Alimentary Pharmacology & Therapeutics

Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibody tests)

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Publication data
Submitted 22 February 2006
First decision 6 March 2006
Resubmitted 17 April 2006
Resubmitted 20 April 2006
Accepted 21 April 2006

SUMMARY

Background
With the appreciation of the high prevalence of coeliac disease there is increasing use of serology in screening asymptomatic people and testing those with suggestive features.

Aim
To compare the sensitivities and specificities of the endomysial antibody and the tissue transglutaminase antibody tests.

Methods
Using electronic databases a search was made for relevant papers using the terms tissue transglutaminase and endomysial antibody.

Results
Both the endomysial antibody and tissue transglutaminase antibody have very high sensitivities (93% for both) and specificities (>99% and >98% respectively) for the diagnosis of typical coeliac disease with villous atrophy. Human recombinant tissue transglutaminase performs much better than guinea pig tissue transglutaminase. Review of studies comparing endomysial antibody with human recombinant tissue transglutaminase antibody shows that endomysial antibody more often has a higher specificity and human recombinant tissue transglutaminase antibody more often has a higher sensitivity.

Conclusion
The human recombinant tissue transglutaminase antibody is the preferred test for screening asymptomatic people and for excluding coeliac disease in symptomatic individuals with a low pretest probability (i.e. <25%) for coeliac disease. Furthermore, it has a number of practical and financial advantages. If the pretest probability is >25%, biopsy is preferred as the post-test probability of coeliac disease with a negative test is still >2%.

Aliment Pharmacol Ther **24**, 47–54

doi:10.1111/j.1365-2036.2006.02967.x

INTRODUCTION

Coeliac disease has assumed increasing importance with the realization of its high prevalence (approximately 1% of the UK population),[1] its association with many other disorders such as type 1 diabetes, primary biliary cirrhosis and dermatitis herpetiformis,[2] and it being a cause of common conditions such as iron deficiency anaemia. Consequently, screening tests have assumed greater importance because histology of small bowel biopsies (still regarded by most as the gold standard) is inconvenient, expensive, unpleasant and not without risk.[3] The first reliable screening test was the endomysial antibody (EMA) devised by Chorzelski *et al*. in 1983.[4] In a systematic review of published studies in 2000,[5] we calculated the pooled sensitivity and specificity to be 94% and 98% respectively. It is hard to think of a better performing screening test for any condition. However, there are problems with the EMA test – it is subjective, labour intensive, and one common substrate (monkey oesophagus) is from an endangered species. In 1997, Dieterich *et al*.[6] found that tissue transglutaminase (tTG) is the antigen recognized by the EMA. A test for detecting antibodies to tTG was soon devised using either guinea pig (*gp*tTG) or human recombinant tTG (*rh*tTG), and it was assumed that this test would perform even better than the EMA test, and at the same time obviate the above problems associated with the EMA test. Certainly the enzyme-linked immunosorbent assay test used is objective and lends itself to automation. Thus, there is a widespread move to replace the EMA test. Before the EMA test disappears, it was thought important to compare the performance of the EMA test with the two types of tTG antibody test with regard to sensitivity and specificity, to make recommendations for the most appropriate screening test and to determine the likelihood ratios (LRs) for the preferred test.

METHODS

A literature search was conducted using PubMed, Medline and Ovid databases up to September 2005 to identify relevant articles in English. The search terms used were tTG and EMA. The reference lists of selected articles were also used to identify other relevant articles. Criteria for inclusion were all of the following:

1 The published study was peer reviewed.

2 The study included untreated coeliac patients and controls.

3 Both EMA and tTG antibody were tested in the same patients and controls.

4 All coeliacs had had a biopsy and the biopsy criteria for diagnosis were given.

5 It was clear which controls were biopsy negative and which had not been biopsied.

It was hoped to exclude studies in which there was ascertainment bias (i.e. where the coeliac group was identified by EMA or tTG antibody tests) but unfortunately very few papers specified how the coeliac patients were identified and thus this criterion was abandoned. This will, inevitably, lead to an overestimation of sensitivity. E-mails were sent to 14 authors of studies using *rh*tTG enquiring about the use of serology to detect the coeliac patients.

For each study, the sensitivity and specificity for EMA and the two types of tTG antibody test (i.e. human recombinant and guinea pig) was calculated. Each study was then assessed to determine whether the tTG antibody test or the EMA test gave the higher sensitivity and specificity in that particular study. The number of studies in which each test gave the higher sensitivity and the higher specificity was then added up. All the subjects were then pooled and the overall sensitivity and specificity were calculated with 95% confidence intervals (CIs) for both tTG antibody and EMA. In addition, the overall sensitivities and specificities were calculated for different types of tests and also separately for studies of adults and for those studies using commercial kits as opposed to in-house tests. From them the positive and negative LR were calculated using the formulae: LR + = sensitivity/100−specificity; LR− = 100−sensitivity/specificity. The LR (positive or negative) indicates how much more likely or less likely is a particular diagnosis if the test is positive or negative. A LR of 1 indicates that the test result makes the diagnosis neither more nor less likely.

RESULTS

Thirty-four studies fulfilled our criteria and the details are shown in Table 1. The histological criterion for diagnosing coeliac disease was partial or more severe villous atrophy in the majority. In four, total villous atrophy was required,[10, 24, 29, 38] and in two[9, 17] an abnormality ranging from an increase in intraepithelial lymphocytes alone to total villous atrophy was allowed. Two studies[32, 35] were vague simply saying that the diagnosis was histologically proven and therefore some degree of villous atrophy was assumed.

Table 1. Summary details of all the relevant studies

Study	Type of tTG assay	Source of antigen	Controls: Biopsy negative*	Controls: Not biopsied*	Controls: tTG Ab positive	Controls: EMA positive	Untreated Coeliac Disease: Total	Untreated Coeliac Disease: tTG Ab positive	Untreated Coeliac Disease: EMA positive	Children, adults or mixed	tTG Ab sensitivity	tTG Ab specificity	EMA sensitivity	EMA specificity
Baudon *et al.*[7]	Eu-tTG	rh	17†	92	3	0	30	28	27	Children	93.3	97.4	90.0	100
Lorente *et al.*[8]	Celikey	rh	64	0	2	2	61	60	43	Mixed	98.4	96.9	70.5	96.9
Tesei *et al.*[9]	Eu-tTG	rh	176	0	9	0	250	225	214	Mixed	90.0	94.9	85.6	100
Tonutti *et al.*[10]	Eu-tTG	rh	6316	0	52	10	737	699	679	Children	94.8	99.2	92.1	99.8
Burgin-Wolff *et al.*[11]	Celikey	rh	157	0	1	0	208	200	201	Mixed	96.2	99.4	96.6	100
Carroccio *et al.*[12]	Eu-tTG	gp	183	0	15	0	24	24	24	Adults	100	91.8	100	100
		rh			6			24			100	96.7		
Wolters *et al.*[13]	In-house	gp	49	0	4	5	52	50	48	Children	96.2	91.8	92.3	89.8
	Celikey	rh			0			50			96.2	100		
Dickey *et al.*[14]	Quanta Lite	gp	58	0	1	2	73	55	59	Mixed	75.3	98.3	80.8	96.6
Bardella *et al.*[15]	Genesis	gp	110	0	2	3	40	40	40	Adults	100	98.2	100	97.3
Dahele *et al.*[16]	In-house	gp	65	0	2	0	114	92	99	Adults	80.7	96.9	86.8	100
Salmaso *et al.*[17]	In-house	gp	106	0	2	0	82	76	80	Mixed	92.7	98.1	97.6	100
Chan *et al.*[18]	unspecified	?	66	0	4	2	9	8	8	Children	88.9	93.9	88.9	97.0
Leon *et al.*[19]	In-house	gp	53	99	13	2	86	82	85	Children	95.3	91.4	98.8	98.7
	Quanta Lite	gp	53	99	2	2	86	84	85		97.7	98.7	98.8	98.7
	Celikey	rh	53	99	1	2	86	85	85		98.8	99.3	98.8	98.7
Fabiani *et al.*[20]	Eu-tTG	rh	186	246	22	0	387	354	365	Mixed	91.5	94.9	94.3	100
Bonamico *et al.*[21]	Eu-tTG	rh	56	0	0	1	62	62	59	Children	100	100	95.2	98.2
	In-house	gp			0			56			90.3	100		
Picarelli *et al.*[22]	Eu-tTG	rh	26	0	0	0	110	110	110	Adults	100	100	100	100
Biagi *et al.*[23]	In-house	gp	52	0	1	0	56	49	53	Adults	87.5	98.1	94.6	100
Vitoria *et al.*[24]	Celikey	rh	28	0	0	0	42	40	42	Children	95.2	100	100	100
Hansson *et al.*[25]	In-house	gp	17	6	1	0	22	22	21	Children	100	95.6	95.5	100
	In-house	rh	17	6	1		22	22			100	95.6		
Baldas *et al.*[26]	In-house	rh	0	196	1	0	70	65	65	Mixed	92.8	99.5	92.8	100
Stern and Working Group on Serologic Screening for Celiac Disease[27]	In-house	rh	89	60	7	1	103	101	96	Mixed	98.1	95.3	93.2	99.3
Sblaterro *et al.*[28]	In-house	rh	20	150	1	0	65	59	60	Mixed	90.8	99.4	92.3	100
		gp			3			53			81.5	98.2		
Gillett and Freeman[29]	In-house	gp	42	86	2	1	21	20	21	?	95.2	98.4	100	99.2
Kaukinen *et al.*[30]	Quanta Lite		30	0	0	0	8	8	7	Adults	100	100	87.7	100

Table 1. (Continued.)

Study	Type of tTG assay	Source of antigen	Controls				Untreated Coeliac Disease			Children, adults or mixed	tTG Ab sensitivity	tTG Ab specificity	EMA sensitivity	EMA specificity
			Biopsy negative*	Not biopsied*	tTG Ab positive	EMA positive	Total	tTG Ab positive	EMA positive					
Cataldo *et al.*[31]	In-house	rh	25	0	0	0	11	11	11	Mixed	100	100	100	100
Koop *et al.*[32]	In-house	gp	23	0	0	0	20	18	19	Mixed	90	100	95	100
Lock *et al.*[33]	In-house	gp	65	0	2	0	27	23	27	Adults	85.2	96.9	100	100
Troncone *et al.*[34]	In-house	gp	63	0	1	1	48	44	42	Children	91.7	98.4	87.5	98.4
Sardy *et al.*[35]	In-house	gp	0	53	2	1	55	51	55	Mixed	92.7	96.2	100	98.1
	In-house	rh	0	53	1	1	55	54	55		98.1	98.1	100	98.1
Vitoria *et al.*[36]	Medipan	gp	1	32	2	0	27	27	27	Children	100	94.1	100	100
Biagi *et al.*[37]	In-house	gp	61	0	5	0	39	37	39	Adults	94.9	91.8	100	100
Bazzigaluppi *et al.*[38]	In-house	gp	0	92	8	1	112	95	109	Children	84.8	91.3	97.3	98.9
Dieterich *et al.*[39]	In-house	gp	0	114	6	0	106	104	105	Mixed	98.1	94.7	99.1	100
Sulkanen *et al.*[40]	In-house	gp	207	0	13	1	136	129	126	Children	94.9	93.7	92.6	99.5

EMA, endomysial antibody; tTG, tissue transglutaminase; Ab, antibody; rh, human recombinant; gp, guinea pig.

* 'Biopsy negative' controls were those controls who had a small bowel biopsy which was negative for coeliac disease on histology. 'Not biopsied' controls were those who did not have a small bowel biopsy to exclude coeliac disease.

† Twenty-four of the controls had small bowel biopsy of which 17 had completely normal histology, six had minor histological changes and one had partial villous atrophy from cow's milk allergy. Ninety-two of the controls had no biopsy.

Table 2. The result of head to head comparisons of tissue transglutaminase (tTG) antibody with endomysial antibody (EMA) in all 42 studies (number of studies in parentheses)

	EMA better	Equal	tTG antibody better
Sensitivity	48% (20)	24% (10)	28% (12)
Specificity	62% (26)	21% (9)	17% (7)

Table 3. The result of head to head comparisons of only recombinant tissue transglutaminase (*rh*tTG) with endomysial antibody (EMA) in all 18 studies (number of studies in parentheses)

	EMA better	Equal	*rh*tTG antibody better
Sensitivity	28% (5)	28% (5)	44% (8)
Specificity	56% (10)	22% (4)	22% (4)

Most studies did not give sufficient information to determine whether there was ascertainment bias. Some, either in the paper or on subsequent email communication,[7, 11, 19, 20, 27] admitted a partial ascertainment bias whereas it was specifically excluded in two.[9, 23]

Nearly all the control groups consisted of patients in whom coeliac disease was suspected for various reasons. Most had symptoms but some were asymptomatic and studied because they had a condition associated with coeliac disease (e.g. type 1 diabetes, iron deficiency anaemia) or were related to patients with coeliac disease.

The sensitivities for both tTG antibody and EMA ranged from 70% to 100%. The specificities for tTG and EMA ranged from 91% to 100% and from 90% to 100% respectively.

The result of head to head studies of EMA with tTG antibody (Table 2) shows that EMA performed better more often for both sensitivity and specificity. However, when only the 18 head to head studies using *rh*tTG are looked at (Table 3), the tTG antibody test performed better more often with regard to sensitivity but not specificity.

In Table 4 all the results are pooled. The total numbers of controls for the tTG antibody and EMA studies were 10 465 and 9741 respectively. The total numbers of untreated coeliac patients for the tTG antibody and EMA studies were 3745 and 3296 respectively. The pooled sensitivities and specificities (with 95% CIs) are given for all tTG antibody and EMA studies and also separately for adults and for the different types of tTG protein (i.e. *rh*tTG or *gp*tTG) and EMA substrates (i.e. monkey oesophagus or human umbilicus). From the sensitivities and specificities the positive and negative LR are also calculated and presented.

The tTG antibody tests perform much better using *rh*tTG rather than *gp*tTG. The sensitivity is higher in adults than in children. The EMA test gives a higher sensitivity using monkey oesophagus and a higher specificity when using human umbilicus as substrate. These differences tend to be more marked in adults.

Table 4. The pooled sensitivities and specificities together with the positive and negative likelihood ratios derived from them

Analysis	No. studies	Sensitivity % (95% CI)	Specificity % (95% CI)	LR+	LR−
All EMA studies	34	93.0 (92.1–93.8)	99.7 (99.5–99.8)	310	0.070
EMA studies; monkey oesophagus	25	93.1 (92.1–94.0)	99.1 (98.8–99.4)	103	0.070
EMA studies; human umbilical cord	9	92.9 (90.7–94.7)	99.7 (99.2–99.9)	310	0.071
EMA studies in adults; monkey oesophagus	4	98.0 (94.2–99.3)	99.3 (97.9–99.8)	140	0.020
EMA studies in adults; human umbilical cord	4	91.5 (86.6–94.7)	100 (97.8–100)	∞	0.085
All tTG studies	42	92.8 (91.9–93.6)	98.1 (97.8–98.4)	49	0.073
*rh*tTG studies	19	93.8 (92.8–94.7)	98.7 (98.5–98.9)	72	0.063
*gp*tTG studies	23	90.4 (88.8–91.9)	92.4 (90.8–93.8)	12	0.103
*rh*tTG studies in adults; commercial	2	100 (97.2–100)	97.1 (93.9–98.7)	34	0
*gp*tTG studies in adults; commercial	3	100 (94.9–100)	94.7 (91.7–96.7)	19	0

EMA, endomysial antibody; tTG, tissue transglutaminase; *rh*tTG, human recombinant tTG; gptTG, guinea pig tTG; LR, likelihood ratio.

The highest positive LR (i.e. the most powerful at confirming a diagnosis of coeliac disease) is provided by EMA especially using human umbilical cord (310) and in adults (infinity). The lowest negative LR (i.e. most powerful at excluding coeliac disease) is provided by the *rh*tTG test (0.063 compared with 0.069 for EMA monkey oesophagus). Both tests tend to perform better in adults but the numbers are too small to be reliable and the 95% CIs are wide.

Most gastroenterologists will be testing adults with commercial tTG antibody kits and such studies were looked at separately. Although there were only two or three such studies the specificities were 100% and the sensitivities were 97.1% for *rh*tTG and 94.7% for *gp*tTG giving very useful LRs.

DISCUSSION

We have shown that the EMA test has greater specificity than the tTG antibody test, whether human umbilicus or monkey oesophagus is used. We have also shown that the tTG antibody test, using human recombinant protein, has greater sensitivity than EMA. The *rh*tTG antibody test is therefore the preferred test to screen asymptomatic people and to exclude coeliac disease in those with symptoms if the pretest probability is low (e.g. <25%). If the pretest probability is higher (i.e. >25%), the post-test probability of coeliac disease with a negative test is >2% (using a negative LR of 0.06 – see below) and therefore small bowel biopsy is still required. Moreover, the *rh*tTG antibody test has a number of practical and financial advantages over the EMA test. The EMA test could be reserved for confirming coeliac disease in those with a positive *rh*tTG antibody test but, as many gastroenterologists would take small bowel biopsies if the tTG antibody test is positive, it would not be necessary unless the patient refused biopsy.

When applying the *rh*tTG antibody test to exclude coeliac disease with a particular pretest probability, a negative LR of 0.06 could be used in conjunction with Fagan's nomogram (Figure 1). This will readily give the post-test probability for coeliac disease. In the example given in the Figure, a patient with iron deficiency anaemia is considered. As we know that the pretest probability (or prevalence) of coeliac disease in iron deficiency anaemia is 5%; the post-test probability of coeliac disease, if the test is negative, is about 0.4%, which effectively excludes the diagnosis. However, it should be borne in mind that most recent studies are likely to give

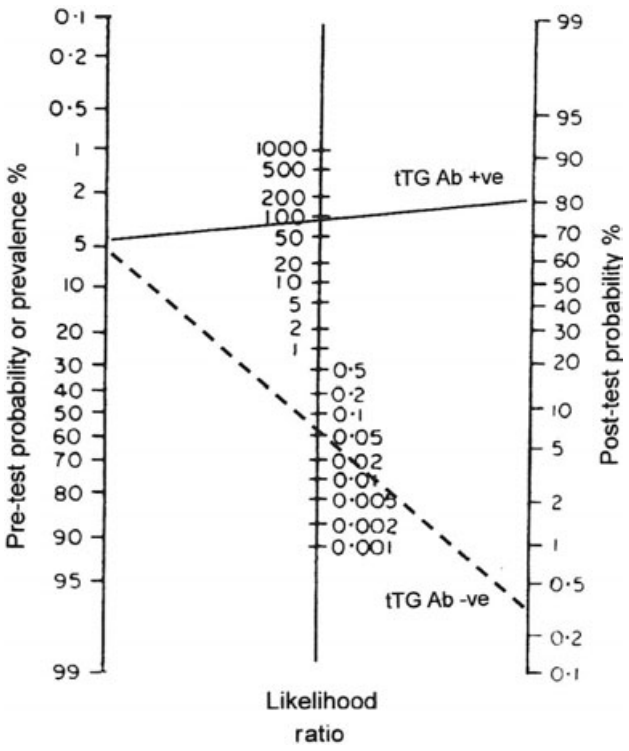

Figure 1. Using Fagan's nomogram to determine the post-test probability of coeliac disease in a patient with iron deficiency anaemia (which has a prevalence of coeliac disease of approximately 5%) for both a negative and a positive human recombinant tissue transglutaminase antibody test (which has a negative likelihood ratio of 0.06 and a positive likelihood ratio of 72).

falsely high sensitivities because of the ascertainment bias, which is inevitable if serology is the main way of detecting coeliac disease. Thus, this and all the other negative LRs given in Table 4 are likely to be lower (i.e. more powerful) than they should be.

When confirming coeliac disease using the EMA test, a positive LR of 300 would be appropriate (or 100 if monkey oesophagus is used), and can be similarly used with Fagan's nomogram in conjunction with the pretest probability to obtain the post-test probability of coeliac disease.

As the detection of at least partial villous atrophy was used to make a diagnosis of coeliac disease in the vast majority of studies, we can't assume that the same LRs apply to coeliac patients with lesser abnormality such as an increase in intraepithelial lymphocytes or electron-microscopic changes only. In fact, if such lesser abnormalities were used as criteria for diagnosing (and excluding) coeliac disease, the sensitivity of the

tests could be lower (i.e. more false negatives), especially since a number of studies suggest that the EMA and tTG antibody tests are less sensitive with lesser degrees of mucosal abnormality.[41–43] On the other hand, many patients have been shown to have positive EMA tests with normal villous and crypt architecture and just an increase in intraepithelial lymphocytes or just electron microscopic changes[44–47] and so the specificity could be higher (i.e. fewer false positives). However, do we want to label people with minor changes as coeliac disease? There is no agreement on what is meant by disease – are symptoms, or an abnormality of structure or function, or an abnormal serological test required? One reason for diagnosing a disease is to offer treatment. Would we want to offer treatment (i.e. a strict lifelong gluten-free diet) to people with just minor abnormalities on biopsy and no symptoms, or even with just positive serology? These questions need answering before embarking on screening of, say, relatives of coeliac patients. When dealing with asymptomatic people many would be reluctant to advise treatment if there is no villous atrophy. Therefore, the LRs given above and obtained from coeliacs predominantly with villous atrophy will be appropriate.

To use these tests for detecting people with minor changes in the small bowel mucosa (such as an increase in intraepithelial lymphocytes or electron-microscopic change), it will be necessary to determine the sensitivity in a large study of such people who had not been selected by positive serology. This may not prove possible with the present widespread reliance on serology and the consequent ascertainment bias.

Another complicating factor is immunoglobulin (Ig) A deficiency which is found in 2% of coeliacs and 0.2% of the general population. Since the usual serology tests (tTG antibody and EMA) are for IgA antibodies, there will be more false negatives thus slightly reducing the sensitivity. It is therefore probably best to follow the advice of Hill *et al.*[48] to test for IgA if low absorbance readings are shown in the tTG assay, and rely on biopsy if IgA deficient, although, alternatively, testing for IgG tTG antibodies has been found useful.[49]

In conclusion, we recommend the use of *rh*tTG antibody test to exclude coeliac disease if the pretest probability is low (e.g. <25%). If the *rh*tTG antibody test is positive we recommend small bowel biopsy to confirm the diagnosis. If for any reason biopsy is precluded then the EMA test could be used to confirm the diagnosis.

ACKNOWLEDGEMENT

No external funding was received for this study.

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