Assessment of a Test for the Screening and Diagnosis of Celiac Disease

Brunetta Porcelli,^{1*} Fabio Ferretti,² Carla Vindigni,³ and Lucia Terzuoli¹

¹Department of Medical Biotechnologies, Biochemistry Division, University of Siena, Siena, Italy

²Department of Medical and Surgical Sciences and Neuroscience, University of Siena, Siena, Italy

³Oncological Department, Pathological Anatomy Division, Siena University Hospital, Siena, Italy

Background: Celiac disease (CD) is an immune-mediated intolerance to dietary gluten, affecting genetically predisposed individuals. ELISA-based serological tests help to decide if further duodenal biopsy is necessary, for this the diagnostic kits have to be accurate, specific, and sensible. In this study, we investigate the performance of an ELISA that uses the purified cross-linked complex of tissue transglutaminase and gliadin, referred as the “neopeptide” (AESKULISA® tTG New Generation), as antigen. **Methods:** We evaluated 41 newly diagnosed celiac patients, 18 celiac patients on gluten-free diet, and 169 controls, comprising healthy subjects, patients affected by other au-

toimmune diseases, and patients affected by several non-autoimmune diseases.

Results and Conclusion: The assay has an excellent performance. Due to its high level of diagnostic accuracy, this assay constitutes a new approach for the screening of celiac patients not only for the diagnosis of CD, but also for monitoring patients on gluten-free diet and their compliance. Moreover, cases of neopeptide-positive subjects who were tested negative with “classical” serological markers could have a predictive value for this pathology. This aspect will require further studies of elaboration. *J. Clin. Lab. Anal.* 30:65–70, 2016. © 2014 Wiley Periodicals, Inc.

Key words: celiac disease; antitissue transglutaminase antibodies; antideamidated gliadin peptides antibodies; anti-cross-linked complex antibodies; neopeptide

INTRODUCTION

Celiac disease (CD) is a syndrome characterized by the damage of the small intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamines) of barley and rye in genetically susceptible subjects (1).

Gliadin is only partially digested in the intestine, leading to proteolysis-resistant peptides (2) that can be transformed by tissue transglutaminase (tTG). This enzyme modifies proteins and peptides by deamidation or transamidation of specific glutamine residues. Deamidation leads to the formation of deamidated gliadin peptides (DGPs) that have a stronger binding to the MHC II molecule resulting in an increased immune response. Transamidation occurs at a higher rate than deamidation (3–5). Thereby, tTG is covalently linked to gliadin peptides leading to complex formation. These gliadin peptide/transglutaminase complexes have been found in vivo in small intestine biopsies of celiac patients (6). The complete pathomechanism of CD and in particular the devel-

opment of autoantibodies against tTG is not completely understood yet. Formation of complexes between gliadin peptides and tTG and their further processing by antigen-presenting cells supports the hypothesis of epitope spreading from neopeptides to gliadin peptides and tTG (7–9).

Widely available serological tests used for detecting CD include detection of anti-tTG antibodies (a-tTG), antien-domysial antibodies (EMA), and antigliadin antibodies (AGA).

Currently, detection of IgA a-tTG is accepted as the first choice test, displaying the highest level of sensitivity (up to 98%) with excellent reproducibility.

*Correspondence to: Brunetta Porcelli, Department of Medical Biotechnologies, Biochemistry Division, Polo Scientifico Universitario di San Miniato, Via Alcide De Gasperi 2, 53100 Siena, Italy. E-mail: brunetta.porcelli@unisi.it

Received 9 November 2013; Accepted 25 August 2014

DOI 10.1002/jcla.21816

Published online in Wiley Online Library (wileyonlinelibrary.com).

IgA EMA, detected by immunofluorescence on sections of monkey esophagus, recognizes the same antigen as a-tTG. The EMA test is highly specific (~100%), but less sensitive than IgA a-tTG (93–96%). Therefore, EMA testing should be used preferably in a-tTG-positive cases as a confirmatory test prior to an intestinal biopsy (10).

Recently, antibodies to gliadin lost in their diagnostic value (perceived performance) because they are neither sensitive nor specific and can also be found in healthy individuals and in patients with other intestinal disorders. With the exception of pediatric patients, increased sensitivity and specificity of a-tTG provide a great improvement in the diagnosis of CD compared to the previously available gliadin testing, and utility of the latter in the diagnosis of CD has been challenged (8). IgG anti-tTG antibodies can only be used as a specific marker in patients with an IgA deficiency, whose risk of developing CD is 10–20 times higher compared to the normal population (11).

Specific ELISA tests for IgA and IgG antibodies against DGPs (a-DGP) show very promising data as second-generation AGA assays (12–19).

An ELISA that incorporates the latest research and utilizes as antigen the purified cross-linked complex, referred as the “neoepitope” (20), has been developed. The neoepitope mimics the physiological antigen and detects antibodies to the cross-linked complex (neoepitope).

In this study, we investigate the performance of this assay in diagnosed CD patients and in a control group composed of healthy subjects, patients affected by other autoimmune diseases, and affected by several non-autoimmune diseases.

MATERIALS AND METHODS

In our study, we included 41 recently diagnosed CD patients: 31 adults (7 males, aged between 19 and 59; 24 females, aged between 18 and 77) and 10 children (3 males, aged between 6 and 9; 7 females, aged between 3 and 13). In addition, sera of 18 previously diagnosed CD patients on gluten-free diet for 8–24 months were included: 8 adults (1 male, aged 37; 7 females, aged between 18 and 42) and 10 children (3 males, aged between 4 and 11; 7 females, aged between 3 and 16). The diagnosis of CD was based on histological and serological criteria, including positive serology tests (a-tTG, EMA).

Intestinal biopsies were performed in the same period as CD serological tests and were classified according to a modified version of Marsh’s classification (21) (Table 1. Examination of all biopsies was performed by the same blinded operator.

We enrolled 169 subjects (60 males, age range: 2–75; 109 females, age range: 3–85 years) as a control population: 24 blood donors as normal controls and 145 disease con-

TABLE 1. Histological Characteristics of Patients at the Time of Diagnosis

Histological characteristics of CD patients	n = 41
Type 3c	26
Type 3b	11
Type 3 a	4
Histological characteristics of CD patients on gluten-free diet	n = 18
Type 3c	9
Type 3b	7
Type 3a	1
Type 1	1

trols: 15 patients with autoimmune hepatopathies positive for anti-smooth muscle (SMA) autoantibodies and/or IgG anti-F-actin autoantibodies; 12 patients with hepatitis/cirrhosis; 35 patients with viral hepatitis/cirrhosis; 83 patients with other gastrointestinal diseases: irritable bowel syndrome (15 patients), gastroesophageal reflux (10 patients), ulcerative colitis (10 patients), cow’s milk and food allergy (18 patients), Crohn’s disease (12 patients), unspecific colitis (8 patients), nonulcerative dyspepsia syndrome (10 patients). Histological diagnosis of only 51 control subjects was also known: 33 biopsies were normal, while 18 showed grade 1 of the Marsh’s classification (21).

In all cases, serum samples were tested using the following:

- IgA a-tTG: ELISA QUANTA Lite™, h-tTG IgA, INOVA Diagnostisc Inc. (San Diego, CA)
- IgG a-tTG: ELISA QUANTA Lite™ h-tTG IgG, INOVA Diagnostics Inc.
- IgA a-DGP: ELISA QUANTA Lite™ Gliadin IgA II, INOVA Diagnostic Inc.
- IgG a-DGP: ELISA QUANTA Lite™ Gliadin IgG II, INOVA Diagnostic Inc.
- EMA: IgA anti-endomysial antibodies (Eurospital, Trieste, Italy).
- tTG-A Neo: AESKULISA® tTG-A New Generation, AESKU Diagnostics (Wendelsheim, Germany).
- tTG-G Neo: Antibodies AESKULISA® tTG-G New Generation AESKU Diagnostics.

The AESKULISA® tTG New Generation is a solid-phase enzyme immunoassay coated with purified neoepitopes resulting from cross-linking between human recombinant tTG and gliadin-specific peptides, which ensures significantly increased sensitivity and specificity of the test.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under the receiver operating characteristic curve (ROC Curve) were calculated for each assay. For INOVA Diagnostics tests,

TABLE 2. Number (Number of Positive Subjects/Number of Total Subjects) and Percentage (%) of Positive Subjects for all Analyzed Parameters

	a-tTG IgA	a-tTG IgG	DGP IgA	DGP IgG	EMA IgA	tTG-A Neo IgA			tTG-G Neo IgG		
Cut-off (U/ml)	>20	>20	>20	>20		>15	>18	>20	>15	>18	>20
CD patients	41/41	32/41	37/41	36/41	41/41	41/41	41/41	41/41	36/41	35/41	33/41
	100%	78%	90%	88%	100%	100%	100%	100%	88%	85%	80%
CD patients on treatment with the gluten free-diet	11/18	10/18	5/18	6/18	9/18	13/18	11/18	11/18	7/18	7/18	7/18
	61%	56%	28%	33%	50%	72%	61%	61%	39%	39%	39%
Disease controls	2/145	29/145	5/145	7/145	0/145	11/145	9/145	5/145	16/145	12/145	12/145
	1%	20%	3%	5%	0%	7%	6%	3%	11%	8%	8%
Normal controls	0/24	1/24	0/24	0/24	0/24	0/24	0/24	0/24	1/24	1/24	1/24
	0%	4%	0%	0%	0%	0%	0%	0%	4%	4%	4%
κ^a	0.89	0.85	0.95	0.95	0.96	0.94	0.95	0.96	0.89	0.94	0.94

In addition, the weighted κ is given for each parameter as a reliability indicator with respect to the diagnoses.

^aA weighted κ for a multiparameter contingency table can be estimated according to Fleiss et al. (24).

we used the cut-off recommended by the manufacturer (for all parameters >20 U/ml). For the *AESKULISA*® tTg New Generation assays, we used besides the recommended cut-off of 15 U/ml, cut-offs of 18 and 20 U/ml. Ninety-five percent confidence intervals were computed for sensitivity, specificity, PPV, and NPV.

For this type of study, usually a “gold standard” reference method for the assessment of case status is included. However, in this case, the EMA was used as “gold standard,” which is not to be considered as an ideal test (22), although additional biopsies validate the diagnosis. Furthermore, EMA presence might be associated with a different interpretation compared to other listed antibodies, or the detection of other autoantibodies might be independent of celiac diagnosis. To address this problem, we tabulated Cohen’s kappa (κ) coefficient (23) as a measure of reliability (or the so-called “inter observer agreement”) with regard to EMA presence. A weighted κ for a multiparameter contingency table can be estimated according to Fleiss et al. (24). The κ value was considered <0.2 inadequate; 0.21–0.40 mediocre; 0.41–0.60 medium; 0.61–0.80 good; 0.81–1 very good agreement grade.

RESULTS

Table 2 reports the number (expressed as a fraction and as a percentage) of samples that were positive for each

parameter analyzed in CD patients and in the control group. Both with cut-off >18 and cut-off >20, tTG-A Neo was positive in all the patients with a diagnosis of CD and in 11 of 18 patients on gluten-free diet (6 adults and 5 children, of which all adults and 4 children also showed positivity for at least one of the other parameters, while one child showed exclusively a weak positivity (23.7 U/ml) for tTG-A Neo. With a cut-off > 18 U/ml, tTG-A Neo was positive in 9 of the 145 disease controls, of which 4 patients also showed positivity for at least one of the other “classical” parameters, and 5 patients showed only a weak positivity for tTG-A Neo, between 18.3 and 38.8 U/ml. When the cut-off was set to >20 U/ml, tTG-A Neo was positive in 5 of the 145 disease controls, of which 3 patients showed positivity for tTG-A Neo and for at least one of the other “classical” parameters, while 2 patients showed a weak positivity only for tTG-A Neo, with values of 31 and 38.8 U/ml (Table 3).

tTG-G Neo was positive in 36 of 41 patients with a diagnosis of CD when cut-off was set at >15 U/ml; in 35 of 41 patients with cut-off >18 U/ml, and in 33 of 41 with cut-off >20 U/ml. tTG-G Neo was also positive in 7 of 18 patients on gluten-free diet (3 adults and 4 children), which were all also positive to the other classical parameters and in 13 subjects in the control population (both with cut-off >18 and cut-off >20). Twelve of these were disease controls (out of 145) and one was a normal control (out

TABLE 3. Characteristics of Control Population Subjects Who Showed Only Positivity for tTG-A Neo

Age (years)	Biopsy	EMA IgA	a-tTG IgA	a-DGP IgA	tTG-A Neo	Diagnosis
36	Normal	Neg	14.80	19.20	19.00	Alcoholic cirrhosis
72	No biopsy	Neg	8.00	12.24	18.30	HCV chronic hepatitis
59	No biopsy	Neg	10.00	16.38	31.00	HCV acute hepatitis
29	Normal	Neg	4.70	8.81	19.00	Unspecific colitis
18	Normal	Neg	12.70	14.98	38.80	Irritable bowel syndrome
11	No biopsy	Neg	9.00	4.1	23.7	Gastroesophageal reflux

TABLE 4. Characteristics of Control Population Subjects Who Showed Only Positivity for tTG-G Neo

Age (years)	Biopsy	a-tTG IgG	a-DGP IgG	tTG-G Neo	Diagnosis
2	No biopsy	13.60	15.28	50.60	Gastroesophageal reflux
3	No biopsy	19.00	15.18	33.70	Gastroesophageal reflux
5	No biopsy	15.00	8.82	31.90	Gastroesophageal reflux
6	No biopsy	19.30	16.34	22.50	Irritable bowel syndrome
9	No biopsy	17.60	15.28	26.5	Irritable bowel syndrome
8	No biopsy	17.80	15.01	74.1	Unspecific colitis
11	No biopsy	13.10	11.00	44.3	Gastroesophageal reflux
30	Normal	19.30	13.14	39.30	Healthy subject

of 24). Five of the disease controls also showed positivity for at least one of the other “classical” parameters. Seven disease controls and the normal control showed only positivity for tTG-A Neo, between 22.5 and 74.1 U/ml (Table 4).

Table 5 reports the number (and percentage) of subjects who were positive for each parameter analyzed in the subgroup of disease controls. In the group of hepatitis/cirrhosis patients, two of them showed a weak positivity (19 U/ml) for the tTG-A Neo (cut-off > 15 and 18 U/ml), with a weak positivity also for the other “classical” parameters only in one patient, while with cut-off >20 U/ml no positivity was observed. In the subgroup with viral hepatitis/cirrhosis only two patients showed a weak positivity for the tTG-A Neo with cut-off >15 and 18 U/ml, while only one patient showed positivity for the tTG-A Neo with cut-off >20 U/ml (31 U/ml). Of the patients with other gastrointestinal diseases, tTG-A Neo was positive in five subjects, of which three also showed positivity for at least one other “classical” parameter and two showed only a weak positivity for tTG-A Neo. With cut-off >20 U/ml, tTG-A Neo was positive in four subjects, of which three also showed positivity for at least one other “classical” parameter, while one showed only positivity for tTG-A Neo (38.8 U/ml).

Of the patients with other gastrointestinal diseases, tTG-G Neo assay was positive for all cut-offs in 12 subjects, of which 5 also showed positivity for at least one other “classical” parameter, and 7 were positive only for tTG-G Neo.

Table 6 shows the sensitivity, specificity, PPV, and NPV of the tests. The diagnostic sensitivity of the tTG-A Neo was 100% in CD patients for all cut-offs used. The specificity was also high and ranged between 93.59% and 97.04% depending on the cut-off. The diagnostic sensitivity of the tTG-G Neo ranged between 87.80% and 80.49%. This is comparable to, or higher than, those of the diagnostic IgG markers analyzed (IgG a-tTG 78.05%, IgG a-DGP 87.80%). The specificity ranged between 89.94% and 92.31%.

DISCUSSION

In this article, we have evaluated the analytical performance of ELISAs for CD that utilize the neoepitope antigen, *AESKULISA*® tTG-A New Generation and *AESKULISA*® tTG-G New Generation. In order to evaluate the performance of the tTG-A Neo and tTG-G Neo assays in terms of sensitivity and specificity, we selected well-characterized populations of CD patients and

TABLE 5. Number (Number of Positive Subjects/Number of Total Subjects) and Percentage (%) of Positive Subjects for Each Parameter Analyzed in the Subgroup of Disease Controls

	a-tTG IgA	a-tTG IgG	DGP IgA	DGP IgG	EMA IgA	tTG-A Neo IgA	tTG-G Neo IgG
Cut-off (U/ml)	>20	>20	>20	>20		>15 >18 >20	>15 >18 >20
Patients with autoimmune hepatopathies	0/15 0%	2/15 13.33%	0/15 0%	0/15 0%	0/15 0%	0/15 0% 0%	0/15 0% 0%
Patients with hepatitis/cirrhosis	1/12 8.33%	3/12 25.00%	1/12 8.33%	0/12 0%	0/12 0%	2/12 16.66% 16.66%	0/12 0% 0%
Patients with viral hepatitis/cirrhosis	1/35 2.85%	6/35 17.14%	0/35 0%	0/35 0%	0/35 0%	2/35 5.71% 5.71%	1/35 2.85% 0%
Patients with other gastrointestinal diseases	0/83 0%	18/83 21.68%	0/83 0%	4/83 4.81%	7/83 8%	5/83 6.02% 6.02%	12/83 14.45% 14.45%

TABLE 6. Sensitivity, Specificity, PPV, and NPV of Tests

	% (95% CI)				
	Sensitivity	Specificity	PPV	NPV	ROC Curve
IgA a-tTG Cut-off > 20 U/ml	100.00	98.82 (98.65–98.98)	95.35 (94.72–95.98)	100.00	1.00
IgG a-tTG Cut-off > 20 U/ml	78.05 (76.78–79.32)	82.25 (81.67–82.82)	51.61 (50.37–52.86)	93.92 (93.53–94.30)	0.91 (0.87–0.95)
IgA a-DGP Cut-off > 20 U/ml	90.24 (89.34–91.15)	97.04 (96.79–97.30)	88.10 (87.12–89.07)	97.62 (97.39–97.85)	0.97 (0.94–0.99)
IgG a-DGP Cut-off > 20 U/ml	87.80 (86.80–88.81)	95.86 (95.56–96.16)	83.72 (82.62–84.82)	97.01 (96.75–97.26)	0.95 (0.90–0.99)
EMA IgA	100.00	100.00	100.00	100.00	1.00
tTG-A Neo Cut-off > 15 U/ml	100.00	93.59 (93.12–93.86)	78.85 (77.74–79.96)	100.00	0.99 (0.001–0.00)
tTG-A Neo Cut-off > 18 U/ml	100.00	94.67 (94.34–95.01)	82.00 (80.94–83.06)	100.00	0.99 (0.001–0.00)
tTG-A Neo Cut-off > 20 U/ml	100.00	97.04 (96.79–97.30)	89.13 (88.23–90.03)	100.00	0.99 (0.001–0.00)
tTG-G Neo Cut-off > 15 U/ml	87.80 (86.80–88.81)	89.94 (89.49–90.39)	67.92 (66.67–69.18)	96.82 (96.54–97.09)	0.96 (0.01–0.00)
tTG-G Neo Cut-off > 18 U/ml	85.37 (84.28–86.45)	92.31 (91.91–92.71)	72.92 (71.66–74.17)	96.30 (96.01–96.59)	0.96 (0.01–0.00)
tTG-G Neo Cut-off > 20 U/ml	80.49 (79.27–81.70)	92.31 (91.91–92.71)	71.74 (70.44–73.04)	95.12 (94.79–95.45)	0.96 (0.01–0.00)

controls. In selecting the control population, we decided to enroll a greater number of subjects with other diseases rather than normal healthy controls alone (145 disease controls and 24 blood donors), in order to assess the specificity of the test in critical conditions such as diseases that may be mixed up with CD or occur together with CD (e.g., autoimmune hepatopathies, hepatitis/cirrhosis, viral hepatitis/cirrhosis, other gastrointestinal diseases).

The results demonstrate that the *AESKULISA*® tTG-A New Generation assay has not only a sensitivity of 100%, but also high specificity, especially with a cut-off >20 U/ml (97.04%). Analytical performance of *AESKULISA*® tTG-G New Generation assay was better when used with a cut-off >18 U/ml, having a sensitivity and specificity higher than IgG a-tTG (85.37% vs. 78.05% and 92.31% vs. 82.25%, respectively), suggesting that it could be an ideal tool for identifying CD in patients with IgA deficiency.

tTG-A Neo was positive in 9 of the 145 disease controls, when the cut-off was set to >18 U/ml and in 5 of the 145 disease controls, when the cut-off was set at >20 U/ml; tTG-G Neo was positive in 12 of the 145 disease controls (both with cut-off >18 and cut-off >20). Some of these “false” positive specimens may be positive: in fact, some of these were found to be positive when tested with one or more “classical” assay for IgA or IgG, a-tTG, and a-DGP. Thus, it appears that the tTG-A Neo and tTG-G Neo perform excellently as screening assays, revealing antibody moieties indicative of putative CD and capable of differentiating from other related diseases. The high κ values indicate a high overall reliability across all tests for all subdiagnoses; therefore, it can be assumed that subsequent tests with different patient panels will give rise to similar results. A high cross-study stability of our results can therefore be expected.

In addition, the test gave positive results in various CD patients on gluten-free diet, indicative of a failure to follow

the diet, an observation also confirmed by the positivity for at least one of the other tests and by Marsh’s 3b and 3c biopsies.

Due to its high level of diagnostic accuracy, the tTG Neo, which detects antibodies to the neoepitope antigen, constitutes a new approach to the screening of CD patients not only for the diagnosis of CD but also for monitoring patients on gluten-free diet. Analysis of the individual analytes (a-tTG, a-DGP, EMA) to confirm positivity needs only be carried out in subjects who are positive with the *AESKULISA*® tTG New Generation assay.

Moreover, this assay is potentially able to identify CD patients who were screened negative with conventional serological markers. As our results show, several subjects enrolled in our study have positivity only for the *AESKULISA*® tTG-A/tTG-G New Generation.

Who are these subjects: silent cases or latent cases? It is known that CD is often atypical or even clinically silent and for this reason, the vast majority of cases remain undiagnosed for many years and are exposed to the risk of long-term complications (25). The use of these sensitive tests using an antigen that mimics the physiological antigen could uncover a large portion of the submerged CD “iceberg,” detecting undiagnosed CD: anti-neoepitope antibodies could constitute the first immunological markers produced, preceding the other “classical” markers by several months, and their determination by these new assays could be helpful for early diagnosis of CD. Recently, Tonutti et al. (26) reported the detection of neoepitope antibodies in two pediatric patients 6 months or more before other parameters showed a positive result. Further studies are necessary to understand if the neoepitope positivity shown in some subjects negative to “classical” serological markers has a predictive value: if this hypothesis can be confirmed, this new marker could be used not only as highly sensitive screening test but also fills the diagnostic gap that constitutes the so-called “celiac iceberg.”

ACKNOWLEDGMENTS

The authors thank AESKU Diagnostics for the donation of the assays.

REFERENCES

1. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: An evolving spectrum. *Gastroenterology* 2001;120:636–651.
2. Shan L, Molberg O, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. *Science* 2002;297:2275–2279.
3. Fleckenstein B, Molberg O, Qiao SW, et al. Gliadin T cell epitope selection by tissue transglutaminase in celiac disease. Role of enzyme specificity and pH influence on the transamidation versus deamidation process. *J Biol Chem* 2002;277:34109–34116.
4. Fleckenstein B, Qiao SW, Larsen MR, Jung G, Roepstorff P, Sollid LM. Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides. *J Biol Chem* 2004;279:17607–17616.
5. Skovbjerg H, Koch C, Anthonsen D, Sjostrom H. Deamidation and cross-linking of gliadin peptides by transglutaminase and the relation to celiac disease. *Biochim Biophys Acta* 2004;1690:220–230.
6. Cicciocioppo R, Di Sabatino A, Ara C, et al. Gliadin and tissue transglutaminase complexes in normal and celiac duodenal mucosa. *Clin Exp Immunol* 2003;134:516–524.
7. Mowat AM. Coeliac disease—a meeting point for genetics, immunology, and protein chemistry. *Lancet* 2003;361:1290–1292.
8. Dieterich W, Esslinger B, Schuppan D. Pathomechanisms in celiac disease. *Int Arch Allergy Immunol* 2003;132:98–108.
9. Dewar D, Pereira SP, Ciclitira PJ. The pathogenesis of coeliac disease. *Int J Biochem Cell Biol* 2004;36:17–24.
10. Tonutti E, Visentini D, Bizzaro N, et al. The role of anti-tissue transglutaminase assay for the diagnosis and monitoring of coeliac disease: A French-Italian multicentre study. *J Clin Pathol* 2003;56:389–393.
11. Korponay-Szabó IR, Dahlbom I, Laurila J, et al. Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for celiac disease in selective IgA deficiency. *Gut* 2003;52:1567–1571.
12. Prince HE. Evaluation of the INOVA diagnostics enzyme-linked immunosorbent assay kits for measuring serum immunoglobulin G (IgG) and IgA to deamidated gliadin peptides. *Clin Vacc Immunol* 2006;13:150–151.
13. Sugai E, Vazquez H, Nachman F, et al. Accuracy of testing for antibodies to synthetic gliadin-related peptides in celiac disease. *Clin Gastroenterol Hepatol* 2006;4:1112–1117.
14. Volta U, Granito A, Fiorini E, et al. Usefulness of antibodies to deamidated gliadin peptides in celiac disease diagnosis and follow up. *Dig Dis Sci* 2008;53:1582–1588.
15. Marietta EV, Rashtak S, Murray JA. Correlation analysis of celiac sprue tissue transglutaminase and deamidated gliadin IgG/IgA. *World J Gastroenterol* 2009;15:845–848.
16. Jaskowski TD, Donaldson MR, Hull CM, et al. Novel screening assay performance in pediatric celiac disease and adult dermatitis herpetiformis. *J Pediatr Gastroenterol Nutr* 2010;51:19–23.
17. Sugai E, Nachman F, Vázquez H, et al. Dynamics of celiac disease-specific serology after initiation of a gluten-free diet and use in the assessment of compliance with treatment. *Dig Liver Dis* 2010;42:352–358.
18. Vermeersch P, Geboes K, Mariën GA, Hoffman I, Hiele M, Bossuyt X. Diagnostic performance of IgG anti-deamidated gliadin peptide antibody assays is comparable to IgA anti-tTG in celiac disease. *Clin Chim Acta* 2010;411:931–935.
19. Villalta D, Tonutti E, Prause C, et al. IgG antibodies against deamidated gliadin peptides for diagnosis of celiac disease in patients with IgA deficiency. *Clin Chem* 2010;56:464–468.
20. Matthias T, Pfeiffer S, Selmi C, Eric Gershwin M. Diagnostic challenges in celiac disease and the role of the tissue transglutaminase-neo-epitope. *Clin Rev Allergy Immunol* 2010;38:298–301.
21. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of celiac disease: Time for a standardized report scheme for pathologist. *Eur J Gastroenterol Hepatol* 1999;11:1185–1194.
22. NIH Consensus Development Conference on Celiac Disease, Bethesda 2004. Available from: <http://www.consensus.nih.gov/cons/118/118cdc.intro.htm>.
23. Cohen J. A coefficient of agreement for nominal scales. *Educ Psychol Meas* 1960;20:37–46.
24. Fleiss J, Cohen J, Everett BS. Large sample standard errors of kappa and weighted kappa. *Psychol Bull* 1969;72:323–327.
25. Green PH, Stavropoulos SN, Panagi SG, et al. Characteristics of adult celiac disease in the USA: Results of a national survey. *Am J Gastroenterol* 2001;96:126–131.
26. Tonutti E, Visentini D, Fabris M, et al. Antibodies to the transglutaminase-deamidated gliadin peptides complex: A new serological approach to the diagnosis of celiac disease, 7th International Congress on Autoimmunity 2010, Ljubljana, Slovenia.