Clinica Chimica Acta 421 (2013) 202–207

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

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

Ada Aita [a], Elisa Rossi [a], Daniela Basso [a,*], Graziella Guariso [b], Dania Bozzato [a], Michela Pelloso [a], Matilde Pescarin [b], Carlo-Federico Zambon [a], Filippo Navaglia [a], Eliana Greco [a], Marco Gasparetto [b], Paola Fogar [a], Andrea Padoan [a], Stefania Moz [a], Mario Plebani [a]

[a] Department of Medicine – DIMED, University of Padova, Italy
[b] Department of Pediatrics, University of Padova, Italy

ARTICLE INFO

Article history:
Received 17 January 2013
Received in revised form 22 February 2013
Accepted 22 March 2013
Available online 28 March 2013

Keywords:
Celiac disease
Gluten free diet
Transglutaminase
Deamidated gliadin peptides
Chemiluminescence
ELISA

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 ($r = 0.263$, $p < 0.0001$, $Exp(B) = 1.0506$, 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.

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].

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

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.

* Corresponding author at: Department of Laboratory Medicine, University-Hospital of Padova, Via Giustiniani 2, 35128 Padova, Italy. Tel.: +39 0498212801; fax: +39 0498211981.
E-mail address: daniela.basso@sanita.padova.it (D. Basso).

0009-8981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cca.2013.03.024

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 ±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 ±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 (LR+) = sensitivity/(1-specificity); negative likelihood ratio (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 $Cb = 0.0787$) 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 Chemiluminence 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