Assessment of a test for the screening and diagnosis of celiac disease

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Running title: Celiac disease: evaluation of a screening test

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Abstract

Background. Celiac disease 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 assay that uses the purified cross-linked complex of tissue transglutaminase and gliadin, referred as the "neo-epitope" (*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 autoimmune diseases and 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 celiac disease, but also for monitoring patients on gluten-free diet and their compliance. Moreover, cases of neo-epitope positive subjects that were tested negative with "classical" serological markers could have a predictive value for this pathology. This aspect will require further studies of elaboration.

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 (5). Thereby, tissue transglutaminase 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 development 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 neo-epitopes to gliadin peptides and tTG (7-9).

Widely available serological tests used for detecting celiac disease include detection of anti-tissue transglutaminase antibodies (a-tTG), anti-endomysial antibodies (EMA), and anti-gliadin 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.

IgA EMA, detected by immunofluorescence on sections of monkey oesophagus, 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 celiac disease 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 deamidated gliadin peptides (a-DGP) show very promising data as second generation AGA assays (12-19).

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

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 19-59 years; 24 females, aged between 18-77 years) and 10 children (3 males, aged between 6-9 years; 7 females, aged between 3-13 years). In addition, sera of 18 previously diagnosed CD patients on gluten-free diet for 8-24 months were included: 8 adults (1 male, 37 years; 7 females, aged between 18-42 years) and 10 children (3 males, aged between 4-11 years; 7 females, aged between 3-16 years).

The diagnosis of CD was based on histological and serological criteria, including positive serology tests (a-tTG, EMA).

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.

tested positive for anti-smooth muscle (SMA) autoantibodies and/or IgG anti-F-actin autoantibodies; 12 patients with autoimmune 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) and 24 blood donors as normal controls. 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:

- 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. (San Diego, CA)
- IgA a-DGP: ELISA QUANTA Lite ™ Gliadin IgA II, INOVA Diagnostic Inc. (San Diego, CA)
- IgG a-DGP: ELISA QUANTA Lite ™ Gliadin IgG II, INOVA Diagnostic Inc. (San Diego, CA)
- 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 (Wendelsheim, Germany).

The *AESKULISA*® tTG New Generation is a solid phase enzyme immunoassay coated with purified neo-epitopes resulting from crosslinking between human recombinant tissue transglutaminase and gliadin-specific peptides in which ensure significantly increased sensitivity and specificity of the test.

Sensitivity, specificity, positive and negative predictive values and area under the receiver operating characteristic curve (AUC) were calculated for each assay. For INOVA Diagnostics tests 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, positive and negative predictive values.

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 k 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 out 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 U/ml and 38.8 U/ml (Table 3).

tTG-G Neo was positive in 36 out of 41 patients with a diagnosis of CD when cut-off was set at >15 U/ml; in 35 out of 41 patients with cut-off >18 U/ml, and in 33 out of 41 with cut-off >20 U/ml. tTG-G Neo was also positive in 7 out 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 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 that were positive for each parameter analyzed in the subgroup of disease controls. In the group of hepatitis/cirrhosis patients 2 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 >20U/ml no positivity was observed. In the subgroup with viral hepatitis/cirrhosis only 2 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 5 subjects, of which 3 also showed positivity for at least one other "classical" parameter and 2 showed only a weak positivity for tTG-A Neo. With cut-off >20 U/ml tTG-A Neo was positive in 4 subjects, of which 3 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 paper we have evaluated the analytical performance of ELISA assays for celiac disease that utilize the neo-epitope 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 assay in terms of sensitivity and specificity, we selected well-characterized populations of CD patients and 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 celiac disease and capable of differentiating from other related diseases. The high κ values indicate a high overall reliability across all tests for all sub-diagnoses; 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 3b and 3c biopsies.

Due to its high level of diagnostic accuracy the tTG Neo, which detects antibodies to the neo-epitope antigen, constitutes a new approach to the screening of CD patients not only for the diagnosis of celiac disease 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 remains undiagnosed for many years and is 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-neo-epitope 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 neo-epitope antibodies in 2 pediatric patients 6 months or more before other parameters showed a positive result. Further studies are necessary to understand if the neo-epitope 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 which constitutes the so-called "celiac iceberg".

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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, Hausch F, Filiz F, Gray GM, Sollid LM, Khosla C. Structural basis for gluten intolerance in celiac sprue. Science 2002; 297:2275-2279.
3. Fleckenstein B, Molberg O, Qiao SW, Schmid DG, von der Mulbe F, Elgstoen K, Jung G, Sollid LM. 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. Ciccocioppo R, Di Sabatino A, Ara C, Biagi F, Perilli M, Amicosante G, Cifone MG, Corazza GR. 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, Caradonna M, Cerni L, Villalta D, Tozzoli R. 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-Szabò IR, Dahlbom I, Laurila J, Koskinen S, Woolley N, Partanen J, Kovacs JB, Maki M, Hansson T. 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, Moreno ML, Mazure R, Smecuol E, Niveloni S, Cabanne A, Kogan Z, Gomez JC, Maurino E, Bai JC. 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, Parisi C, Piscaglia M, Pappas G, Muratori P, Bianchi FB. 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, Wilson AR, Hill HR, Zone JJ, Book LS. 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, Váquez H, González A, Andrenacci P, Czech A, Niveloni S, Mazure R, Smecuol E, Cabanne A, Mauriño E, Bai JC. 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, Koletzko S, Uhlig HH, Vermeersch P, Bossuyt X, Stern M, Laass MW, Ellis JH, Ciclitira PJ, Richter T, Daehnrich C, Schlumberger W, Mothes T. 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. Educational and Psychological Measurement 1960; 20:37-46.
24. Fleiss J, Cohen J, Everett BS. Large sample standard errors of kappa and weighted kappa. Psychological Bulletin 1969; 72:323-327.
25. Green PH, Stavropoulos SN, Panagi SG, Goldstein SL, Mcmahon DJ, Absan H, Neugut 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, Blasone N, Molinaro P, Pavan E, Tozzoli R, Villalta D, Bizzaro N. 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.

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

TABLE 2. TABLE 2. Number (n. positive subjects/n. total subjects) and percentage (%) of positive subjects for all analysed parameters. In addition, the weighted κ is given for each parameter as a reliability indicator with respect to the diagnoses.

	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%
K*	0.89	0.85	0.95	0.95	0.96	0.94	0.95	0.96	0.89	0.94	0.94

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

TABLE 3. Characteristics of control population subjects which showed only positivity for tTG-A Neo.

Age	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 which showed only positivity for tTG-G Neo.

Age	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

TABLE 5. Number (n. positive subjects/n. 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%	0/15 0%	0/15 0%	0/15 0%	0/15 0%	0/15 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%	2/12 16.66%	0/12 0%	0/12 0%	0/12 0%	0/12 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%	2/35 5.71%	1/35 2.85%	0/35 0%	0/35 0%	0/35 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%	5/83 6.02%	4/83 4.81%	12/83 14.45%	12/83 14.45%	12/83 14.45%

TABLE 6. Sensitivity, specificity, PPV and NPV of tests.

	% (95% CI)				
	Sensitivity	Specificity	PPV	NPV	AU ROC
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 (82.6 2 - 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)