

Chemiluminescence and ELISA-based serum assays for diagnosing and monitoring celiac disease in children: A comparative study



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ABSTRACT

Background: Anti-transglutaminase (tTG) or anti-deamidated gliadin peptides (DGP) serum determination is the first step in diagnosing celiac disease (CD). Our aims were to: compare the performance of novel chemiluminescent tool in the detection of tTG and DGP (Q-Flash®, Inova) with that of the established ELISA (Q-Lite®, Inova) methods; identify the more reliable index for making a sound diagnosis and monitoring therapy.

Methods: Using Q-Flash® and Q-Lite®, IgA and IgG class tTG and DGP were measured in the sera of 155 CD pediatric patients and 166 healthy age-matched controls. Forty-two of the patients had a follow-up one year after starting gluten free diet (GFD).

Results: Q-Flash® IgA tTG, the more accurate (intra-assay CV for low, intermediate and high values: 2.2%, 1.6%, and 1.1%; inter-assay CV: 2.8%, 4%, and 3%), sensitive (96.1%) and specific (97%) test for diagnosing CD, was the only variable to be significantly correlated with CD at binary logistic regression analysis ($r = 0.263$, $p < 0.0001$, $\text{Exp}(B) = 1.0506$, 95% CI = 1.0286–1.0731). Q-Flash® IgA tTG or DGP screen were more accurate than Q-Lite® IgA tTG in monitoring CD patients on GFD ($p = 0.003$).

Conclusion: Q-Flash® IgA tTG measurement is an extremely precise, sensitive and specific index for not only diagnosing CD, but also monitoring therapy.

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1. Introduction

Celiac disease (CD), a common autoimmune enteropathy occurring following gluten ingestion in children and adults with a genetic predisposition [1,2], affects ~1% of most populations, although this percentage is probably an underestimation since the incidence of CD has dramatically increased in recent decades, the condition often being left undiagnosed [2–5]. The early diagnosis of the disease and treatment with a gluten free diet (GFD) are mandatory, decreasing the risk of complications, malignancies, and mortality [1].

Abbreviations: CD, celiac disease; GFD, gluten free diet; tTG, anti-transglutaminase antibodies; EMA, anti-endomysial antibodies; DGP, anti-deamidated gliadin peptides antibodies; CV, coefficient of variation; CS, control subjects; DPR, differential positive rate; LR+, positive likelihood ratio; LR-, negative likelihood ratio; CI, confidence interval; ROC, receiver operating characteristics; PPV, positive predictive value; NPV, negative predictive value; U, units; CU, chemiluminescence units.

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In 2004 the National Institutes of Health Consensus [6], and in 2005 the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition [7], stated that the first step in diagnosing CD is a serologic test, confirmatory histology being required. Although biopsy is still considered a prerequisite for confirming a diagnosis of CD in a subset of selected patients, in the recent European Society of Paediatric Gastroenterology and Nutrition guidelines, it is stated that histology may be omitted in symptomatic patients with high IgA anti-tissue transglutaminase (tTG) levels (10-fold the upper normal limit), verified by anti-endomysium (EMA) positivity, and positive findings for HLA-DQ2 and/or HLA-DQ8 heterodimer [8]. This approach was prompted by evidence based data showing that the sera of CD patients contain highly specific autoantibodies directed against the common CD autoantigen tTG, the deamidated gliadin peptides (DGP) and endomysium, and by the observation that more than 95% of patients with CD share the HLA-DQ2 and/or HLA-DQ8 heterodimer [8–11].

The majority of commercially assays available for the detection of tTG and DGP are ELISA-based tests. The ELISA determination of DGP allows us to obtain sensitive and specific results that are more reliable than those obtained by measuring anti-gliadin antibodies, but slightly less reliable than tTG based findings [10]. At meta-analyses the

sensitivity and specificity of IgA tTG are higher than 92% and 96% respectively [10–13], and those of IgA DGP range from 80.7% to 95.1% and 86.3% to 93.1%, respectively [10,12]. The measurement of IgG class antibodies against tTG or DGP should be confined to patients with total IgA deficiency [14,15], which is found in about 2% of CD cases [16].

EmA and tTG recognize the same antigen, tTG [17], have the same diagnostic accuracy [10], the difference found between these two antibodies being relative, and mainly based on variations in techniques used to assess them [18]. Therefore, since the determination of EmA is based on indirect immunofluorescence (a low through-put and operator-dependent technique), we, in agreement with several other research groups, have replaced EmA with tTG [19–23]. However EmA was recently proposed as the confirmatory test for a diagnosis of CD in cases showing tTG levels of above 100 U/mL since it spares patients from undergoing a confirmatory biopsy [8]. This threshold should be considered the decision limit for tTG, being almost five-fold that of the 20 U/mL reference value [24–27]. In CD patients, tTG, DGP and EmA span a wide range of values, which correlate well with the severity of mucosal atrophy in adults and children, values comprised between the upper normal limit and the decision limit often being associated with type I-type II intestinal lesions [24–30]. Thanks to the association between the levels of serum antibody and the severity of intestinal lesions, antibody measurement is a useful tool for monitoring CD patients on GFD, and is widely preferred to biopsy [31–33]. The return of tTG and/or DGP levels within the reference range after starting a GFD usually takes more than six months in compliant cases but, in others, it can take several years thus leading to clinical doubt concerning patient compliance [33,34].

The aim of the present study was to compare the analytical and clinical performance of new chemiluminescent assays in detecting IgA and IgG class tTG and DGP with that of respective ELISAs. A large retrospective cohort of children and adolescents with or without a histologically proven CD diagnosis was evaluated in a clinical setting, and serial measurements were taken in a group of CD patients on a GFD in order to identify the most reliable index for monitoring therapy.

2. Materials and method

2.1. Laboratory assays

The following chemiluminescent assays were analyzed: Q-Flash® IgA and IgG tTG, Q-Flash® DGP IgA and IgG, Q-Flash® DGP screen (Q-Flash® INOVA Diagnostics, San Diego, CA, USA). Q-Flash® DGP screen allows the detection of IgA and IgG DGP in one run. The following ELISA assays were analyzed: Q-Lite® IgA tTG, Q-Lite® IgA and IgG DGP (Q-Lite® INOVA Diagnostics, San Diego, CA, USA).

Chemiluminescent assays were performed on the automated BIO-FLASH® instrument (Inova Diagnostics, San Diego, CA, USA). ELISAs were performed using the DSX™ Four-Plate Automated ELISA Processing System (Dynex Technologies, Chantilly, VA). For all of the studied chemiluminescence assays, analytical imprecision was estimated by the analysis of intra and inter-assay coefficients of variation (CV). Three different serum samples with high, intermediate and low values were analyzed ten times in the same run to obtain intra-assay CV, and ten times in ten different runs to obtain day-to-day imprecision (inter-assay CV).

2.2. Patients

Five chemiluminescence assays were performed in a series of 321 sera (previously stored at -20°C) drawn from children and adolescents who consecutively underwent upper gastrointestinal endoscopy for suspected gastrointestinal diseases from January 2001 to December 2009. Fasting blood samples, collected immediately before endoscopy, were centrifuged at 3000 g for 5 min to obtain sera, respective parts of

which were used to measure total IgA (immunonephelometric assay, Siemens, Marburg, Germany), the remaining parts being aliquoted, immediately frozen and stored at -20°C . Approval for the study protocol was obtained from the Local Institutional Review Board Committee. In all cases, duodenal biopsies were taken for histology, which confirmed CD in 155 children (60 boys and 95 girls; mean age 7.2 years $\pm$ 0.35; median 6 years) and ruled out the disease in the remaining 166 children (control subjects [CS] 69 boys, 97 girls; mean age 9.2 years $\pm$ 0.34; median 10 years). The Marsh-Oberhuber classification of celiac lesions was available for 150 CD children; of these, two had type I, 20 type II, 39 type IIIa, 17 type IIIb and 72 Marsh type IIIc lesions. Q-Lite® IgA tTG assay was performed in all sera. Q-Lite® IgA and IgG DGP were analyzed in 93 CD and 80 CS. Forty-two of the 155 CD patients had follow-up with blood sampling 4 and 12 months after starting GFD.

2.3. Data analysis

The statistical analysis of data was performed using the MedCalc and SPSS version 9.0 statistical software. Passing and Bablok regression and Bland-Altman plots were used to compare methods. The Cusum test was performed to evaluate linearity of paired data. Receiver operating characteristics (ROC) curve analysis was undertaken to detect the best cut-off values associated with the highest sensitivity and specificity by calculating the differential positive rate (DPR). To calculate post-test probability of disease, the following formulas were used: positive likelihood ratio ($\text{LR}+$) = sensitivity/(1-specificity); negative likelihood ratio ($\text{LR}-$) = (1-sensitivity)/specificity. One-way analysis of variance, Bonferroni's test for pairwise comparisons, binary logistic regression analysis and repeated measures analysis of variance were used.

3. Results

3.1. Imprecision studies

Table 1 shows the results for analytical imprecision, and reports intra- and inter-assay CV for low, intermediate and high antibody values measured with chemiluminescence assays.

IgA tTG Q-Flash® (chemiluminescence) and IgA tTG Q-Lite® (ELISA) were compared by analyzing the concordance correlation coefficient (Pearson $p = 0.817$, Bias correction factor $C_b = 0.0787$) and Passing and Bablok regression (Cusum test: $p < 0.01$, Intercept = -25.38 ; 95% CI: -41.47 – 21.91 ; Slope: 8.95, 95% CI: 7.80–10.74). At Passing and Bablok regression analysis, a constant and proportional difference between the two methods was found: the 95% CI for the intercept did not contain the value zero, thus indicating that the methods differ to a constant degree; nor did the 95% CI of the slope contain the value one, this indicating a proportional difference between the two. These observations were borne out by the Bland-Altman difference plot obtained (Fig. 1), which documented a clear concentration-dependent positive difference between Chemiluminescence and ELISA IgA tTG measurements. Supplementary Table 1 shows the results of method comparison on considering IgA and IgG DGP measurements. Only a subset of 173 subjects, for whom ELISAs were available, entered these analyses.

3.2. Chemiluminescence IgA tTG measurement: the more sensitive and specific CD biomarker

ROC curve analyses of the new chemiluminescence assays and of IgA tTG Q-Lite® were performed (Fig. 2). IgA tTG Q-Lite® under the ROC curve area, 0.976 ± 0.009 (95% CI: 0.952 to 0.990), was not significantly different from that of IgA tTG Q-Flash® ($p = 0.664$). Supplementary Table 2 reports the areas under the ROC curves of Q-Flash® assays and the results of the statistical comparison between them. For each assay, the best threshold (best delta positive rate) was calculated based on ROC curve analyses, and the results for sensitivity, specificity, positive and negative predictive values and for positive and negative

Table 1

Imprecision of chemiluminescence assays. Three sera with low, intermediate and high antibody levels were analyzed ten times in a single run to obtain intra-assay coefficients of variation (CV_{intra}), and ten times in ten different runs to obtain day-to-day imprecision (CV_{inter}). The mean results from any ten series of measurements are expressed in arbitrary chemiluminescence units (CU).

	Low			Intermediate			High		
	Mean CU	CV_{intra}	CV_{inter}	Mean CU	CV_{intra}	CV_{inter}	Mean CU	CV_{intra}	CV_{inter}
Q-Flash® IgA tTG	8.9	2.2	2.8	22.6	1.6	4.0	59.3	1.1	3.0
Q-Flash® IgG tTG	4.2	5.5	4.9	27.0	3.8	4.0	87.6	1.9	3.1
Q-Flash® IgA DGP	7.5	2.7	5.3	25.7	11.5	7.5	73.7	11.7	7.6
Q-Flash® IgG DGP	6.1	3.7	2.7	29.9	18.0	4.2	81.2	4.0	2.8
Q-Flash® DGP Screen	5.4	3.8	3.0	23.9	8.1	1.9	50.9	3.5	2.0

likelihood ratios were then obtained (Table 2). The results for Q-Lite® IgG tTG, IgA and IgG DGP are reported in Supplementary Table 3.

Of the 166 children without histological evidence of CD, eight had elevated IgA tTG Q-Lite® results and four also had elevated IgA tTG Q-Flash® (Table 3). One child (Control 8) showed an increase in IgA tTG Q-Flash®, but not in IgA tTG Q-Lite®. The other Q-Flash® results in these nine children are detailed in Table 3, which shows that six CD children had false negative IgA tTG Q-Lite®, and/or false negative Q-Flash® IgA tTG results; three of these seven children (CD1, CD2 and CD6) had total IgA deficiency and had positive results for antibodies of the IgG class, two (CD3 and CD4) had negative findings for all antibodies, while the remaining two had some positive and some negative test results.

Among 155 CD children, five had two repeated endoscopies at one-year intervals prior their inclusion in the study. Three cases had typical CD findings at both endoscopies, while the other two cases presented no lesions in the first endoscopy, but had developed typical lesions by the second. Supplementary Fig. 1 shows the patterns for IgA tTG Q-Lite® and Q-Flash® biomarkers obtained in these five children in correspondence with the two endoscopic evaluations.

The five Q-Flash® results were submitted for the multivariate binary logistic regression analysis considering CD diagnosis as outcome (Table 4). This model allowed correct prediction in 94% of CD and 98% of CS cases.

On evaluating the diagnostic performances of Q-Flash® IgA tTG using a threshold 160 CU (10-fold higher than the established cut-off), sensitivity was 86.5%, specificity 100%, positive predictive value 100%, and negative predictive value 88.8%.

3.3. Chemiluminescence (IgA tTG or DGP Screen) is more reliable than Elisa IgA tTG for monitoring CD patients on GFD

On measuring IgA tTG Q-Lite® and all Q-Flash® biomarkers in serum samples of the 42 children with a one-year follow-up after starting GFD, it was found that each CD biomarker had significantly decreased at the twelfth month (Student's t test for paired data: $p < 0.0001$), although values above the established cut-offs were found to range from 14% (Q-Flash® DGP Screen) to 45% of cases (Q-Flash® IgA tTG) at one year follow-up (Supplementary Fig. 2). We calculated the percentage decline over time of the different assays, considering 100% the individual values at diagnosis. Repeated measures analysis of variance was performed considering follow-up data at 4 and 12 months (within-subjects effects: $F = 3.77$, $p < 0.0001$; between subjects effects: $F = 3.73$, $p = 0.003$). A statistically significant difference was found between results of Q-Flash® IgA tTG (Bonferroni's test for pairwise comparisons; $p < 0.0001$) or Q-Flash® DGP Screen ($p = 0.005$) and Q-Lite® IgA tTG (Fig. 3).

4. Discussion and conclusions

Measurement of IgA class antibodies that recognize human recombinant or purified tTG antigen is recommended as the first step in the diagnostic work-up for symptomatic patients with suspected CD [6–8]. Total IgA or direct testing for IgG tTG or DGP is advisable for identifying cases with total IgA deficiency [8]. Transglutaminase autoantibody measurement “to demonstrate a decrease in antibody titer as an indirect indicator of dietary adherence and recovery” is

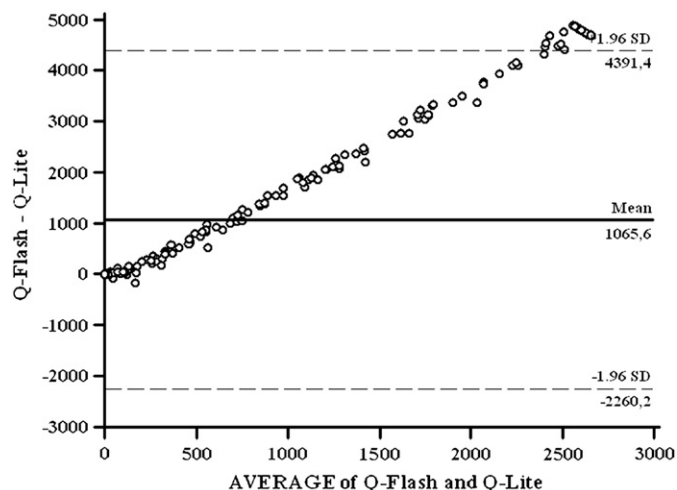


Fig. 1. Bland-Altman plot. Method comparison for the detection of IgA tTG. Q-Flash®: IgA tTG chemiluminescence detection; Q-Lite®: IgA tTG ELISA detection.

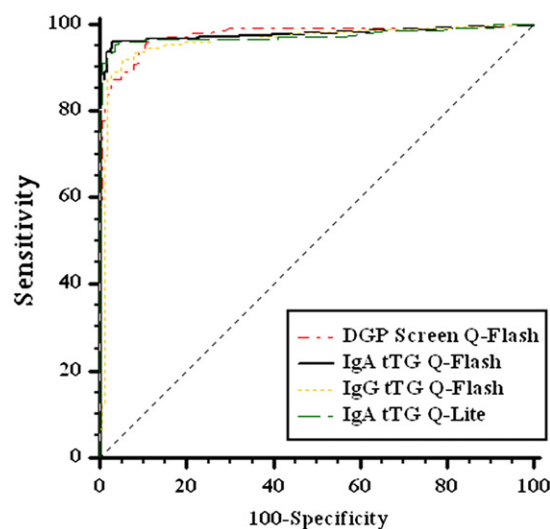


Fig. 2. Receiver Operating Characteristics (ROC) curves of IgA tTG Q-Flash (black line), IgG tTG Q-Flash (yellow line), DGP Screen Q-Flash (red line) and of IgA tTG Q-Lite (green line).

Table 2

Cut-off, sensitivity, specificity, positive (PPV) and negative (NPV) predicted values, positive (LR+) and negative (LR-) likelihood ratios of the CD marker Q-Lite® IgA tTG and of all Q-Flash® CD biomarkers analyzed. U = units; CU = chemiluminescence units; in brackets, the 95% confidence intervals (CI).

	Cut-off	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ (95% CI)	LR- (95% CI)
Q-Lite® IgA tTG	20 U	96.1 (91.4–98.4)	95.2 (90.4–97.7)	94.9 (89.9–97.6)	96.3 (91.8–98.5)	19.9 (10.1–39.2)	0.04 (0.02–0.09)
Q-Flash® IgA tTG	16 CU	96.1 (91.4–98.4)	97.0 (92.7–98.9)	96.8 (92.2–98.8)	96.4 (92.0–98.5)	31.9 (13.5–75.7)	0.04 (0.02–0.09)
Q-Flash® IgG tTG	10 CU	91.6 (85.8–95.3)	94.6 (89.6–97.3)	94.0 (88.6–97.0)	92.3 (87.0–95.7)	16.9 (8.9–32.0)	0.09 (0.05–0.15)
Q-Flash® IgA DGP	6 CU	90.3 (84.3–94.3)	92.2 (86.7–95.6)	91.5 (85.6–95.2)	91.1 (85.4–94.7)	11.5 (6.8–19.5)	0.11 (0.06–0.17)
Q-Flash® IgG DGP	21 CU	92.3 (86.6–95.8)	90.4 (84.6–94.2)	90.0 (84.0–94.0)	92.6 (87.1–96.0)	9.6 (6.0–15.3)	0.09 (0.05–0.15)
Q-Flash® DGP Screen	14 CU	95.5 (90.6–98.0)	88.6 (82.5–92.8)	88.6 (82.6–92.8)	95.5 (90.5–98.0)	8.3 (5.5–12.8)	0.05 (0.02–0.22)

Table 3

False positives and negatives. The results with Q-Lite® IgA tTG and all five Q-Flash® biomarkers, and total IgA in serum are shown. U = units; CU = chemiluminescence units; false positives and negatives are in bold.

Diagnosis	Q-Lite® IgA tTG U	Q-Flash® IgA tTG CU	Q-Flash® IgG tTG CU	Q-Flash® IgA DGP CU	Q-Flash® IgG DGP CU	Q-Flash® DGP Screen CU	Total IgA g/L
Control 1	31	6	4	5	4	3	1.18
Control 2	100	125	9	7	21	15	1.71
Control 3	34	2	5	5	3	1	1.01
Control 4	50	96	519	5	34	22	0.66
Control 5	28	4	5	14	3	8	1.19
Control 6	52	48	5	8	21	14	1.64
Control 7	35	14	4	33	23	31	3.30
Control 8	7	33	6	5	6	5	1.19
Control 9	20	130	1058	16	267	193	1.43
CD 1 (Type 3a)	3	2	1334	5	2000	1465	0.06
CD 2 (Type 3c)	3	2	2560	5	144	94	0.06
CD 3 (Type 3a)	4	2	4	5	3	4	1.12
CD 4 (Type 2)	2	2	4	6	3	1	0.97
CD 5 (Type 2)	10	54	6	5	28	19	0.77
CD 6 (Type 3c)	2	2	2600	5	794	630	0.05
CD 7 (Type 3c)	87	4	141	5	321	222	0.5

also recommended for monitoring CD in patients on GFD [7]. These recommendations are supported by the availability of biochemical tests that are analytically robust and extremely accurate from a clinical viewpoint. The majority of tTG and/or DGP are ELISA-based assays, while only a few commercially available tests are chemiluminescence-based [20]. Among the advantages of this laboratory technique over ELISAs are its higher sensitivity and dynamic range, which allows a clearer distinction between positive and negative results, and an easier and faster sample processing work-up, these characteristics making chemiluminescence an attractive tool for diagnosing CD [35].

On comparing the results from our evaluation of a panel of five chemiluminescence assays proposed for CD diagnosis and monitoring with results obtained using the well established IgA tTG ELISA [7,8,12,13,36], the Q-Flash® IgA tTG assay was found to have high precision; the intra- and inter-assay coefficients of variations were less

than 4% for high, low and intermediate values, the latter being of critical importance in distinguishing between positive and negative results. Not only was Q-Flash® IgA tTG imprecision lower than that reported for 10 different ELISAs [37], but it was also lower than that previously reported by us on using another chemiluminescent IgA tTG assay [20]. Measurements using Q-Flash® IgA or IgG DGP were more imprecise, attaining an intra-assay CV of more than 10% for intermediate values. Q-Flash® IgA tTG levels were constantly and proportionally higher than those

Table 4

Binary logistic regression analysis. Dependent: CD diagnosis. Predictors: Q-Flash® IgA and IgG tTG, IgA and IgG DGP and DGP Screen.

	Exp(B)	95% CI for Exp(B)	p-value
Q-Flash® IgA tTG	1.0506	1.0286–1.0731	<0.0001
Q-Flash® IgG tTG	1.0005	0.9988–1.0022	0.560
Q-Flash® IgA DGP	0.9915	0.8337–1.1792	0.923
Q-Flash® IgG DGP	1.0495	0.9388–1.1732	0.396
Q-Flash® DGP Screen	0.9489	0.8171–1.1020	0.492

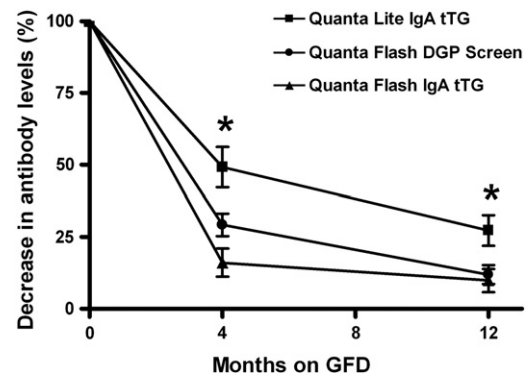


Fig. 3. Decrease in antibody levels at 4 and 12 months after initiating gluten free diet (GFD). * Bonferroni's test for pairwise comparisons: $p < 0.0001$ with respect to Quanta Flash IgA tTG, and $p = 0.005$ with respect to Quanta Flash DGP Screen.

obtained with ELISA determination. We believe that this phenomenon is mainly due to the higher dynamic range of chemiluminescence, since both assays (supplied by the same manufacturer) employ the same human purified transglutaminase antigen.

As expected, Q-Flash® IgA and IgG tTG and DGP levels in CD patients were significantly higher than in controls. However differences between the studied CD biomarkers for sensitivity and specificity were found when results were split into positive or negative (based on the ROC curves calculated cut-offs). The most sensitive and specific CD biomarker was Q-Flash® IgA tTG, the results obtained being more reliable than those obtained with Q-Flash® DGP and Q-Lite® IgA tTG (ELISA). Q-Flash® IgA or IgG DGP were less reliable CD biomarkers, their sensitivity and specificity being lower than 93%, whereas the percentage for Q-Flash® IgA tTG exceeded 95, thus further supporting the claim that DGP measurements should not be considered in the initial diagnostic work-up for the diagnosis of CD [6–8]. Despite the very high diagnostic precision of Q-Flash® IgA tTG, some false negative and false positive results were recorded. Three CD patients had negative findings because they had total IgA deficiency and, accordingly, they had elevated IgG class antibody levels. One child with CD was negative at Q-Lite® IgA tTG but positive at Q-Flash® IgA tTG, Q-Flash® IgG DGP and DGP Screen; this reflects the higher sensitivity of chemiluminescence—especially for low antibody titers. The negative Q-Flash® IgA tTG findings in two CD patients, who were also negative for all the studied biomarkers, are more difficult to explain. Possibly the sera of both these false negative patients contained auto-antibodies that recognized autoantigens and/or epitopes that were different from those employed in the present series of assays [38,39]. Elevated Q-Flash® IgA tTG values were found in five children without a proven histological diagnosis of CD. Three of these five children had elevated Q-Lite® IgA tTG, and two had also elevated Q-Flash® IgG tTG and/or DGP. These concordant findings suggest that these patients probably had latent CD, as did the two children who underwent repeat endoscopy: the first yielded normal findings and the second evidenced CD. However, it cannot be ruled out that negative histological findings may depend on errors in the pre-analytical phase (biopsy site and orientation, staining) or on a patchy distribution of the lesion itself [40].

New diagnostic tests introduced into clinical practice are often used in addition to assays that are already available, perhaps based on the premise that “bigger is better”. However, different assays may either yield the same or discordant clinical results, thus increasing clinical doubt rather than resolving it. The multivariate binary logistic regression analysis performed by us in order to establish which of the new Q-Flash® biomarkers is correlated with CD (independent of all other tests) demonstrated that none of the studied biomarkers provided any useful clinical information over and above that provided by Q-Flash® IgA tTG, the only variable in the analysis to be found significant. Moreover, the finding of Q-Flash® IgA tTG results that exceeded the cut-off value by ten-fold, confirmed that the positive predictive value of the test was 100% for the diagnosis of CD, thus further supporting the proposed ESPGHAN algorithm for the diagnosis of this condition [8].

Once diagnosed, CD must be treated with GFD, and biomarkers are useful for monitoring diet compliance and gut recovery. After one year on a GFD, about 25% of our patients still had abnormal values for almost all studied CD biomarkers, with the exception of Q-Flash® DGP Screen, which was elevated in only 13.9% of the cases. These data suggest that gluten withdrawal first leads to a lowered production of antibodies that recognize the modified DGP gluten antigens, while autoantibody production may persist for longer. Since the starting levels of DGP and tTG antibodies presented inter-individual variability, we evaluated whether the percentage decline over time was different for the different assays. Interestingly, the most marked and early percentage variations were found for Q-Flash® IgA tTG and Q-Flash® DGP Screen, which performed better than Q-Lite® IgA tTG.

In conclusion, the results obtained in the present study confirm that, in cases of suspected CD, chemiluminescence is preferable to

ELISA as a diagnostic tool for the detection of IgA tTG in view of its greater precision and accuracy. Q-Flash® IgA tTG appears also to be a reliable tool for monitoring CD patients on GFD.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.cca.2013.03.024>.

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