Development of a novel rapid non-invasive screening test for coeliac disease

V Baldas, A Tommasini, C Trevisiol, I Berti, A Fasano, D Sblattero, A Bradbury, R Marzari, G Barillari, A Ventura, T Not

Abstract

**Background**—Coeliac disease is one of the commonest underdiagnosed diseases in general practice. The autoantigen recognised by the sera of patients with coeliac disease has recently been identified as tissue transglutaminase.

**Aims**—We evaluated a simple non-invasive immunological dot blot assay for coeliac disease, suitable for use by the general physician in the ambulatory setting. The sensitivity and specificity of this dot blot assay based on recognition of recombinant human transglutaminase were compared with those of antiendomysial antibodies and an enzyme linked immunosorbent assay.

**Methods**—Serum samples were analysed from 64 healthy controls, 58 first degree relatives of coeliacs, 74 diseased controls, and 70 biopsy confirmed untreated patients with coeliac disease. Dot blot assay and enzyme linked immunosorbent assay were performed using recombinant human transglutaminase as antigen.

**Results**—The dot blot assay, which can be performed in 20 minutes, was positive in all 70 untreated coeliacs (sensitivity 100%). Among the three control groups, there were three false positive tests by dot blot (specificity 98%), all belonging to the group of healthy subjects. The antiendomysial antibodies test missed five untreated coeliac patients (sensitivity 93%) and was negative in all three control groups (specificity 100%). The specificity of the immunosorbent assay was 99% for IgA and 98% for IgG, while sensitivity was 93% for IgA, 47% for IgG, and 100% for IgA and IgG combined.

**Conclusions**—The dot blot assay is highly accurate in detecting untreated subjects with coeliac disease and can be performed in the general physician’s medical office during the course of a routine examination. This innovative test is a practical, reliable alternative to both the immunofluorescent based antiendomysial test and immunosorbent assay for detection of transglutaminase antibodies for the diagnosis of coeliac disease.

Keywords: coeliac disease; dot blot immunobinding assay; recombinant human tissue transglutaminase

Coeliac disease (CD) is one of the commonest underdiagnosed diseases in general practice, estimated to occur in 0.4% of the general population. This is mainly due to its often atypical presentation, which in recent surveys have ranged from vague tiredness associated with anaemia to the consequences of associated autoimmune diseases (for example, thyroid disease or insulin dependent diabetes mellitus). Although determination of IgA antiendomysium antibodies (AEA) and IgA and IgG anti-transglutaminase antibodies have been suggested as valid alternatives to duodenal biopsy, everyday clinical practice is beginning to consider reducing its dependency on biopsy as the gold standard for the definitive diagnosis of CD. The easy availability of such tests has expanded the range of subjects tested and has revealed that CD is far more common than previously suspected. Recently, the antigen recognised by AEA has been identified as tissue transglutaminase (tTG), a finding which has been exploited to develop a number of enzyme linked immunosorbent assay (ELISA) tests based on guinea pig tTG, but also on human tTG (h-tTG). The use of the human recombinant transglutaminase antigen has proved to be more sensitive than the AEA test and just as specific. Although far faster and easier to perform than intestinal biopsy, these tests have remained within the realm of the specialised diagnostic laboratory. Given the high prevalence of the disease and its protean nature, a simple diagnostic test which could be used in the general practitioner’s office would represent a great step forward in the rapid diagnosis of CD. Here we present a dot blot test based on detection of anti-h-tTG antibodies in serum or in one drop of whole blood that can be carried out in 20 minutes. We evaluated the sensitivity and specificity of our new test in comparison with AEA and a h-tTG ELISA technique for the diagnosis of CD.

Material and methods

**PATIENTS AND HEALTHY CONTROLS**

We examined serum samples from 70 untreated CD patients (30 males and 40 females, median age 12 years (range 2–60)) diagnosed between June 1997 and October 1999, following the revised ESPGHAN criteria for CD. Controls comprised 58 first degree relatives of CD patients and 74 subjects suffering from

Abbreviations used in this paper: AEA, antiendomysium antibodies; CD, coeliac disease; ELISA, enzyme linked immunosorbent assay; h-tTG, human tissue transglutaminase; ELISA, enzyme linked immunosorbent assay; TBS, Tris buffered saline; TTBS, Tween 20 Tris buffered saline; PBS, phosphate buffered saline.
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Serum IgA and IgG anti-h-tTG antibodies (Valencia, California, USA). Denaturising conditions using IMAC (Qiagen, USA), expressed, and purified under non-denaturising conditions using IMAC (pET28b-Novagen, Madison, Wisconsin, USA) were coated with either 1:3000 phosphatase conjugated antihuman IgA and IgG immunoglobulin (Sigma A-3062, A-8542) diluted 1:2500 in PBS-0.3% Tween 20. The squares were then washed and the immunocomplexes were revealed by substrate solution. The strips were dried and examined, and the results expressed as a colorimetric reaction.

The immunological tests were performed by four operators (VB, AT, CT, IB) who were unaware of the clinical and laboratory findings of the subjects tested.

**HLA-DQ TYPING**

The known susceptibility alleles for CD were determined by polymerase chain reaction with allele specific primers identifying DQ2 and DQ8, carried out with a Dynal Classic SSP DQ kit (Dynal AS, Oslo, Norway).

**Results**

An example of the colorimetric reaction using our dot blot assay is shown in fig 1. Serum and whole blood samples from both CD patients and controls showed similar results with a clear discrimination between positives and negatives.

All 70 CD patients tested positive to both the dot blot test and the ELISA (taking into account the results of specific responses to IgG and IgA, sensitivity was 100%, specificity for ELISA IgA was 92.8%, and for ELISA IgG 47%). In contrast, the antiendomysial IgA test missed five patients (sensitivity 92.8%) either because of IgA deficiency (two patients) or the presence of IgG anti-h-tTG antibodies alone, which the IgA AEA test was unable to detect (three patients). Among the 64 healthy blood donors, 58 first degree relatives of CD patients and 74 diseased controls, three healthy blood donors tested positive by dot blot (specificity 98.4%) and ELISA (specificity for IgA 99.4%, for IgG 98.9%, for IgA and IgG combined 98.4%), predominantly due to the presence of anti-h-tTG IgG antibodies. In contrast, all controls were negative by AEA (table 1).

None of the three subjects who tested positive for anti-h-tTG antibodies by both dot
blist and ELISA had a clinical history or symptoms associated with CD (such as gastrointestinal complaints, unexplained anaemia, dental enamel defects, autoimmune disorders, or central nervous system involvement). We analysed HLA DQ2 and DQ8 of three healthy controls and only one had the DQA1 *0501 and DQB *0201 haplotypes. The blood donor positive for the CD related allele refused intestinal biopsy to confirm the diagnosis of CD, even after being informed of the possible risks involved in not treating the disease.

We extended our investigations to the small subset of subjects who had provided samples of whole blood as well as serum (table 2). Similar results were obtained: of 17 biopsy confirmed CD patients, all tested positive by h-tTG dot blot, while eight healthy blood donors, 22 disease controls, and 13 CD patients on a gluten free diet all tested negative. The dot blot test using whole blood performed better than the AEA test. Of the 17 patients with CD, the AEA assay identified only 14 subjects as two were IgA deficient and one tested positive only for anti-h-tTG IgG. As shown here and previously by others, some CD patients with normal levels of serum IgA class immunoglobulin lack IgA antibodies to tTG but have anti-tTG IgG antibodies. It is noteworthy that our rapid test identified those patients with serum IgA deficiency which is found more frequently in CD patients.

The test described here is quick and requires minimal handling; its cost is low (1 EURO per sample) and in view of its high sensitivity and specificity could easily be introduced into the general physician’s armoury of medical tests for patients complaining of vague symptoms of tiredness, as well as those with more classic gastroenterological symptoms. This is in line with a recent report that chronic tiredness, and not gastrointestinal symptoms, were the commonest presentation of CD, when this was considered as a possible diagnosis in a large number of patients tested using AEA serology.

In view of the risks accompanying prolonged untreated CD, which include autoimmune diseases, depression, multiple miscarriage, cerebellar ataxia, drug resistant epilepsy with occipital calcification, and intestinal lymphoma, and given the increased prevalence of CD among close relatives of CD patients and sufferers from autoimmune diseases, testing for CD should become a routine part of many clinical workups; the quick and easy test described here makes this a practical reality.

This work was supported by grant 31/98 from Istituto per l’Infanzia IRCCS “Burlo Garofolo” Trieste, Italy. We thank the nursing staff of the gastrointestinal investigation suite: Mrs S Ferrara, Mrs F Balsermin, and Mrs E Filferro.

Table 1  Dot blot results on serum samples

<table>
<thead>
<tr>
<th>Study group</th>
<th>Human tTG ELISA</th>
<th>IgA</th>
<th>IgG</th>
<th>IgA AEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 Patients with CD</td>
<td>70</td>
<td>0</td>
<td>65</td>
<td>2', 3'</td>
</tr>
<tr>
<td>64 Healthy controls</td>
<td>3</td>
<td>61</td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td>58 First degree CD relatives</td>
<td>0</td>
<td>58</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>64 Disease controls</td>
<td>0</td>
<td>64</td>
<td>0</td>
<td>64</td>
</tr>
<tr>
<td>10 Patients with Crohn's</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

1Subjects with IgA serum deficiency.
2Subjects with IgA antidiomysium negative tests but with positive human tTG IgG ELISA and dot blot.
3CD, coeliac disease; human tTG, human tissue transglutaminase.

Table 2  Dot blot results on whole blood samples

<table>
<thead>
<tr>
<th>Study group</th>
<th>Human tTG dot</th>
<th>IgA AEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 Patients with CD</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>13 Patients on GFD</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>22 Disease controls</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>8 Healthy controls</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

1Subjects with IgA serum deficiency.
2Subjects with IgA antidiomysium negative tests but with positive human tTG IgG ELISA and dot blot.
3CD, coeliac disease; human tTG, human tissue transglutaminase, GFD, gluten free diet.

Discussion

This simple dot blot test diagnosed all CD patients as effectively as the tTG ELISA and with greater sensitivity than the commonly used AEA test. This is likely to be due to the fact that while both tests detect tTG specific antibodies, AEA can only detect IgA, whereas the dot blot detects both IgA and IgG. As shown here and previously by others, some CD patients with normal levels of serum IgA class immunoglobulin lack IgA antibodies to tTG but have anti-tTG IgG antibodies. It is noteworthy that our rapid test identified those patients with serum IgA deficiency which is found more frequently in CD patients.

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