The role of antitissue transglutaminase assay for the diagnosis and monitoring of coeliac disease: a French–Italian multicentre study

E Tonutti, D Visentini, N Bizzaro, M Caradonna, L Cerni, D Villalta, R Tozzoli, and the French–Italian Laboratory Study Group on Coeliac Disease

Aims: Tissue transglutaminase (tTG) was recently identified as the major autoantigen in coeliac disease. The aim of this multicentre study was to evaluate the impact of a new immunoenzymatic assay for the detection of IgA anti-tTG antibodies.

Methods: Seventy-four Italian and French clinical laboratories participated in this study; anti-tTG IgA with an enzyme linked immunosorbent assay (ELISA) method using guinea pig liver extract as the coating antigen, anti-endomysium IgA autoantibodies (EMA), and total serum IgA were determined in 7948 patients, 1162 of whom had coeliac disease (737 untreated cases and 425 on a gluten free diet). A proportion of the sera were then sent to a reference laboratory for anti-tTG retesting with an ELISA method using recombinant human tTG antigen.

Results: Seven thousand four hundred and fifty eight (93.8%) sera were EMA/antiguinea pig tTG concordant (positive or negative); 490 (6.2%) were non-concordant. The sensitivity of EMA and antiguainea pig tTG in the 737 untreated patients with coeliac disease was 92.1% and 94.8%, respectively, and the specificity was 99.8% and 99.2%, respectively. Retesting of the discordant sera showed that of the 162 sera classified as EMA positive/antiguinea pig tTG positive, only 49 were positive for human recombinant anti-tTG, and that 39 of these were also EMA positive. Furthermore, of the 36 sera classified as EMA positive/antiguinea pig tTG negative, only two were confirmed as EMA positive.

Conclusions: The antiguainea pig tTG assay is more sensitive but less specific than EMA, whereas the antihuman recombinant tTG assay is far more specific and just as sensitive as antiguainea pig tTG. Testing for EMA presents considerable interpretative problems and is difficult to standardise.

Coeliac disease (CD) is a gluten dependent disorder characterised by small intestinal damage with loss of absorptive villi.1 2 IgA and IgG antigliadin (AGA), IgA antireticulin (ARA), and IgA anti-endomysium antibodies (EMA) are valuable serological tests for the identification of patients with CD and treatment control.3 4 However, the final requirement for the diagnosis of CD is the histological finding of a characteristic mucosal abnormality in an intestinal biopsy specimen.5 6 The enzyme tissue transglutaminase (tTG) was recently identified as the major autoantigen in CD,7 8 and the antigenic target recognised by EMA.9 10 tTG induces the deamination of gluten peptides and the formation of novel epitopes that, in association with human leucocyte antigen class II antigens, drive the antibody response to both gliadin and tTG.11 12 Newly available enzyme linked immunosorbent assay (ELISA) methods to identify IgA anti-tTG antibodies have increased the sensitivity of laboratory tests in detecting CD, but data regarding their specificity are, at times, contradictory.11 12 Probably because of differences in the antigenic source used (purified extracts from guinea pig liver or human tissues).10 12

"The enzyme tissue transglutaminase was recently identified as the major autoantigen in coeliac disease, and the antigenic target recognised by anti-endomysium antibodies"

The main aim of our study was to evaluate the diagnostic impact of an ELISA assay that uses guinea pig liver extract as the coating antigen (tTGgp) for the identification of anti-tTG autoantibodies, in a large number of Italian and French clinical laboratories, and to compare the results with the EMA method. The subsequent availability of a new ELISA assay with human recombinant tTG (tTGhr) when our study was already in progress made it possible to re-evaluate some sera samples in a reference laboratory, and to compare the performances of the anti-tTGgp and anti-tTGhr assays.

PATIENTS AND METHODS
Seventy-four laboratories (64 Italian and 10 French) participated in our study. The sera, which were collected from January to October 2000, were obtained from: (1) patients with a clinical diagnosis of CD, who were either untreated or on a gluten free diet (GFD), and (2) patients without a previous diagnosis of CD, whose sera were sent for EMA and AGA testing.

Demographic characteristics (name, address, date of birth, and sex), in addition to serological and clinical data (symptoms, diagnosis of active coeliac or non-coeliac disease both confirmed by intestinal biopsy, or patient with CD on diet), were collected on a standard report form. Only patients who fulfilled the diagnostic criteria of the European Society...
for Paediatric Gastroenterology, Hepatology, and Nutrition\(^7\) were defined as being affected by CD.

In total, 7948 patients (male to female ratio, 1/1.28) were recruited. These were further subdivided into three age groups (< 2 years, between 2 and 14 years, and > 14 years) to discern the possible presence of different antibody reactivities in different age groups (table 1).

IgA anti-tTG, IgA EMA, and total serum IgA values were determined in all subjects. IgA and IgG AGA were tested in all children < 2 years of age, and in all patients with CD. All laboratories tested for IgA anti-tTG using a commercial ELISA (Eu-tTG\(^®\) IgA; Eurospital, Trieste, Italy) that uses tTGgp as the coating antigen, and anti-human IgA peroxidase conjugate as the secondary antibody; as indicated by the manufacturer, sera with a concentration > 5 arbitrary units (AU)/ml were considered positive. IgA EMA, IgA and IgG AGA, and total serum IgA antibodies were determined by the method used routinely in each laboratory. All sera were tested immediately and then stored at \(-20{}^\circ\text{C}\) for further investigation.

One hundred and ninety eight available sera for which discordant results were obtained by the participating laboratories (162 EMA negative and anti-tTGgp positive, and 36 EMA positive and anti-tTGgp negative) were retested in a reference laboratory for EMA (antiendomysium; Eurospital) by indirect immunofluorescence (IIF) on monkey oesophagus and for anti-tTG using both the above assay and a new ELISA test (Eu-tTG-humana\(^®\) IgA; Eurospital) that uses tTGhr as the coating antigen; for this last method, the cut off point was set at 7 AU/ml, as proposed by the manufacturer.

Furthermore, to verify the consistency of the preliminary test results and to compare the sensitivity and specificity of the anti-tTGgp and anti-tTGhr assays, 400 randomly selected sera for which concordant results had been obtained (300 EMA and anti-tTGgp negative, and 100 EMA and anti-tTGgp positive) were also retested in the reference laboratory. Two different operators independently interpreted the immunofluorescence pattern of EMA.

Statistical analysis was carried out by calculating the sensitivity of each method in identifying sera of patients with CD; the specificity of each method was calculated in the group of patients in which CD was excluded. The \(\chi^2\) test was used to see whether there were differences between the assays in the different age groups, and a \(p\) value < 0.05 was considered significant. Confidence intervals (CI) were calculated where appropriate.

All the patients gave their informed oral consent to be included in our study. Results of the assays were given to individual patients. When there were discrepancies between the anti-tTG and EMA assays, the patients were notified that confirmatory tests were in progress. Results obtained in the reference laboratory were communicated to the patients and proper counselling was given.

**RESULTS**

Of the 7948 patients studied, 1162 had CD (737 were untreated, and 425 were on a GFD), and 6316 were classified as non-CD; in 470 (5.9%) cases the diagnosis remained undefined because of a lack of clinical information or refusal to undergo intestinal biopsy, or because biopsy findings were not discriminant (latent CD).\(^8\) The patients were then divided into two groups based on EMA and anti-tTGgp results: 7458 (93.8%) were EMA and anti-tTGgp concordant (group A), and 490 (6.2%) were EMA and anti-tTGgp discordant (group B) (fig 1).

**Group A: EMA/anti-tTGgp concordant results**

This group of 7458 patients was further subdivided in two subgroups, according to whether the EMA and anti-tTGgp tests were both negative (group A1) or both positive (group A2).

**Group A1:** concordant negative results were obtained in 7106 patients, of whom 6528 had untreated CD, 110 had CD and were on a GFD, and 470 cases were undefined.

**Group A2:** concordant positive results were obtained in 332 patients, of whom 293 had untreated CD, 110 had CD and were on a GFD, and 150 cases were undefined.

**Group A2:** concordant positive results were obtained in 930 patients, of whom 670 had untreated CD, 110 had CD and were on a GFD, and 150 cases were undefined.

---

**Table 1** Number of patients studied and male to female ratio for age group

<table>
<thead>
<tr>
<th>Age</th>
<th>Number</th>
<th>Male:female ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2 years</td>
<td>1246</td>
<td>1:1.10</td>
</tr>
<tr>
<td>2–14 years</td>
<td>4254</td>
<td>1:1.16</td>
</tr>
<tr>
<td>&gt;14 years</td>
<td>2438</td>
<td>1:1.66</td>
</tr>
<tr>
<td>Total</td>
<td>7948</td>
<td>1:1.28</td>
</tr>
</tbody>
</table>

---

**Figure 1** Flow diagram of test results according to the clinical diagnosis. CD, coeliac disease; EMA, anti-endomysium antibodies; GFD, gluten free diet; tTGgp, guinea pig tissue transglutaminase.
Group B: EMA/anti-tTGgp discordant results
The 490 sera with discordant results for EMA and anti-tTG testing were also subdivided into two subgroups.

Group B1: 54 patients were EMA positive/anti-tTGgp negative (nine untreated CD, eight with CD on a GFD, 10 not CD on biopsy, and 27 undefined).

Group B2: 436 patients were EMA negative and anti-tTGgp positive (29 untreated CD, 62 with CD on a GFD, 52 no CD on biopsy, and 293 undefined).

The sensitivity of anti-tTGgp in the 737 untreated patients with CD was higher (94.8%; CI, 93 to 96) than that of EMA (92.1%; CI, 90 to 94) (table 2), whereas the specificity of anti-tTGgp in the 6316 non-CD subjects was 99.2% (CI, 98.9 to 99.4) and that of EMA was 99.8% (CI, 99.6 to 99.9) (table 3). In the 425 patients with CD on a GFD, EMA was positive in 27.7%, and anti-tTGgp was positive in 39.5%. It must be noted that clinically undefined cases were excluded from the statistical analysis, and that sensitivity and specificity values were calculated using the data provided by the participating laboratories and not after retesting in the reference laboratory (see below).

AGA IgA and IgG
AGA IgA and IgG were positive in 6% and 17.3%, respectively, of the 6254 discordant negative patients, in 70% and 74.1%, respectively, of the 737 untreated patients with CD, and in 43.5% and 55.5%, respectively, of the patients with CD who were on a GFD, thus showing that these tests are both less sensitive and less specific than EMA and anti-tTG. However, an excellent sensitivity (95.9%; CI, 91 to 100) of AGA IgG was seen in the 74 patients with CD who were < 2 years old (table 2).

Results of sera retesting in a reference laboratory
Five hundred and ninety-eight sera were re-evaluated: 300 were EMA and anti-tTGgp concordant negative (group A1), 100 were EMA and anti-tTGgp discordant positive (group A2), 36 were EMA positive and anti-tTGgp negative (group B1), and 162 EMA negative and anti-tTG positive (group B2).

The 300 concordant negative and the 100 discordant positive sera were all confirmed by EMA and anti-tTGhr retesting. All 36 sera of group B1 (EMA positive/anti-tTGgp negative) were confirmed negative when retested by the anti-tTGgp assay, but one sample was positive for anti-tTGhr. More noteworthy, EMA positivity was confirmed in only two of the 36 sera (table 4), showing that most of the discordant results in this group were caused by a false positive interpretation of the EMA patterns.

In group B2 (EMA negative/anti-tTG positive), 152 of the 162 sera were confirmed positive when retested for anti-tTGgp, but only 49 were anti-tTGhr positive, showing that the tTG assay using the human recombinant antigen had a higher specificity. Moreover, 39 of these 49 sera were also EMA positive (table 5), indicating once again that the false negatives resulted from the misinterpretation of the EMA test. Thus, although the data are biased by the fact that not all the samples were retested by the anti-tTGhr assay, after retesting, specificity increased from 99.8% to 99.9% for EMA, and decreased from 99.2% to 94.7% for the anti-tTGgp assay.

DISCUSSION
The incidence of CD in white individuals is very high (1/100–200 people). Its clinical manifestations vary greatly and there are often no specific gastrointestinal symptoms. Therefore, it is very important to have sensitive and specific diagnostic tests that can enable the accurate and early identification of patients with CD so that a GFD can be instituted.
thus avoiding a wide range of associated pathological conditions.22–25 For the past 10 years, IgA EMA has been the reference test for the diagnosis and monitoring of patients with CD because of its high sensitivity and specificity.26 This test is based on the identification of specific antibodies on monkey oesophagus sections by IIF; however, it requires skilled personnel for the correct interpretation of the fluorescence pattern and its reproducibility is poor.11 For this reason, several laboratories measure EMA in association with AGA IgA and IgG, and ARA, and this involves a large expenditure of time and resources.10

Assays for anti-tTG detection have been developed recently and have been proposed as an alternative method. Our study was designed to verify the diagnostic accuracy of the anti-tTGpp test, and its role as a possible substitute for the older tests.31

We found that the anti-tTGpp test has a higher sensitivity than the EMA test, which in turn is slightly more specific, and is more efficient than EMA in monitoring patients with CD who are on a GFD (39.5% v 27.7% sensitivity, respectively). A more sensitive method to monitor patients on a GFD is needed, because recent research has shown that a negative EMA test does not always indicate a normalisation of the intestinal mucosa.32 In this context, the anti-tTG test can be useful even at lower cut off values than those used for diagnostic purposes.

The IgA and IgG AGA assays had a lower sensitivity and specificity, in line with those reported in previous studies.33–35 However, the IgG AGA test was positive in 95.9% of patients with CD aged less than 2 years; this indicates that an IgG class marker can be very useful in situations where low amounts or a deficiency of IgA is suspected.36 Furthermore, it was shown recently that some patients with CD may be positive for the IgG class only, even though they do not have IgA deficiency.37 Thus, if anti-tTG replaces the AGA assay, both the IgA and IgG isotypes should be determined.

Another interesting feature of the anti-tTG test relates to the 47 patients who were affected by IgA deficiency, and in whom IgA anti-tTGpp values were constantly below 1 AU; this aspect could be useful for the selection of sera to test for total IgA.

The need for continuous monitoring of assay accuracy by external quality assessment programmes also emerged from this multicentre survey, especially for the detection of EMA, because of its subjectivity of interpretation and standardisation problems. The use of anti-tTG testing, particularly if a human recombinant antigen is used, will probably lead to a more precise diagnosis of CD.

**ACKNOWLEDGEMENTS**

This study was sponsored by Eurospital S.r.l, Trieste, Italy.

**APPENDIX 1**

The following investigators contributed to this trial from Italy: F Ferrara, M Barraco, Agrigento; E Migali, D Mariotti, Arezzo; G Danzi, ML Martino, M Danzi, Aversa (CE); G Baldassarre, G Di Bitonto, M Ciccarelli, Bari; D Rielo, G Bertiato, Belluno; G Pedicini, RC Bocchino, F Mocia, Benevento; G Alessio, P Amboni, C Ottomano, Bergamo; U Volta, A Granito, Bologna, N Carabellace, Brescia; R Amato, G Aurina, C Spagnolo, Catalturin (CT); P Clemen, Castelfranco Veneto (TV); F Coppola, G Spagnoletti, Castelmare di Stabia (NA); M Spina, T Trigilia, F Branciforte, Catania; I Giancotti, M Apollini, Catanzaro; B Malamisaura, A Sofia, M Boifardi, Cava dei Tirreni (SA); F Antico, Chioggia (VE); P Arigliano, M Garch, Cologna Veneta (VR); E Sala, M Li, Grassi, G Giana, Como; G Staffa, V Cova, M Martinelli, Faenza (RA); A Calabò, D Renzi, D Nigro, Firenze; D Macchia, M Manfredi, E Cammelli, Firenze; G Castellucci, I Ferraro, Foligno (PG); I Marchetti, G Garelli, M Colombo, Garbagnate-Rho (MI); E Castellano, M Cingolani, Genova; A Sabatino, A Di Blasi, Grosseto; M Golato, A Carlucci, Lanciano (CH); G Spagnolo, M Trivisonno, V Castelli, Lariano (CB); S Babbini, V Marrè, Lavagna (GE); G Melli, S Amoroso, Licata (AG); M Moniesanti, E Mei, Lucca; S Armelloni, C Gerosa, C Marcellini, Milano; G Gallo, R Pizzoli, Milano; M Peracchi, MT Bardella, Certo Croatto, Milano; VS Arosio, L Malberti, Monza (MI); F Rea, MR Di Domenico, A Sergio, Napoli; P Iardino, V Formicola, G Tamborino, Napoli; A Massari, M Cicella, E Rondinella, Napoli; A Pignero, D Scognamiglio, Napoli; S Spagnolo, S Orfice, V Romano, Napoli; B Pennucci, M Maglione, S Lavvecchia, Napoli; A Rubino, O Leone, Napoli; N Cantieri, F Michelutti, Negrar (VR); G Guariso, D Basso, Padova; S Terses, E Gucciardino, M Di Gregorio, Palermo; MA Trippedi, P Greco, R Guadagna, Palermo; E Malvese, Palermo; T Iimbastano, G Lombardi, Palermo; A Rossi, E Savi, L Spada, Piacenza; D Villalba, Polignano (BA); F Tabellini, M Saccarola, Portogruaro (VE); P Palumbo, G Marinucci.
Take home messages

- The tissue transglutaminase (tTG) antibody assay is more sensitive than the antigliadin and anti-endomysium assays in the diagnosis of coeliac disease (CD).
- The tTG antibody assay using human recombinant antigen is more specific than the guinea pig tTG antibody assay.
- The most specific test for the diagnosis of CD is the anti-endomysium antibody assay.
- We suggest that samples should be screened with the human recombinant tTG antibody assay and positive results confirmed with the anti-endomysium antibody assay.

References