Low Sensitivity of Simtomax Point of Care Test in Detection of Celiac Disease in a Prospective Multicenter Study

Paul Tangermann,[*] Federica Branchi,[*] Alice Itzlinger,[*] Jens Aschenbeck,[‡] Stefan Schubert,[§] Jochen Maul,[§] Thomas Liceni,[§] Andreas Schröder,[‖] Frank Heller,[¶] Wolfgang Spitz,[#] Ulrich Möhler,[#] Ulrich Graefe,[#] Michael Radke,[**] Stefan Trenkel,[**] Markus Schmitt,[‡‡] Christoph Loddenkemper,[§§] Jan C. Preiß,[*] Reiner Ullrich,[*] Severin Daum,[*] Britta Siegmund,[*] Christian Bojarski,[*] and Michael Schumann[*]

*Department of Gastroenterology, Infectious Diseases and Rheumatology, Campus Benjamin Franklin, Charité – Universitätsmedizin Berlin and Berlin Institute of Health, Berlin, Germany; [‡]Praxis Dr. Aschenbeck, Berlin, Germany; [§]Praxis für Gastroenterologie am Bayerischen Platz, Berlin, Germany; [‖]Gemeinschaftspraxis Hohenzollerndamm, Berlin, Germany; [¶]Praxis Heller/Mayr, Berlin, Germany; [#]Gastroenterologie am Mexikoplatz, Berlin, Germany; **Ernst von Bergmann Klinikum, Potsdam, Germany; [‡‡]Evangelisches Krankenhaus Ludwigsfelde-Teltow, Ludwigsfelde, Germany; and [§§]PathoTres Praxis für Pathologie und Neuropathologie, Berlin, Germany

BACKGROUND & AIMS: **Point of care tests (POCTs) might be used to identify patients with undiagnosed celiac disease who require further evaluation. We performed a large multicenter study to determine the performance of a POCT for celiac disease and assessed celiac disease prevalence in endoscopy centers.**

METHODS: **We performed a prospective study of 1055 patients (888 adults; median age, 48 yrs and 167 children; median age, 10 yrs) referred to 8 endoscopy centers in Germany, for various indications, from January 2016 through June 2017. Patients were tested for celiac disease using Simtomax, which detects immunoglobulin (Ig)A and IgG antibodies against deamidated gliadin peptides (DGP). Results were compared with findings from histologic analyses of duodenal biopsies (reference standard). The primary aim was to determine the accuracy of this POCT for the detection of celiac disease, to identify candidates for duodenal biopsy. A secondary aim was to determine the prevalence of celiac disease in adult and pediatric populations referred for outpatient endoscopic evaluation.**

RESULTS: **The overall prevalence of celiac disease was 4.1%. The POCT identified individuals with celiac disease with 79% sensitivity (95% CI, 64%–89%) and 94% specificity (95% CI, 93%–96%). Positive and negative predictive values were 37% and 99%. When we analyzed the adult and pediatric populations separately, we found the test to identify adults with celiac disease (prevalence 1.2%) with 100% sensitivity and 95% specificity. In the pediatric population (celiac disease prevalence 19.6%), the test produced false-negative results for 9 cases; the test therefore identified children with celiac disease with 72% sensitivity (95% CI 53%–86%). Analyses of serologic data revealed significantly lower DGP titers in the false-negative vs the true-positive group.**

CONCLUSIONS: **In a study of more than 1000 adults and children, we found the Simtomax POCT to detect celiac disease with lower overall levels of sensitivity than expected. Although the test identifies adults with celiac disease with high levels of sensitivity and specificity, the prevalence of celiac disease was as low as 1.2% among adults. The test's lack of sensitivity might be due to the low intensity of the POCT bands and was associated with low serum DGP titers. Study ID no: DRKS00012499.**

Keywords: Noninvasive; Gluten; Gastroscopy; Diagnostic.

Abbreviations used in this paper: **CI, confidence interval; DGP, deamidated gliadin peptide; FN, false negative; POCT, point-of-care test; tTG, antitransglutaminase antibodies.**

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Celiac disease is a chronic inflammatory disorder of the small bowel, characterized by an abnormal immune response triggered by the ingestion of gluten in genetically susceptible individuals.[1] It is the most common chronic enteropathy in Western countries with an estimated prevalence up to 1% in Europe.[2] Data from population screenings suggest that a large percentage of celiac patients remains undiagnosed.[3] The wide spectrum of celiac disease manifestations causes the diagnosis to be challenging: the classic manifestation with malabsorption syndrome, weight loss, and diarrhea represents nowadays only a small proportion of cases, whereas many patients report mild gastrointestinal symptoms or single-nutrient deficiency.[4] In view of this, physicians evaluating patients with nonspecific gastrointestinal symptoms that are common in many functional and organic disorders face the risk of missing a celiac disease diagnosis.[1,5] Particularly, patients referred for gastroscopy are not regularly tested for celiac disease serology, such as antitransglutaminase antibodies (tTG; sensitivity 95% and specificity 95%[5]) or deamidated gliadin peptides antibodies (DGP; sensitivity 82%–84% and specificity 80%–99%[6]). Considering the reported lack of visual endoscopic markers pointing toward the diagnosis,[7] a trigger to acquire duodenal biopsies is frequently missing. Routine duodenal biopsies during all gastroscopies could improve diagnosis rates but would result in a high burden in terms of costs and resources and increase the risk of complications.[8–10] In this setting, the availability of a simple point-of-care test (POCT) for the detection of specific serum antibodies would be of great value in aiding the diagnostic process. Particularly, a test with an optimal sensitivity and a reasonable specificity could contribute to select patients with suspected celiac disease in whom to perform duodenal biopsies. To date, different POCTs have been evaluated as possible screening in primary or secondary care.[11–17] Simtomax (Tillotts Pharma AG, Rheinfelden, Switzerland) is a POCT developed to detect serum IgA and IgG antibodies specific for DGP.[18] In several previous single-center studies, Simtomax was applied to patient populations undergoing gastroscopy for both suspected celiac disease and unspecific GI symptoms and showed sensitivity and specificity similar to routine tTG testing.[17–23]

With these premises, the aim of our study was to evaluate the sensitivity and specificity of Simtomax in a clinical endoscopy setting. Our goal was to establish whether the accuracy of this POCT for the detection of celiac disease is sufficient to propose its introduction in routine endoscopic practice as a screening test to guide duodenal biopsy. A secondary aim of our study was to obtain prevalence data for celiac disease in an adult and a pediatric population referred for outpatient endoscopic evaluation.

What You Need to Know

Background

The diagnosis of CD can be challenging with high numbers of undiagnosed cases nowadays. Rapid, easy-to-use POCTs are currently under investigation to uncover CD cases, showing diagnostic performance close to standard serology in previous monocentric studies.

Findings

This large multi-center-study showed an unreported lack of sensitivity of the Simtomax-POCT. The resulting false negative tests are linked to weakly colored test-bands and low antibody titers.

Implications for patient care

At this stage the POCT does not qualify to be introduced as a screening test into routine clinical practice.

Materials and Methods

Study Design

This prospective, investigator-initiated multicenter study was carried out in 8 endoscopy centers within the greater Berlin area in Germany, including 5 adult outpatient endoscopy services and 3 hospital-associated endoscopy divisions, of which 1 is a tertiary center for adult gastroenterology and 2 are pediatric centers. The recruiting centers were coordinated by the Department of Gastroenterology, Infectious Diseases and Rheumatology (Campus Benjamin Franklin, Charité-Universitätsmedizin Berlin).

Patients

Between January 2016 and June 2017, patients referred to the participating centers for gastroscopy were prospectively evaluated for participation in the study. The inclusion criteria were the presence of informed consent for participation and clinical indication to gastroscopy. Exclusion criteria were a known diagnosis of celiac disease, an ongoing gluten-free diet, and the presence of bleeding disorders including the intake of anticoagulant medications.

Point-of-Care-Test

Simtomax is a lateral-flow immunochromatographic test developed to detect IgA and IgG class DGP antibodies (Figure 1). The POCT was performed and interpreted according to the manufacturer's instructions. For further details, refer to the Supplementary Methods section.

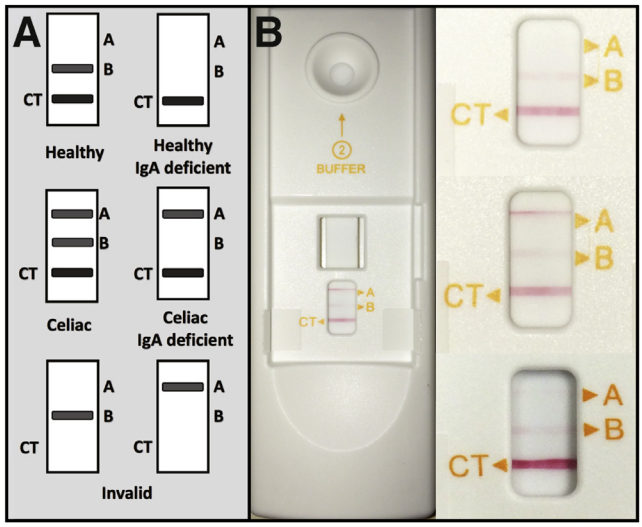

Figure 1. Simtomax POCT. (*A*) Instructions to read test results (adapted from the manufacturer's instructions leaflet). *Band A* is for DGP antibodies detection, *Band B* is for total IgA (presence of a visible line indicates normal IgA levels), and *Band CT* is a control band (absence of a visible line indicates invalid result). (*B*) Example of POCT tests with representative results of negative (*above*), positive (*middle*), and weakly positive (*below*) POCT in 3 subjects with normal IgA levels.

Histology

During gastroscopy, 6 duodenal biopsy specimens were collected from the duodenum, including 1 biopsy from the duodenal bulb, according to current guidelines.[1,5,24] Biopsies were fixed in buffered formalin and sent to the pathology services of the recruiting centers. According to practice standards, the specimens were oriented and successively embedded in paraffin wax. Sections 1–2 μm thick were stained with hematoxylin-eosin and periodic acid–Schiff and evaluated by pathologists blinded to the POCT result. The presence of intraepithelial lymphocytosis, villous atrophy, and crypt hyperplasia was graded according to the Marsh-Oberhuber classification.[25] A prestudy briefing with participating pathology centers ensured adherence to adequate standards of histopathology analysis and reporting.

Diagnosis of Celiac Disease

The diagnosis of celiac disease was confirmed or excluded based on the criteria established by international guidelines, namely the presence of a Marsh-graded enteropathy and positive tTG (or, in accordance to the ACG and European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines, positive DGP antibodies in children younger than 2 years).[5,24] In all cases with Marsh other than 0 or with a positive POCT, tTG and/or DGP serology were performed. In case of discrepant results (ie, incoherence of tTG-IgA and Marsh grade), an expert pathologist blinded to POCT results and to the site pathology report reviewed the duodenal histology and verified quantification of intraepithelial lymphocytes by immunohistochemistry (CD3, CD8) and the study clinicians re-evaluated the cases according to clinical presentation. In particular, patients with Marsh lesions grade 1 and negative antibody titers were not classified as celiac disease. The DQ2/8 status and/or the clinical response to a gluten-free diet were taken into account in those cases remaining unclear.

Ethics

The study was approved by the Ethics Committees of Charité Universitätsmedizin Berlin and of Brandenburg, Cottbus, Germany (protocol numbers EA4/140/15 and AS83(bB)/2016, respectively). It was conducted in accordance with the principles of the Helsinki Declaration. All authors had access to the study data and reviewed and approved the final manuscript. The trial was registered in the German Clinical Trials Register (DRKS, approved WHO Primary Register, www.drks.de, Study ID: DRKS00012499).

Statistical Analysis

A sample size calculation was performed before study start with the Software nQuery-3. It was initially determined that a minimum of 560 cases were required to investigate the performance of the test at an estimated celiac disease prevalence of 5% in a setting of adult gastroscopy services. This prevalence turned out to be overestimated, therefore an amendment to the protocol was approved for the recruitment of further participants including children, because it was anticipated that pediatric services have a substantially higher percentage of celiac disease cases. Within the new study population of adults and children, with an estimated celiac disease prevalence of 3.5%, the total sample size required was calculated as at least 988 subjects (to ensure a precision of 10% for an estimated sensitivity of 90%, with a 2-tailed confidence interval [CI] of 95%).

The diagnostic performance of the POCT was calculated as sensitivity, specificity, and positive and negative predictive value and reported with 95% CI. Descriptive statistics (median, range, and interquartile ranges) were calculated, and chi-square or Fisher exact test was used to compare proportions. Mann-Whitney test was used to determine significance of tTG and DGP serology. *P* values were considered significant when $<.05$. Analyses were performed with the software GraphPad Prism version 6.0 for Mac (GraphPad Software, La Jolla, CA).

Table 1. Characteristics of Study Participants

	Adults (n = 888)	Children (n = 167)	Overall (n = 1055)	*P* value
Median age, *y*	48 (18–87)	10 (0–18)	42 (0–87)	<.0001
Female/male	538/350 (61/39)	93/74 (56/44)	631/424 (60/40)	ns
Indication for gastroscopy				
Abdominal pain/dyspepsia	542 (61)	98 (59)	640 (61)	ns
Anemia	28 (3)	1 (1)	29 (3)	ns
Weight loss/malabsorption	22 (2)	5 (3)	27 (3)	ns
Diarrhea[a]	61 (7)	9 (5)	70 (7)	ns
Gastroesophageal reflux	183 (21)	34 (20)	217 (21)	ns
Dysphagia	27 (3)	4 (2)	31 (3)	ns
Obstipation	—	2 (1)	2 (0.2)	.025
Failure to thrive	—	5 (3)	5 (1)	< .0001
Other[b]	25 (3)	9 (5)	34 (3)	ns

NOTE. Data are expressed as median (range) or number (percentage).
[a]Mean stool frequency within diarrhea was 5/day for adults, 2/day for children, and 4/day overall.
[b]Other indications included Crohn's disease, arthralgia, esophageal varices, gastric cancer, autoimmune gastritis, endoscopy before gastric bypass/fundoplication, skin rash, and screened positive for Crohn's disease by antitransglutaminase antibodies (first-degree relatives, diabetes mellitus type I, other autoimmune disorder).

Results

Characteristics of the Enrolled Patients

Within the study period, a total of 1097 patients were enrolled, of whom 1055 were suitable for analysis by POCT and gastroscopy. Characteristics of the study participants are summarized in Table 1. Among the 888 adults (538 females; median age, 48 years) and the 167 children (93 females; median age, 10 years), the main indications for referral to gastroscopy were chronic abdominal pain/dyspeptic symptoms in both groups, followed by gastroesophageal reflux symptoms.

The contribution of each center to the overall recruitment is depicted in Supplementary Figure 1. Each of the adult gastroenterologic centers enrolled between 3% and 32% of all adult participants, whereas the 2 pediatric centers enrolled 19% and 81% of all children.

Performance of the Point-of-Care Test

After collection of POCT data, the results were compared with duodenal histology and with tTG and/or DGP antibodies titers. Figure 2 and Supplementary Figure 2 summarize the results of the POCT toward the diagnosis of celiac disease, and Supplementary Table 1 describes the approach to the unclear cases.

Apart from 8 cases of invalid POCT (0.8%), 1047 POCT results were compared with the respective pathology reports. The POCT was found positive in 91 of 1047 cases (56 adults and 35 children), of whom 34 were diagnosed with celiac disease. Among the 956 negative POCTs, 9 individuals were diagnosed with celiac disease (9 false negatives [FN]). These data allowed for the calculation of specificity and sensitivity of the POCT (Table 2). In the whole cohort, the POCT showed a good specificity (94%; 95% CI, 93%–96%) but a suboptimal sensitivity (79%; 95% CI, 64%–89%).

When analyzing the adult and the pediatric population separately, a sensitivity and specificity of 100% and 95%, respectively, were found in the adult group, where no celiac disease cases were diagnosed among patients with a negative POCT. Nevertheless, the 95% CI for sensitivity was considerably wide, as a consequence of the comparatively small number of celiac disease cases identified in the adult population, so that the performance of the POCT was not significantly different. Sensitivity in children was as low as 72% (95% CI 53%–86%) because of the presence of 9 FN, whereas the specificity was 91% (95% CI, 85%–95%).

All false-positive cases showed clinical characteristics similar to the patients with true-negative POCT results (54x Marsh 0, 3x Marsh 1, all tTG negative with normal IgA levels).

Analysis of False-Negative Results

The 9 celiac disease cases with a negative POCT were further analyzed to better understand the reason why FN POCT results occurred (Table 3). Notably, all cases were found in the same pediatric center, although it must be emphasized that this center recruited almost 5 times more participants than the second pediatric center (see Supplementary Table 2 for details on celiac disease prevalence and diagnostic performance of POCT in each center). As a consequence, and because of the high prevalence of celiac disease among children, more than half of all cases included in our study (25 of 43) were identified in this center. Importantly, FN results were generated by different investigators over the whole recruiting period. As soon as the study board realized the occurrence of FN cases, a further training session was

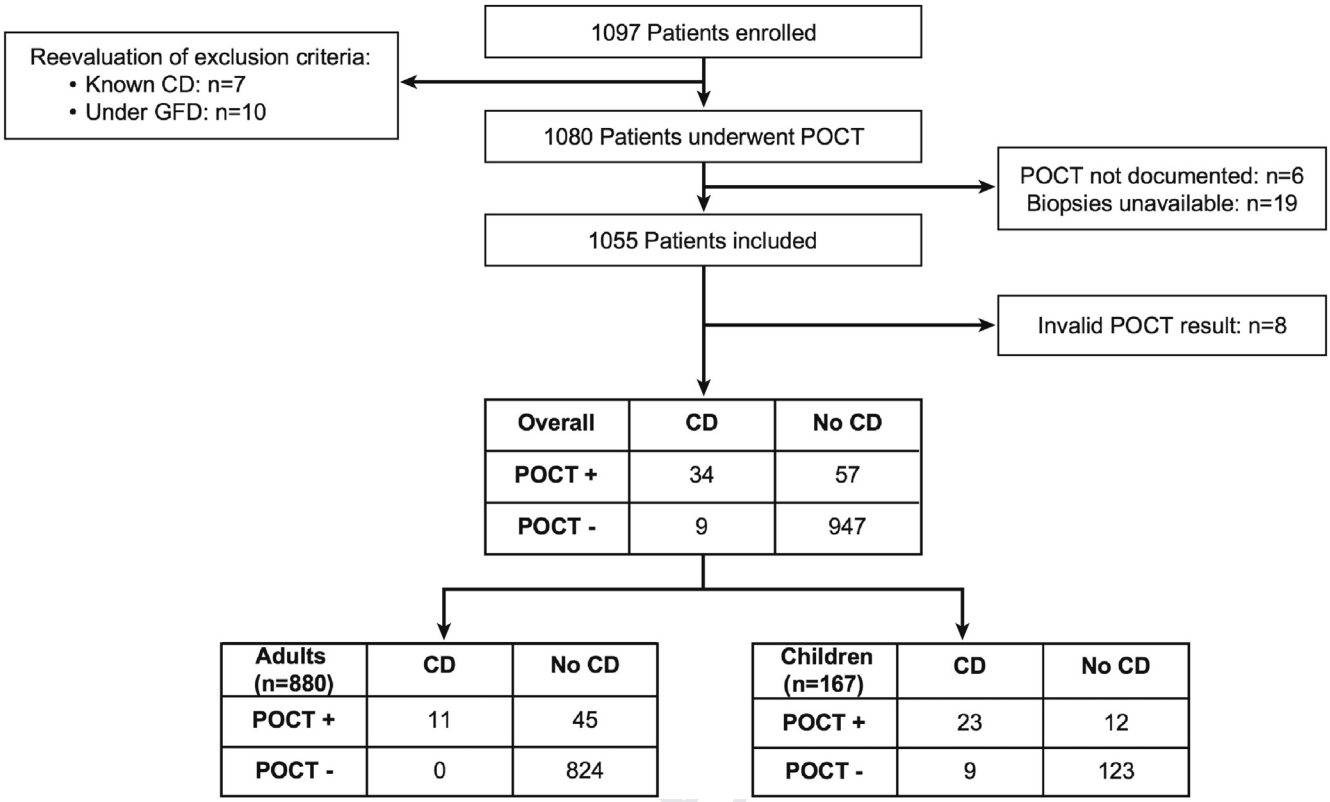

Figure 2. Results of the POCT integrated into the study algorithm. *POCT not documented*: POCT results were not documented in the clinical report form. *Biopsies unavailable*: collection of biopsies was not performed.

scheduled with the involved operators to ensure that analysis of POCT results was adequate. Moreover, remaining tests in the center were exchanged to a new lot. However, neither intervention affected the rate of FNs. To identify a possible reason that contributes to a FN test result we examined histology and serology of participants with FN POCTs. No significant differences where observed with regards to the Marsh grading of duodenal histology in FN cases as compared with true positive, as shown in Supplementary Table 3. Additionally, no significant age difference was found between FN and true positive within the pediatric population, suggesting that there is no particular age cutoff predictive for POCT failure (Supplementary Figure 3). Because the largest pediatric center routinely determined tTG and DGP serology in children undergoing gastroscopy, serology results were compared with POCT results. Of note, the median DGP and tTG titers of the FN were significantly lower compared with the titers of the pediatric true-positive patients (Figure 3).

Celiac Disease Prevalence in Endoscopy Centers

Overall, 43 cases of celiac disease (11 in adults, 32 in children) were diagnosed. The identified celiac disease cases were distributed among recruiting centers with prevalences of 0%–2.7% in the outpatient clinics, 2.1% in the adult tertiary center, and 18.4% and 22.6% in the pediatric centers. According to our results, the prevalence of undiagnosed celiac disease among patients referred to gastroscopy was 1.2% in the adult population and 19% in children (overall prevalence, 4.1%). Details on the prevalence of celiac disease according to symptoms at presentations are presented in Supplementary Figure 4. Of note, within the whole study collective only 13 children and 1 adult presented to the gastroscopy appointment with celiac disease serology results, underlining the potential for a diagnostic tool that would guide the endoscopist in the decision to collect biopsies.

Discussion

The ability to immediately witness the result and react accordingly makes POCTs 1 of the fastest growing sector of modern medical diagnostics. The introduction of these noninvasive, simple diagnostic tests has been viewed as a major improvement in the approach to various clinical situations including celiac disease, a disease with a high prevalence but also a high number of undiagnosed cases.[3]

Specifically, POCTs in the field of celiac disease may allow guiding the decision whether to collect duodenal biopsies during a gastroscopy, but might also respond to the need to implement routine diagnostics in primary care. Because POCTs are developed as screening tools to allocate patients and/or trigger confirmative follow-up diagnostics, emphasis when developing or evaluating a

Table 2. Operative Statistics of the POCT in the Whole Cohort and in the Adult and Children Populations

Cohort	CD prevalence, %	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Overall n = 1047	4.1 (3–5.5)	79 (64–89)	94 (93–96)	37 (28–48)	99 (98–100)
Adults n = 880	1.2 (0.7–2)	100 (68–100)	95 (93–96)	20 (11–33)	100 (99–100)
Children n = 167	19.2 (14–26)	72 (53–86)	91 (85–95)	66 (48–80)	93 (87–97)

NOTE. 95% confidence intervals are reported in brackets.
CD, celiac disease; NPV, negative predictive value; POCT, point-of-care test; PPV, positive predictive value.

POCT needs to be placed not only on the immediate availability of the result but also on sensitivity as close as possible to standard laboratory testing.[26]

Our study on the diagnostic performance of Simtomax is so far the largest and furthermore the first multicenter study on this POCT. In accordance to the outlined field of application of POCTs in celiac disease, our study was designed to evaluate test performance in an appropriately sized group of patients referred to gastroscopy for various reasons. Within this population, the test was able to predict celiac disease diagnosis with an overall sensitivity and specificity of 79% and 94%, respectively.

Previous studies investigating POCTs included tTG-based tests, such as the Biocard and Celiac-Quick-Test, and DGP antibody-based tests, such as Simtomax. A comparative study suggested the Simtomax test to be superior for predicting celiac disease with a sensitivity of 92.7% and a specificity of 85% in a large adult cohort with high celiac disease prevalence of 13.4%,[18] and 100% sensitivity in other cohorts.[17,21,22] Another study by Polanco et al[20] showed a sensitivity of 95.8% compared with diagnosis according to European Society for Pediatric Gastroenterology, Hepatology, and Nutrition criteria in a cohort of 100 pediatric patients with 48% celiac disease prevalence. A very recent study involving Simtomax revealed a test sensitivity in the range of the standard tTG serology.[23] Contrary to these data, our study showed lower diagnostic performances for this POCT, with a sensitivity as low as 79%. The comparably poor performance of Simtomax observed in our study was rather unexpected; therefore, possible factors influencing these results were taken into consideration.

Although a single-center bias cannot be absolutely excluded, we believe this to be unlikely considering the repeated adequate instructions and feedback received by the operators of the pediatric center. As previously mentioned, this is the first multicenter study designed to assess the performance of this POCT. The design of our study resulted in a "real world scenario" regarding 2 key aspects: the prevalence of celiac disease was closer to the normal celiac disease prevalence compared with previous Simtomax studies[2]; and various operators working in independent endoscopy centers needed to interpret test results, as opposed to single operator reading of test results in most previous studies on Simtomax. Importantly, the pediatric center, where FN were generated,

Table 3. Characteristics of Study Participants Displaying a False-Negative POCT Result

						tTG cutoff 10 U/mL (antibody titers U/mL)		DGP cutoff 10 U/mL (antibody titers U/mL)	
No.	Age, sex	Symptoms	POCT	Marsh	IELs	IgA	IgG	IgA	IgG
1	9 y, F	Failure to thrive, obstipation	Negative	1	45	pos (18.8)	neg (4.2)	neg (2.1)	neg (9.8)
2	16 y, F	Abdominal pain, iron deficiency, and known IgA deficiency	Negative	3A	40	neg (0.1)	pos (>200)	neg (0.1)	pos (57.3)
3	6 y, F	Abdominal pain	Negative	3B	70	pos (187)	neg (8.2)	neg (4.7)	pos (41.9)
4	1.5 y, F	Failure to thrive, gastroesophageal reflux, minor IgA deficiency	Negative	3A	50	neg (0.5)	neg (1.2)	neg (0.5)	pos (13.2)
5	5 y, F	Abdominal pain and diarrhea	Negative	3B	50	pos (>200)	pos (10.8)	neg (n.a.)	pos (16.5)
6	15 y, F	Diarrhea, screened tTG pos because of diabetes mellitus type I	Negative	3B	60	pos (90)	pos (29)	pos (12)	pos (10.2)
7	5 y, M	Abdominal pain	Negative	3A	40	neg (n.a.)	neg (n.a.)	pos (84.3)	neg (n.a.)
8	12 y, F	Asymptomatic, screened tTG pos because of autoimmune thyroiditis	Negative IgA deficiency	3B	40	pos (40.3)	pos (25.7)	pos (20.9)	n.a.
9	10 y, M	Failure to thrive	Negative	3B	40	pos (81.5)	pos (>200)	n.a.	pos (>100)

NOTE. Antibody titers are reported in brackets when available: in some cases, serology results were reported as "positive" or "negative" (ie, without quantitative titers).
DGP, deamidated gliadin peptides; IELs, intraepithelial lymphocytes (number/100 enterocytes); n.a., not available; neg, negative; POCT, point-of-care test; pos, positive; tTG, tissue transglutaminase antibodies.

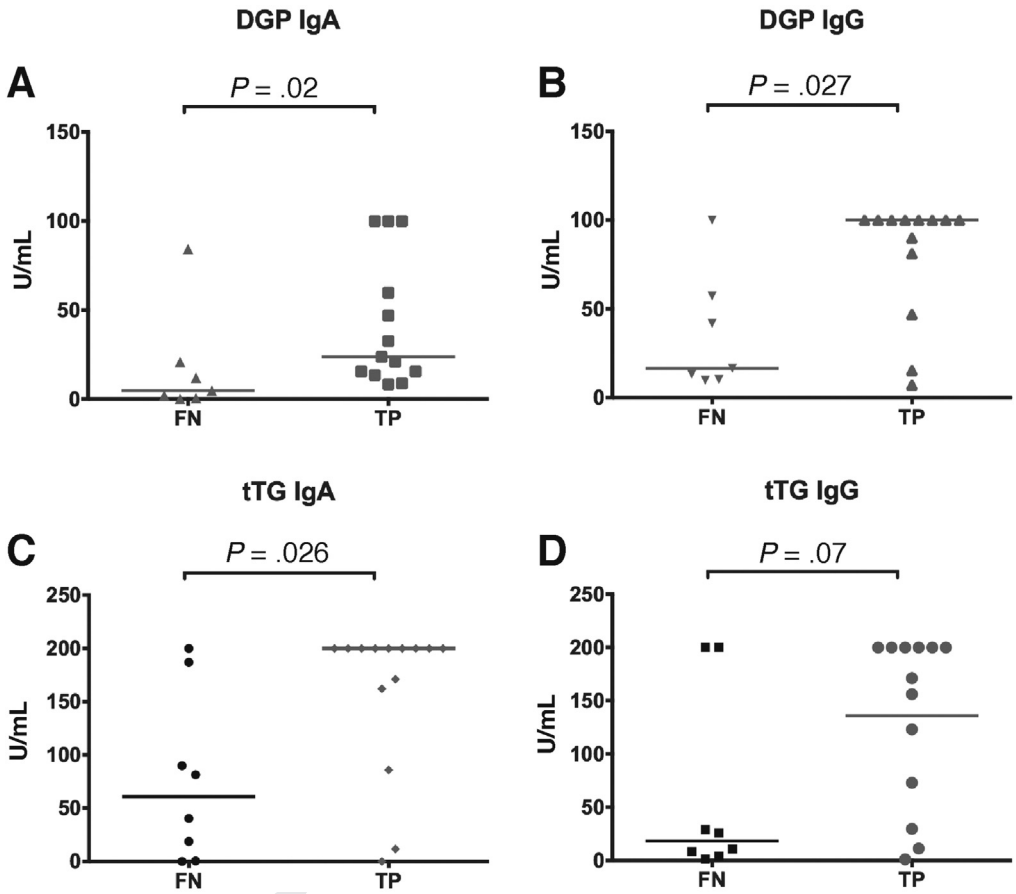

Figure 3. (*A*, *B*) Comparison of titers of DGP antibodies levels in the serum of FN and true-positive (TP) participants. Cutoff for normal values was 10 U/mL for both DGP IgA and IgG. Of note, 1 of 9 (11%) patients with FN POCT also had FN DGP serology (both DGP IgA and IgG <10 U/mL), as compared with 1 of the 16 POCT TP patients (6%). (*C*, *D*) Comparison of serum tTG antibodies in the same participants. Cutoff for normal values was 10 U/mL for both tTG IgA and IgG. Two of 9 (22%) patients with FN POCT also had FN tTG serology as compared with 1 of the 16 (6%) POCT TP patients. Medians are presented as *horizontal lines*.

was also the center where most celiac disease diagnoses were made (23 out of 43 diagnoses); thus, the high prevalence of celiac disease in this subpopulation may account for unmasking a previously unreported sensitivity problem.

Moreover, the analysis of available data on tTG and DGP serology allowed us to identify significantly lower DGP and tTG titers in patients with FN POCT results as compared with those with true-positive results. Because of the technical characteristics of the test, it can be expected that moderately elevated DGP titers might elicit an immunochromatographic reaction resulting in bands being too faint to be reliably read as positive. In line with this argumentation, all operators involved in this study reported frequent difficulties in interpreting results because of the low intensity of the bands, which might contribute to the occurrence of FN results in patients with low antibody titers (Figure 1*B*).

The evidence for a lower-than-expected sensitivity questions the benefits of introducing this POCT into routine clinical practice (eg, for directing the decision to obtain duodenal biopsies). Especially in pediatric populations, where the pretest probability of celiac disease before a gastroscopy is high (secondary to a limited number of indications for gastroscopy), this study suggests that the decision to perform duodenal biopsies guided by clinical suspicion might not be inferior to the introduction of a POCT into the diagnostic algorithm, even in case of unavailable standard serology. Moreover, 0.7% of the POCTs performed in our study were analyzed as "invalid," which requires cost- and time-intensive repetition.

With regard to celiac disease prevalence in our cohort, the 1.2% prevalence in adult endoscopy centers was lower than what was anticipated in view of the rising figures of celiac disease incidence reported in literature.[4] However, a recent German study reported a 0.9% prevalence of positive tTG serology in more than 12,700 children and adolescents, whereas a diagnosis of celiac disease was already known in only 0.07%, which corroborates the hypothesis of a high number of unknown cases in the population.[27] Data from other cohorts suggest a celiac disease prevalence up to 5 times higher in children than in adults.[28] In view of that, a 1.2% prevalence of undiagnosed celiac disease in the selected population of adult patients referred for gastroscopy (and 19% in children) can be considered accurate.

In conclusion, the results of our study question the benefit of the use of Simtomax for the screening of adult patients in the pregastroscopy setting, to identify those who require duodenal biopsies and reduce the number of unnecessary histologic analyses. The multicenter design of the study allowed identifying a previously unreported lack of sensitivity. Thus, we suggest caution before introducing this POCT into routine clinical practice at this stage. Moreover, our data suggested that FN

results are linked to lower DGP antibody titers. In line with this, the operators involved in the evaluation of POCT results expressed the need for a stronger intensity of the colored bands to ease the interpretation of test results and thereby to improve test sensitivity.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2018.09.032.

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Reprint requests
Address requests for reprints to: Michael Schumann, MD, Medizinische Klinik für Gastroenterologie, Infektiologie und Rheumatologie, Campus Benjamin Franklin der Charité - Universitätsmedizin Berlin, Hindenburgdamm 30, 12200 Berlin, Germany. e-mail: michael.schumann@charite.de; fax: +49 (0)30 450-514962.

Conflicts of interest
The authors disclose no conflicts.

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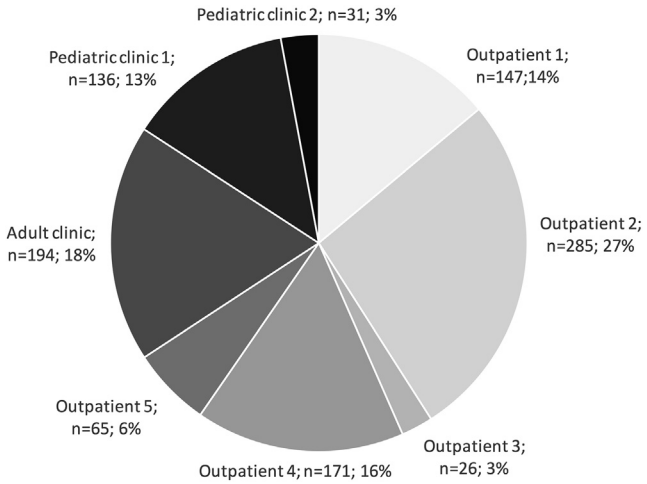
Supplementary Methods

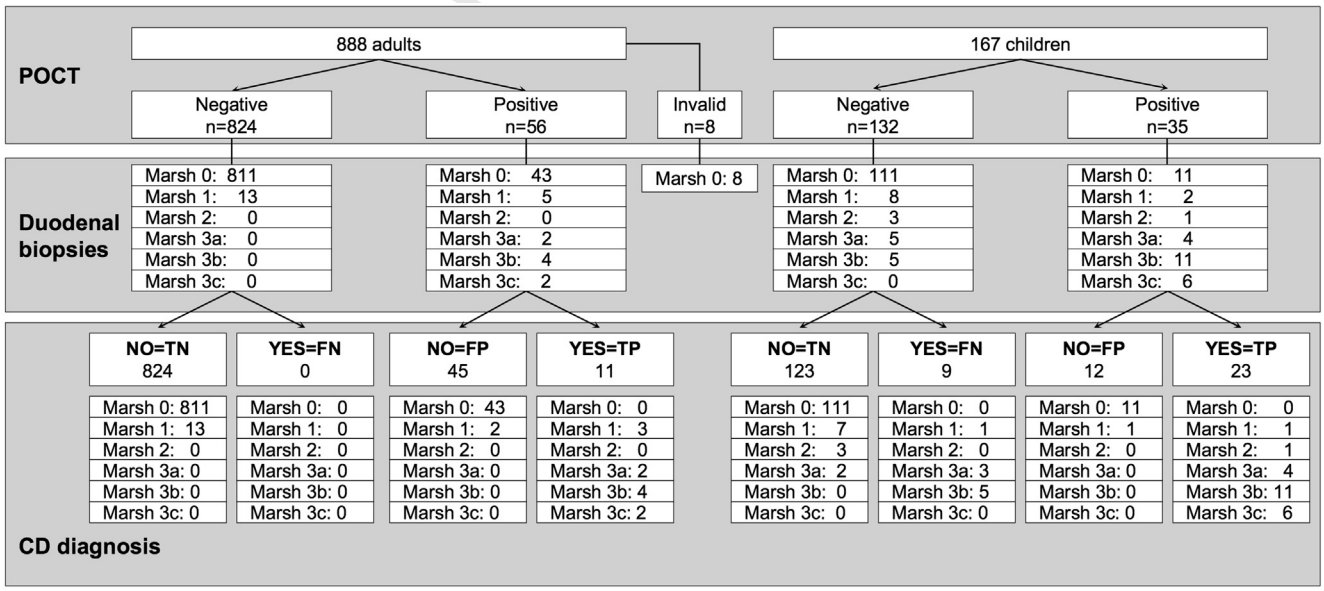
Point-of-Care-Test

The POCT was performed in the endoscopy center shortly before gastroscopy. Simtomax is a lateral-flow immunochromatographic test developed to detect IgA and IgG class DGP-antibodies (Figure 1). The test is performed with a small amount (25 μL) of capillary whole blood from a finger prick, which is placed on a test field and filtered so that only proteins pass through onto a nitrocellulose membrane. A synthetic DGP is attached to the membrane at the test site "A" for detection of IgA and IgG anti-DGP.[17] At site "B," attached antihuman IgA allows total IgA detection. A running buffer provided in the test kit contains secondary gold-conjugated antibodies that bind to the patient's antibodies: detectable complexes are formed at site "A" in case DGP antibodies are present in the patient's blood. The detection of total IgA at site "B" serves as internal validation to identify potential false-negative results caused by IgA deficiency, a condition associated with celiac disease.[5] At the control site "CT," attached goat antimouse antibodies react with the secondary gold-conjugated antibodies of the buffer. The control line does not appear if the buffer did not diffuse properly onto the strip; in that case the test is considered invalid. The POCTs were performed according to the manufacturer's instructions and were read after at least 10 minutes of exposure time by specifically instructed operators. In case of a positive POCT, tTG serology was also obtained. In 1 of the pediatric centers, tTG and DGP serology was performed in all children referred to gastroscopy.

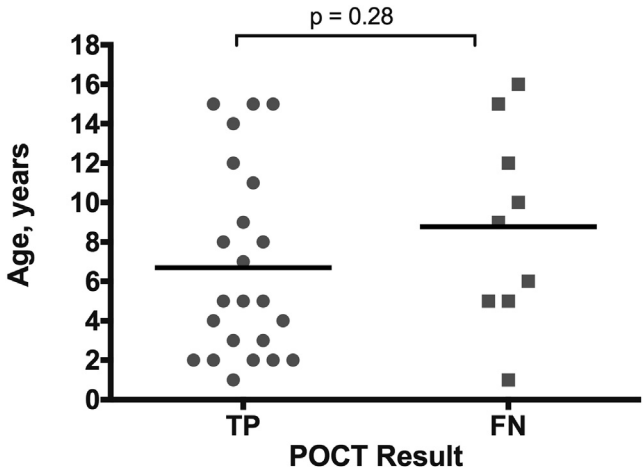
Because these study results suggest CD diagnosis to be a rare outcome in an adult gastroscopy center, it was interesting to us to identify the clinical symptomatology that contributed to the decision to perform an endoscopy. As regards adults, CD was diagnosed in 3 of 61 (5%) patients presenting with the symptom "diarrhea" making it the symptom associated with a higher proportion of CD diagnoses, whereas in children the symptoms "failure to thrive" and "unexplained anemia" were associated with CD diagnosis in 4 out of 5 cases (80%) and 1 out of 1 case (100%), respectively. The most prevailing symptom leading to gastroscopy was abdominal pain/dyspepsia in children and adults. However, it was associated with CD only in 18 of 98 (18%) children and 6 of 542 (1%) adults. Of note, in 3 cases CD was found in asymptomatic patients screened positive for tTG secondary to the previous diagnosis of other autoimmune disorders. CD, celiac disease.



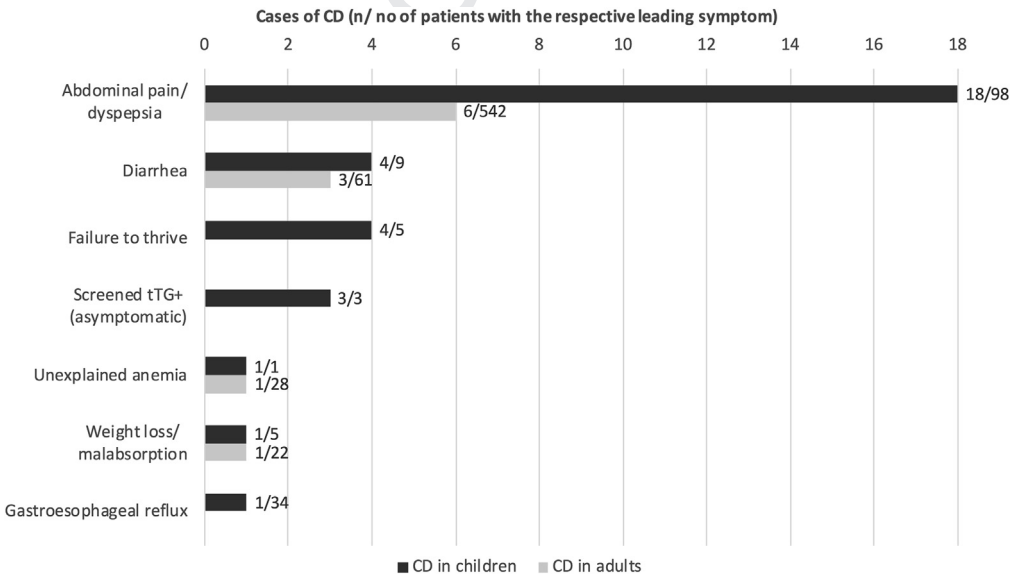
Supplementary Figure 1. Contribution of the participating centers to the study (n, %).



Supplementary Figure 2. Results of the POCT with listing of histology. CD, celiac disease; FP, false positive; M0-3c, Marsh grading; TN, true negative; TP, true positive.



Supplementary Figure 3. Age distribution of pediatric CD cases according to test performance (TP vs FN). Means are shown as *horizontal lines*. TP, true positive.



Supplementary Figure 4. Cases of celiac diagnoses found stratified according to the leading symptom. Within the groups "dysphagia" and "obstipation" no CD diagnoses were made.

Supplementary Table 1. Clinical Approach to Unclear Cases

Age, sex	Symptoms	POCT	Marsh, (IELs)	tTG		DGP		HLA	GFD	CD	Follow-up
				IgA	IgG	IgA	IgG				
1.5, F	Failure to thrive, minor IgA deficiency	neg	3A (50)	neg (0.5)	neg (1.2)	neg (0.5)	pos (13.2)	nd	Response	Yes	Percentile of weight: - before GFD = 5 - on GFD = 14 Histology after 2 y: - on GFD: Marsh 0
1, F	Weight loss, minor IgA deficiency	neg	2 (45)	neg (0.9)	pos (17.8)	neg (0.5)	pos (12.8)	neg	No response	No	No changes in percentile of weight while on GFD
13, F	Abdominal pain, gastritis	neg	3A (42)	neg (n.a.)	neg (n.a.)	neg (n.a.)	neg (n.a.)	nd	nd	No	Lost to follow-up Resolution of symptoms with proton pump inhibitors
16, F	Abdominal pain, gastritis	neg	3A (41)	neg (n.a.)	neg (n.a.)	neg (n.a.)	neg (n.a.)	pos	nd	No	Resolution of symptoms with proton pump inhibitors Histology after 1 y on gluten-containing diet: Marsh 0
5, M	Abdominal pain	neg	3A (40)	neg (n.a.)	neg (n.a.)	84.3 (pos)	neg (n.a.)	pos	Response	Yes	Clear improvement on general state of health while on GFD Intensity of abdominal pain (mean): - before GFD: VAS 8 - on GFD: VAS 2

NOTE. Antibody titers (U/mL) are reported in brackets when available: in some cases, serology results were reported as positive or negative, without quantitative titers (cutoff 10 U/mL).
CD, celiac disease; DGP, deamidated gliadin peptides; FN, false negative; GFD, gluten-free diet; HLA, human leucocyte antigen (positive if HLA-DQ2 and/or DQ8 is present, negative if both alleles are absent); IELs, intra-epithelial lymphocytes (number/100 enterocytes); n.a.; nd, not done; neg, negative; POCT, point-of-care test; pos, positive; TN, true negative; tTG, tissue transglutaminase antibodies; VAS, visual analogue scale. Q8

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Supplementary Table 2. Characteristics of the Participating Centers

Study center	N	CD cases	Sensitivity, %	Specificity, %
Outpatient 1	147	4	100 (40–100)	92 (85–95)
Outpatient 2	285	1	100 (5–100)	99 (97–100)
Outpatient 3	26	0	—	100 (84–100)
Outpatient 4	171	2	100 (20–100)	95 (90–98)
Outpatient 5	65	0	—	97 (87–99)
Adult clinic	194	4	100 (40–100)	89 (84–93)
Pediatric clinic 1	136	25	64 (43–81)	93 (86–97)
Pediatric clinic 2	31	7	100 (56–100)	83 (62–95)

95% confidence intervals are reported in brackets. CD, celiac disease.

Supplementary Table 3. Stratification of FN and TP Patients With CD According to Marsh Grading

	CD patients		
Marsh	FN (n = 9)	TP (n = 34)	*P* value
1	1 (11)	4 (12)	ns
2	0 (0)	1 (3)	ns
3	8 (89)	29 (85)	ns
3a	3 (33)	6 (18)	ns
3b	5 (56)	15 (44)	ns
3c	0 (0)	8 (24)	ns

Data are expressed as number (%). CD, celiac disease; FN, false negative; ns; TP, true positive. Q9