Endomysial antibody: is it the best screening test for coeliac disease?

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Abstract

The sensitivities and specificities of the IgA and IgG antigliadin antibody and the IgA antireticulin antibody have been compared with the recently described endomysial antibody directed against the basement membrane of smooth muscle in monkey oesophagus. One hundred and seventeen patients with adult coeliac disease (21 untreated), 84 patients with inflammatory bowel disease, systemic lupus erythematosus and rheumatoid arthritis (comprising the disease control group), 47 normal controls and a miscellaneous group of 29 patients, who were selected because of a positive reticulin staining pattern, were investigated. These results were correlated with the degree of abnormality of the intestinal mucosa in patients with adult coeliac disease. Endomysial antibodies were found in all patients with untreated coeliac disease and subtotal villous atrophy and in 47% of patients on a non-strict gluten free diet. One patient on a strict gluten free diet was positive and had partial villous atrophy while all patients in disease control groups were negative. Results were variable with the antireticulin and anti-gliadin antibodies. Sensitivity and correlation with subtotal villous atrophy in the untreated patients was 100%. It is concluded that the endomysial antibody is superior to other current antibody tests and should be used in preference for the diagnosis of coeliac disease.
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The 'gold standard' for the diagnosis of coeliac disease remains the jejunal biopsy. Over the last 30 years, however, most workers have been searching for a less invasive test for both screening and diagnostic purposes. Tests looking for circulating antibodies to gluten, gliadin or reticulin, using immunofluorescence or the enzyme linked immunosorbent assay (ELISA) techniques[1-13] have been considered potential screening tests for coeliac disease. The sensitivities and specificities of these tests have varied from centre to centre but have been improved by using class specific immunoglobulin.[14-16] This variability has been a drawback to their diagnostic use.

Chorzelski *et al*[17] described a new antibody directed against the membrane of smooth muscle bundles of primates. This endomysial antibody was present in patients with dermatitis herpetiformis and coeliac disease. This antibody may be closely related to the reticulin antibody. The antibody titres have been shown to correlate with the severity of the histopathological abnormali-

ties in the jejunal mucosa[18] although the numbers of patients investigated are small.

The aim of our study was to compare the sensitivities and specificities of the antigliadin antibody, antireticulin antibody and endomysial antibody using ELISA and immunofluorescent techniques in a variety of patient groups.

Methods

PATIENTS

One hundred and seventeen patients with adult coeliac disease (21 untreated, 81 treated with a strict gluten free diet and 15 on a non-strict gluten free diet were studied. The age range was 15–78 years (median age 38) and these patients were compared with 47 normal controls (median age 46 years) from the hospital staff population and matched as closely as possible for age and sex with the coeliac population. The diagnosis of coeliac disease was based on the typical histological appearance of the jejunal mucosa followed by an improvement on gluten withdrawal. Eight patients originally diagnosed on histological grounds did not improve on gluten withdrawal but have been included for interest. All 21 untreated patients have had repeat jejunal biopsies which have shown morphological improvement. The disease control group consisted of 21 patients with Crohn's disease (median age 26 years) and 10 patients with ulcerative colitis (median age 40 years); 33 patients with systemic lupus erythematosus (median age 35 years) and 20 patients with rheumatoid arthritis (median age 60 years) were also included as these conditions have an immunological aetiology. Another 29 patients were included as their initial immuno-fluorescence screen with polyclonal antisera gave a positive reticulin pattern. These sera had been sent to the laboratory for routine screening but their clinical diagnosis was unknown at the time.

All untreated patients with coeliac disease had intestinal biopsies, 14 of 15 patients on a non-strict gluten free diet and 23 of the 81 patients on a strict gluten free diet also had biopsies. These were performed using a Crosby capsule either alone or barrel loaded on endoscopes and were taken from the duodenojejunal flexure.

Blood was taken from the patients on the day of the jejunal biopsy or within two weeks and aliquots of the sera were stored at $-30°C$. A new aliquot was thawed for each test. Some patients were assayed twice but these specimens were at least three months apart. In total 21 assays were performed on 21 untreated patients, 19 assays on 15 patients on a non-strict gluten free diet and 91

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assays on 81 patients treated with a strict gluten free diet.

The strictness of the gluten free diet was assessed by at least two of the following methods: visual analogue scale,[18] review by dietitian, and the patients' information to the physician. Patients on an estimated 60–80% gluten free diet (approximately 3–10 g gluten per day) were considered to be 'non-strict.'

IMMUNOFLUORESCENCE

This was used for both the endomysial and antireticulin antibody assays. The method described by Beutner *et al*[19] was used with minor modifications. Tissue sections (rat kidney for antireticulin antibodies and Marmoset monkey oesophagus for endomysial antibodies were mounted on microscope slides. Serum diluted 1:2·5 with phosphate buffered saline was applied to slides which were incubated for 30 minutes at room temperature (positive sera were further diluted), this titre was chosen because no residual staining was seen at this or higher titres. After washing with phosphate buffered saline the sections were covered with 1:20 fluorescein conjugated rabbit anti-human IgA (Dako) for 30 minutes, washed with phosphate buffered saline and examined by fluorescence microscopy. Sera were considered positive if fluorescence was seen at a dilution of 1:2·5 or greater.

Sections of Marmoset monkey oesophagus were used for the endomysial antibody estimations and compared with the commercially available oesophageal sections (BioDiagnostics, Upton upon Severn). Positive and negative controls were used for each batch and reproducibility of the tests was checked on the same day and on different days.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Antibodies to gliadin were estimated using the enzyme-linked immunosorbent assay[10] following modification (Lloyd Davies *et al* unpublished data). A strongly positive serum was used to produce a standard curve, this serum had been given an arbitrary value of 100 units at a specific dilution (IgG 1:500, IgA 1:100). The IgA and IgG antigliadin antibody distribution within a normal population had been established by comparing absorbances of a large number of samples generated from a blood bank population to the standard curve of the strongly positive serum and a reporting regime established. Studies were carried out on reproducibility during modification of the ELISA, the method was found to be highly reproducible. Negative values were 0–34 units for IgG and 0–9 for IgA; positive values 35–59 and 10–49 and strongly positive values were 60+ or 50+ respectively.

JEJUNAL MUCOSA

This was assessed histologically and graded into three broad categories: 'normal' (no inflammation, normal intraepithelial lymphocyte counts, normal villi): 'partial villous atrophy' (mild to moderate villous flattening, an increase in intraepithelial lymphocytes and some crypt

TABLE I *Serology results*

Patient groups	Assays	EmA IgA % Positive	ARA IgA	AGA IgA	AGA IgG
Coeliacs					
Untreated	21	100	90	90	76
GFD non-strict*	19	47	47	47	42
GFD strict†	91	1	8	11	23
Disease controls					
Crohn's	21	0	0	25	21
UC	10	0	0	10	10
SLE	33	0	0	6	6
RA	20	0	0	15	10
Miscellaneous	29	7	7	17	18
Healthy controls	47	0	0	15	11

GFD=gluten free diet; *=19 assays on 15 patients; †=91 assays on 81 patients; EmA=endomysial antibody; ARA=antireticulin antibodies; AGA=antigliadin antibodies; UC=ulcerative colitis; SLE=systemic lupus erythematosus; RA=rheumatoid arthritis.

hyperplasia): 'Sub-total villous atrophy' (severe villous flattening with crypt hyperplasia, inflammation and increased intraepithelial lymphocytes).

Results

A comparison was made between positive results for all the assays (Table I).

COELIAC DISEASE

All 21 patients with untreated coeliac disease had positive endomysial antibody. Both antireticulin and IgA antigliadin antibodies were found in only 90% of these patients. Only one patient on a strict gluten free diet had a positive endomysial antibody, but 8% were positive for antireticulin antibodies and 11% were positive IgA antigliadin antibodies. As expected the results were intermediate in those patients on a non-strict gluten free diet.

Eight previously untreated patients were followed up after treatment with a gluten free diet and after six months only one patient (on a strict gluten free diet, mentioned above) was positive for endomysial, IgA and antireticulin antibodies.

DISEASE CONTROLS

No patients had positive endomysial or antireticulin antibodies. The IgA antigliadin antibodies, however, were positive in 6–25% of the disease control groups whilst the IgA antigliadin antibodies were positive in 6–21%.

NORMAL CONTROLS

No positive results were obtained for either endomysial or antireticulin antibodies. Fifteen per cent of patients, however, were positive for IgA antigliadin antibodies and 11% for IgG antigliadin antibodies.

MISCELLANEOUS CONTROLS

Only two patients (7%) were positive for endomysial antibody. One patient had insulin dependent diabetes mellitus and one had thyroiditis.

TABLE II Biopsy/serology correlation

Patient (n)	Patient (n)	EmA positive	IgA ARA positive
Untreated (21)	SVA-20	20	18
	PVA-1	1	1
Strict GFD (23)	PVA-1	1	0
	Normal-23	0	0
Non-strict GFD (14)	PVA-9	6	4
	Normal-5	1	1

PVA=partial villous atrophy; SVA=sub total villous atrophy; GFD=gluten free diet.

TABLE III Sensitivity/specificity and predictive values for all assays

	EmA IgA	ARA IgA	AGA IgA	AGA IgG
Sensitivity	100	91	91	76
Specificity	99	99	85	88
Positive predictive value	91	91	45	46
Negative predictive value	100	99	99	97

For abbreviations see Table I.

CORRELATION OF ANTIBODIES WITH JEJUNAL MORPHOLOGY (Table II).

Coeliac disease

Untreated patients. Of these 21 patients 20 had subtotal villous atrophy and one had severe partial villous atrophy and all were endomysial antibody positive.

Strict gluten free diet. Twenty three patients had normal mucosa; all were endomysial antibody and antireticulin antibody negative but two patients had positive antigliadin antibodies (IgA, IgG). One patient with partial villous atrophy had a weakly positive endomysial antibody.

Non-strict gluten free diet. In patients on a 'non-strict' gluten free diet the positive results were as follows: five patients had a normal mucosa and only one patient was positive for endomysial and antireticulin antibodies, nine patients had partial villous atrophy, six were endomysial antibody positive and four were positive for antireticulin antibodies.

SENSITIVITY/SPECIFICITY
The sensitivity and specificity of each test was calculated from the results and are shown in Table III with the predictive values for the tests.

Discussion
The description of the endomysial antibody by Chorzelski *et al* in 1983[17] has reawakened the search to find a less invasive reliable screening test for coeliac disease. The endomysial antibody was initially found in patients with coeliac disease and dermatitis herpetiformis and has been found to have a greater specificity.[8 11 16 17 20 22] It has never been found to be positive in healthy controls[16 21-23] and has so far been negative in patients with Crohn's disease, ulcerative colitis[16 21-23] and in other gastrointestinal diseases. The endomysial antibody therefore promises to be an excellent non-invasive screening test with a high specificity. However, it has not always been shown to have 100% sensitivity for untreated coeliac disease, as the positivity has varied between 91 and 100%.[11 16] Results of patients on gluten free diets have varied from 0% positivity[11 22 23] to 67·9%.[8 22-24]

The aim of our study was to compare and contrast the sensitivities and specificities of the endomysial antibody compared with antireticulin and antigliadin antibodies. We measured these antibodies using the immunofluorescent test for the endomysial and antireticulin antibodies and the ELISA test for the antigliadin antibodies. The immunofluorescent test for the antigliadin antibody was not used as it has been shown to be unreliable. Our results show that the antibody was 100% sensitive for patients with untreated coeliac disease. The antireticulin antibody although not so sensitive, showed a very high degree of specificity. Both the endomysial and IgA antireticulin antibodies showed a positive predictive value for detecting the disease in approximately 91% of patients; the latter figure for the endomysial antibody is not 100% as two patients (with insulin independent diabetes mellitus and Hashimoto's thyroiditis disease) were shown to be positive. These patients had no recorded symptoms of malabsorption and have refused further investigation. It is known, however, that there is an increased frequency of coeliac disease in patients with insulin dependent diabetes mellitus[25 26] and autoimmune diseases are also seen with increased frequency in patients with coeliac disease[27]; it is therefore possible that these patients may have coeliac disease.

Our results are not in accordance with those of McMillan *et al*[28] who found that 89% of their patients with untreated coeliac disease had a positive endomysial antibody (measured by immunofluorescence) compared with 100% positivity obtained with gliadin IgA using an ELISA test. The titres of patients' sera used in both tests were not given in their report. We used a titre of 1:2·5 for the endomysial antibody immunofluorescence as no residual staining was seen at this titre and with the ELISA there was a definite cut off point between positive and negative. Our IgA antigliadin antibody only showed a 91% positivity in patients with untreated coeliac disease while the endomysial antibody was 100% positive.

Rossi *et al*[29] assessed the relationship of the endomysial antibody to the severity of the mucosal lesions. They found that all patients with detectable endomysial antibody at the time of biopsy exhibited the more advanced changes of villous atrophy. Endomysial antibody was not detected in 160 subjects with diarrhoea, 18 of whom had mild villous atrophy. Their conclusion was that endomysial antibody appeared to be specifically correlated to the intestinal histopathology of coeliac disease and did not appear to be a non-specific marker for mucosal atrophy. One of their patients, however, who was initially positive for endomysial antibody, had an intestinal infestation with *Giardia lamblia*, an

intestinal biopsy performed after treatment for the parasite showed normal histology and the patient negative endomysial antibody. Volta et al[10] in their recent report noted that some of their patients were endomysial antibody positive although their jejunum had recovered; endomysial antibody was seen at a higher frequency in children rather than adults and in their study the prevalence of IgA endomysial and antigliadin antibodies were not statistically different. In our non-strict group although six of nine patients with partial villous atrophy were endomysial antibody positive we were surprised to find three patients who were negative. The kinetics of the endomysial antibody are not yet known and it is possible that these patients may not have had a recent ingestion of gluten. Our results, however, were obtained from a predominantly adult population and indicate that endomysial antibody is found in all untreated patients.

The exact nature of the endomysial antibody is still unknown, recently Kárpáti et al[31] have observed IgA antibodies from patients with dermatitis herpetiformis that bind to the normal human jejunum. These jejunal antibodies showed similarities to both IgA antireticulin antibodies and endomysial antibody. They showed that the binding of these antibodies to intestinal tissue was comparable with the IgA deposits seen in small bowel biopsy samples from patients with dermatitis herpetiformis and coeliac disease.[32] In a further study Kárpáti et al[11] showed that 13 of 14 untreated children with coeliac disease had jejunal antibodies and only one of 21 were positive after a gluten free diet although 90% of patients on a gluten challenge showed a reappearance of the jejunal antibodies. This antibody could not be demonstrated in 53 disease control patients with normal jejunal morphology or in three patients with coeliac disease with a selective IgA deficiency. In their studies they simultaneously determined concentrations of antigliadin, endomysial and jejunal antibodies. Using absorption studies with jejunal and antigliadin antibodies they found that jejunal antibodies and antigliadin antibodies were different whereas jejunal antibodies and endomysial antibodies were probably identical. The authors suggested that IgA jejunal antibodies could be the 'target organ related' autoantibody in coeliac disease. A report this year from Kárpáti et al[33] further corroborates their suggestion that the endomysial antibody and jejunal antibody are identical.

Hälström et al,[16] however, was unable to distinguish between IgA antigliadin antibodies reacting with human tissues and endomysial antibodies reacting with monkey oesophagus in his absorption studies. We found that the endomysial antibody was very similar but not identical to the antireticulin antibody shown on rat kidney.

Antigliadin antibodies in our study have again shown that they are not specific for the disease. They were found in all disease control groups as well as in normal controls and the specificity was only slightly improved with the IgA antigliadin antibody. The results are disappointing and reiterate results in previous studies.

We conclude that in our hands the endomysial

and antireticulin antibodies are very similar, although the endomysial antibody is more sensitive. The endomysial antibody fluorescence is cleaner and easier to read than the antireticulin antibodies and therefore we suggest that this is the optimum screening test for use within a routine laboratory. Tissue sections are available commercially and actual technique is routine in most immunology laboratories.

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