Celiac disease (CD), an immune enteropathy caused by gluten-containing foods in genetically susceptible individuals, is usually diagnosed during childhood, but delayed diagnosis in adulthood is not uncommon (1). One-half of cases show atypical forms of the disease, e.g., iron-deficient anemia unresponsive to iron or persistent hypertransaminasemia (2–4).

Patients with CD often have high circulating concentrations of anti-endomysium antibodies (EMAs), the main disease marker (5), and anti-gliadin antibodies (AGAs), the most effective marker for children <3 years (6, 7). Tissue transglutaminase (tTG) has been identified as the autoantigen of CD (8).

The first assays for tTG antibodies used antigen from guinea pigs (9–15). Assays that used recombinant human tTG (rh-tTG) improved sensitivity and specificity (16–21), but it is not well established in prospective studies whether the clinical effectiveness of rh-tTG is similar for children and adults.

The aims of this study were (a) to evaluate the potential utility of rh-tTG IgA (rh-tTGA) compared with EMAs and AGAs for CD diagnosis in children and adults; (b) to analyze the concordance between rh-tTGA and small intestine biopsy (IB); and (c) to analyze the association of hypertransaminasemia and ferropenia with untreated CD.

We prospectively selected 2570 patients with clinical suspicion of CD. Most patients (73.4%) were referred by the Pediatrics Department. From this cohort of patients we analyzed consecutively all patients fulfilling the inclusion and exclusion criteria. Inclusion criteria included histologic analysis of an IB and determination of serum markers for CD; the exclusion criterion was IgA deficiency. Both the IB and the immunologic markers were analyzed blindly and independently.

The patients were classified into two groups. Group I consisted of 61 patients diagnosed with classic CD based on the results of the IB and obvious clinical and serologic response to a gluten-free diet. Group II contained 64 patients with clinical suspicion of CD but a normal IB and was considered the control group. The most common presentations in the control group were failure to thrive (29%) and gastrointestinal symptoms (27%).

We also included 86 first-degree relatives of celiac patients, all asymptomatic. In this group we diagnosed six new cases of CD, who were then incorporated in group I.

In our series 146 IBs were performed. Full-thickness jejunal biopsies were obtained by endoscopy or Watson–Crosby capsule (children). The histopathologic findings were classified according to internationally accepted criteria as normal mucosa, partial villous atrophy (slight, moderate, or severe), and subtotal villous atrophy (22).

We considered all cases with at least a moderate villous atrophy as diseased, and biopsies showing slight atrophy or unspecific changes were considered as normal.

rh-TGA (expressed in the eukaryotic baculovirus system) was determined by enzyme immunoassay (Celikey; Pharmacia Diagnostics). The antibody concentration was expressed in arbitrary units (AU/mL).

IgA-AGAs were measured by fluorescent enzyme immunoassay (Pharmacia Diagnostics). IgA-EMAs were measured by indirect immunofluorescence with monkey esophagus as substrate, and IgA was measured by turbidimetry in a Hitachi-Modular (Roche Diagnostics). Aspartate aminotransferase, alanine aminotransferase, and ferritin were measured in the same analyzer.

A descriptive analysis (frequencies, medians, and percentiles) was performed; we also compared the quantitative variables (Mann–Whitney U-test) and analyzed correlation with the Spearman ρ coefficient. The concordance rate was estimated with the κ index. We constructed ROC curves and calculated sensitivity, specificity, and likelihood ratios (LRs) at selected marker concentrations.

In our series, similar to other reports (2), 67% of the CD cases were women. Forty-seven patients (77%) were children (31 females and 16 males; age range, 1–17 years), and 14 (23%) were adults (10 women and 4 men; age range, 19–70 years). The frequency of CD among patients’ relatives was 7%, similar to the rates reported in other studies (23).

Serum rh-tTGA was significantly higher in celiac patients [median, 100 (5th–95th percentiles, 21.9–100) AU/mL] than in controls [0.3 (0.1–4.4) AU/mL] or asymptomatic relatives [0.33 (0.1–1.7) AU/mL; Fig. 1].

The specificity, sensitivity, and LRs obtained for the serologic markers of CD are summarized in Table 1. We detected two false-positive (FP) results for rh-tTG at a cutoff of 7.5 AU/mL. One was a 1-year-old boy with long-standing diarrhea (EMA titer, 1/5; AGA concentration, 1.1 mg/L; rh-tTG concentration, 9.6 AU/mL); IB showed intraepithelial lymphocytosis without villous atrophy or crypt hyperplasia. The final diagnosis was a postenteritis syndrome with transitory intolerance to lactose. The rh-tTGA value normalized to 1.9 AU/mL after 7 months. The other FP was an 8-year-old girl with hypertransaminasemia of undetermined etiology (rh-tTGA concentration, 23 AU/mL; EMA titer, 1/40; AGA concentration, 2.6 mg/L) with a normal IB. The serologic markers and the transaminases were within reference values 3, 6, and 18 months later.
The sensitivities (100% and 97.8%) and specificities (90% and 97.8%) of rh-tTGA were similar in children and adults, EMAs were as specific as rh-tTGA, whereas AGAs were less specific in patients over 3 years of age (P < 0.001; see the table in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue2/).

In CD patients, we did not find the described inverse correlation between age and rh-tTGA (ρ = −0.123; not significant) (12). However, we did find a weak statistically significant correlation with AGAs (ρ = −0.248; P = 0.02) that could justify the different specificities of this marker in different age subgroups.

The area under the curve for rh-tTGA was 0.998 (5th–95th percentiles) values: CD, 100.0 (21.9–100.0) AU/mL; no CD, 0.3 (0.1–4.4) AU/mL; relatives, 0.33 (0.1–1.7) AU/mL.

The concordance rate between the IB and serum concentrations of rh-tTGA was excellent (κ = 0.95; 5th–95th percentile, 0.9–1.0). All patients with villous atrophy had rh-tTGA concentrations >3.5 AU/mL. Three patients without atrophy (described above) had initial rh-tTGA concentrations that were in a gray zone or slightly increased.

Forty-nine percent of CD patients had significantly increased serum aspartate aminotransferase and alanine aminotransferase (P < 0.001) compared with controls and relatives (25). After initiation of a gluten-free diet, the transaminases normalized. Ferritin was low in 70% of the patients and in 39% of the controls (2, 4). The median ferritin concentration was significantly lower in patients with CD than in controls (21.5 vs 33.5 μg/L; P < 0.001).

Our results are consistent with those reported in the literature (15, 17–21) (see the online Data Supplement) and seem to indicate that rh-tTGA is the most effective marker in all age subgroups. The lower sensitivity of anti-tTG reported in children can be explained by the use of guinea pig tTG (9, 12).

We could consider rh-tTGA and EMAs almost interchangeable in children and adults. The only discordance was seen in a woman, mother of a daughter with CD, who entered the family study and had positive results for rh-tTGA and AGAs and negative results for EMAs, in whom biopsy confirmed CD. AGA results offer less clinical information (12), and we consider their use questionable in children >3 years and in adults because of the lower specificity.

We feel it necessary to establish a gray zone for rh-tTGA (3.5–7.5 AU/mL) in which results are hard to interpret. In patients with low clinical suspicion of CD, other immunologic markers, preferably EMAs, should be measured before an IB is performed.

The concordance with IB confirms that rh-tTGA is a good marker for the presence or absence of intestinal lesions. Some authors have proposed that rh-tTGA could replace IB in some cases (15). Diagnostic tests with positive LRs >10 or negative LRs <0.1 (11) could indicate significant changes in the probability of disease and are important for clinical decision-making. A negative rh-tTGA result would nearly exclude a diagnosis of CD in

### Table 1. Comparison for the serologic markers of CD.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity (presence of CD)</th>
<th>Specificity (no CD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% (95% confidence interval)</td>
</tr>
<tr>
<td>rh-tTGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutoff, 7.5 AU/mL (ROC curve)</td>
<td>60/61</td>
<td>98.4 (90–100)</td>
</tr>
<tr>
<td>Cutoff, 3.5 AU/mL (ROC curve)</td>
<td>61/61</td>
<td>100 (92–100)</td>
</tr>
<tr>
<td>EMA titer ≥1/5</td>
<td>43/44</td>
<td>97.7 (86.5–100)</td>
</tr>
<tr>
<td>AGAs (cutoff, 3 AU/mL)*</td>
<td>51/61</td>
<td>84° (72–92)</td>
</tr>
</tbody>
</table>

* Manufacturer’s information.

P < 0.004 for sensitivity of AGAs compared with rh-tTGA and EMAs.

P < 0.001 for specificity of AGAs compared with rh-tTGA and EMAs.
cases with a low probability of disease, whereas high concentrations indicate a high probability of disease.

The FP rh-tTGA results in patients with slight histologic abnormalities or a normal IB may represent either true FPs or be predictive of patients with risk of progressing to CD (so-called celiac condition). In these cases it would be advisable to perform serial rh-tTGA determinations until definitive diagnosis. In our series, the two patients who were FP in the test had normalized values in subsequent samples (up to 18 months later), and both patients are healthy on a non-gluten-free diet. Nevertheless, one is a carrier of DQ2 and is still being monitored clinically.

rh-tTGA measurements are also suitable for the diagnosis of atypical forms of CD, mainly in adults with nonspecific extraintestinal symptoms (2, 4, 26). The recombinant human assay can reduce the higher FP rate obtained with guinea pig tTGA in patients with hypertransaminasemia (27).
rh-tTGA may be suitable for the diagnosis of CD in first-degree relatives of patients (5–10% in our study) because in these cases biopsy is not adequate for diagnosis.

In summary, the rh-tTGA assay indicated the presence of CD in both children and adults and the test could be used as an alternative to EMA assays in the diagnosis of the disease.

We acknowledge no conflict of interest for the present study.

References

DOI: 10.1373/clinchem.2003.024976