Diagnostic Efficacy of the ELISA Test for the Detection of Deamidated Anti-Gliadin Peptide Antibodies in the Diagnosis and Monitoring of Celiac Disease

Elio Tonutti,1* Daniela Visentini,1 Alessia Picierno,1 Nicola Bizzaro,2 Danilo Villalta,3 Renato Tozzoli,4 Graziano Kodermaz,4 Antonio Carroccio,5 Giuseppe Iacono,6 Saverio Teresi,7 Stella Maria La Chiusa,8 and Ignazio Brusca8
1Immunopatologia e Allergologia Azienda Ospedaliero-Universitaria S. Maria della Misericordia di Udine, Udine, Italy
2Laboratorio di Patologia Clinica, Ospedale di Tolmezzo, Udine, Italy
3Immunologia Clinica e Virologia, Azienda Ospedaliera S. Maria degli Angeli, Pordenone, Italy
4Laboratorio Analisi, Ospedale di Latisana, Udine, Italy
5Dipartimento di Medicina Interna, Università di Palermo, Palermo, Italy
6U.O. di Gastroenterologia A.R.N.A.S, Ospedale dei Bambini di Palermo, Palermo, Italy
7U.O. di Patologia Clinica A.R.N.A.S, Ospedale dei Bambini di Palermo, Palermo, Italy
8U.O. di Patologia Clinica, Ospedale Bucccheri la Ferla F.B.F, Palermo, Italy

Background and Aim: We evaluated the diagnostic performance of an ELISA test for anti-gliadin IgA and IgG antibodies, which uses synthetic deamidated gliadin peptides (anti-gliadin antibodies, AGAs) as coating; the results were compared with a test that uses extracted gliadin (AGAe).

Methods: The study was conducted on the sera of 144 patients suffering from celiac disease (CD), including 20 patients with IgA deficiency and 9 who were following a gluten-free diet (GFD), and 129 controls.

Results: In the 115 CD patients (without IgA deficiency), the sensitivity of AGAe IgA and IgG was 32.2 and 60.9%, whereas that of AGAs IgA and IgG was 59.1 and 72.2%. The specificity for AGAe IgA and IgG, and AGAs IgA and IgG was 93.8 and 89.9%, and 96.9% and 99.2%, respectively. Of the 20 patients with CD and IgA deficiency, 7 tested positive for AGAe IgG and 14 for AGAs IgG. The test using deamidated gliadin peptides performed better in terms of sensitivity and specificity than the AGA tests with extracted antigen.

Conclusions: The very high specificity of the AGAs IgG test (99.2%) also suggests that patients who test positive with this assay require a thorough followup, even if the anti-tissue transglutaminase antibodies (anti-tTG) and anti-endomysial autoantibodies (EMA) assays are negative. J. Clin. Lab. Anal. 23:165–171, 2009.

Key words: celiac disease; deamidated gliadin peptides; anti-gliadin antibodies; anti-transglutaminase antibodies

INTRODUCTION

Gluten is a complex protein constituted by the gliadins and glutenins present in wheat, barley, and rye. It is the trigger factor of celiac disease (CD), as it triggers an immune response by the T lymphocytes with subsequent production of cytokines, anti-gliadin antibodies (AGAs) and anti-tissue transglutaminase antibodies (anti-tTG) (1–4). This cascade of events requires some predisposing situations, namely (in order of importance): intake of gluten with the diet, increased permeability of the intestinal mucosa, a predisposing genetic makeup characterized by the presence of HLA molecules of class II DQ2 or DQ8 (5–7). The tTG

*Correspondence to: Elio Tonutti, Immunopatologia e Allergologia Azienda Ospedaliero-Universitaria S. Maria della Misericordia di Udine, P.zza S. Maria della Misericordia, 33100 Udine, Italy. E-mail: tonutti.elio@aoud.sanita.fvg.it
Received 3 December 2008; Accepted 5 February 2009
DOI 10.1002/jcla.20313
Published online in Wiley InterScience (www.interscience.wiley.com).

© 2009 Wiley-Liss, Inc.
enzyme is a calcium-dependent enzyme able to deamidate the glutamine residues in which gliadin is rich, forming glutamic acid residues, which are very efficient in promoting bonding of the modified peptides with the HLA class II DQ2/DQ8 complex (8). The complex formed by deamidated gliadin peptides, tTG and DQ2/ DQ8 is recognized by specific intestinal T cells. The end result of this immune response to gluten is reversible damage to the enterocytes, with flattening of the intestinal villi and consequent malabsorption (9–11). A diagnosis of CD is based on the typical signs and symptoms associated with the presence of specific anti-tTG or anti-endomysial autoantibodies (EMA) (12–14). AGA testing currently has little diagnostic significance, because the methods used to identify them present lower levels of sensitivity and specificity than tests for anti-tTG or EMA (15,16). However, AGA testing is still considered useful in patients under 5 years old who test negative for anti-tTG (17).

The peptide regions of gliadin recognized by the T lymphocytes of genetically predisposed individuals were recently identified (2). These peptides have a relatively constant aminoacid pattern consisting of the sequence QPEQPFP, where the triplet PEQ is the epitopic core. Some studies demonstrate that immunoenzyme tests (ELISA) that use the sequence QPEQPFP as antigen have greater sensitivity and specificity than ELISA tests with extracted gliadin antigen (18,19).

The purpose of our study was to evaluate the diagnostic accuracy of a new commercial ELISA test for the identification of AGA IgA and IgG, which uses synthetic deamidated gliadin peptides as antigen, and to compare the results with those obtained with tests using extracted gliadin, and with ELISA and indirect immunofluorescence tests for the determination of anti-tTG and EMA, respectively. We used a large number of patients with a histological diagnosis of CD for the study, together with controls.

MATERIAL AND METHODS

Patients

A total of 273 sera were tested: 144 from patients with a diagnosis of CD, confirmed by a duodenal biopsy in all cases, and 129 subjects not suffering from CD as control group.

Of the 144 patients with CD, 115 were newly diagnosed, 70 of whom were aged 5 years or over (range, 5–72 years; 32 males and 48 females) and 45 aged under 5 years (range, 0–4 years; 22 males and 32 females); 20 patients had an absolute IgA deficiency (range, 4–38 years; 8 males and 12 females) and 9 were celiac patients who had been following a gluten-free diet (GFD) for 4–17 months (range, 9–46 years; 2 males and 7 females).

The 129 subjects in the control group consisted of 60 healthy blood donors (range 18–52 years; 36 males and 24 females) and of 69 patients affected by different clinical conditions: 16 lactose intolerance, 24 hepatic cirrhosis, 15 Crohn’s disease, and 14 ulcerative colitis (range 2–71 years; 29 males and 40 females).

The patients and controls were selected in 2004–2007 by the Immunopathology and Allergology Department of Udine Hospital, the Clinical Immunology Department of Pordenone Hospital, the Gastroenterology Department of the Palermo Children’s Hospital, and the Clinical Pathology Laboratory of S. Dona’ di Piave Hospital. The sera were frozen at −80°C and thawed only once for the performance of tests for specific AGA antibodies.

Antibody Assays

In all the sera (CD and controls) the test for AGA IgA and IgG was conducted with an ELISA test using gliadin of extracted origin (AGAe) (Quanta-Lite Gliadin IgA I and IgG I, INOVA Diagnostics, Inc., San Diego, CA) and with an ELISA test using synthetic deamidated gliadin peptides (AGAs) containing the sequence PEQ (Quanta-Lite Gliadin IgA II and IgG II, INOVA). Both tests were conducted in accordance with the manufacturer’s instructions. The same sera were also tested for anti-tTG IgA with ELISA using recombinant human antigen (Orgentec Diagnostika, Mainz, Germany); the EMA IgA test was conducted by the indirect immunofluorescence method on monkey esophagus (INOVA), and the total IgA assay was conducted by the nephelometric method (Dade Behring, Marburg, Germany). Sera with an absolute IgA deficiency (IgA <0.5 g/L) were tested for anti-tTG antibodies in the IgG class (Orgentec; normal value <10 AU).

Statistics

The sensitivity and specificity of the assays for AGAe and AGAs in the IgA and IgG classes were first calculated using the manufacturers’ cutoffs, and then using cutoffs corresponding to a specificity of 95%, as obtained by receiver operating characteristic (ROC) curves. When the ROC curves for AGAe and AGAs were conducted, the 9 patients with CD on a diet and the 20 patients with IgA deficiency were excluded. The area under the curve (AUC), with a 95% confidence interval (95%CI), was also calculated for each kit. Statistical analysis was performed using the SPSS 11.0 for Windows statistical package (SPSS, Chicago, IL). Two-sided P values of less than 0.05 were considered significant throughout.
RESULTS

**Anti-tTG and EMA Assays**

The 70 patients with CD and an age of ≥5 years all tested anti-tTG IgA positive (range 9–4200 AU) and EMA IgA positive (range 1:5–1:5120). Of the 45 patients with CD aged <5 years, 37 tested anti-tTG IgA positive (range 8–200 AU) and EMA IgA positive (range 1:5–1:2560), whereas 8 tested anti-tTG and EMA IgA negative. Of the 20 CD patients with an IgA deficiency, 19 tested anti-tTG IgG positive (range 15–4200 AU) and 1 tested anti-tTG IgG negative. The 9 patients on a GFD tested anti-tTG IgA positive in four cases (range 8–11 AU) and negative in five cases.

All 129 control sera tested negative for anti-tTG IgA (Table 1).

**AGA Assays**

At the manufacturer’s cutoff, in the 70 patients with CD ≥5 years the sensitivity of AGAe IgA and IgG was 45.7 and 64.3% and that of AGAs IgA and IgG was 70 and 78.6%. In the 45 patients aged <5 years, the sensitivity of AGAe IgA and IgG was 11.1 and 55.6%, and that of AGAs IgA and IgG was 44.4 and 62.2% (Table 1). On the whole, in the 115 patients with newly diagnosed CD without an IgA deficiency and not on a GFD, the sensitivity of AGAe IgA and IgG was 32.2 and 60.9% and that of AGAs IgA and IgG was 59.1 and 72.2%.

Of the 20 patients with an IgA deficiency, 7 (35%) tested positive for AGAe IgG and 14 (70%) for AGAs IgG.

Of the 9 celiac patients on a GFD, none tested positive for AGAe IgA and 2 (22%) tested positive for AGAe IgG, while 3 (33%) tested positive for AGAs IgA and 3 (33%) for AGAs IgG (one positive serum for each antibody class).

The results of the AGAe and AGAs tests in the control groups showed negligible percentages of false positives with the manufacturer’s cutoff (20 AU), with two exceptions: of the 24 patients with cirrhosis of the liver, 4 (16.6%) tested positive for AGAe IgA, and of the 16 patients with intolerance of cow’s milk, 11 (68.7%) tested positive for AGAe IgG with a high titer (Table 2).

The overall specificity evaluated in the control group was 93.8 and 89.9% for AGAe IgA and IgG and 96.9 and 99.2% for AGAs IgA and IgG (Table 1).

Analysis of the ROC curves showed an area under the curve (AUC) of 0.812 and 0.849 for AGAe IgA and IgG and 0.928 and 0.945 for AGAs IgA and IgG (both differences were statistically significant: \( P = 0.04 \) for IgA and \( P = 0.03 \) for IgG). The cutoffs corresponding to 95% specificity were 23.3 and 66.2 AU, respectively for AGAe IgA and IgG, and 14.8 and 9.6 AU for AGAs IgA and IgG.

If the sensitivity data between the manufacturer’s cutoff and a cutoff with predefined specificity of 95% are compared, the AGAs tests are clearly superior to the AGAe tests in the 115 newly diagnosed patients, and in the 20 cases with an IgA deficiency (only for the class IgG tests) and the 9 patients following a diet (Table 3).

In the 14 anti-tTG negative patients (8 with a diagnosis <5 years, 5 on a diet, and 1 patient with an IgA deficiency) a significant difference in sensitivity was observed between the IgA AGAe and IgG AGAs tests (0 and 4 positive cases, respectively), whereas for the IgG class tests, the sensitivity did not vary significantly;

**TABLE 1. Results of the Test for Anti-Gliadin Antibodies IgA and IgG with extracted antigen (AGAe) and With Synthetic Peptide Antigen (AGAs) in Patients Suffering From Celiac Disease and Controls**

<table>
<thead>
<tr>
<th></th>
<th>Anti-tTG pos</th>
<th>Anti-tTG neg</th>
<th>IgA AGAe N (%)</th>
<th>IgG AGAe N (%)</th>
<th>IgA AGAs N (%)</th>
<th>IgG AGAs N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Celiac patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5 years</td>
<td>70</td>
<td>70</td>
<td>0</td>
<td>32 (46%)</td>
<td>45 (64%)</td>
<td>49 (70%)</td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>45</td>
<td>37</td>
<td>8</td>
<td>5 (11%)</td>
<td>25 (56%)</td>
<td>20 (44%)</td>
</tr>
<tr>
<td>IgA deficient</td>
<td>20</td>
<td>*19</td>
<td>1</td>
<td>ND</td>
<td>7 (35%)</td>
<td>ND</td>
</tr>
<tr>
<td>On gluten free diet</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>2 (22%)</td>
<td>3 (33%)</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>130</td>
<td>14</td>
<td>37 (26%)</td>
<td>79 (55%)</td>
<td>72 (50%)</td>
</tr>
<tr>
<td>(B) Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>60</td>
<td>0</td>
<td>60</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Milk intolerance children</td>
<td>16</td>
<td>0</td>
<td>16</td>
<td>2 (12%)</td>
<td>11 (69%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Hepatic cirrhosis</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td>4 (17%)</td>
<td>0</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>IBD (15 Crohn’s disease, 14 UC)</td>
<td>29</td>
<td>0</td>
<td>29</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td>129</td>
<td>8</td>
<td>8 (6%)</td>
<td>13 (10%)</td>
<td>4 (3%)</td>
</tr>
</tbody>
</table>

The cutoff considered was that recommended by the manufacturer (20 AU for the four tests).

*Patients with an IgA deficiency were evaluated with the anti-tTG IgG test. IBD: inflammatory bowel disease UC: ulcerative colitis.

a substantial increase in sensitivity emerged for AGAs IgG with the cutoff obtained from ROC curves (Table 4).

**DISCUSSION**

The excellent diagnostic performance of tests for anti-tTG and EMA antibodies has enabled the hidden part of the celiac iceberg to be identified in recent years (20,21). The current prevalence of CD in the Western countries is estimated at between 1:80 and 1:100 (16,20–24). Identification of anti-tTG and EMA antibodies as a marker for CD has caused AGA to lose the diagnostic role they had held since the early 80s (25). Today, the clinical usefulness of AGA is restricted to specific diagnostic ambits: in the early years of life (possible negativity for anti-tTG), in patients with an IgA deficiency (determination of AGA IgG in association with anti-tTG IgG), and in all cases in which anti-tTG or EMA antibodies do not give clear results (i.e., borderline values) (26,27). However, the use of the quantitative ELISA assay to test for AGA IgA and IgG is still very widespread. Statistics supplied by the European Diagnostic Manufacturers’ Association demonstrate that expenditure on the purchase of AGA tests in 2006 was only slightly less than the total expenditure on EMA and anti-tTG tests. There are several reasons for this: little knowledge of the greater diagnostic accuracy of the new tests, inappropriate requests that involve the performance of AGA in association with anti-tTG and EMA tests, and administrative aspects (reimbursement of the tests). The persistence of demand for AGA tests has led some manufacturers to develop new AGA ELISA tests that use deamidated gliadin peptides as antigen. Some studies have demonstrated that the immune response to gliadin in CD patients is directed against limited portions of the protein structure, and that the epitopic core of these sequences is constituted by the tripeptide PEQ (28,29). The aim of our study was to compare the diagnostic performance of a new ELISA test using synthetic gliadin peptides and an ELISA test using extracted gliadin for assaying AGA IgA and IgG in a group of celiac patients and controls with different clinical and serological characteristics.

The specificity evaluation, conducted on a heterogeneous population of normal subjects and patients with disorders of the gastroenteric tract other than CD, demonstrates that AGAs tests are far more specific (IgA 96.9%, IgG 99.2%) than the analogous AGAe tests (IgA 93.8%, IgG 89.9%). In particular, the AGAe IgG test gave false positives with high titers in 11 out of 16 cases in pediatric patients with milk intolerance, whereas only one of these patients tested AGAs IgG positive. This aspect is important for two reasons, firstly because it demonstrates that patients with intestinal disorders other than CD can synthesize high titers of AGA whose epitopic targets are different from those of the AGA of CD patients. The second aspect is clinically significant because the use of AGA IgG is recommended specifically in pediatric patients where a negative anti-tTG IgA test may not rule out CD.

As regards sensitivity, our study demonstrates the clear superiority of AGAs over AGAe in all categories of CD patients studied. In particular, the excellent sensitivity of the AGAs IgG test should be noted; with the cutoff at 9.6 AU (determined on the basis of the ROC curves with 95% specificity) the test was positive.
### TABLE 3. Number and Percentage of Positive Cases for Extracted AGA IgA and IgG (AGAe) and AGA Synthetic Peptide (AGAs) Tests, at the Manufacturer’s Cutoff and at a Cutoff Corresponding to 95% Specificity

<table>
<thead>
<tr>
<th>Celiac patients</th>
<th>No.</th>
<th>IgA AGAe Cutoff 20 AU spec 93.8%</th>
<th>Cutoff 23.3 AU spec 95%</th>
<th>IgG AGAe Cutoff 20 AU spec 89.9%</th>
<th>Cutoff 66.2 AU spec 95%</th>
<th>IgA AGAs Cutoff 20 AU spec 96.9%</th>
<th>Cutoff 14.8 AU spec 95%</th>
<th>IgG AGAs Cutoff 20 AU spec 99.2%</th>
<th>Cutoff 9.6 AU spec 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 5 years</td>
<td>70</td>
<td>32 (45.7%)</td>
<td>29 (41.4%)</td>
<td>45 (64.3%)</td>
<td>16 (22.8%)</td>
<td>49 (70%)</td>
<td>60 (85.7%)</td>
<td>55 (78.6%)</td>
<td>66 (94.3%)</td>
</tr>
<tr>
<td>&lt; 5 years</td>
<td>45</td>
<td>5 (11.1%)</td>
<td>5 (11.1%)</td>
<td>25 (55.6%)</td>
<td>13 (28.9%)</td>
<td>20 (44.4%)</td>
<td>24 (53.3%)</td>
<td>28 (62.2%)</td>
<td>36 (80%)</td>
</tr>
<tr>
<td>IgA deficient</td>
<td>20</td>
<td>N.D</td>
<td>ND</td>
<td>7 (35%)</td>
<td>4 (20%)</td>
<td>ND</td>
<td>ND</td>
<td>14 (70%)</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>On gluten free diet</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>2 (22.2%)</td>
<td>0</td>
<td>3 (33.3%)</td>
<td>3 (33.3%)</td>
<td>3 (33.3%)</td>
<td>7 (77.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>37 (29.8%)</td>
<td>34 (27.4%)</td>
<td>79 (54.9%)</td>
<td>33 (22.9%)</td>
<td>72 (58.1%)</td>
<td>87 (70.2%)</td>
<td>100 (69.4%)</td>
<td>125 (87.7%)</td>
</tr>
</tbody>
</table>

The data are shown both at the manufacturer’s cutoff and at a cutoff corresponding to 95% specificity, as determined by ROC curves.

### TABLE 4. Positive Results Obtained by Extracted AGA IgA and IgG (AGAe) and AGA Synthetic Peptides (AGAs) in Anti-tTG-Negative CD Patients

<table>
<thead>
<tr>
<th>Anti-tTG negative CD patients</th>
<th>No.</th>
<th>IgA AGAe Cutoff 20 AU spec 93.8%</th>
<th>Cutoff 23.3 AU spec 95%</th>
<th>IgG AGAe Cutoff 20 AU spec 89.9%</th>
<th>Cutoff 66.2 AU spec 95%</th>
<th>IgA AGAs Cutoff 20 AU spec 96.9%</th>
<th>Cutoff 14.8 AU spec 95%</th>
<th>IgG AGAs Cutoff 20 AU spec 99.2%</th>
<th>Cutoff 9.6 AU spec 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5 years</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>IgA deficient</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>On gluten free diet</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

The data are shown both at the manufacturer’s cutoff and at a cutoff corresponding to 95% specificity, as determined by ROC curves.
in 94.3% of the 70 CD patients aged >5 years and in 80% of the 45 CD patients aged <5 years (8 of these were anti-tTG IgA negative).

An important factor is the role of AGA class IgG in patients with an IgA deficiency. In the 20 patients with an IgA deficiency, the AGAs IgG test was positive in 70% of cases as against 35% for AGAe IgG. Patients with an IgA deficiency (total IgA <0.5 mg/L) are known to have a significantly higher risk than the normal population of developing CD and, in the absence of IgA, it is necessary to test for class IgG antibodies. However, recent studies report unfavorable data relating to the performance of commercial anti-tTG IgG tests in terms of both sensitivity and specificity (21). The possibility of combining the anti-tTG IgG test with a sensitive, specific AGA IgG test may therefore prove very useful in diagnostic practice (30). The diagnostic usefulness of the AGAs IgA and IgG tests was demonstrated in the group of 14 CD patients who tested anti-tTG IgA or anti-tTG IgG negative. These data confirm that the AGA test may have a diagnostic role in all cases in which there is a valid clinical suspicion of CD, but the anti-tTG or EMA tests are negative. Our series of CD patients following a GFD includes too few patients to yield definite conclusions. Nevertheless, we have observed in these patients a greater sensitivity of AGAs than AGAe. Positivity with a low titer of AGAs could suggest that the diet should be reassessed, even if the anti-tTG test is negative: the introduction of small quantities of gluten into the diet of celiac patients on a GFD could damage the intestinal mucosa, and be associated with positivity for AGA but not for anti-tTG (31).

To sum up, our study confirms the preliminary data published by other authors, in which it is evident that AGA tests using deamidated gliadin peptides give excellent results in the diagnosis of CD patients (18,19,32). Our very heterogeneous case study demonstrates the particular diagnostic efficiency of the AGAs class IgG tests. In view of its performance in all groups of patients, AGAs IgG demonstrates high sensitivity, suggesting its use in routine diagnostic practice in all cases in which anti-tTG tests are inconclusive. The very high specificity of the test (99.2%) also suggests that a thorough followup is necessary if the values are even slightly above the cutoff, even if the other markers are negative, because the patient may develop CD. This test could therefore be used together with other laboratory tests to increase the diagnostic sensitivity of tests for CD, bearing in mind that in the coming years, cases of CD presenting a typical picture from the clinical and serological standpoint will decline as a result of the greater attention paid to this disorder by pediatricians, gastroenterologists, and general practitioners, whereas cases with atypical or mild symptoms will increase. Pathologists will have to interpret more and more often anti-tTG/EMA tests that are negative or present a low titer in patients with vague clinical symptoms, and will consequently require additional tests that either support the diagnosis or rule out the disease.

REFERENCES
20. Lock RJ, Gilmour JE, Unsworth DJ. Anti-tissue transglutaminate, anti-endomysium and anti-R1-reticulin autoantibodies: The