EDUCATION IN PRACTICE

Positive tissue transglutaminase antibodies with negative endomysial antibodies: coeliac disease or not?

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Introduction

Coeliac disease (CD) is a chronic autoimmune disorder of the small intestine induced in genetically susceptible individuals by the ingestion of gluten, the major storage protein of wheat, rye and barley.¹ It has been estimated that overall prevalence of CD in the general population is as high as 1/160 or 0.62%.² The current diagnostic algorithm for CD includes initial serological screening test, followed by a confirmatory small intestinal biopsy showing the autoimmune insult typical of CD.^{3,4} In following such an approach, serological screening is primarily used to identify those individuals in need of a diagnostic endoscopic biopsy.^{3,5}

The most sensitive and specific serological tests for the diagnosis of CD are based on the detection of immunoglobulin A (IgA) antibodies against human tissue transglutaminase (tTGA) enzyme and connective tissue elements covering individual smooth muscle fibres, endomysium antibodies (EMA).^{1,3,6}

Detection of tTGA is currently proposed as the first choice serological test in screening for CD.^{3,4} This recommendation has been based on high sensitivity (up to 98%) and high specificity (around 96%) reported for the tTGA assay.^{5,7-9} The tTGA test also demonstrates a remarkable negative predictive value approaching 100% and, therefore, represents an excellent tool in excluding CD in both high and low risk groups.^{8,9} In contrast, positive predictive value of the tTGA test is rather poor with values between 28.6% and 60.2% being reported in several studies.¹⁰ Performance of the alternative assay detecting EMA as a screening tool for CD is characterised by extremely high specificity values close to 100% and

positive predictive value values approaching 80%.^{5,10} However, compared with tTGA, sensitivity of EMA is somewhat lower and does not exceed 90%.^{5,7}

Therefore, a screening strategy locally has been to use tTGA initially, and then confirm positive results using EMA. However, this then identifies a group of patients with divergent serological results.

Aims

The aim of this study is to determine the proportion of patients with positive tTGA and negative EMA with a final diagnosis of CD following histological confirmation and to identify characteristics within this cohort that may help in identifying patients with an increased probability of having CD.

Methods

Patients

One hundred and twenty-five consecutive patients from April 2007 with positive tTGA and negative EMA, who subsequently underwent endoscopy with at least two biopsies from the second part of the duodenum, were identified. A positive tTGA was taken as greater than 15 U/ml. Patients who were known to have CD at the time of serology testing were excluded. Patient notes were then reviewed to determine:

- Indications for CD serological screening, including the presence of iron deficiency anaemia, symptoms such as diarrhoea or weight loss, and family history of CD. Diarrhoea was defined as a bowel frequency of more than three times a day. A patient was deemed as having lost weight if this was detailed in the referral or clinic letter.

b) Histological evidence of CD on subsequent duodenal biopsies plus Marsh grading (table 1).

In cases where the histology was equivocal, the overall clinical impression of a consultant gastroenterologist was used. Equivocal histology included minimal/mild increase in intraepithelial lymphocytes of not more than 30 per 100 enterocytes and without villous atrophy, plus mild villous blunting with no increase in intraepithelial lymphocytes.

Patients were categorised into two groups:

1. CD negative: no histological evidence of CD on duodenal biopsies or equivocal histology plus overall clinical impression of CD absence;
2. CD positive: histological evidence of CD on duodenal biopsies or equivocal histology plus overall clinical impression of CD presence.

Serological tests

To measure IgA anti-tTGA antibody we used a commercially available enzyme linked immunosorbent assay (Aeskulisa; Aesku Diagnostics GmbH, Wendelsheim, Germany). This assay employs human recombinant transglutaminase cross-linked with gliadin-specific peptides and detects IgA antibodies recognising neopeptides of tTGA in human serum. As recommended by the manufacturer, a titre of >15 U/ml was interpreted as positive.

IgA anti-EMA was detected by the standard immunofluorescent method using commercial slides of monkey oesophagus sections (Euroimmun, Euroimmun AG, Lübeck, Germany). Conjugated sheep antihuman IgA was used as a secondary antibody (Instrumentation Laboratory UK Ltd, Warrington, UK).

The laboratory's performance for both tTGA and EMA was continuously evaluated by participation in external quality assurance scheme (NEQAS, UK).

Statistical analyses

Continuous variables were assessed for normality by visual inspection of the histogram and by Kolmogorov-Smirnov test. Age and TTG were not normally distributed and therefore non-parametric tests were carried out. Descriptive statistics was used to characterise the patient sample, using proportions for categorical variables or medians with IQR for non-parametric variables. Bivariate comparisons were performed for disease presence and disease absence using χ^2 test

(gender, diarrhoea, etc) and Mann-Whitney U test for age and tTGA values. A p value of <0.05 was taken to be statistically significant.

Results

One hundred and thirteen patients (90.4%) were categorised into group 1. Of these, 102 patients had no histological features of CD, and 11 patients had equivocal histology plus an overall clinical impression of not having CD. Twelve patients (9.6%) were categorised into group 2. Of these, 10 patients had positive histology, and two patients had equivocal histology plus an overall clinical impression of having CD. Of those with positive histology, 17% were Marsh grade I, 8% were Marsh grade II, 33% were Marsh grade IIIa, 17% were Marsh grade IIIb and 25% were Marsh grade IIIc. Those who had CD were more likely to be older and to have a higher tTGA level. There was no difference between the groups in any clinical parameter (table 2).

Discussion

Our laboratory uses tTGA test as a primary screening for CD owing to its strong sensitivity (98%) and specificity (96%). When the result is positive, that is, greater than 15 U/ml, our laboratory also confirms this with EMA testing. Unsurprisingly, we are seeing patients with divergent serology, that is, tTGA positive and EMA negative. Our results show that in this group there is a low probability of CD, approximately 10%. This probability increases however with increasing absolute tTGA value and also with increasing age of the patient. The presence of diarrhoea, weight loss or iron deficiency anaemia is not significantly associated with a diagnosis of CD in this cohort.

There was a significant difference between tTGA values in patients in group 2 (74.0 U/ml) compared with patients in group 1 (28.8 U/ml). The higher the tTGA level the more likely the patient was to have positive histological features of CD.

We found that age was significantly positively correlated with tTTG (spearman's $\rho=0.231$, $p=0.012$) and a positive biopsy result (spearman's $\rho=0.252$, $p=0.006$). Interestingly all patients with histological evidence of CD on duodenal biopsies who were between the ages of 16 and 55 years had an absolute tTGA value of 100 U/ml or more. All patients with positive histology, but a tTGA value of less than 100 U/ml, were outside of

Table 1 Marsh classification of intestinal coeliac lesions

I	II	III			IV
Infiltration stage	Hyperplasia stage	Destruction stage: villous atrophy			Hypoplasia
		IIIa	IIIb	IIIc	
Increase of intraepithelial lymphocytes more than 30 per 100 enterocytes	Increase of intraepithelial lymphocytes more than 30 per 100 enterocytes with crypt hyperplasia (branching and elongation of crypts). Architecture of villi is normal.	Partial villous atrophy	Subtotal villous atrophy with individual villi still being detectable	Total villous atrophy	Flat atrophic mucosa with minimal inflammation and lack of crypt hyperplasia. Changes are often irreversible.

Table 2 Results

		Group 1 (coeliac negative)	Group 2 (coeliac positive)
Age, median (IQR)		45 (40)*	65 (29)*
tTGA U/ml, median (IQR)		28.8 (29.9)†	74.0 (129.7)†
Gender	Female (%)	75 (66.4%)‡	8 (66.7%)‡
	Male (%)	38 (33.6%)‡	4 (33.3%)‡
Diarrhoea	No (%)	64 (56.6%)‡	10 (83.3%)‡
	Yes (%)	49 (43.4%)‡	2 (16.7%)‡
Weight loss	No (%)	93 (82.3%)‡	10 (83.3%)‡
	Yes (%)	20 (17.7%)‡	2 (16.7%)‡
Anaemia	No (%)	78 (69%)‡	9 (75%)‡
	Yes (%)	35 (31%)‡	3 (25%)‡

*Significant difference in age between group 1 and group 2 (U=426; p=0.038).

†Significant difference in tTGA between group 1 and group 2 (U=380; p=0.013).

‡No significant difference on χ^2 test for any of the categorical variables. tTGA, tissue transglutaminase.

this age range. Therefore we suggest that adult patients under the age 55 years with divergent serology need not undergo endoscopy and duodenal biopsy unless the absolute tTGA value is greater than 100 U/ml. However, patients over the age of 55 years with divergent serology should undergo endoscopy and duodenal biopsy regardless of the absolute tTGA value.

We have divided those with equivocal histology into groups 1 and 2 for the sake of simplicity and to avoid too many groups with small number of patients. However, if these patients are excluded the results are broadly similar with the same conclusions.

Conclusion

Approximately 10% of patients with a positive tTGA and negative EMA were subsequently diagnosed with CD in this study. Histological features of CD on D2

biopsies were more likely with higher values of tTGA. Advancing age was a risk factor for CD in patients with positive tTGA and negative EMA.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

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