# IgA-class transglutaminase antibodies in evaluating the efficacy of gluten-free diet in coeliac disease

Katri Kaukinen[a], Satu Sulkanen[b], Markku Mäki[b] and Pekka Collin[a]

***Objective*** **Serum IgA-class tissue transglutaminase antibody has proved effective in screening for coeliac disease. The response to a gluten-free diet has been assessed on the basis of small-intestinal morphology. We investigated whether the tissue transglutaminase antibody test could substitute biopsy in this respect, and whether the test is better than the endomysial antibody test in follow-up.**

***Design*** **Controlled cross sectional, and follow-up study.**

***Methods*** **Serum IgA-class tissue transglutaminase antibodies and endomysial antibodies were determined in 87 coeliac adults on a gluten-free diet. All underwent small bowel biopsy, and the mucosal morphology was interpreted along with Marsh's grading 0–3. In 30 patients histological and serological data could be analysed before and after adopting the diet; Marsh 3 was considered inadequate mucosal recovery during the diet.**

***Results*** **Of the 87 coeliac patients 27 showed Marsh 3 villous atrophy on gluten-free diet; of these 27, tissue transglutaminase antibody was within normal limits in 16 (59%) and endomysial antibody in 20 (74%). Two (7%) out of 29 with normal mucosa (Marsh 0) had positive tissue transglutaminase antibodies. Six (55%) out of 11 admitting regular dietary lapses remained tissue transglutaminase antibody negative. In the follow-up, serum IgA-class tissue transglutaminase antibody was initially positive in 28 (93%) out of 30 untreated patients; even a significant decrease in tissue transglutaminase antibody did not guarantee mucosal recovery.**

***Conclusions*** **A substantial number of coeliac patients with negative tissue transglutaminase or endomysial antibodies may still have manifest mucosal villous atrophy. Small bowel biopsy is therefore still necessary to ensure that the gluten-free diet is adequate.** ***Eur J Gastroenterol Hepatol*** **14:311–315 © 2002 Lippincott Williams & Wilkins**

**European Journal of Gastroenterology & Hepatology** 2002, **14**:311–315

**Keywords: coeliac disease, gluten-free diet, transglutaminase antibodies, endomysial antibodies**

Departments of [a]Internal Medicine and [b]Paediatrics, Tampere University Hospital, Tampere; and Medical School, University of Tampere, Tampere, Finland

Correspondence to Dr Pekka Collin, Medical School, FIN-33014 University of Tampere, Finland
Tel: +358 3 247 7869; fax: +358 3 215 8402; e-mail: pekka.collin@uta.fi

**Received** 28 June 2001 **Revised** 12 September 2001
**Accepted** 30 October 2001



## Introduction

The sensitivity of the IgA-class endomysial antibody (EmA) test is 85–100%, and the specificity close to 100% in untreated coeliac disease [1–4]. However, the test is not as reliable in the assessment of dietary compliance or small bowel mucosal recovery. EmA may be normal even when dietary transgressions are evident [5,6]. Similarly, negative EmA has been found despite persisting small bowel mucosal atrophy in patients maintaining a gluten-free diet [5–8]. On the other hand, some patients showing normal small bowel mucosal morphology have still remained EmA-positive [2,9].

Recently, tissue transglutaminase was recognized as being the major antigen of EmA [10]. An enzyme-linked immunosorbent assay (ELISA) test for IgA-class tissue transglutaminase antibodies (tTG-ab) has since been developed, and may be even more sensitive than EmA in detecting coeliac disease [11,12]. During a gluten-free diet tTG-ab values seem to decrease significantly [11–14], but there are no studies comparing tTG-ab to small bowel mucosal histology during a gluten-free diet. Provided that a negative tTG-ab invariably signifies adequate morphological recovery, the test could substitute the biopsy in dietary assessment. We tested this hypothesis in a number of adult coeliac patients who had been on a gluten-free diet for variable periods. For comparison, an EmA test was carried out concomitantly.

## Patients and methods

### Patients

Eighty-seven consecutive adult coeliac disease patients on a gluten-free diet were enrolled for the study (Table 1). In all cases the diagnosis was initially based on small bowel mucosal villous atrophy and crypt hyperplasia. Patients with selective IgA deficiency were not included. A dietician evaluated dietary compliance by means of an interview and a 3 day record of food intake. The gluten-free diet was regarded as strict when no additional gluten intake was shown. Patients were considered to have diet-

**Table 1** **Demographic data on the 87 treated coeliac disease patients**

| | |
|---|---|
| Female, *n* (%) | 63 (72%) |
| Median age (range) | 49 years (22–73 years) |
| Median duration of gluten-free diet (range) | 1 year (1–18 years) |
| Symptoms leading to the diagnosis of coeliac disease, *n* (%) | |
| Abdominal complaints | 61 (71%) |
| Anaemia with deficiency of iron or folic acid | 9 (10%) |
| Arthritis | 3 (3%) |
| Neurological disorders | 2 (2%) |
| Serological screening in risk groups* | 12 (14%) |

*First-degree relatives of coeliac patients, patients with diabetes mellitus, autoimmune thyroid disorders, Sjögren's syndrome, osteoporosis.

ary transgressions when admitting every week or at least once a month occasional consumption of gluten-containing foods, such as bread or cake. Histological and serological data were further analysed on 30 (six male, 24 female, median age 46 years, range 22–69 years) of these 87 patients also before the introduction of the diet. This was in order to survey whether the overall decrease of antibodies in serial testing would improve the value of the tests.

### Serology

Serum IgA-class tTG-ab was determined by ELISA (Inova Diagnostics, San Diego, California, USA) [11]; an arbitrary unit value (U) $\geqslant 20$ U for tTG-ab was considered positive. An indirect immunofluorescence method was used in the determination of serum IgA-class EmA, using human umbilical cord [15] as antigen. A screening dilution of 1:5 was considered positive, and positive sera were further diluted 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000, 1:4000 and 1:8000. IgA-class gliadin antibody (AGA) was not determined, because its sensitivity and specificity were expected to be low [6,11].

In the present study tTG-ab was investigated by using guinea pig liver tissue transglutaminase as antigen. Recently, a tTG-ab test by human recombinant tissue transglutaminase has been introduced. For comparison, afterwards we tested 105 serum samples outside the original study protocol with Inova's test and with a human recombinant tTG-ab test (Celikey, Pharmacia, Uppsala, Sweden).

### Small bowel biopsy

Biopsy specimens were taken from the distal part of the duodenum upon endoscopy. Specimens were stained with haematoxylin–eosin, studied under light microscopy and classified according to Marsh [16,17]. The lesion was scored as type 0 when there were normal finger-like villi with a normal crypt depth and no excess of intraepithelial lymphocytes. In type 1–2 lesions intraepithelial lymphocytosis was seen in normal villous structure without (= Marsh 1) or with (= Marsh 2) hyperplastic crypts. A type 3a lesion comprised partial villous atrophy with crypt hyperplasia, type 3b of subtotal, and 3c of total villous atrophy with crypt hyperplasia. A Marsh 3a–c lesion was considered an inadequate dietary response.

In the follow-up study, comparison was carried out to determine how the overall decline in tTG-ab units on a gluten-free diet was seen as an improvement in small bowel mucosal morphology.

### Statistics

Fisher's exact test was used in cross-tabulations. *P* values lower than 0.05 were considered statistically significant.

### Ethics

The protocol of the study was approved by the ethics committee of Tampere University Hospital. All subjects gave informed consent.

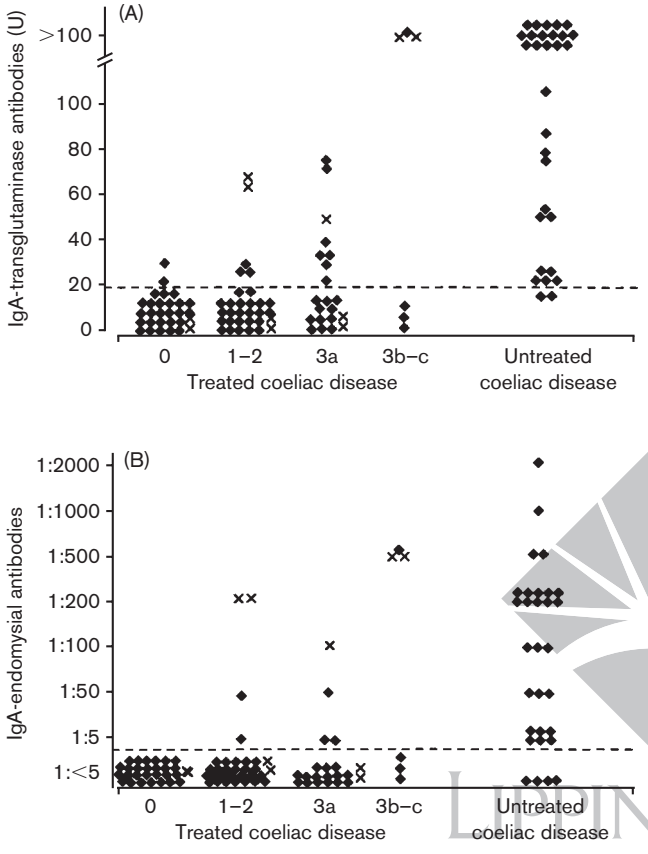
## Results

tTG-ab were negative in 16 (59%) and EmA in 20 (74%) out of 27 coeliac patients showing persistent small bowel mucosal villous atrophy (Marsh 3a–c) under dietary treatment (Fig. 1) ($P = 0.12$). Thus, the sensitivity of tTG-ab in revealing a severe mucosal lesion was only 41% (Table 2). The majority of patients showing Marsh 1–2 type lesions were antibody negative.

In the follow-up study, tTG-ab values declined within 1 year of gluten-free treatment in all 30 patients (Fig. 2). tTG-ab were consistently within reference values when adequate mucosal recovery was obtained (Fig. 2A and B). However, five patients showed a Marsh 3 lesion despite normal antibody levels, and notably, the overall decrease in tTG-ab units had been substantial in most of them (Fig. 2C).

In the dietary assessment, 76 (87%) out of 87 coeliac disease patients adhered to a strict gluten-free diet. Eleven patients were found to have occasional dietary transgressions, such as consumption of gluten-containing bread weekly or at least once a month; both serum tTG-ab and EmA were negative in six of them (Fig. 1A

**Fig. 1**




Serum tissue transglutaminase (A) and endomysial (B) antibodies in treated coeliac disease patients divided according to small bowel mucosal Marsh type. Results are compared to those in untreated patients. Cases with dietary lapses are depicted with a cross (X).

**Table 2** **Characteristics of the tissue transglutaminase and endomysial tests used for determining clinically significant (Marsh 3) mucosal lesions in patients on a gluten-free diet**

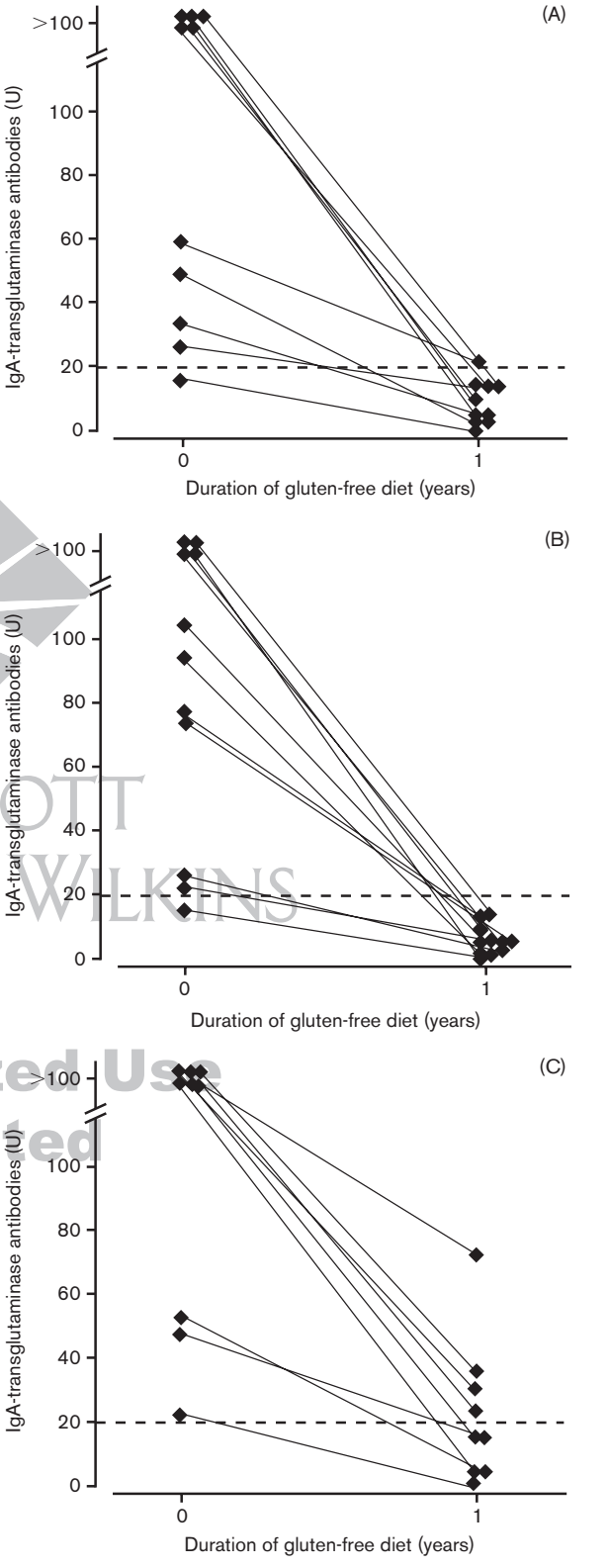
| Characteristic | tTG-ab | EmA |
|---|---|---|
| Sensitivity (%) | 41 | 26 |
| Specificity (%) | 88 | 93 |
| Positive predictive value (%) | 61 | 63 |
| Negative predictive value (%) | 77 | 74 |

tTG-ab, tissue transglutaminase antibody; EmA, endomysial antibody. Individual data are depicted in Fig. 1.

and B). Upon inquiry eight patients admitted suffering from mild abdominal complaints; none of them had dietary lapses or positive coeliac antibodies.

When we compared our tTG-ab test (Inova) to the human recombinant test (Celikey, Pharmacia), 44 out of 105 sera were positive and 51 negative by both tests; 10 sera were slightly positive by Inova's test and nega-

**Fig. 2**




Serum tissue transglutaminase antibody levels before and after 1 year's gluten-free diet in coeliac patients showing adequate (A, Marsh 3 > Marsh 0; B, Marsh 3 > Marsh 1–2) and inadequate (C, Marsh 3 > Marsh 3) histological response within the same period.

tive by Pharmacia's test. No sera were negative by Inova's test and positive by Celikey, Pharmacia.

## Discussion

In coeliac disease, the assessment of dietary response has been based on morphological recovery of small bowel mucosa during follow-up. A serological test able to substitute biopsy ought to reveal effectively such patients who still have ongoing mucosal damage, especially a Marsh 3 lesion. False-positive test results would not be as problematic as negative ones, since positive cases can well be verified by biopsy. We showed for the first time that in many cases, seroconversion of tTG-ab during a gluten-free diet does not necessarily ally with morphological recovery. Even a significant decrease in absolute units can occur in the absence of unequivocal mucosal improvement (Fig. 2C). Thus, the test cannot replace small bowel biopsy in interpreting the adequacy of a gluten-free diet. In fact, tTG-ab was not definitely superior to EmA in this respect.

Dickey and associates [8] suggested that even though EmA does not disappear concomitantly with mucosal recovery, the test may be of value in monitoring dietary compliance; four out of their five patients with dietary lapses were EmA positive. By contrast, in the present study most (55%) coeliac patients found to have repeated, albeit occasional, dietary transgressions remained both tTG-ab- and EmA negative. This indicates that the normalization of antibodies does not always indicate adequate dietary compliance. Figure 1 shows, however, that occasional dietary lapses strongly indicate the presence of villous atrophy: a Marsh 3 lesion was present in five out of nine such cases.

On the other hand, when serum tTG-ab or EmA remained positive after one year on a gluten-free diet, there was consistently ongoing disease activity in the small bowel mucosa (Fig. 2). In other words, positive values seem to indicate inadequate mucosal response. Therefore, provided that the limitations of negative test results are recognized, the antibody tests are beneficial in respect of timing biopsy. It can further be seen in Figure 2 that even on a strict gluten-free diet complete mucosal recovery may sometimes take 1 year or even longer, as also previously noted [18].

The antibody assays seemed to work no better in long-term treated patients than in the whole group: in patients adopting gluten-free diet for more than 5 years ($n = 28$), four had Marsh 3 small bowel mucosal villous atrophy, and two of them were both tTG-ab and EmA negative. Furthermore, nine out of 28 had dietary transgressions, but only four were positive for these antibodies.

IgA-class AGA has been reliable in detecting small bowel villous atrophy, but the low sensitivity of the test has been problematic [19]. Moreover, AGA has been poor in predicting villous atrophy in treated coeliac disease patients [6]. Therefore, we did not use IgA-class AGA in the follow-up of dietary treatment.

Our tTG-ab test was based on guinea pig liver tissue transglutaminase as antigen. It seems unlikely that the latest tTG-ab tests based on a human recombinant technique would be significantly superior for detecting poor small bowel mucosal recovery. As expected, the test we used showed somewhat more marginally positive findings. However, as earlier stated, a positive result can be verified by intestinal biopsy. It is of note that our test was in no case negative when human recombinant test was positive.

To conclude, we consider that tTG-ab (or EmA) has some use in evaluating the adequacy of a gluten-free diet. However, the test is not sensitive enough to reveal dietary transgressions or poor small bowel mucosal recovery in coeliac disease, and hence cannot substitute the control biopsy.



## Acknowledgements

The Coeliac Disease Study Group is supported by the Sigrid Juselius Foundation, the Research Fund of the Finnish Coeliac Society, the Research Fund of AstraZeneca, the Finnish Medical Society Duodecim, the Academy of Finland (grant no. 51547) and the Medical Research Fund of Tampere University Hospital.

## References

1 Hällström O. Comparison of IgA-class reticulin and endomysium antibodies in coeliac disease and dermatitis herpetiformis. *Gut* 1989; **30**: 1225–1232.
2 Volta U, Molinaro N, Fusconi M, Cassani F, Bianchi FB. IgA antiendomysial antibody test. A step forward in celiac disease screening. *Dig Dis Sci* 1991; **36**:752–756.
3 McMillan SA, Haughton DJ, Biggart JD, Edgar JD, Porter KG, McNeill TA. Predictive value for coeliac disease of antibodies to gliadin, endomysium, and jejunum in patients attending for jejunal biopsy. *BMJ* 1991; **303**:1163–1165.
4 Ferreira M, Davies SL, Butler M, Scott D, Clark M, Kumar P. Endomysial antibody: is it the best screening test for coeliac disease? *Gut* 1992; **33**:1633–1637.
5 Troncone R, Mayer M, Spagnuolo F, Maiuri L, Greco L. Endomysial antibodies as unreliable markers for slight dietary transgressions in adolescents with celiac disease. *J Pediatr Gastroenterol Nutr* 1995; **21**:69–72.
6 Sategna-Guidetti C, Grosso S, Bruno M, Grosso SB. Reliability of immunologic markers of celiac sprue in the assessment of mucosal recovery after gluten withdrawal. *J Clin Gastroenterol* 1996; **23**:101–104.
7 Sategna-Guidetti C, Grosso SB, Bruno M, Grosso S. Is human umbilical cord the most suitable substrate for the detection of endomysium antibodies in the screening and follow-up of coeliac disease? *Eur J Gastroenterol Hepatol* 1997; **9**:657–660.
8 Dickey W, Hughes DF, McMillan SA. Disappearance of endomysial antibodies in treated celiac disease does not indicate histological recovery. *Am J Gastroenterol* 2000; **95**:712–714.
9 Feighery C, Weir DG, Whelan A, Willoughby R, Youngprapakorn S, Lynch S, *et al*. Diagnosis of gluten-sensitive enteropathy: is exclusive reliance on histology appropriate? *Eur J Gastroenterol Hepatol* 1998; **10**:919–925.
10 Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, *et al*. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nature Med* 1997; **3**:797–801.
11 Sulkanen S, Halttunen T, Laurila K, Kolho K-L, Korponay-Szabo I, Sarnesto

A, *et al*. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology* 1998; **115**:1322–1328.
12 Dieterich W, Laag E, Schopper H, Volta U, Ferguson A, Gillett H, *et al*. Autoantibodies to tissue transglutaminase as predictors of celiac disease. *Gastroenterology* 1998; **115**:1317–1321.
13 Troncone R, Maurano F, Rossi M, Micillo M, Greco L, Auricchio R, *et al*. IgA antibodies to tissue transglutaminase: an effective diagnostic test for celiac disease. *J Pediatr* 1999; **134**:166–171.
14 Biagi F, Ellis H, Yiannakou J, Brusco G, Swift G, Smith P, *et al*. Tissue transglutaminase antibodies in celiac disease. *Am J Gastroenterol* 1999; **94**:2187–2192.
15 Stern M. Comparative evaluation of serologic tests for celiac disease: a European initiative toward standardization. Working Group on Serologic Screening for Celiac Disease. *J Pediatr Gastroenterol Nutr* 2000; **31**:513–519.
16 Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). *Gastroenterology* 1992; **102**:330–354.
17 Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999; **11**:1185–1194.
18 Grefte JM, Bouman JG, Grond J, Jansen W, Kleibeuker JH. Slow and incomplete histological and functional recovery in adult gluten sensitive enteropathy. *J Clin Pathol* 1988; **41**:886–891.
19 Volta U, Corazza GR, Frisoni M, Valentini RA, Molinaro N, Bianchi FB, *et al*. IgA antigliadin antibodies and persistence of jejunal lesions in adult coeliac disease. *Digestion* 1990; **47**:111–114.