB supernatant, and these samples were unsuitable for assessment. Thus, 312 samples were evaluated.

The sensitivity, specificity and diagnostic accuracy of GSE diagnoses were calculated for patients with definite GSE (Table 3) and are also provided separately for the Marsh III and Marsh I groups. A complete agreement was observed between tTGA-positive serum and CDB samples in patients with atrophy, showing a progressive increase from Marsh IIIa (85%) to Marsh IIIc (100%). In

Marsh I patients, there was a slight increase in the percentage of positive tTGA in CDB samples compared to serum samples, in both definite and likely GSE patients. However, in two cases with definite GSE and in one case with likely GSE, the serum samples were positive for tTGA while the CDB samples were not. Thus, combined measurement of tTGA in serum and CDB samples from Marsh I patients increases the serological sensitivity from 19% to 30% (8/27) in definite GSE patients, and increases the sensitivity from 14% to 22% (11/50) in likely GSE patients. The percentages of samples positive for tTGA were similar in Marsh I patients with definite or likely GSE in both the serum (definite GSE 19%, likely GSE 14%; Fisher's exact test: $P = 0.744$) and CDB samples (definite GSE 22%, likely GSE 20%; Fisher's exact test: $P = 0.819$). In the control group, one DQ2/DQ8-negative patient with systemic lupus erythematosus and collagenous colitis was positive for tTGA in their CDB sample (45 U/mL). No patients with LE unrelated to gluten had positive tTGA samples. The kappa coefficient of the tTGA measurements between the CDB and serum samples was 0.79 (95% CI: 0.75–0.84; $P < 0.0001$). While the concordance in Marsh III patients was absolute, the percentage of Marsh I patients with CDB positive for tTGA was significantly higher

Table 2. Comparison of tTGA detection in serum and CDB samples ($n = 312$)

Groups	Histology	tTGA in serum	tTGA in CDB
Definite GSE ($n = 70$)	27 Marsh I	5/27 (19%)	6/27 (22%)
	1 Marsh II	1/1 (100%)	1/1 (100%)
	42 Marsh III:		
	13 Marsh IIIa	11/13 (85%)	11/13 (85%)
	18 Marsh IIIb	17/18 (94%)	17/18 (94%)
Likely GSE ($n = 51$)	11 Marsh IIIc	11/11 (100%)	11/11 (100%)
	50 Marsh I	7/50 (14%)	10/50 (20%)
	1 Marsh II	0/1 (0%)	0/1 (0%)
Risk group ($n = 141$)	141 Marsh 0	1/141 (1%)*	9/141 (6%)
Control group ($n = 39$)	39 normal	0/39 (0%)	1/39 (3%)†
LE unrelated to gluten ($n = 11$)	11 LE	0/11 (0%)	0/11 (0%)

Results are expressed as the number of positive samples over the total number of samples. Values in brackets represent the per cent sensitivity.

Serum cut-off = 2 U/mL, CDB cut-off = 0.17 U/mL, DA cut-off = 1.1 U/mL.

Results equal or greater than the cut-off were considered positive.

* First-degree relative of a CD case with 2.3 U/mL of tTGA in serum, 14 U/mL of tTGA in CDB and positive HLA-DQ2. See follow-up (case 1, Table 4).

† Patient with systemic lupus erythematosus and collagenous colitis (CDB tTGA value = 45 U/mL) and negative CD genetic markers.

GSE, gluten-sensitive enteropathy; DA, duodenal aspirate; tTGA, antitissue transglutaminase antibodies; CDB, cultured duodenal biopsy; LE, lymphocytic enteritis.

Group	Sample	Sensitivity	Specificity	PPV	NPV	Accuracy
Marsh III (<i>n</i> = 42)	Serum	92.9	100	100	94.3	96.7
	CDB	92.9	98	97.5	94.2	95.7
Marsh I (<i>n</i> = 27)	Serum	18.5	100	100	69.4	71.4
	CDB	22.2	98	85.7	70	71.4
Total definite GSE (<i>n</i> = 70*)	Serum	64.3	100	100	66.6	79.2
	CDB	65.7	98	97.9	67.1	79.2

Table 3. Accuracy of tTGA assessment in serum and CDB samples

Results are expressed as percentages, taking into account 70 true positive cases (definite GSE) and 50 true negative cases (control group + lymphocytic enteritis cases unrelated to gluten).

PPV, positive predictive value; NPV, negative predictive value; GSE, gluten-sensitive enteropathy; tTGA, antitissue transglutaminase antibodies; CDB, cultured duodenal biopsy.

* Total definite GSE including one Marsh II patient.

than the percentage of patients with positive serology (CDB: 21%; serum: 16%; McNemar's test: $P = 0.004$).

Follow-up of patients from the risk group with normal biopsies and positive tTGA in their CDB and/or DA samples at baseline

Eleven patients had normal duodenal biopsies and were positive for tTGA in their CDB and/or DA samples during their baseline evaluations. Eight of these patients agreed to be retested. In Table 4, the serology results and tTGA measurements in their CDB and DA samples are shown for both the initial testing and follow-up. All patients were on a gluten nonrestricted diet. Four patients (50%) showed a progression of intestinal damage from normal to Marsh I. Six of eight patients (75%) showed either persistence of tTGA positivity in one of the assessed samples or evidence of intestinal damage. Only one patient was positive for tTGA in both serum and CDB samples (Table 4, case 1).

DISCUSSION

In this study, patients with duodenal villous atrophy ($n = 42$) had 93% positive serologies with sensitivity values ranging from 85% in Marsh IIIa patients to 100% in Marsh IIIc, which is in agreement with previously published results.^{1, 2} In addition, the sensitivity of serological tests falls sharply in Marsh I patients.^{3, 4, 30} This observation was confirmed by our data where only 19% of Marsh I patients showed positive serology.

A recent study demonstrated the relevance of diagnosing mild forms of CD, as they may cause notable

morbidity.⁴ This is often a difficult task as LE can have multiple aetiologies.^{6, 7} LE occurs in mild forms of CD where it is important to find new diagnostic techniques to identify definite cases of GSE. One study assessed the use of tTGA measurements for diagnostic accuracy in CDB samples, which included 191 CD patients and 82 controls (a mixed group of normal histology, LE and mild atrophy). The results demonstrated an increased sensitivity of tTGA detection in CDB samples compared to serum samples (98% vs. 85%). However, most of GSE patients with positive serologies had severe histological forms of disease (60% of Marsh IIIb–c). Thus, this method provided a diagnostic benefit for Marsh IIIa patients. In contrast, there were limited data on the usefulness of tTGA measurements on CDB samples from LE patients. In the previous study, only seven Marsh I patients with negative serologies were included, and two of these patients were positive for tTGA in their CDB samples.³¹

This study provides additional information on the diagnostic value of tTGA assessment in CDB samples. We included a representative sample of all degrees of the CD spectrum with a high proportion of Marsh I patients with negative serologies, who are the most frequently misdiagnosed target group. In fact, in the group of 88 patients with LE, only 43% were ultimately classified with definite GSE or LE unrelated to gluten when using the clinical criteria and GFD response. The rest of the patients were categorized as likely but not definite GSE, with no possible confirmation. There was an improvement in GSE detection when using combined tTGA measurements from both serum and CDB samples (30%) in LE patients with gluten sensitivity

Table 4. Follow-up of eight patients with normal histology and positive tTGA in CDB and/or DA samples at their initial evaluation

Case	Initial testing				Time elapsed between tests (months)	Follow-up			
	Main reason for testing	Serology	tTGA CDB	tTGA DA		Serology	tTGA CDB	tTGA DA	Follow-up biopsy
1	First-degree relative	tTGA: 2.3 EmA: neg	14	D	46	tTGA: 3.4 EmA: weak+	5.51	D	Marsh I (25% IEL)
2	Anaemia	tTGA: neg EmA: neg	0.42	Neg	40	tTGA: neg EmA: neg	0.29	Neg	Marsh I (53% IEL)
3	Type I diabetes	tTGA: neg EmA: neg	0.22	D	41	tTGA: neg EmA: neg	Neg	D	Normal
4	Type I diabetes	tTGA: neg EmA: neg	0.50	D	41	tTGA: neg EmA: neg	0.54	D	Normal
5	First-degree relative	tTGA: neg EmA: neg	Neg	1.3	42	tTGA: neg EmA: neg	Neg	1.6	Marsh I (68% IEL)
6	Chronic diarrhoea	tTGA: neg EmA: neg	0.18	NS	22	tTGA: neg EmA: neg	NS	3.0	Normal
7	Epilepsy and previous tTGA+ and EmA+	tTGA: neg EmA: neg	Neg	1.2	50	tTGA: neg EmA: neg	Neg	Neg	Marsh I (35% IEL)
8	Chronic diarrhoea	tTGA: neg EmA: neg	Neg	1.2	36	tTGA: neg EmA: neg	Neg	Neg	Normal

tTGA, antitissue transglutaminase in serum (cut-off = 2 U/mL); EmA, antiendomysium antibodies in serum (cut-off = 1/10 titre); tTGA CDB, antitissue transglutaminase antibodies in cultured duodenal biopsy (cut-off = 0.17 U/mL); tTGA DA, antitissue transglutaminase antibodies in duodenal aspirate (cut-off = 1.1 U/mL); IEL, intraepithelial lymphocytes (cut-off = 25% IEL); D, samples under spontaneous degradation. No reliable results; NS, no sample; Neg, negative; DA, duodenal aspirate; tTGA, antitissue transglutaminase antibodies; CDB, cultured duodenal biopsy. Results equal or greater than the cut-off were considered positive.

compared to detection by serology alone (19%). Taking into account the high specificity of positive tTGA, its detection in patients with likely GSE has a great diagnostic value and helps to take a therapeutic decision.

In the risk group of patients with potential or latent CD, we observed that 1% of patients were positive for tTGA in serum, and 6% were positive for tTGA in CDB. These results suggest that local production of autoantibodies may precede subsequent histological damage. In fact, the follow-up of eight of the 11 patients with positive tTGA in their CDB or DA samples, demonstrated a progression from normal mucosa to Marsh I lesions in half of them. In contrast, tTGA was not detected in the follow-up in two patients, and the appearance of their duodenal mucosa remained normal. Similar evolutions towards worsening or spontaneous remissions have been reported in GSE patients.³²⁻³⁴ These results highlight the necessity for strict follow-ups in individuals within the risk group, who have normal mucosa but who are positive for

tTGA in their serum and/or duodenal samples. In addition, the present study demonstrates that local production of autoantibodies can be detected in the DA collected during diagnostic endoscopy, along with detection in CDB samples. The analysis of tTGA in the DA has never been used in clinical practice. We optimized a previously used noncommercial ELISA method to use the technique for GSE diagnoses and we also ensured that a commercially available ELISA kit (Varrelisa Celikey™, Phadia AB) provided the same reliable results. The main disadvantages of DA collection is that the endoscopy procedure is lengthened and more complex, and there are processing difficulties, which are likely because of sample degradation. For these reasons, collection of DA samples was justified in only a subgroup of the recruited individuals in our study. However, because of the incomplete level of agreement between the evaluated samples (kappa coefficient <1), the use of tTGA detection in all three samples (serum, CDB and DA) may provide a higher sensitivity in the

diagnoses of mild forms of CD compared with detection methods using serum alone (11–56% in definite and likely GSE). In clinical practice, combined tTGA assessment should be restricted to cases of doubtful diagnosis.

We required a high specificity for tTGA in duodenal samples, at the expense of lowering sensitivity, to avoid prescribing a GFD to patients with LE unrelated to gluten. In fact, a GFD should only be advised for those patients with LE that is likely because of gluten sensitivity and those who have disabling symptoms. The long-term benefit, if any, of preventing complications, such as osteoporosis, autoimmune disorders and mortality, in patients with mild forms of GSE is at present unknown.³⁵

In conclusion, the sensitivity, specificity and accuracy of tTGA detection in both serological and CDB samples

are equivalent in severe forms of CD (villous atrophy). In patients with LE, the sensitivity is slightly improved by using tTGA detection in CDB samples compared to serological analysis alone. Finally, as LE may arise out of causes other than gluten sensitivity, duodenal detection of CD-specific autoantibodies may have a significant diagnostic value and therapeutic impact.

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