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ORIGINAL CONTRIBUTIONS

Looking for Celiac Disease: Diagnostic Accuracy of Two Rapid Commercial Assays

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BACKGROUND: Early diagnosis and treatment with gluten-free diet reduces mortality and the prevalence of associated disorders in celiac disease (CD). A simple "in the office" test of anti-transglutaminase antibodies might be of great help in first-line screening for CD.

AIMS: We evaluated the sensitivity and specificity of two commercial kits based, respectively, on rapid detection of IgA-IgG anti-human-transglutaminase antibodies (anti-h-tTG) in serum and IgA anti-h-tTG antibody in one drop of whole blood. These assays were compared to a well-established enzyme-linked immunosorbent assay technique.

METHODS: Serum samples were analyzed from 114 biopsy-confirmed celiacs, 120 healthy controls, 20 first-degree relatives of celiacs, and 75 diseased controls. The whole blood samples were analyzed from 51 biopsy-confirmed celiacs and 100 controls.

RESULTS: The serum-based test was positive in all 114 celiacs (sensitivity 100%). Among the controls there were seven healthy blood donors, one first-degree relative, and three diseased controls who tested positive (specificity 94.9%). The blood drop-based assay testing IgA antibodies was positive in 46 of 51 (sensitivity 90.2%), and since three of the five patients testing negative had total IgA deficiency, the sensitivity value can be increased to 95.8%. All 100 controls tested negative (specificity 100%).

CONCLUSIONS: The commercial kits described here produce high values of sensitivity and specificity, offering the general practitioner who suspects a possible case of CD the real possibility to look for anti-h-tTG antibodies in his own medical office during a standard visit at a satisfyingly low cost.

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Celiac disease (CD) is a gluten-dependent autoimmune disease, estimated to occur in 1% of the population (1). In pediatric and adult case-finding studies (2, 3), general physicians have found that the prevalence of CD increases to 12–23% among patients with anemia, to 9% among children with Down syndrome, to 7% among first-degree relatives of CD patients, to 7.6% with isolate stature growth defect, to 5% among patients with autoimmune diseases, and to 1.8% among patients suffering from chronic fatigue. The later the diagnosis of CD, the more likelihood of serious illness and excess mortality (4, 5). Early diagnosis and treatment with gluten-free diet, on the other hand, reduces mortality and the prevalence of CD-associated disorders. Although definitive diagnosis of CD is still based on certain characteristic histological changes in the jejunal intestine, serological tests for CD screening based on detection of anti-human-transglutaminase antibodies (anti-h-tTG) are cheaper and less invasive, with excellent sensitivity (98%) and specificity (95%) (6). Given the high prevalence of the disease and the implications of missing it (or detecting it late), two prototypes of simple immunoassays were developed as a first step toward speeding up CD diagnosis in the physician's office (7, 8). Here we present two commercial test kits, based, respectively, on detection of IgA-IgG anti-h-tTG antibodies in serum (Stick CD1, Operon S.A., Saragoza, Spain) and IgA anti-h-tTG antibodies in one drop of whole blood (Biocard^{TM} Celiac Disease Stick, Ani Biotech Oy, Vantaa, Finland), both performable in 5 min. We prospectively evaluated the sensitivity and specificity of these assays, and interobserver variability in the interpretation of results compared with a well-established enzyme-linked immunosorbent assay (ELISA) technique for the diagnosis of CD.

SUBJECTS AND METHODS

We consecutively examined serum samples from 114 biopsy-proven untreated CD patients (74 were female, 40 were male, median age 6 yr, range 1–53) diagnosed between January 2004 and September 2005 following the revised ESPGHAN criteria for CD (9). All the patients were referred to our

third-level referral clinic for pediatric gastroenterology by family doctors for intestinal biopsy following positive serological tests for CD and/or strongly suspected CD. Clinical characteristics were as follows: 20 patients were suffering from chronic diarrhea, 36 from failure to thrive, 30 from sideropenic anemia, 4 from total serum IgA deficiency, and 13 from autoimmune disorders, while 29 had no symptoms but were first-degree relatives of CD patients. Biopsy specimens were analyzed according to Oberhuber's classification (10), showing a type 1 lesion in 2 patients, type 3a lesion in 19, type 3b in 39, and type 3c in 54. All patients were confirmed as positive for IgA and/or IgG anti-h-tTG using our ELISA assay (11). Controls comprised 120 healthy blood donors, 20 first-degree relatives of CD patients, and 75 patients suffering from various diseases (48 Crohn's disease, 12 autoimmune diseases, 15 recurrent abdominal pain) but testing negative for IgA-IgG anti-h-tTG by ELISA assay. The control groups comprised 125 female subjects and 90 male subjects (median age 9 yr, range 2–45).

Eight months into the study (from September 2004), with the availability of a new assay kit based on whole blood drop, it was possible to extend the investigation to include blood as well as sera; whole blood samples were consecutively collected and examined from a total of 51 untreated celiacs (36 were female, 15 were male, median age 7 yr, range 1–43; clinical characteristics: 10 patients suffered from chronic diarrhea, 10 from failure to thrive, 15 from sideropenic anemia, 3 from total serum IgA deficiency, 7 from autoimmune disorders, while 10 had no symptoms but were first-degree relatives of CD patients; intestinal lesions: type 1 in 1 patient, type 3a in 8, type 3b in 19, type 3c in 23) and from 100 nonceliac controls (60 were female, 40 were male, median age 15 yr, range 3–50; 23 healthy blood donors, 35 patients with Crohn's disease, 2 liver transplant patients, 12 failure to thrive, 18 recurrent abdominal pain, and 10 CD first-degree relatives).

The serum samples were diluted 1:5 for the *StickCD1* assay; for the *Biocard*™ *Celiac Disease Stick* a drop of whole blood was diluted 1:50. The sticks were incubated with the samples and the result was readable after 5 min. Positive results appear as varying shades of red or gray bands, negative results remain white (Fig. 1). The reactions were interpreted by three operators (GN, VB, AT) blind to the subjects' histories and laboratory findings. Interobserver variability in the interpretation of the immunochromatographic results were computed using k statistic including the 95% confidence interval.

Serum IgA-IgG anti-h-tTG antibodies were measured by ELISA in plates coated with 1 μg/well of human-tTG. Serum samples diluted 1:100 were incubated for 1 h, and then for 1 h more with anti-human IgA or IgG. Absorbance was read at 405 nm. The results were expressed as percentages of the positive control serum. The specificity and the sensitivity of the assay were 95% and 98% as previously reported (11). This assay was performed 4–7 days after the immunochromatographic assays by two operators (AD, AC) blind to the clinical and laboratory findings of all the sick and healthy subjects tested.

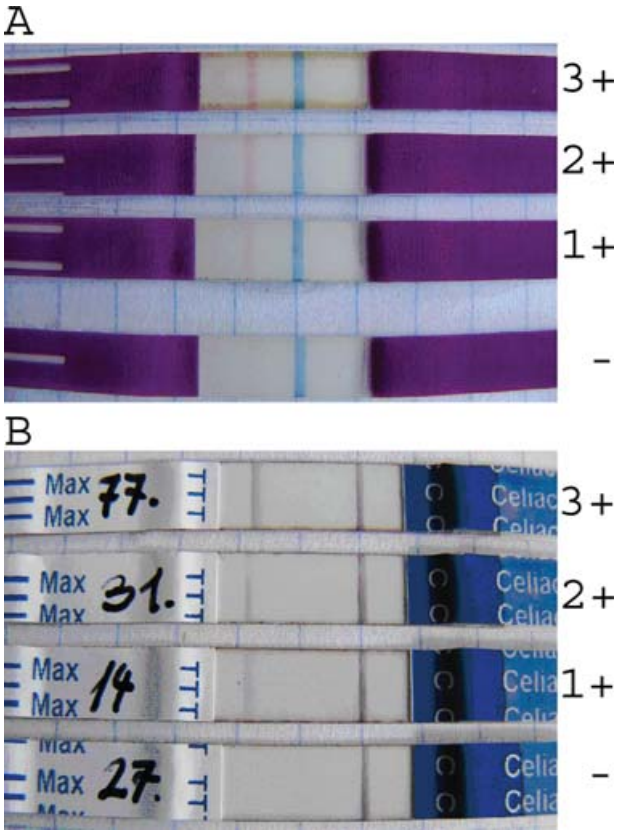

Figure 1. In both immunoassays the first band represents the result of the test, whereas the right intense band must always appear as technical control. (*A*) Serum-based *StickCD1* assay. This assay identified celiac patients in three different shades of red bands (color reactions: 3+ in the first strip, 2+ and 1+ in the second and third strip). No red band was detected in the fourth strip incubated with serum tested negative for IgA and IgG anti-human-transglutaminase antibodies. (*B*) Blood drop-based *Biocard*™assay. Different shades of gray bands identified celiac patients (color reaction: 3+ in the first, 2+ and 1+ in the second and third strip). No gray band was detected in the fourth strip incubated with serum tested negative for IgA anti-human-transglutaminase antibody.

RESULTS

StickCD1 (serum-based, detecting IgA-IgG anti-h-tTG) results. All of the 114 CD patients tested positive to the *StickCD1* assay (stick sensitivity was 100%) and to the ELISA assay (110 of 114 testing positive for IgA and 72 of 114 testing positive for IgG; all 4 patients with serum IgA deficiency were positive for IgG) (Fig. 2A). Among the controls, 7 of 120 healthy blood donors, 1 of 20 first-degree relatives and 3 diseased controls (1 with Crohn's disease, 2 with type 1 diabetes) tested positive (specificity 94.9%); all these positive results had a weak 1+ color reaction. The 3 diseased controls and the first-degree relative also tested positive to IgG anti-h-tTG ELISA assay, while the 7 blood donors tested negative to ELISA assay. The 3 diseased controls underwent intestinal biopsy but no CD-related lesions were found, while

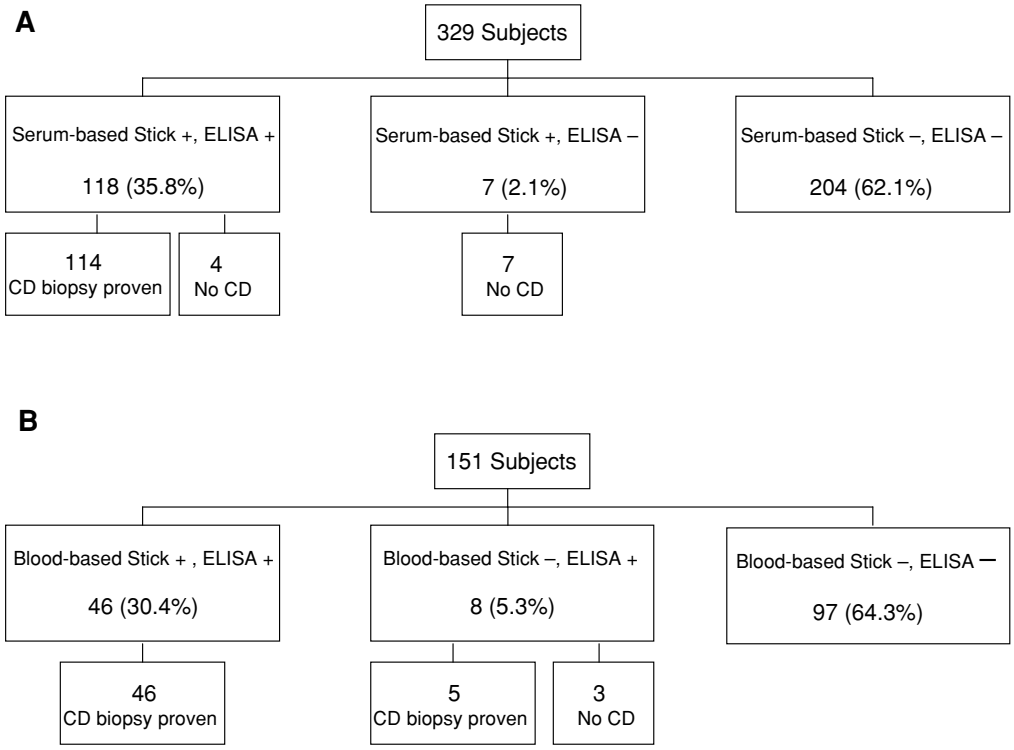

Figure 2. Immunochromatographic assay results to both serum (*A*) and blood drop samples (*B*) in comparison with the enzyme-linked immunosorbent assay results. The IgA-IgG anti-transglutaminase antibodies serum-based assay identified all the untreated celiac patients (sensitivity 100%), and produced 11 false positive results (specificity 94.9%). The IgA anti-transglutaminase antibody blood-based assay identified 46 of 51 untreated celiac patients (sensitivity 90.2%), 3 of whom had total serum IgA deficiency (sensitivity 96%). No controls tested positive (specificity 100%).

the asymptomatic first-degree relative who tested negative for the CD-related HLA DQ2/8 refused intestinal biopsy. The 7 blood donors were negative for clinical history of immunological or gastrointestinal disorders and 8 months later were confirmed negative for anti-tTG ELISA assay and tested negative for the *StickCD1* assay.

Biocard™ *Celiac Disease Stick* (blood drop-based, detecting IgA anti-h-tTG) results. Of 51 biopsy-confirmed CD patients, 46 tested positive (sensitivity 90.2%). Among the *Biocard* study group, 3 patients suffering from total IgA deficiency tested negative (Fig. 2B). If these 3 patients are excluded from the sensitivity calculation we obtain a sensitivity of 96%. All the controls tested negative (specificity 100%). ELISA assays were positive in all the 51 untreated patients (48 of 51 testing positive for IgA and 33 of 51 testing positive for IgG; all 3 CD patients with serum IgA deficiency were positive for IgG). Two diseased controls with Crohn's disease and 1 first-degree relative tested positive to IgG anti-h-tTG ELISA assay.

The negative and positive results of all three operators on both stick assays were perfectly in agreement (125+ and 204− among the serum-based assay; 46+ and 105− among the blood drop-based assay). Interobserver variability analysis in the interpretation of the varying degrees of positivity among the three operators to the serum-based sticks and to blood-based sticks produced a mean k value of 0.830 ± 0.140 standard deviation (95% CI 0.743–0.917) and of 0.763 ± 0.168 (95% CI 0.592–0.930), respectively.

DISCUSSION

The serum-based test (*StickCD1*) was highly sensitive and identified all four CD patients with total serum IgA deficiency. The latter result is particularly satisfying because it proves that the IgG-reagent component is able to pick out those cases with IgA deficiency, a condition present in about 8% of all CD patients (12). It would also seem that the assay works well regardless of the grade of intestinal lesion; patients with type 1 lesions tested positive as well. One of the limits of the test lies in the need for a serum sample, which may be small but still involves venous puncture, not always practicable in the medical office. The need for a centrifuge may also represent a drawback, since basic laboratory equipment is often not available in a family doctor's office, but more and more are being equipped with similar biomedical devices (13), making serological testing possible. These two limitations should not exist in hospital facilities at any level, especially in outpatient clinics, which could finally free themselves of their dependence upon centralized laboratories, and provide evidence-based diagnosis in the course of one visit.

The blood-based test (*Biocard*™) does indeed possess all the prerequisites needed for a busy doctor's office, but sensitivity was slightly lower (96%) than the serum-based test and it failed to identify the three patients with IgA deficiency. Until *Biocard* can measure also anti-h-tTg IgG, it is advisable to perform simultaneous total serum IgA assay in subjects at risk for CD who test negative for this assay.

Although the two assays are operator-dependent with respect to how the colors on the reactive bands are read, test reproducibility was very high, both as regards determining of positive and negative results and identifying the different degrees of color reaction. Although this very encouraging result was obtained in a large series and in a prospective study, it obviously needs to be confirmed on a larger scale and by others, especially by family doctors in their day-to-day practice. It must be stressed, however, that despite the excellent sensitivity and good specificity of these kits (comparable to the well-established ELISA test currently used in accredited laboratories) (14), intestinal biopsy is still the gold standard for a firm diagnosis of CD.

In conclusion, the commercial kits described here are indeed quick and easy, with high values of sensitivity and specificity, giving the physician who suspects a case of CD the opportunity to test for anti-h-tTG in his own office during a standard visit at a satisfyingly low cost (around ◦ 6 per test, *vs.* ◦ 16 for IgA-IgG ELISA assay). Since all CD patients can be spotted by the serum-based assay (regardless of the clinical form of the disease), cases can be quickly identified, thereby avoiding late diagnosis, especially risky among patients with atypical symptoms (about 60% of celiacs) (15). We are convinced that this new way of testing for CD can be used on a large scale for daily practice in case-finding situations (such as anemia, autoimmune diseases, and fatigue), bearing in mind that CD is not only treatable, but also carries the risk of serious, though preventable, long-term complications.

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STUDY HIGHLIGHTS

What Is Current Knowledge

- Screening tests for celiac disease generally require a series of blood assays sent to a reference lab resulting in a delay of 1–5 days in identifying patients potentially at risk for celiac disease.

What Is New Here

- A simple "in the office" test of antitransglutaminase antibodies is very helpful in immediately screening patients for the presence of celiac disease with a sensitivity of 95.8% and a specificity of 100%.

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