Evaluation of a point-of-care test based on deamidated gliadin peptides for celiac disease screening in a large pediatric population

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Objectives. Celiac disease (CD) is nowadays known to be a common chronic enteropathy that is becoming a growing public health concern. Yet, it is estimated that more than 90% of patients remain undiagnosed. A point-of-care diagnostic test can be a rapid and cost-effective solution in the first-line screening of CD. The aim of this study is to evaluate the performance of a novel point-of-care screening test in a large pediatric population.

Materials and methods. Serum samples were collected from a cohort of 250 children presenting either an increased risk or a clinical suspicion of CD. All sera were tested using the point-of-care test detecting IgA and IgG antibodies against a combination of three different deamidated gliadin peptides as well as total IgA. The results of the screening test were compared with an enzyme-linked tissue transglutaminase immunosorbent assay and with histology resulting from intestinal biopsies performed in patients with elevated titers of antitissue transglutaminase antibodies.

Results. The point-of-care test showed highly concordant results with the laboratory immunoassay, yielding a sensitivity of 93.1% (78–98.1%) and a specificity of 95% (91.2–97.2%), with a diagnostic accuracy of 94.8% (91.3–96.9%) and a negative predictive value of 99.1% (96.6–99.7%). The screening test identified all patients with celiac-type histology findings on biopsy, as well as all patients with concomitant IgA deficiency.

Conclusion. With a high diagnostic accuracy, this novel point-of-care approach is an efficient tool for CD case finding in pediatric populations. It has the potential to improve the management of celiac patients in primary care by providing faster counseling and treatment.

Keywords: celiac disease, deamidated gliadin peptides, point-of-care diagnostic test

Introduction. Celiac disease (CD) is a T-cell mediated gluten-sensitive chronic enteropathy, defined by characteristic changes observed on intestinal biopsy. Recent epidemiological studies have reported that CD is a very common disease affecting approximately 1% of the population not only in Europe and North America but also in many other parts of the world, and is becoming a widespread public health concern [1]. In some countries such as Finland, this figure has more than doubled to up to 2.7% in the last two decades [2,3]. Different environmental factors such as cereal consumption, breast-feeding, and early infections have been suggested to play a role in this increasing prevalence [4].

Current diagnosis relies on CD-related serology before confirmation through a small intestinal biopsy histopathologic examination. Serology markers have evolved over the years with the identification of more specific antibodies. Endomysial and antitissue transglutaminase (tTG) IgA autoantibodies are considered nowadays to be among the most reliable [5]. Although these markers show a high sensitivity and specificity, their accuracy remains controversial for young children, for adult patients with a minor degree of mucosal damage, and for the follow-up of CD patients on a gluten-free diet [6,7]. Very recently, a new generation of assays on the basis of the detection of antibodies against deamidated gliadin peptides (DGP) has shown very high sensitivity, with a diagnostic accuracy for CD that is at least equivalent to established assays [8,9]. tTG and DGP are important functionally related antigens that play a crucial role in the pathogenesis of CD. Indeed, tTG was found to catalyze deamidation of specific native gliadin residues, enhancing T-cell reactivity [10].

Left untreated, CD can lead to long-term health issues such as infertility, osteoporosis, and cancers of the digestive tract [11]. Yet, it is estimated that more than 90% of patients remain undiagnosed [12]. Increasing the diagnosis rate of CD can be quite challenging for physicians, as the clinical presentation is often highly variable, with patients being either asymptomatic or...
presenting typical gastrointestinal symptoms or extraintestinal manifestations. This results in delays in diagnosis that can be as long as 14 years [13].

Because CD can be treated effectively with a gluten-free diet, it is important to identify celiac patients as to provide treatment and therefore to improve the overall health of the community.

A number of studies have reported that screening programs are cost-effective strategies that can lead to a significant reduction in medical costs over time [14,15]. An accurate rapid, point-of-care serology test for CD would have the potential to markedly reduce the turnaround time for the delivery of results and allow faster counseling and treatment.

The aim of the present study is to evaluate the performance of a new rapid point-of-care serologic screening test on the basis of DGP in a large pediatric population in identifying patients who would require confirmatory biopsy. The results of the point-of-care test were compared with a well-established tTG-based enzyme-linked immunosorbent assay (ELISA) technique and with the results of intestinal biopsy histology.

**Materials and methods**

Serum samples are sent to the Immunology Laboratory of the Lyon-Sud University Hospital for centralized analyses. A cohort of 250 children (125 females, 125 males; mean age: 8.3 years, median: 8.2, range 0.2–20.3) was studied. The age distribution showed that the majority of the population (n = 190) was between 2 and 16 years old at the time of diagnosis, 49 children were younger than 2 years of age, and 11 were older than 16 years. The latter were either first-degree relatives of a celiac patient or patients monitored in our laboratory for CD serology since the discovery of type I diabetes in childhood.

The children selected presented either a clinical suspicion of CD (n = 149) or a risk factor (n = 101). Among the children with suggestive symptoms of CD, 73 experienced gastrointestinal manifestations, another 69 presented with growth failure, and seven had extraintestinal symptoms. The remaining 101 children had a risk factor for CD such as type I diabetes (n = 92), the presence of another autoimmune condition (n = 6), or first-degree relatives with either CD, type I diabetes, or an autoimmune disease (n = 3).

All serum samples were tested using a well-established IgA anti-tTG ELISA (Celikey, IgA Varelisa; Phadia, Uppsala, Sweden) and total IgA (BNII system; Siemens Dade Behring, Milton Keynes, UK) assay, performed according to the manufacturers’ instructions. IgG anti-tTG immunoassays (Celikey, IgG Varelisa; Phadia) were performed systematically in patients with total IgA deficiency (total IgA levels <0.06 g/l). The laboratory IgA and IgG anti-tTG ELISA reference assay cut-off values were established at 3 and 5 U/ml, respectively.

In the case of a positive laboratory result, intestinal biopsies were performed by upper gastrointestinal endoscopy within less than a month before the introduction of a gluten-free diet. A minimum of four biopsies was sampled from the bulb up to the distal duodenum. The mucosal biopsy sections were analyzed by an experienced histopathologist to assess for pathologic features of CD that included villous atrophy, crypt hyperplasia, chronic inflammation in the lamina propria, and increased intraepithelial lymphocytes with a cut-off exceeding 30 per 100 enterocytes. A diagnosis of CD was made using the modified Oberhuber–Marsh classification [16].

Venous blood sampling was performed by phlebotomy in clinical units, before centrifugation, to obtain serum samples. All serum (20 µl) was tested using the novel point-of-care screening test, Simtomax (Augurix SA, Monthey, Switzerland). Interestingly, different samples can be tested on the rapid test such as venous whole blood, serum, plasma (EDTA or heparin treated), and capillary blood from a simple finger prick. The assay is based on lateral flow immunochromatographic technology using colloidal gold antihuman antibodies as a signal detector. Simtomax simultaneously detects both IgA and IgG antibodies against DGP, as well as total IgA. The DGP marker used is a combination of three different nonapeptides conjugated on a carrier protein. The three analyses combined on the rapid test are essential in routine screening to detect IgA-deficient patients, a hereditary condition present in approximately 2% of celiac patients [17]. In these CD patients with IgA deficiency and consequently with negative IgA anti-DGP, the serologic diagnosis of CD will be established on the single positivity of IgG anti-DGP on line A (Fig. 1). An internal control system is also integrated into the test to ensure its proper function. The samples tested with Simtomax were visually read after 20 min. The test result is CD positive when both the CT control line and the A line can be seen. The absence of a B line indicates that the patient is IgA-deficient. Representative devices run with samples are shown in Fig. 1.

Statistical analyses were carried out using Open Epi, version 2.3.1 (Emory University, Atlanta, Georgia, USA) [18]. Performance of Simtomax was evaluated by calculating the sensitivity, specificity, positive predictive value, and negative predictive value compared with the outcome of the laboratory immunoassay results and biopsy. Ninety-five percent confidence intervals were determined using the Wilson method.

**Results**

We screened a total of 250 children. Within this cohort, 29 children (21 girls and 8 boys) were found to have elevated titers of anti-tTG. Twenty-seven had elevated IgA
anti-tTG and two were found to have high titers of IgG anti-tTG. Among these 29 patients, 25 underwent intestinal biopsies. We found that a total of 24 children had positive pathological findings. Only one patient was found to have a normal intestinal architecture, but had been on a gluten-free diet for several weeks between the biological assay and the endoscopy procedure. The elevated titers of IgA anti-tTG (> 100 U/ml) had been measured before the introduction of the gluten-free diet. Four children with positive titers of anti-tTG did not undergo biopsy. One patient, an 18-month-old girl, had very high levels of IgA anti-tTG ( > 100 U/ml) and IgG anti-tTG (65.9 U/ml) with a typical clinical presentation of CD. The three remaining children had levels of IgA anti-tTG close to the threshold (5.6, 4.5, and 5.1 U/ml), given at 3 U/ml. Two of them were screened for CD in the context of type I diabetes, and for one of the children, a positive HLA-DQ2 genetic background was found. The last patient presented with a failure to thrive. For this patient, no further follow-up was carried out.

Of the 29 seropositive patients, two (0.8%) were IgA deficient, with mean titers of anti-IgG antibodies of 59.3 U/ml. Both had positive biopsies.

The rest of the 221 patients were found to be seronegative for tTG.

All CD patients were newly diagnosed, and none were on a gluten-free diet by the time of the laboratory serology analyses and testing with Simtomax. The prevalence of biopsy-confirmed CD in the investigated cohort was 9.6%. The frequency of tTG seropositivity was slightly higher at 11.6%.

Point-of-care testing using Simtomax identified all 24 CD patients, including the two IgA-deficient patients, who had villous atrophy. The test also correctly evaluated 210 negative samples, thus achieving a 95% agreement with the reference laboratory tTG immunoassay (Table 1). No invalid rapid test devices were reported. These results yielded a specificity and a sensitivity of Simtomax of 95.0% (91.2–97.2) and 93.1% (78–98.1), respectively. Its diagnostic accuracy was 94.8% (91.2–96.9). Positive and negative predictive values were also computed, yielding 71% (55.2–83) and 99.1% (96.6–99.7), respectively, when comparing Simtomax with tTG laboratory results. As the positive predictive value is highly dependent on the disease prevalence of the population, a likelihood ratio (LR) was calculated, yielding a high positive LR of 18.71 (15.57–22.47).

A total of 13 discordant results were observed between the rapid test screening and the laboratory tTG-based immunoassay (Fig. 2). Two patients were found to be CD-negative with Simtomax, whereas they were slightly CD positive, with values of IgA anti-tTG at 5.1 and 5.6 U/ml, close to the cut-off of 3 U/ml. The first had type I diabetes. A serological follow-up after a few months showed a spontaneous regression to normal IgA anti-tTG values. The second patient was screened in the context of a growth failure. No confirmatory intestinal biopsies were performed for either one of them. Eleven patients were found to be CD positive using Simtomax whereas they were CD-negative using the laboratory immunoassay. Of these 11 children, five had type I diabetes and were being screened for CD on a regular basis. Three children presented with a growth failure. One child was diagnosed with a Helicobacter
A intestinal biopsy was performed, showing an increased intraepithelial lymphocytosis initially believed to be because of the infection, but a diagnosis of CD was confirmed several months later by another biopsy. One patient was found to have a liver cytolysis. One child was screened for CD in the context of multiple food allergies. An additional genetic analysis did not indicate the presence of either HLA-DQ2 or DQ8.

**Discussion**

CD has now been acknowledged to be a common medical condition, with an estimated prevalence close to 1% worldwide [19]. In this context, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) has formulated new guidelines for the diagnosis of CD on the basis of scientific and technical developments in an evidence-based approach [20]. Different reference parameters are used to diagnose CD such as the presence of gluten-dependent symptoms, CD-specific antibody levels, HLA-DQ2 and/or DQ8 positivity, and evidence of mucosal lesions on intestinal biopsy. However, traditional CD management requires several consultations. Primary care physicians could play a crucial role in the management of patients presenting with symptoms potentially related to CD. In this specific setting, there is an unmet clinical need for an accurate and user-friendly point-of-care test that could facilitate the screening procedure, in particular to exclude CD.

The need to refer to secondary care for further investigation could be limited to patients found positive on the rapid test. Thus, the availability of CD screening in primary care could have the potential to reduce healthcare resource utilization and increase the rate of diagnosis. Moreover, a high negative predictive value of 99.1% compared with laboratory assay is an argument for the use of Simtomax in screening.

In this study, we have tested the performance of a novel point-of-care test based on DGP in a large pediatric population. Antibodies against DGP have already been shown to be accurate biomarkers of childhood CD [21]. The point-of-care screening test showed highly concordant results with the laboratory anti-tTG immunoassay, with a sensitivity and a specificity of 93.1 and 95%, respectively. Furthermore, when considering the population confirmed with the assertion diagnostic analysis (intestinal biopsy), the point-of-care test identified all patients found to have positive CD-type histology findings. Importantly, the rapid test also correctly identified the two IgA-deficient patients, results that need to be confirmed in a larger cohort of patients. This is an important feature for CD case finding as selective IgA deficiency is found more often in celiac patients [22].

However, a total of 11 CD-positive patients using Simtomax were found among the CD-negative laboratory immunoassay results. Indeed, the rapid test appears to be

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**Fig. 2**

**Discordant results**

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<tr>
<th>Rapid test+/laboratory−</th>
<th>Rapid test−/laboratory+</th>
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Diabetes type I
- Boy 4.7 years
- Girl 5.4 years
- Girl 13.5 years
- Girl 16.5 years
- Boy 17.1 years

Growth failure
- Boy 1.3 years
- Boy 13.2 years (bone pain)

Gastrointestinal
- Boy 3.3 years
- (Helicobacter Pylori infection)

Other
- Girl 0.8 years (food allergies)
- Girl 1.6 years (liver cytolysis)

Clinical symptoms in patients with discordant results between the rapid test and the laboratory reference.

*Pylori* infection. An increased intraepithelial lymphocytosis initially believed to be because of the infection, but a diagnosis of CD was confirmed several months later by another biopsy. One patient was found to have a liver cytolysis. One child was screened for CD in the context of multiple food allergies. An additional genetic analysis did not indicate the presence of either HLA-DQ2 or DQ8.
less specific in children with type I diabetes. The cohort studied presented a high percentage (36%) of type I diabetes. These patients are regularly monitored as they are at risk of developing a CD [23]. In a previous study, it was shown that anti-tTG antibodies are closely correlated with intestinal mucosal lesions in these patients and that CD is most often present before the onset of diabetes [24]. Unfortunately, intestinal biopsies were not performed systematically in these patients. It would be interesting to follow up this population and correlate the results of the rapid test with intestinal biopsy. Interestingly, the child with a \textit{H. Pylori} infection with a Simtomax-positive result was confirmed to have CD.

In this context, as it has been reported recently, anti-DGP antibodies could be better and more sensitive predictors of early-stage CD than anti-tTG [25]. In a screening perspective, a test with a high sensitivity value is preferred over one with a high specificity with the risk of yielding a high number of false-positive results when a condition has a low prevalence [26]. However, a high positive LR was found for Simtomax, indicating an increased certainty of a positive diagnosis.

The present study showed that this novel point-of-care DGP-based approach is an efficient tool for CD case finding in high-risk pediatric populations. It would be deemed necessary to assess its performance at a population level with a lower prevalence of CD. Unfortunately, a considerably higher fraction of false-positive results could be predicted in this case, and this is why this test should not be used as a home test. However, the test format was found to perform robustly, and has been described as easy to use and interpret. As such, it would fit the needs for CD screening in primary care by facilitating diagnostic work-up in a high-risk pediatric population. Simtomax strengthens the suspicion of CD in the case of positive results, but could become essentially an interesting tool in an exclusion diagnostic strategy. It would reduce the need for referral to secondary care to only positive patients requiring a confirmatory quantitative laboratory assay for anti-tTG IgA. In the case of high titer results and clinical symptoms suggestive of CD, the clinician could choose to follow the new ESPGHAN guidelines [20] and avoid an unnecessary intestinal biopsy. Healthcare resource utilization would therefore be reduced, and ultimately result in cost savings. The budgetary impact of using the assessed DGP-based point-of-care test in a screening process has been evaluated economically and could lead to substantial savings [27].

**Conclusion**

The use of a DGP point-of-care test has the potential to improve the management of patients who show symptoms likely to be related to CD in primary care. It allows either to provide faster counseling and treatment to newly diagnosed celiac patients, or to rapidly rule out CD in the diagnostic approach.

**Acknowledgements**

**Conflicts of interest**

Dr Cécile Besson Duval sits in the board of Augurix SA, a company based in Monthey, Switzerland, who supported the study by a grant. For the remaining authors there are no conflicts of interest.

**References**