Clinica Chimica Acta 411 (2010) 931–935

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Diagnostic performance of IgG anti-deamidated gliadin peptide antibody assays is comparable to IgA anti-tTG in celiac disease

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ARTICLE INFO

Article history:
Received 19 January 2010
Received in revised form 16 February 2010
Accepted 16 February 2010
Available online 19 February 2010

Keywords:
Celiac disease
Sensitivity and specificity
Gliadin
Deamidated gliadin peptide
Tissue-type transglutaminase
IgG

ABSTRACT

Background: Detection of IgG antibodies against deamidated gliadin peptides (DGP) is more sensitive and more specific for celiac disease than detection of IgG antibodies against native gliadin. Our aim was to evaluate the technical performance and diagnostic accuracy of four commercial IgG anti-DGP assays.
Methods: Commercial IgG anti-DGP assays from Euroimmun, Inova, Phadia and The Binding Site were evaluated and their diagnostic accuracy (sensitivity and specificity) compared to other serologic assays for celiac disease (3 IgA and 2 IgG anti-tTG assays, 1 IgA and 1 IgG anti-gliadin assay, 1 IgA anti-DGP assay). The study population consisted of 86 consecutive CD patients and 741 disease controls.
Results: The technical performance (linearity, interference and imprecision) of the IgG anti-DGP assays was acceptable. The sensitivity of the IgG anti-DGP assays varied between 76.7% and 86.0% at the cut-off recommended by the manufacturer and between 74.4% and 86.0% at the cut-off that corresponded to a specificity of 98%. The specificity varied between 97.3% and 99.3%. The diagnostic accuracy of the IgG anti-DGP assays was comparable to the diagnostic accuracy of the IgA anti-tTG assays. The sensitivity of the IgG anti-DGP assays was significantly better than sensitivity of the IgG anti-tTG assays ($p<0.05$) and the specificity was significantly better than the IgA and IgG anti-gliadin assays ($p<0.05$).
Conclusions: The overall performance of the four IgG anti-DGP assays was acceptable and the diagnostic accuracy comparable to the three IgA anti-tTG assays.

1. Introduction

Celiac disease is an autoimmune disorder characterized by a heightened immunological responsiveness to ingested gluten (from wheat, barley or rye) in genetically susceptible individuals [1,2]. The exact mechanisms responsible for celiac disease are not fully understood. It is thought that selective deamidation of gliadin (which is rich in glutamine residues) by tTG to deamidated gliadin peptides (DGP) facilitates binding to MHC class II molecules, and thereby triggers a T cell-mediated immune response [3]. Furthermore, it has been shown that deamidation of gliadin increases the binding of anti-gliadin antibodies in serum of CD patients, but not of non-CD patients [4,5].

The definitive diagnosis of celiac disease requires a small intestinal biopsy examination [6]. The detection of autoantibodies or antibodies against gliadin has often been used as first-line test to identify individuals who require a duodenal biopsy. Detection of IgA antibodies directed against endomysium or tissue transglutaminase (tTG), the target of anti-endomysial autoantibodies, is preferred over detection of IgG antibodies against endomysium, tTG or gliadin because of the higher sensitivity and specificity [7]. This was confirmed in two systematic reviews [8,9]. A drawback of the detection of IgA antibodies for celiac disease, however, is that patients with a selective IgA deficiency will test false-negative.

In 2004, Schwertz et al. showed that the detection of antibodies against DGP could be a valuable tool for the diagnosis of celiac disease [10]. IgG anti-DGP antibodies are more sensitive and more specific for CD than IgG anti-gliadin antibodies[11] and their performance is at least as good as that of IgA anti-DGP antibodies [11–13]. The performance of IgG anti-DGP antibodies is also reported to be comparable to the performance of IgA anti-tTG [12,13], although large studies examining this in consecutive adult and pediatric patients are lacking.

There are currently four manufacturers of commercially available IgG DGP assays: Euroimmun, Inova, Phadia and The Binding Site. Several authors have evaluated the performance of the Inova IgG anti-DGP assay [11–16] and the Euroimmun assay in children [17], but there are no studies that have evaluated the assays from The Binding

Abbreviations: CD, celiac disease; DGP, deamidated gliadin peptide; tTG, tissue transglutaminase.

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doi:10.1016/j.cca.2010.02.060

Site or Phadia or that have compared the performance of the different IgG anti-DGP assays. Previous studies also had limitations with respect to patient selection. In some studies, for example, only patients with more severe histological changes of the small bowel were included [11,16]. Other studies included only a limited number of disease control patients or included selected control patients such as healthy blood donors [14,15,18]. These methodological flaws were highlighted in a recent systematic review on the performance of IgA anti-tTG and IgG anti-DGP [19]. Most studies were also restricted either to adults or children [12,13,15,17,20].

The aim of this study was to compare the performance of the four IgG anti-DGP assays to other serologic assays for celiac disease including 3 IgA anti-tTG assays in a large cohort of 86 consecutive CD patients and 741 consecutive disease control patients. The study population consisted of 599 adults and 228 children. All CD and control patients had a duodenal biopsy.

2. Materials and methods

2.1. Study population

Patients attending the University Hospitals Leuven who had serologic tests for celiac disease and underwent an intestinal biopsy were identified retrospectively. The main reasons for performing these tests were diarrhea, weight loss, abdominal pain, anemia, anorexia, failure to thrive and small stature. Consecutive CD patients for whom serum was available were recruited over a 100-month period (between August 1st 2000 and November 31st 2008) and consecutive disease control patients over a 42-month period (May 1st 2004 and October 31st 2007). CD patients were recruited over a longer period in order to have a sufficient number of CD patients. Only newly diagnosed CD patients were included. Patients who were previously diagnosed with celiac disease or who were on a gluten-free diet were excluded.

The diagnosis of celiac disease was based on a combination of characteristic, though not specific, changes on duodenal biopsy and the clinical presentation. Intestinal biopsies were graded by a pathologist with more than 30 years experience (KG) who was unaware of the serologic results. The diagnosis of CD by the treating physician was considered confirmed in patients with Marsh IIIa to IIIc lesions on intestinal biopsy and in patients with Marsh I or II lesions on intestinal biopsy who responded to a gluten-free diet serologically or on intestinal biopsy. The diagnosis of non-CD was considered confirmed when intestinal biopsy showed a Marsh 0 at initial presentation or when intestinal biopsy showed Marsh I or Marsh II and the morphologic lesion could be explained by another disease according to the treating physician such as Helicobacter pylori gastritis or giardiasis.

The patient characteristics of the CD and non-CD group are given in Table 1. The CD group consisted of 86 consecutive newly diagnosed patients and included 1 patient with a selective IgA deficiency (IgA<0.05 g/L) and 1 adult patient with a decreased IgA concentration (0.22 g/L, reference interval 0.82–4.53 g/L). The disease control group consisted of 741 consecutive patients diagnosed as non-CD with no IgA-deficiency aged 2 years to 89 years.

The study was approved by the Institutional Ethics Committee of the University Hospital of the University of Leuven.

2.2. IgA measurement

Total serum IgA was measured by immunonephelometry on an Immage nephelometer (Beckman Coulter). A patient was considered non IgA-deficient when the IgA concentration was equal to or higher than the age-adjusted mean minus 2 standard deviations [21].

Table 1
Characteristics of the patients diagnosed as celiac disease and non celiac disease (non-CD).

	CD	Non-CD
Demographic data		
Number of patients	86	741
Male/female, *n*	27/59	305/436
Mean age (years)	29.9	28.6
≥16 years old	58	541
<16 years old	28	200
Biopsy results		
Marsh 0	0	703
Marsh I	8	37
Marsh II	7	1
Marsh IIIa	22	0
Marsh IIIb	20	0
Marsh IIIc	29	0

2.3. Serologic assays for celiac disease

Serologic assays were obtained from the following companies: Euroimmun (Lübeck, Germany), Genesis (Cambridgeshire, United Kingdom), Inova Diagnostics (San Diego, CA), Phadia (Uppsala, Sweden) and The Binding Site (Birmingham, United Kingdom). The assays from Euroimmun, Genesis, Inova Diagnostics and The Binding Site are enzyme immunoassays (ELISA) that were carried out on an automated ELISA instrument, the PhD (BioRad, Hercules, CA). The assays from Phadia are enzyme fluoroimmunoassays that were carried out on an automated ImmunoCAP 250 analyser (Phadia, Uppsala, Sweden). The manufacturer's recommended cut-off values as well as the cut-off values corresponding to a specificity of 98% were used to calculate the diagnostic accuracy for each test. So-called dubious results were treated as positive results.

The only results available to physicians as part of our routine laboratory testing for celiac disease were the results of the IgA anti-tTG assay from Genesis starting from May 2004. All other assays were performed on stored serum samples. Samples were stored at −20 °C.

2.4. Technical performance of the IgG anti-DGP assays

To evaluate interference by hemoglobin and unconjugated bilirubin, various concentrations of hemoglobin from lysed red blood cells and unconjugated bilirubin (Sigma, Saint Louis, MO) were added to a sample that did not contain IgG anti-DGP antibodies. Final concentrations were 0, 5, 10, 20, 35 and 50 g/L hemoglobin and 0, 50, 100, 200 and 300 mg/L unconjugated bilirubin. To evaluate interference by triglycerides, a sample with a low triglyceride content was mixed with a sample with a high triglyceride content to obtain final concentrations of 0.78, 1.84, 2.90, 3.97, and 5.03 g/L.

2.5. Statistical analysis

ROC plot analysis and linear regression was calculated using Analyse-it version 2.12 (Analyse-it Software Ltd., Leeds, UK). Sensitivity and specificity were compared using a Fisher's exact test since the expected value was sometimes less than 10. Confidence intervals for positive likelihood ratios were calculated by use of the methodology described by Simel et al. [22].

3. Results

3.1. Technical performance of the four IgG-DGP assays

3.1.1. Linearity

We determined the linearity by diluting a sample that contained a high concentration of IgG anti-DGP antibodies with increasing amounts (from 0% to 100%) of a sample that was negative for IgG