Are positive serum-IgA-tissue-transglutaminase antibodies enough to diagnose coeliac disease without a small bowel biopsy? Post-test probability of coeliac disease

Fernando Fernández-Bañares, Montserrat Alsinab, Inés Modolell, Xavier Andújar, Marta Piquerasc, Roger García-Puigd, Benjamín Martíne, Mercé Rosinach, Antonio Salas, Josep Maria Viver, Maria Esteve

Department of Gastroenterology, Hospital Universitari Mutua Terrassa, University of Barcelona, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Terrassa, Barcelona, Spain
Department of Immunology, CATLAB, Viladecavalls, Barcelona, Spain
Department of Gastroenterology, Consorci Sanitari, Terrassa, Barcelona, Spain
Department of Paediatrics, Hospital Universitari Mutua Terrassa, University of Barcelona, Terrassa, Barcelona, Spain
Department of Paediatrics, Consorci Sanitari, Terrassa, Barcelona, Spain
Department of Pathology, Hospital Universitari Mutua Terrassa, University of Barcelona, Terrassa, Barcelona, Spain

Received 28 December 2011; received in revised form 19 January 2012; accepted 19 January 2012

KEYWORDS
Anti-transglutaminase antibody; Small bowel biopsy; Celiac disease; Post-test probability; Endomysial antibody

Abstract

Background: It has been suggested that high titres of tTG are associated with elevated positive predictive values (PPV) for celiac disease. However, the PPV of a strongly positive tTG will depend on the celiac disease prevalence in the different risk groups of the disease.

Aims: To assess the PPV of a strongly positive tTG for celiac disease. In addition, to calculate the post-test probability for celiac disease of a strongly positive tTG in a setting of routine clinical practice.

Methods: 145 consecutive celiac disease patients with positive tTG, and with a small bowel biopsy were included. The PPV for different cut-off points of tTG levels for the diagnosis of celiac disease was assessed. In addition, the cut-offs associated with higher PPV were used to calculate the positive likelihood ratio. A simulation in a setting of routine clinical practice was performed to calculate the post-test probability of celiac disease.

Results: No cut-off level was associated with a PPV of 100%. A cut-off of 80 U/mL (11.4× upper normal limit) was associated with the higher PPV value of 98.6%.
situations, which in general have a pre-test probability <10%, the post-test probability after having a strongly positive tTG was 90% or less.

Conclusions: A strongly positive tTG should not be enough to diagnose celiac disease in the most frequent clinical situations, small bowel biopsy remaining as the gold standard in these cases.

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1. Introduction

Systematic reviews have concluded that the assays for the detection of serum-IgA-tissue-transglutaminase antibodies (tTG), using human recombinant tissue transglutaminase-2 as antigen, have a high sensitivity and specificity in children and adult for identifying those with coeliac disease (CD). However, diagnostic accuracy in clinical practice is not as high as that reported in research laboratories, being the consequence of several causes recently reviewed.

While in most studies results of tTG are regarded as qualitative, i.e., positive or negative, some authors have recently taken into account tTG as a quantitative parameter. In this sense, several studies have suggested that high titres of tTG have a high specificity and are associated with elevated positive predictive values (PPV) for CD. In this situation, the requirement for small bowel biopsy to establish the diagnosis of CD in every case has been questioned. In pediatric populations, strongly positive tTG (≥100 U) were predictive of Marsh 3a or greater changes. In another study performed in patients older than 15 years, the PPV of tTG levels higher than 30 U/ml (≥10×ULN) for CD was 100%. If this was the case, it should not have false positives and small bowel biopsy might be avoidable. However, there are studies describing patients with strongly positive tTG but with normal or near normal small bowel histology (Marsh 0 to Marsh 1).

On the other hand, the prevalence of a disease among the population of interest has an important influence on the PPV of a diagnostic test. The same diagnostic tests will have variable PPV in different populations. With increasing disease prevalence, the more likely it becomes that a person with a positive test result has the disease, and the less likely it becomes that a positive result is a false positive. Conversely, when the prevalence of the disease is low, the PPV will also be low, even using a test with high sensitivity and specificity. Thus, without taking into account the disease prevalence in the population of interest, PPV cannot be accurately estimated. In the case of CD, the PPV of a strongly positive tTG will depend on the CD prevalence in the different risk groups of the disease.

The aim of the present study was to assess the PPV of a strongly positive tTG for CD. In addition, to calculate the post-test probability for CD of a strongly positive tTG in a situation of routine clinical practice, taking into account the pre-test probability, which corresponds to the prevalence of the disease in different situations of CD risk.

2. Patients and methods

2.1. Patients

All adult and pediatric CD patients with tTG ≥7 U/ml, measured in the period between January 2008 and December 2010, in whom a small bowel biopsy was performed, were recruited from the two participating centers. All patients were on a gluten-containing diet at inclusion, and in all of them, the tTG determination was performed in the same laboratory, and immediately (less than 6 weeks) prior to biopsy. The study was approved by the Research and Ethical Committees of the participating hospitals.

2.2. Coeliac serology

Serum IgA-tissue transglutaminase antibody (tTG) was analyzed in serum using a quantitative automated ELISA method by means of a commercially available detection kit (Varelisa Celi-keyTM, Phadia AB, Freiburg, Germany) using recombinant human tTG as antigen (recommended cut-off by the manufacturer >8 U/ml). Total serum IgA was measured using rate nephelometry (BN II, Siemens Healthcare Diagnostics SL, Marburg, Germany); no patient was IgA deficient in the present study. In a previous paper of our group, over 98% of individuals in a general population sample had tTG levels below 2 U/ml. Values of tTG between 2 and 7 U/ml were considered as doubtful positive, and those of 7 U/ml or more as positive. Thus, a cut-off of ≥7 U/ml was used in the present study.

Serum IgA-endomysial antibody (EmA) was determined by indirect immunofluorescence (IFI) assay in serum samples at 1/5 dilution, as previously described. Commercial sections of monkey distal esophagus (BioMedical Diagnostics, France) were used as the IFI substrate.

The laboratory performing tTG and EmA assays (CATLAB, Viladecavalls, Barcelona, Spain) has a documented experience and high standards in immunohistochemistry, and participates in international quality control programs for celiac disease serology (UKNEQAS, Sheffield, UK).

2.3. HLA genotyping markers

Standard techniques for DNA extraction, PCR amplification and product detection were used. To purify genomic DNA from whole blood, a commercial reagent Generation® Capture Column Kit (Genta Systems, Minneapolis, MN, USA) was used. 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) on a GeneAmp PCR 2400 System (Applied Biosystems, Foster City, CA, USA). PCR products were detected by electrophoresis on 2% agarose gel and were visualized under UV light. Analysis of HLA-DQ8 haplotype was performed only on those patients with negative DQ2.

2.4. Histological studies

Four endoscopic biopsies from the 2nd–3rd portions of the duodenum were obtained in the index endoscopy. Additional
bulb biopsies were taken under the criteria of the physician performing the endoscopy. Duodenal samples were processed using haematoxylin/eosin staining and CD3 immunophenotyping. The number of intra-epithelial lymphocytes (IEL), the architecture of villi, and the inflammatory cell infiltration of the lamina propria were assessed. Histopathological changes were classified according to the Marsh-Oberhuber criteria.16 Lymphocytic enteropathy (Marsh 1 lesion) was defined as 25 or more IEL per 100 epithelial nuclei, and normal villous architecture. 17 We have validated this cut-off in our area in 15 consecutive patients who underwent upper gastrointestinal endoscopic examination due to heartburn or as a follow-up of Barret esophagus in whom gastric and duodenal mucosa were macroscopically normal; all were consuming gluten and had no relatives with coeliac disease. Mean IEL count in this control group was 14.1% and mean + 2SD was 24.9%.

All biopsy samples were reviewed by an expert gastrointestinal pathologist (A.S.). Particular attention was paid with those Marsh 0 and Marsh 1 patients with high tTG titres.

2.5. Diagnosis of celiac disease

Diagnosis of CD was based on evidence of villous atrophy on duodenal biopsy, increased IEL and crypt hyperplasia, and on the clinical and serological response when on a strict gluten-free diet, according to the ESPGAN and AGA criteria.18,19

2.6. Statistical analysis

The McNemar’s test was used to assess the concordance degree between the titres of both tTG and EmA. The null hypothesis was that the two serological tests give either high or low titres of the antibody at the same rate. Kappa coefficient was calculated as a measure of agreement between the two serological tests.

The PPV for different cut-off points of tTG levels for the diagnosis of CD was assessed. The PPV expresses the proportion of those patients with positive test results who truly have the disease, and is calculated by the quotient of true positives (numerator) and the sum of all positives, either true positives or false positives (denominator).

The cut-offs associated with higher PPV were used to calculate the positive likelihood ratio (LR+). Since in the present study patients with negative tTG were not biopsied, a simulation in a setting of routine clinical practice was performed. For that the following assumptions were considered: a) patients with positive tTG results were those observed in the present study (n=145); b) patients with negative tTG were extrapolated to reach a final sample size of 500 individuals taking into account a sensitivity and specificity of tTG of 90% and 94%, respectively, and a percentage of seronegative CD of 10%, as it can be observed in routine clinical practice studies4 (Table 1). The cut-offs of the tTG levels used to calculate the LR+ were 50 U/mL and 80 U/mL, since they were the associated with higher PPV for CD in the present study.

Likelihood ratios are independent of disease prevalence. LR+ is defined as the ratio of the true positive rate (sensitivity) divided by the false-positive rate (1-specificity). LR+ expresses how much more likely the patient is to actually have the disease after a positive test result. Post-test probability was calculated by means of Fagan nomogram, which is based on Bayes’ theorem, knowing the pre-test probability (Post-test odds = Pre-test odds × Likelihood ratio). Because of the theorem’s mathematical properties, the likelihood ratio was used with odds rather than per cent probability of the disease. Pre-test probability corresponds to the known prevalence in each risk group of CD, 1% for general population, 3% for general population who have HLA-DQ2+, 5–10% for most clinical situations, 10% for first-degree relatives, 20–30% for first degree-relatives with positive celiac genetics (the same HLA-DQ2 or DQ8 that the index case).20

3. Results

3.1. Patients

Small bowel biopsy results were available in 145 patients (67% women; median age, 15 years, IQ range, 6 to 37 years) who had a tTG value ≥ 7 U/mL. Histological results were 13 Marsh 0, 10 Marsh 1, 20 Marsh 3a, 52 Marsh 3b, and 50 Marsh 3c. HLA-DQ2 and/or HLA-DQ8 were available in 107 of the 145 patients, being positive in 104 (100 HLA-DQ2, 4 HLA-DQ8). In the other 3, an allele of the HLA-DQ2 haplotype was positive.

3.2. Comparison of tTG and EmA titres

EMA titres were assessed in 106 patients with a tTG value ≥ 7 U/mL, all with positive celiac genetic study. EmA was negative in one out of the 106 patients, who had a tTG of 19 U/mL and a Marsh 3b on small bowel biopsy. We consider high titres of tTG those of 80 U/mL or more, and high titres of EmA those over 1/80. Concordance results are described in Table 2. Discordant data were observed in 21 patients (18%): 16 patients with tTG below 80 U/mL had high titres of EmA, and 5 patients with high titres of tTG had EmA of 1/80 or below. There were significant differences in the concordance degree of both tests as assessed by the McNemar’s test (p=0.026). The Kappa coefficient was 0.59 indicating a moderate agreement.

3.3. Positive predictive value for different cut-off levels of tTG

In Table 3 the cumulative data at each of the tTG cut-offs expressed as PPVs for the diagnosis of CD is described. No cut-off level was associated with a PPV of 100%. Eighty three patients (57.2%) had tTG titres ≥ 50 U/mL, of whom 80 had a Marsh 3, two a Marsh 1, and one a Marsh 0 lesion (tTG values

![Table 1](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAAIAAAACgCAYAAAAJiL0gAAAgAElEQVR42m2Y2h+8w/...)

<table>
<thead>
<tr>
<th>Coeliac disease</th>
<th>Not coeliac disease</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>tTG+</td>
<td>122</td>
<td>23</td>
</tr>
<tr>
<td>tTG−</td>
<td>14</td>
<td>341</td>
</tr>
<tr>
<td>Totals</td>
<td>136</td>
<td>364</td>
</tr>
</tbody>
</table>

*a A 10% seronegative CD and a sensitivity and specificity of tTG of 90% and 94% were considered (see explanation for these assumptions in the statistical section).
in Marsh 1 and 0 of 57, 57, 107 U/mL, respectively). On the other hand, 72 patients (49.6%) had tTG titres ≥ 80 U/mL, of whom 71 had a Marsh 3 and one a Marsh 0 lesion (tTG value of 107 U/mL). All the three patients with Marsh 0 to 1 lesion and tTG of 50 U/mL or more had positive EmA (titres of 1/40, 1/80 and 1/160) and positive HLA-DQ2. Two of them were 2- and 6-year-old girls with a typical clinical presentation of CD; the third patient was a 32-year-old man with dermatitis herpetiformis. In all three a gluten-free diet (GFD) was started with an excellent clinical and serological response.

### 3.4. Assessment of positive likelihood ratio and the post-test probability of CD

Using the cut-off of 50 U/mL, the sensitivity, specificity, PPV and LR+ of the test were 52.2%, 99.7%, 98.6%, and 190, respectively. Using the cut-off of 80 U/mL, the sensitivity, specificity, PPV and LR+ of the test were 58%, 99.2%, 96.3%, and 71, respectively.

Post-test probability of the disease for each pre-test probability, using both cut-off levels of tTG, is described in Fig. 1. A strongly positive tTG was 90% or less, arguing against of avoiding the diagnosis of CD almost certain in these cases. In the present study, with a similar design to that of Hill et al., showed that there was no cut-off of tTG levels associated with a PPV of 100%. A cut-off of 80 U/mL (11.4×upper normal limit) was associated with the higher PPV of 98.6%. This figure is similar to other previous studies, in which PPV of high tTG titres was elevated but not of 100%. Considering the prevalence of CD in the risk groups, we observed that the post-test probability of CD after a tTG of 80 U/mL or more approached 98% only for pre-test probabilities of 30% or more, like those seen in symptomatic patients with a first-degree relative sharing the same HLA-DQ2 and/or HLA-DQ8. In the most frequent clinical situations, which in general have a pre-test probability <10%, the post-test probability was 90% or less, arguing against of avoiding the diagnostic small bowel biopsy in these cases.

These PPV were calculated assuming that CD is only villous atrophy. In addition to the present study, there are other authors in the literature reporting a 2–7% of Marsh 0 to 2 lesions in patients with strongly positive tTG, in both adults and children. In some of these studies these strongly positive tTG levels were considered as false-positive serological results. Other studies, however, they were considered as an early CD or as having patchy lesions missed on biopsy. In fact, having all the following, strongly positive IgA tTG, positive IgA EmA, high baseline risk for CD, and positive celiac genetics make the probability of CD almost certain in these cases. In the present study, the three patients observed with a Marsh 0 to 1 lesion showed a complete serologic response after a GFD. If these patients were considered as CD, the PPV of a tTG of 80 U/mL or more would be 100%. However, there is still a lack of reliable evidence that all seropositive patients with mild enteropathy require treatment, and likewise, to ascertain if a patient has either villous atrophy of a milder lesion before starting a GFD might be of relevance at follow-up, mainly in cases with either incomplete or no response.

The need for a basal small bowel biopsy also comes from studies of follow-up biopsies after starting a GFD. Several studies have suggested that in 25–45% of adult patients mucosal recovery was not achieved after two years on a GFD, despite normalization of serology and symptoms improvement. These patients with persisting mucosal atrophy degrees might be at risk for subsequent severe complications. In pediatric patients, this fact has been less evaluated, disclosing persisting atrophy between 2 and 22% at 2 years on a GFD. These observations suggest the need for a follow-up biopsy, at least in adults, and in this sense initial diagnostic biopsy seems indispensable. Furthermore, some patients with strongly positive tTG, in both present series and previous studies, had partial villous

### Table 3 Positive predictive value (PPV) for the different cut-off levels of tTG.

<table>
<thead>
<tr>
<th>tTG cut-off U/mL</th>
<th>xULN</th>
<th>Coeliac disease</th>
<th>Not coeliac disease</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 7</td>
<td>1</td>
<td>122</td>
<td>23</td>
<td>84</td>
</tr>
<tr>
<td>≥ 20</td>
<td>2.8</td>
<td>104</td>
<td>7</td>
<td>93.7</td>
</tr>
<tr>
<td>≥ 30</td>
<td>4.3</td>
<td>97</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>≥ 50</td>
<td>7.1</td>
<td>80</td>
<td>3</td>
<td>96.3</td>
</tr>
<tr>
<td>≥ 80</td>
<td>11.4</td>
<td>71</td>
<td>1</td>
<td>98.6</td>
</tr>
</tbody>
</table>

Note: tTG is expressed as U/mL and as multiples of the upper limit of normal (ULN).

For Marsh 1 and 1 Marsh 0 with tTG values of 57, 57, 107 U/mL, respectively.
atrophy (Marsh 3a), thus a follow-up biopsy disclosing a Marsh 3a lesion without knowing the basal mucosal status would be misleading concerning histological recovery.

Finally, agreement between titres of both tTG and EmA was only moderate. In fact, there were significant differences between both serological tests in the frequency of either high or low positive titre rates. Previous studies have shown an excellent agreement when comparing tTG and EmA, but it was on a binary scale (positive or negative results).30,31 In addition, a highly significant correlation between tTG and EmA titres using Spearman's test has been described.31,32 However, a high correlation does not mean that two methods have a high degree of agreement,33 and this was not assessed in those studies. Discrepancies between both serological tests were mainly due to patients with low positive titres of tTG who had high EmA titres, this observation is in agreement with those papers suggesting that EmA has the highest impact among celiac antibodies.21

In conclusion, there was no cut-off level of IgA tTG associated with a PPV for CD of 100%. In the most frequent clinical situations, which in general have a pre-test probability <10%, the post-test probability of CD after having a strongly positive tTG was 90% or less. Therefore, a strongly positive tTG should not be enough to diagnose CD in the most frequent clinical situations, small bowel biopsy remaining as the gold standard in these cases.

Conflict of interest

No conflicts of interest exist.

Funding

There was no financial support for the study.

Acknowledgments

The 'Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd)' is an initiative of the Instituto de Salud Carlos III, Spain.

All authors have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, and (3) final approval of the version to be submitted.

References