Serology and endoscopy in coeliac disease: applications and limitations

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Tissue transglutaminase antibody testing in clinical practice

SENSITIVITY OF TISSUE TRANSGlutaminase ANTIBOdIES FOR COELIAC DISEASE: COMPARISON WITH ENDOMYSIAL ANTIBOdIES

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Submitted for publication
Abstract

**Background:** Initial studies suggest that serum antibodies to tissue transglutaminase (anti-tTG) have high sensitivity and specificity for coeliac disease. However, they correlate closely with endomysial antibodies (EmA), present in most patients included in these studies. Their performance among patients who are EmA negative is uncertain.

**Methods:** We used a commercial ELISA kit to test for IgA class anti-tTG in sera from a population of 70 untreated coeliac patients with normal serum IgA and a high percentage (20%) EmA negative, using 58 patients with normal duodenal biopsies as controls. EmA was measured using indirect immunofluorescence.

**Results:** Considered separately, sensitivity of anti-tTG and EmA were similar (76% v. 80%) and both has high specificity (2% v. 3%). However, 9 patients were anti-tTG positive only and 12 had EmA only. Using the presence of either antibody to select patients for biopsy would have had a sensitivity of 93% (65 of 70).

**Conclusions:** Although the ELISA anti-tTG assay is more convenient than EmA testing, it offers no advantages in sensitivity or specificity if used in isolation. However, incomplete concordance between EmA and anti-tTG positivity means that combination screening with both assays offers higher sensitivity. As a minority of coeliac patients are both EmA and anti-tTG negative, biopsies should still be considered in high risk individuals.
Introduction

In 1997 Dieterich et al identified the primary autoantigen of coeliac disease as tissue transglutaminase (tTG) [1]. They demonstrated that serum IgA from coeliac patients reacted with tTG, an enzyme widely distributed in human organs and thought to be released from intracellular vesicles within fibroblasts and endothelial cells during tissue damage. Extracellular tTG was proposed to catalyse cross-linking of gliadin, resulting in gliadin-gliadin and gliadin-tTG complexes which act as antigenic neoepitopes and initiate the immune response in susceptible individuals typical of coeliac disease. Dieterich et al later developed an enzyme-linked immunosorbent assay (ELISA) for the detection of serum antibodies to tTG (anti-tTG) which they proposed could be used for screening and case-finding [2]. Initial studies suggest that anti-tTG correlates well with endomysial antibodies (EmA) as an indicator of small bowel villous atrophy [2,3]. ELISA techniques are also technically less demanding and less subjective than the indirect immunofluorescence methods required to detect EmA. However, the value of anti-tTG in identifying EmA-negative coeliac patients, who are commoner than supposed [4,5], is uncertain. We assessed the performance of a commercial anti-tTG kit using sera from a coeliac population with high EmA-negative rates.

Methods

We used residual sera, which had been stored at -20°C, from routine blood tests of patients with small bowel villous atrophy, crypt hyperplasia and increased intraepithelial lymphocytes. All sera had been obtained before patients had started gluten-free diets. Patients with IgA deficiency were excluded.

IgA-class anti-tTG was assayed using a commercial ELISA kit (Quanta Lite: Inova Diagnostics, San Diego, California). This used tTG antigen purified from guinea pig liver. Recommended ranges expressed as ELISA units (EU) were negative (<20), weak positive (20-30) and moderate to strong positive (>30). IgA-class EmA was tested by indirect immunofluorescence on primate (Maccaca fascicularis) oesophagus substrate (Biodiagnostics, Upton-upon-Severn, England), with a positive titre of 1:5 or greater. Duodenal histology was reviewed by DFH without knowledge of serological results and classified as partial
(PVA), subtotal (STVA) or total (TVA) according to standard criteria [4,5].

Results

Of seventy patients included in the study (42 female, 28 male; ages 13-72 years), 21 (30%) had PVA and 49 (70%) ST/TVA. Primary indications for biopsy were anaemia in 20 patients, diarrhoea (16), dermatitis herpetiformis (5) and others in 29 patients. None had IgA deficiency, an important cause of false negative EmA testing. Sera from 58 patients who had normal duodenal biopsies were tested as controls. Indications for biopsy were diarrhoea (18 patients), anaemia (15), family history of coeliac disease (7) and others in 18.

Although sensitivities of anti-tTG ≥20 EU and of EmA for VA irrespective of grade were individually similar (76% v. 80%, Table), nine patients were anti-tTG positive only and 12 were EmA positive only. A combination protocol using the presence of either antibody to select patients for biopsy would have had a sensitivity of 93%, failing to recognise 5 of 70 patients. The sensitivity of anti-tTG ≥30 EU for VA was only 64% (45 of 70), and in combination with EmA 90% (63 of 70), with no change in specificity.

Table. Sensitivity (with 95% confidence intervals) of endomysial (EmA) and anti-tissue transglutaminase (anti-tTG) antibodies for villous atrophy

<table>
<thead>
<tr>
<th></th>
<th>EmA +ve</th>
<th>Anti-tTG +ve</th>
<th>EmA and/or tTG +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVA</td>
<td>18/21 (86%; 64-97)</td>
<td>15/21 (71%; 48-89)</td>
<td>21/21 (100%; 84-100)</td>
</tr>
<tr>
<td>ST/TVA</td>
<td>38/49 (78%; 63-88)</td>
<td>38/49 (78%; 63-88)</td>
<td>44/49 (90%; 78-97)</td>
</tr>
<tr>
<td>All VA</td>
<td>56/70 (80%; 69-89)</td>
<td>53/70 (76%; 64-85)</td>
<td>65/70 (93%; 84-98)</td>
</tr>
<tr>
<td>Controls</td>
<td>2/58 (3%; 0-12)</td>
<td>1/58 (2%; 0-9)</td>
<td>2/58 (3%; 0-12)</td>
</tr>
</tbody>
</table>

Discussion

Our results suggest that combined EmA and anti-tTG testing offers better sensitivity than
either individually, as many patients (21 of 70, 30%) were positive for one antibody only. Dieterich et al [2] obtained a sensitivity for anti-tTG of 98% for EmA positive PVA or STVA and specificity of 95%. Sulkane et al [3] tested sera from adults and children (median age 11 years) with severe VA. Anti-tTG had 95% sensitivity, compared with 93% for EmA and 85% for AGA in patients with severe VA. Lock et al [6] studied sera from 27 patients with “typical” coeliac histology: anti-tTG had a sensitivity of 85% and specificity of 92%, compared with 100% sensitivity and specificity for EmA. Biagi et al. [7] obtained a sensitivity and specificity of 95% and 90% respectively for anti-tTG in an exclusively adult population including 39 untreated coeliacs for whom EmA had 100% sensitivity and specificity. However, full validation of anti-tTG requires study of EmA-negative coeliac patients, who are commoner than previously supposed and may go unrecognised if undue reliance is placed on EmA results for biopsy selection. We previously reported a sensitivity of EmA for VA of 78%, with no difference between PVA and ST/TV A [4], while Rostami et al [5] obtained sensitivities for TVA, STVA and PVA of 100%, 70% and 31% respectively [4]. The higher sensitivity of EmA reported in other studies may reflect a bias towards only performing biopsies in EmA positive patients, the “self-fulfilling prophecy” described by Rostami et al. [8], or a failure to recognise PVA as a manifestation of coeliac disease. A follow-up study by Rostami et al [9] in a small number of coeliac patients from a population with high EmA negative rates obtained sensitivity of tTG for PVA of only 20% (2 of 10 patients), STVA of 66% (8/12) and TVA of 100% (5 of 5) compared with 40%, 66% and 100% respectively of EmA. In contrast to previous studies, our results show incomplete concordance between EmA and anti-tTG in a population with 20% false negative EmA rates. However, this allows the possibility of improved sensitivity for serological case-finding and screening using a combined approach, while high specificity for both anti-tTG and EmA will make biopsy for false positive serology unlikely. Thus, although the ELISA assay for anti-tTG is less subjective and more convenient than indirect immunofluorescence for EmA, a combination protocol should be used. As a minority of patients without IgA deficiency are negative for both antibodies (7% in this series), biopsies should still be considered in seronegative patients with classical symptoms or a family history of coeliac disease, or unexplained anaemia. Most studies to date have used guinea pig tTG as antigen for ELISA. Recombinant human tTG-based ELISA may have superior sensitivity [10] and further research is needed for confirmation and assessment of its potential role for diagnosis.
References