

# A comparison of antibodies to tissue transglutaminase with conventional serological tests in the diagnosis of coeliac disease

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**Background** Tissue transglutaminase is now recognized as the autoantigen for antiendomysial antibodies. Antibodies to tissue transglutaminase have been proposed as a valuable test for coeliac disease.

**Objective** To determine the value of antibodies to tissue transglutaminase in the diagnosis of coeliac disease in our outpatient population.

**Methods** Patients who underwent serological tests for coeliac disease during the first 18 months of the tissue transglutaminase antibody assay were retrospectively identified from the regional serology laboratory database. Patients' symptoms were noted, along with serological results and duodenal histology in those patients who underwent duodenal biopsy.

**Results** In total, 586 patients were identified as having been serologically tested for coeliac disease, of whom 92 patients (33 men; mean age 51.7 years) had been followed up with duodenal biopsies. Of these 92 patients, 29 (31%; 14 men; mean age 52.5 years) had histological features of coeliac disease. The 63 patients with normal histology (19 men; mean age 51.8 years) acted as controls. Weight loss was more frequent in coeliac disease patients compared to controls (7 vs 5;  $P = 0.04$ ) whereas the frequency of

anaemia ( $P = 0.85$ ) and diarrhoea ( $P = 0.74$ ) did not differ significantly between the two groups. The sensitivity and specificity of tissue transglutaminase antibodies (86%; 84%) were compared to those for antiendomysial antibodies (90%; 98%) and antigliadin antibodies (76%; 79%).

**Conclusions** The diagnostic value of tissue transglutaminase antibodies was intermediate between that of antiendomysial antibodies and antigliadin antibodies. However, duodenal biopsy remains the gold standard diagnostic test for coeliac disease. *Eur J Gastroenterol Hepatol* 15:1001–1004 © 2003 Lippincott Williams & Wilkins

*European Journal of Gastroenterology & Hepatology* 2003, 15:1001–1004

**Keywords:** transglutaminase, coeliac disease, duodenal biopsy

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Received 20 June 2002 Revised 24 February 2003  
Accepted 26 March 2003

## Introduction

Tissue transglutaminase is now recognized as the autoantigen for antiendomysial antibodies [1], although other autoantigens may also have a role to play in the pathogenesis of coeliac disease [2]. Antibodies to tissue transglutaminase have been proposed as a valuable test for coeliac disease, since they have a sensitivity of 85–100% and a specificity of 76–100%, which compares favourably with the respective values for antiendomysial antibodies (sensitivity 86–100%; specificity 94–100%) [2–7]. Tissue transglutaminase antibodies may also be of particular diagnostic value in antiendomysial antibody-negative subjects [8]. Combining tests for antiendomysial and tissue transglutaminase antibodies offers a higher sensitivity for detecting coeliac disease, since almost a third of patients may have only one of these two antibodies present [9].

The aim of this study was to determine the value of anti-tissue transglutaminase antibodies in the diagnosis of coeliac disease in our hospital outpatient population.

## Patients and methods

Consecutive adult patients, who had undergone serological tests for coeliac disease during the first 18 months of use of the tissue transglutaminase antibody assay, were retrospectively identified from the regional serology laboratory database. Patients were recruited from five local hospitals in Northern Ireland. These hospitals serve as secondary referral centres in a mainly urban and suburban population. Patients' symptoms (diarrhoea, anaemia and weight loss) were noted along with histological results in those patients who underwent duodenal biopsy. Coeliac disease was defined as severe partial villous atrophy, sub-total or total villous atrophy.

Serum was analysed for immunoglobulin A (IgA)-antigliadin, antiendomysial antibodies and anti-tissue transglutaminase antibodies. Determination of IgA antibodies to gliadin was carried out using a commercial enzyme-linked immunosorbent assay (ELISA) (Immco Diagnostics, Buffalo, New York, USA). Results were expressed in ELISA units with a normal reference range of 0–15 U (97.5th centile). Antiendomysial antibodies in the IgA class were detected by indirect immunofluorescence using monkey oesophagus tissue (BioDiagnostics Ltd, Worcestershire, UK) as the antigen. Positivity was taken as positive staining at a titre of 1:5 or greater. Anti-tissue transglutaminase antibodies were determined by commercial ELISA (Immco Diagnostics). Results were expressed in ELISA units with a normal reference range of 0–25 U (97.5th centile). The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of the three serological tests were compared.

## Results

In total, 586 patients (220 men; mean age 46.6 years) were identified from the laboratory database as having been serologically tested for coeliac disease. Of these, 92 patients (33 men; mean age 51.7 years) had been followed up with duodenal biopsies and were therefore identified as the study group. All patients were recruited from five local hospitals in Northern Ireland. Of the 92 patients in the study group, 29 (31%; 14 men; mean age 52.5 years) had histological features of coeliac disease and the 63 patients (19 men; mean age 51.8 years) with normal histology acted as controls. The most frequent symptoms in the 92 patients were diarrhoea ( $n = 21$ ), anaemia ( $n = 18$ ) and weight loss

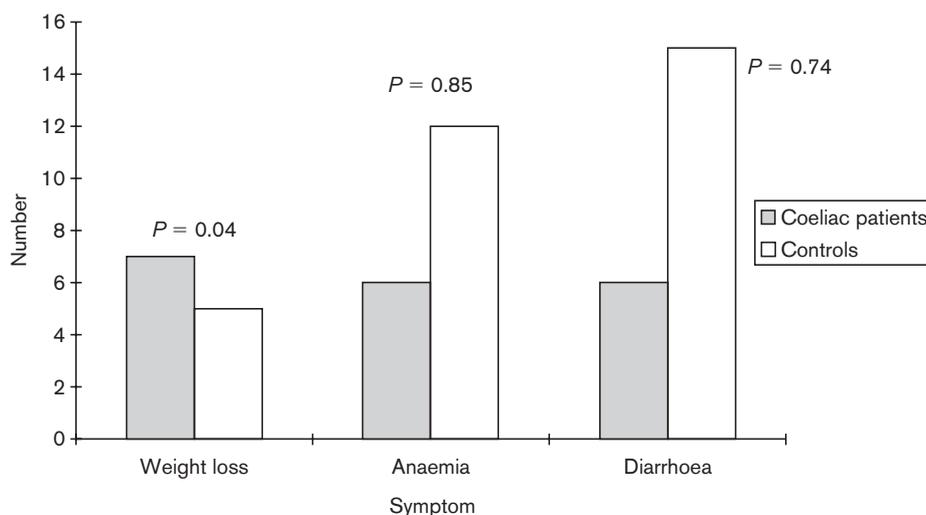
( $n = 12$ ). Weight loss was more frequent in coeliac patients compared to controls (7 vs 5;  $P = 0.04$ ), whereas the frequency of anaemia (6 vs 12;  $P = 0.85$ ) and diarrhoea (6 vs 15;  $P = 0.74$ ) did not differ significantly between the two groups (Fig. 1).

A comparison of the serological markers and their relationship to duodenal histology is given in Table 1. The sensitivity, specificity, NPV and PPV of tissue transglutaminase antibodies (86%; 84%; 93% and 71%, respectively) were compared to those for antiendomysial antibodies (90%; 98%; 95%; 96%, respectively) and antigliadin antibodies (76%; 79%; 88%; 63%, respectively) (Fig. 2). A combination of either antiendomysial or tissue transglutaminase antibody positivity gave a sensitivity of 93%, specificity of 83%, PPV of 71% and NPV of 96%. When the study group was restricted to the 55 patients in whom a decision was made to proceed with duodenal biopsy on clinical grounds only (unaffected by whether the serology was positive or not), the sensitivity and specificity for the three serological tests were comparable with the original results in Table 1.

## Discussion

The prevalence of adult coeliac disease in Northern Ireland is known, as a result of our screening study of the general adult population for antigliadin and antiendomysial antibodies, to be approximately 1:122 [11]. The advent of a further serological test for coeliac disease (tissue transglutaminase antibodies) introduces the possibility that a superior assay may obviate the need for duodenal biopsy in the diagnosis of coeliac disease. During the 18 months prior to this study, the

Fig. 1

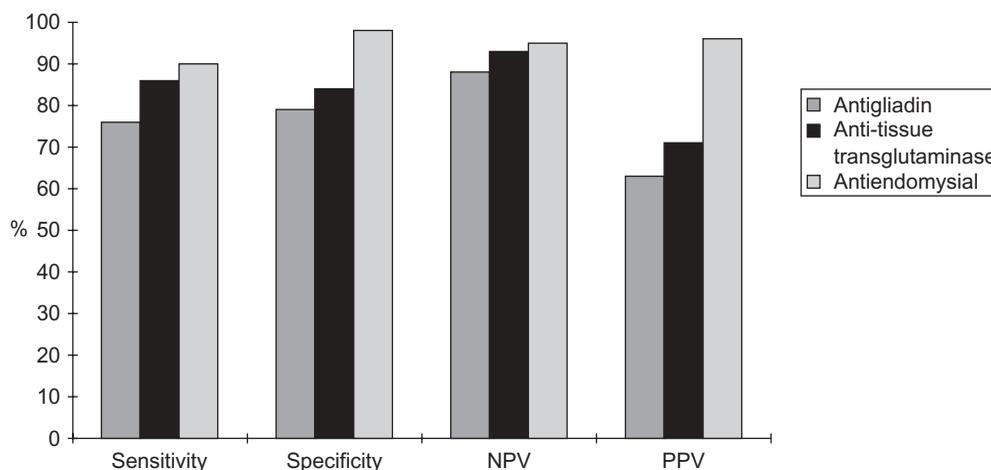


A comparison of the frequency of symptoms between coeliac patients and controls.

**Table 1** A comparison of the sensitivity and specificity of the three serological tests for coeliac disease

	(n = 92)		(n = 55)	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Antitransglutaminase antibodies	86	84	86	76
Antiendomysial antibodies	90	98	95	100
Antigliadin antibodies	76	79	76	76

(n = 55) refers to those in whom we had clinical grounds to carry out a biopsy, e.g. diarrhoea, weight loss, anaemia, positive family history of coeliac disease etc; (n = 92) refers to the original study group.

**Fig. 2**

A comparison of the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of the three serological tests for coeliac disease.

regional serology laboratory introduced the tissue transglutaminase antibody assay. This laboratory performs all the serological assays for the Northern Ireland region (population 1.6 million). It was therefore necessary to evaluate the usefulness of the tissue transglutaminase antibody assay in comparison to the standard serological tests (antigliadin and antiendomysial antibodies), which were in use prior to its introduction.

We included an initial estimation of the sensitivity and specificity of the serological assays in the group of 92 patients undergoing duodenal biopsy, regardless of whether this was on the grounds of clinical symptoms or serological results. By restricting the evaluation of sensitivity and specificity to the group of 55 patients in whom a decision was made to proceed with duodenal biopsy on clinical grounds only, a more accurate estimation of sensitivity and specificity may be obtained. The most notable difference was that the specificity of the tissue transglutaminase assay fell from 84% to 76%; the other values were not significantly altered.

Dickey *et al.* demonstrated a lower sensitivity (75% vs 86%) and a higher specificity (97% vs 84%) for tissue

transglutaminase antibodies than in the current study, despite the fact that the same regional serology laboratory was used in both studies [9]. This may relate to the fact that Dickey *et al.* performed duodenal biopsies when there was a suspicion of endoscopic stigmata suggestive of coeliac disease, rather than on the basis of patients' symptoms or serology.

Comparing the use of tissue transglutaminase antibodies in our study with that in other reports, we have found the sensitivity and specificity of this assay to be somewhat lower than those in recent publications, although the reason for this is not entirely clear [2–7]. However, we have confirmed that the sensitivity and specificity of tissue transglutaminase antibodies are intermediate between those of the conventional serological tests for coeliac disease (antiendomysial and antigliadin antibodies).

McMillan *et al.* [10] previously reported a series of 96 patients investigated for coeliac disease, of whom 28 fulfilled the criteria for diagnosis of this disease based on their histology. The sensitivity and specificity for antigliadin antibodies (both 100%) and antiendomysial

antibodies (89% and 100%, respectively) from McMillan's study were higher than those in our present study, the difference being more marked for the antigliadin antibodies assay than the antiendomysial antibodies assay. This may relate to differences in the commercial assay used to measure IgA-antigliadin antibodies (Labmaster, Turku, Finland in McMillan's study and Immco Diagnostics' kit in the present study). Although we only compared a small group of coeliac patients, it was apparent that symptoms of malabsorption, including diarrhoea and anaemia, were no more frequent in coeliac patients than in controls, whereas weight loss was more common in the untreated coeliac patients. It is well recognized that many patients with coeliac disease do not present with marked symptoms of malabsorption, and indeed many patients are mono-symptomatic or asymptomatic.

Our first experience of using antibodies to tissue transglutaminase indicates that it is not as valuable in the diagnosis of coeliac disease as some previous reports have proposed, being intermediate between that for antigliadin and antiendomysial antibodies. In fact, based on the results of this study, the addition of tissue transglutaminase antibodies to the serological evaluation of coeliac disease adds little to the present combination of antiendomysial and antigliadin antibodies, which has been our laboratory standard for the past 8 years. Only one coeliac patient who was negative for antiendomysial antibodies was positive for both tissue transglutaminase and antigliadin antibodies.

Although these serological tests are a useful diagnostic aid, they are unreliable on their own when making a diagnosis of coeliac disease. In particular, they are not useful in patients with IgA deficiency. Although we did not measure IgA in our study, a previous paper from our reference laboratory demonstrated a prevalence of IgA deficiency of 1:500 [12]. It is possible that some patients with coeliac disease may have been missed in our study, if coeliac serology alone was relied upon. In practice, a combination of clinical symptoms, coeliac serology and duodenal biopsies indicating villous atrophy, followed by a satisfactory response to dietary gluten exclusion, are used to make a diagnosis of coeliac disease. Therefore, duodenal biopsy remains the gold standard diagnostic test for coeliac disease.

#### Conflict of interest

None declared.

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