Serology and endoscopy in coeliac disease: applications and limitations
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Chapter 5

EmA sensitivity in routine clinical practice

RELIANCE ON SERUM ENDOMYSIAL ANTIBODY TESTING UNDERESTIMATES THE TRUE PREVALENCE OF COELIAC DISEASE BY ONE FIFTH

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Abstract

Background
Though IgA endomysial antibody (EmA) is currently the serological test of choice in selecting suspected coeliac patients for duodenal biopsies, false negative cases have been reported and may be more common than previous studies suggest. We assessed the sensitivity of EmA for patients with biopsy-confirmed villous atrophy (VA).

Methods
We studied 89 patients without IgA deficiency and for whom biopsy had not been primarily prompted by a positive EmA result. VA was graded as partial, subtotal or total (PVA, STVA, TVA). Serum EmA was assayed by indirect immunofluorescence.

Results
Sensitivity of EmA for VA was 78% (69 of 89) and was similar for PVA (79%) and ST/TVA (77%). Only four of the 20 EmA negative patients had raised serum IgA class antigliadin antibody measured by ELISA. All seronegative patients who complied with dietary gluten exclusion responded clinically, with histological improvement after 12 months in 8 (67%) of 12 patients who had follow-up biopsies.

Conclusions
EmA negative coeliac disease is common. Reliance on EmA testing to select patients for biopsy will result in significant underdiagnosis.
Introduction

Serum IgA class endomysial antibody (EmA) assay is currently the serological test of choice in selecting patients with suspected coeliac disease for duodenal biopsy. It is superior to IgA class antigliadin antibody (AGA), with high specificity for villous atrophy (VA), though reported sensitivity varies from 74% to 98% [1-5]. EmA testing with human umbilical cord instead of primate oesophagus similarly had 85% sensitivity for coeliac disease [6]. Recently Rostami et al [7] reported disappointing sensitivity of EmA, particularly for partial villous atrophy (PVA). Because of our own concerns about false negative serological testing, we use a duodenal biopsy protocol which is largely based on clinical presentation rather than EmA results. This allowed a prospective study of EmA sensitivity and of the prevalence of seronegative VA.

Materials and Methods

Duodenal biopsy was part of our investigation protocol for anaemia, diarrhoea, weight loss, and dermatitis herpetiformis, and when duodenal appearance during routine endoscopy suggested villous atrophy (VA) (nodular or mosaic mucosa, scalloping or loss of duodenal folds [8]). In these cases we performed biopsy without prior EmA testing, or disregarded the result if it had already been done by the referring clinician. We arranged EmA testing as an initial investigation and used it to select patients for biopsy only if clinical presentations were non-specific (e.g. abdominal cramps/wind, chronic fatigue, arthralgia, or abnormal liver biochemistry). Three biopsies were taken from the second part of duodenum by standard forceps during upper gastrointestinal endoscopy and carefully orientated and mounted on filter paper before submission in formalin. They were assessed by a consultant histopathologist (DFH) using criteria defined by Marsh [9], for evidence of an excess of intraepithelial lymphocytes, crypt hyperplasia, and villous atrophy (VA). VA where present was classified as partial, subtotal or total villous atrophy (PVA, STVA, TVA), as described by Rostami et al [7]. Patients with VA on duodenal biopsy had serum EmA testing before starting dietary gluten exclusion, if not already done by referring clinicians. EmA was tested by indirect immunofluorescence using primate (Maccaca fasciularis) oesophagus (Biodiagnostics, Upton-upon-Severn, England) as substrate, with a titre of 1:5 or greater.
taken as positive. Total serum IgA was measured in all patients to exclude deficiency as a cause of false negative EmA [3,10]. AGA was also tested using a commercial ELISA kit (Labmaster, Turku, Finland), with a positive result taken as greater than 100 ELISA units (EU).

Results

Over a 39 month period, one hundred and two patients aged 14 or over had first-time duodenal biopsies showing VA. Two EmA negative patients had IgA deficiency and were excluded, as were eleven patients with non-specific symptoms whose biopsies were a direct consequence of positive EmA testing. Primary indications for biopsy in the remaining 89 patients were anaemia in 26, diarrhoea (30), dermatitis herpetiformis (9), endoscopic duodenal abnormality (22), and weight loss (2). Sixty-five patients (73%) had ST or TVA and 24 (27%) had PVA. The Table shows EmA prevalences for different clinical groups, the overall sensitivity being 78%. Sensitivity was not significantly different for ST/TVA compared with PVA, patient age under 50 years v. 50 or older, or male patients compared with female. Twenty-nine (42%) of the 69 EmA positive and six (30%) of the 20 EmA negative patients had had testing performed before referral to us.
Table. Patient subgroups and EmA sensitivity.

<table>
<thead>
<tr>
<th>Patient subgroup</th>
<th>No. of patients</th>
<th>EmA positive (%)</th>
<th>P (Fisher’s exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>89</td>
<td>69 (78%, 64-87)</td>
<td>Not significant</td>
</tr>
<tr>
<td>ST/T VA</td>
<td>65</td>
<td>50 (77%, 65-87)</td>
<td></td>
</tr>
<tr>
<td>P VA</td>
<td>24</td>
<td>19 (79%, 58-93)</td>
<td></td>
</tr>
<tr>
<td>Primary presentation*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>30</td>
<td>22 (73%, 54-88)</td>
<td></td>
</tr>
<tr>
<td>Anaemia</td>
<td>26</td>
<td>22 (85%, 65-96)</td>
<td></td>
</tr>
<tr>
<td>Endoscopic abnormalities</td>
<td>22</td>
<td>18 (82%, 60-95)</td>
<td></td>
</tr>
<tr>
<td>Dermatitis herpetiformis</td>
<td>9</td>
<td>5 (56%, 21-86)</td>
<td></td>
</tr>
<tr>
<td>Age ≥50</td>
<td>46</td>
<td>32 (70%, 54-82)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Age &lt;50</td>
<td>43</td>
<td>37 (86%, 72-95)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>22 (76%, 57-90)</td>
<td>Not significant</td>
</tr>
<tr>
<td>Female</td>
<td>60</td>
<td>47 (78%, 66-88)</td>
<td></td>
</tr>
</tbody>
</table>

*Excluding weight loss as a category (applied to 2 patients only).

Of the twenty EmA negative patients 15 had ST/T VA and 5 PVA. Four (20%) had raised AGA (> 100 EU). All of 18 patients who complied with dietary gluten exclusion reported symptomatic improvement. Twelve patients had follow-up biopsies after at least twelve months of dietary treatment, which showed complete villous recovery in seven (all five with PVA plus two with ST/TVA: 58%), improvement from STVA to PVA in one (8%), and persistent ST/TVA in four (33%).

Twelve month follow-up biopsy results were available for 49 EmA positive patients (37 with ST/TVA, 12 with PVA). There was complete villous recovery in 20 (41%), improvement
from ST/TVA to PVA in 17 (35%), and no change in histological grade in 12 (24%). Nine (75%) of the 12 patients with EmA positive PVA had complete villous recovery.

Discussion

Rostami et al [7] reported a sensitivity of EmA for TVA of 100%, STVA of 70%, and PVA of only 31%. They suggested that the high sensitivities reported previously may have been due to selection bias from EmA testing before biopsy, or a failure to consider PVA as part of the spectrum of coeliac disease. Although our threshold for small bowel biopsy was not influenced by EmA testing except for non-specific presentations, prior EmA testing by referring clinicians may have biased their referral patterns. Our sensitivity of 78%, which was similar for PVA and ST/TVA, may therefore be an overestimate even though we excluded patients with non-specific symptoms whose EmA result had selected for biopsy. Two groups of patients were completely free of referral bias: those who had biopsies because of duodenal abnormalities seen during routine endoscopy, and those with dermatitis herpetiformis. The former showed comparatively high EmA sensitivity and we cannot exclude the possibility that endoscopically visible enteropathy is more likely to be EmA positive, although a previous study showed high sensitivity of endoscopic abnormality for all grades of VA [8]. The number of patients with dermatitis herpetiformis in our study was too small for comment.

The sensitivity of EmA for PVA in our study was similar to that for ST/TVA and much higher than reported from the Netherlands [7]. While this is most likely due to variation in patient populations studied, another possibility is that the amount of gluten in the diet of untreated coeliac patients may influence EmA positivity on a regional or individual basis. Soda bread, a staple of the Northern Ireland diet, has a much higher gluten content than other breads [11] and may with other high-gluten foods not only increase the overall prevalence of gluten-sensitive enteropathy but also the likelihood of a positive EmA result in patients with milder histological abnormality. EmA levels are dependent on gluten intake, as it is known that a gluten-free diet causes disappearance of EmA before any villous recovery [12,13].

Our results suggest that EmA testing fails to identify at least one-fifth of patients even after accounting for IgA deficiency. There is little doubt that the seronegative patients with VA
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had true coeliac disease given the clinical improvement in all patients having gluten exclusion and histological improvement in two-thirds of those who had follow-up biopsy at twelve months: this response was noted for PVA as well as ST/TVA. AGA testing in parallel has been used to increase diagnostic yield [14] but would have been of little help in identifying the EmA negative patients in this study, of whom 80% had normal AGA levels. It remains to be determined whether serological testing for antibody to tissue transglutaminase improves diagnosis, either as a replacement for EmA or as an assay to be run in parallel [15]. EmA testing has been used by non-gastroenterologists to select patients for gastroenterology referral [16] and for case finding in patients with diseases associated with coeliac disease such as insulin dependent diabetes mellitus [17] and primary biliary cirrhosis [18]. It will remain valuable in these situations and will identify some patients with PVA as well as with ST/TVA. Some EmA positive patients may also have milder degrees of enteropathy with intact villi [5]. However, clinicians need to be aware that seronegative VA is common and duodenal biopsy should be considered if the clinical picture is suggestive. Biopsy is also more likely to identify milder degrees of enteropathy without VA [9]. It is also likely that the prevalence figures of coeliac disease determined by EmA screening of the general population [19,20], though much higher than previously supposed, are underestimates.
Chapter 5

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