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Coeliac disease case finding and diet monitoring by point-of-care testing

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SUMMARY

Background: Immunoglobulin A class transglutaminase autoantibodies are highly predictive markers of active coeliac disease, a disorder difficult to recognize solely on clinical grounds.

Aims: To develop and evaluate a simple rapid test for point-of-care detection of coeliac autoantibodies.

Methods: The novel whole blood test utilizes the patient's endogenous transglutaminase in red blood cells for detection of transglutaminase-specific immunoglobulin A antibodies present in the blood sample, with normal plasma immunoglobulin A detection as positive test control. We evaluated 284 patients under suspicion of coeliac disease and undergoing jejunal biopsy, and 263 coeliac patients on a gluten-free diet, 383 being tested prospectively in a point-of-care setting. Results were compared with histology, conventional serum autoantibody results and dietary adherence.

Results: The rapid test showed 97% sensitivity and 97% specificity for untreated coeliac disease, and identified all immunoglobulin A-deficient samples. Point-of-care testing found new coeliac cases as efficiently as antibody tests in laboratory. Coeliac autoantibodies were detected onsite in 21% of treated patients, while endomysial and transglutaminase antibodies were positive in 20% and 19%, respectively. The positivity rate correlated with dietary lapses and decreased on intensified dietary advice given upon positive point-of-care test results.

Conclusions: Point-of-care testing was accurate in finding new coeliac cases and helped to identify and decrease dietary non-compliance.

INTRODUCTION

Coeliac disease is an autoimmune gastrointestinal disorder induced by ingestion of gluten found in wheat, rye and barley.[1, 2] The active disease is characterized by gluten-dependent autoantibodies against endomysium (EMA), a complex connective tissue structure surrounding smooth muscle cells, and more precisely, against the protein type 2 ('tissue') transglutaminase (TG2), the coeliac autoantigen anchored to endomysial collagen by fibronectin.[3, 4] Detection of these autoantibodies in the serum is a useful means of identifying new coeliac patients presenting with only mild gastrointestinal symptoms, non-specific general complaints or extraintestinal manifestations, or in populations in general.[1, 5–8] A further important application of serological tests is the regular monitoring of dietary adherence in treated patients, as the autoantibodies

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disappear from the serum on a strict gluten-free diet.[9] There is thus call for quick, easy-to-perform, economical and widely accessible coeliac antibody tests which can be carried out at the first care-taking level locally.

Currently, coeliac-specific serum antibody tests are centralized in specialized laboratories to ensure appropriate sensitivity and specificity.[9] Testing is costly and the turnaround time of results may be relatively long.

Natural human TG2 protein is also found within the red blood cells,[10] and thus in any diagnostic blood specimens comprising whole blood. This easily available endogenous TG2 antigen has, after liberation by haemolysis, the potential to bind to and thereby detect coeliac autoantibodies present in the same sample without need for purified, external TG2 antigen,[11] serum separation, and possibly even without a laboratory reader. This innovative means of detection thus offers an opportunity for point-of-care testing (POCT), defined as performing a diagnostic procedure in a variety of environments outside the central laboratory.[12]

In the present study, we showed a simple self-TG2-based rapid whole blood test to be accurate in detecting untreated coeliac disease. The performance of the test was further evaluated in point-of-care (POC) settings in finding new cases and monitoring treatment.

METHODS

Patients

The patients included in the present study were investigated at the Department of Gastroenterology-Nephrology, Heim Pál Children's Hospital, Budapest and Department of Paediatrics, University of Debrecen, Debrecen, Hungary and at Tampere University Hospital, Tampere, Finland in 2002–2004.

To assess (a) whether the self-TG2-based rapid whole blood test detects antibodies to TG2, 164 stored samples from patients undergoing small intestinal biopsy because of gastrointestinal symptoms were evaluated in blinded fashion in the laboratory. Results were compared with small bowel histology as the gold standard. Coeliac disease was diagnosed in 99 patients (median age 10.2 years, range: 1.4–59) according to European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) criteria and based on the presence of Marsh type III histological lesions.[13, 14] The 65 patients without villous atrophy (median age 14.7 years, range: 3.3–67) were diagnosed with conditions shown in Table 1. The blood samples had been collected with ethylene diamineteraacetic acid (EDTA) or sodium citrate and stored frozen as whole blood at −20 °C until use. Serum immunoglobulin (Ig) A EMA, TG2 antibodies and total serum IgA were determined independently. Patients with total serum IgA < 0.05 g/L were considered IgA-deficient.

To evaluate (b) whether POCT can be used for finding new coeliac cases, 165 new patients (median age 13 years, range: 1.2–72) were prospectively enrolled. This cohort comprised (i) 46 patients with gastrointestinal symptoms admitted to the secondary level referral centre with a high suspicion of coeliac or other enteral disease, (ii) 84 subjects at risk for coeliac disease (patients with various autoimmune diseases, diabetes mellitus, eating disorders, first-degree relatives of known

Table 1. Clinical diagnoses in control patients with normal jejunal histology results

Diagnosis	Number of patients
Gastro-oesophageal reflux disease	19 (6)
IBD*	16 (6)
Nutritive allergy	8 (8)
Lactase deficiency	5 (1)
Postinfectious disaccharidase deficiency	5 (3)
Congenital sucrase–isomaltase deficiency	12 (10)
Familiar adenomatosus polyposis	5 (2)
Helicobacter pylori infection/duodenal ulcer disease	5 (2)
Recurrent abdominal pain	4 (2)
Irritable bowel syndrome	2
Cystic fibrosis	2 (2)
Shwachman-Diamond syndrome	1 (1)
Intestinal lymphangiectasia	1 (1)
Helminthiasis	1
Duodenum stenosis	1
Bacterial overgrowth syndrome	1
Meckel diverticulum bleeding	1 (1)
Myopathy	1 (1)
Autoimmune disease†	4 (4)
Non-specific diarrhoea/dyspepsia	35 (6)
Non-specific rash	3
First-degree relatives of known coeliac patients	6 (4)
No gastrointestinal disease	9 (5)
Total	147 (65)

Values in parentheses indicate patients studied on stored blood samples.
* Crohn's disease: 15, ulcerative colitis: 1.
† Type 1 diabetes mellitus: 3, autoimmune thyreoiditis: 1.
IBD, inflammatory bowel disease.

coeliac patients) and (iii) 35 consecutive adult primary care patients coming to open-access endoscopy who had low suspicion of coeliac disease. All consumed normal, gluten-containing food. Patients with previously known EMA or TG2 antibody results were excluded. Serum antibody measurements were carried out as in the previous group. Patients with clinical suspicion of upper gastrointestinal disease underwent endoscopy and small intestinal biopsy irrespective of the antibody results.

(c) In the prospective evaluation of POCT to monitor dietary compliance, 263 consecutive patients (median age 13 years, range: 2.8–76) with previously diagnosed biopsy-proven coeliac disease and known serum total IgA levels took part. They had followed a gluten-free diet for 2 months to 21.4 years (median: 3.9 years).

Point-of-care testing

Out-patient or ward staff performed the rapid whole blood test on drawn EDTA blood after receiving the patients' consent. The test result was read on site and was always available before that of the serum antibody tests.

Point-of-care testing was similarly performed on the 263 treated coeliac patients at their scheduled check-up visit. Dietary compliance was estimated prospectively at the time of the interview on the basis of a structured questionnaire, discussion with the patient, clinical findings and history as follows: (i) strict adherence to the diet for <6 months, (ii) strict diet over 6 months, (iii) suspected but not admitted lapses and (iv) admitted dietary lapses. Diet failure was suspected clinically in patients with persistent iron deficiency, gastrointestinal complaints, retarded growth or known psychosocial problems or who previously had positive antibody results despite a diet followed for over 1 year.

Dietary intervention

If POCT gave positive results in treated patients, means of improving the diet were immediately discussed with the patient, this also involving a dietician. POCT was repeated after 3–6 months following the dietary intervention. As controls for the intervention, we used serum antibody-positive coeliac subjects who had had their check-up visit in the same year before the POCT study began and who received their results and the instructions to improve the diet by mail. The control coeliac patients were also offered a consultation with a dietician when the positive antibody results became available.

Ethical permission for the study was obtained from the Ethical Committee of the Heim Pál Children's Hospital, Budapest and of Tampere University Hospital, Tampere.

Self-TG2-based coeliac antibody testing from whole blood

In previous laboratory studies, we found that in whole blood samples anticoagulated with EDTA or sodium citrate, antibodies against TG2 form complexes with self-TG2 liberated from red blood cells upon haemolysis. These complexes can be detected by binding TG2 to a solid surface using capture proteins such as fibronectin or gelatine (denatured collagen) which binds fibronectin.[11] Based on this principle, a rapid coeliac antibody test was developed into a Nunc-Immunostick (Nunc A/S, Roskilde, Denmark) format (Figure 1), and gives results in approximately 30 min. The test requires only minimal handling and no laboratory expertise in its execution, as all reagents can be prepared in advance. Two wings of the stick were precoated with gelatine (0.05% in 0.3 M bicarbonate buffer, pH 9.6) to capture self-TG2/anti-TG2 antibody complexes from the haemolyzed patient blood sample,[11] and one wing is coated with antibodies against human IgA (Boehringer, Mannheim, Germany) diluted 1:4000 in 0.3 M bicarbonate buffer (pH 9.6) to react with normal plasma IgA as a positive control. The fourth, uncoated wing serves as negative control. For testing, one drop of whole blood (approximately 25 μL) was delivered into the haemolyzing solution (hypotonic saline with 0.05 M Tris, 0.01 M EDTA and 0.1% Tween 20) and incubated with the stick for 15 min. The stick was then washed under tap water, immersed for another 15 min in peroxidase-labelled antihuman IgA solution (Dako, Glostrup, Denmark), diluted in 1:2000 of 0.05 M Tris (pH 7.4), washed again, inserted into a gel-containing colorigenic substrate, 3,3′,5,5′-tetramethylbenzidine (Sigma-Aldrich, Steinheim, Germany) and stirred with 0.12 g/mL of Sephadex 100 (Pharmacia, Uppsala, Sweden), and evaluated on site by inspection. The test was read as negative, if only one quadrant (the IgA-sensitive part) developed a blue colour, and positive if also both gelatine-coated (altogether three) quadrants became blue within 5 min. If no colour developed, the sample was labelled IgA-deficient and the test invalid (Figure 1). The substrate was stable for up to 1 month at +4 °C, the conjugate was made up freshly each morning.

To investigate whether the colour developing was only due to binding of specific antibodies to TG2, 10 EMA-

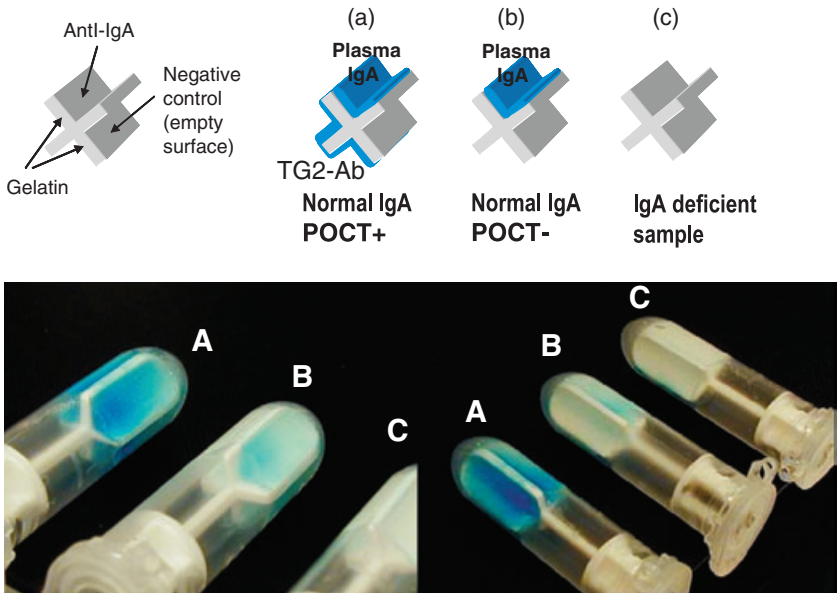

Figure 1. Layout of the self-transglutaminase (TG2)-based rapid whole blood test stick and interpretation of the point-of-care testing (POCT) result. Two quadrants of the stick are coated with gelatine to capture TG2–coeliac antibody (Ab) complexes from haemolyzed patient blood, one quadrant is coated with anti-immnoglobulin A to detect normal plasma IgA. The fourth quadrant is uncoated and serves as negative control. (a) Positive POCT result in an IgA-competent patient with blue colour reaction for TG2-Ab complexes and plasma IgA. (b) Negative POCT result in a subject with normal plasma IgA. (c) Invalid POCT result (IgA-deficient sample).

positive coeliac serum samples were mixed 1:1 with washed red blood cells from normal or TG2-null mice[15] and then tested in the same manner as the patient whole blood samples.

For the assessment of interobserver variation, 30 randomly selected EDTA blood samples from the patient cohorts evaluated at the POC were tested again in the laboratory in a blinded fashion. Further, quality control evaluations were conducted with whole blood samples thawed after various lengths (1–36 months) of storage at −20 or −80 °C and with Nunc-Immunosticks stored at +4 °C for up to 9 months after coating.

Serum antibody measurements

The IgA class serum antibodies against TG2 were measured with human recombinant TG2 using the Celikey (Pharmacia Diagnostics, Freiburg, Germany) enzyme-linked immunosorbent assay (ELISA), according to manufacturer's instructions. Cut-off for positivity was 5 U/mL. EMA was determined on monkey oesophagus sections by indirect immunofluorescence as described elsewhere.[16] Samples reactive at a serum dilution of 1:≥2.5 were considered positive.

Statistical analysis

The McNemar test was used to determine that differences observed between assays were not due to chance. A probability of <0.05 was considered significant. The degree of agreement between any two tests or between rapid test results by two different observers was calculated with fourfold contingency tables using κ-statistics. A κ-value of >0.75 indicates excellent, 0.4–0.75 good and <0.4 poor agreement. Serum TG2 antibody levels before and after dietary intervention were compared by the Wilcoxon signed rank test.

RESULTS

Use of the POCT kit to detect antibodies to TG2

The endogenous TG2-based whole blood rapid test showed 97.0% sensitivity and 96.9% specificity for untreated coeliac disease when applied to the stored 164 samples (Table 2), and performed comparably with serum EMA and TG2 antibody measurements. The results were reproducible in 94% of testings when seven positive, three weakly positive and 10 negative samples were investigated five times by altogether three observers. All eight coeliac blood samples stored after collection frozen without thawing for 24–36 months gave positive results upon thawing, and there was no interference with haemolysis if repeated freezing and thawing was avoided. Immunosticks coated with gelatine were working even after 9 months of storage at +4 °C.

All the 10 serum samples from coeliac patients containing EMA and TG2 autoantibodies tested negative if applied without red blood cells (thus without TG2 antigen) or together with TG2-deficient red blood cell lysate derived from TG2 knockout mice. Nonetheless, the test was positive if normal mouse erythrocytes were

Table 2. Positivity of the whole blood rapid test, serum IgA endomysial antibody (EMA) and serum IgA transglutaminase antibody (TG2-Ab) test in untreated coeliac disease patients and controls

	Rapid test+	Rapid test−	EMA+*	EMA−	TG2-Ab+†	TG-Ab−
Stored samples tested at the laboratory						
Untreated coeliac disease ($n = 99$)	96	3	98	1	98	1
Controls ($n = 65$)	2	63	0	65	0	65
Total ($n = 164$)	98	66	98	66	98	66
Sensitivity	97.0% (93.6–100)		99.0% (97.0–100)		99.0% (97.0–100)	
Specificity	96.9% (92.7–100)		100%		100%	
Positive predictive value	98.0%		100%		100%	
Negative predictive value	95.5%		98.5%		98.5%	
Prospectively tested patients (point-of-care case finding)						
Untreated coeliac disease ($n = 38$)	37	1‡	37	1‡	37	1‡
Controls ($n = 82$)	2	80	0	82	0	82
Total ($n = 120$)	39	81	37	83	37	83
Sensitivity	97.4% (94.3–100)		97.4% (94.3–100)		97.4% (94.3–100)	
Specificity	97.6% (94.5–100)		100%		100%	
Positive predictive value	94.9%		100%		100%	
Negative predictive value	98.8%		98.8%		98.8%	

95% confidence intervals are shown in parentheses.
* Positive if binding is seen at a serum dilution of 1:2.5 or more.
† Cut-off for positivity: 5 U/mL.
‡ Patient with selective immunoglobulin A deficiency.
There were no significant differences by the McNemar test for the rapid test results between point-of-care and laboratory testing and vs. EMA or TG2-Ab.

used. This shows that the antigen specifically recognized in the rapid test was TG2, and other blood components or potential antibodies to them did not contribute to the colour reaction even in coeliac subjects.

Use of POCT for finding new coeliac cases

As laboratory evaluation showed the rapid whole blood test to recognize patients with coeliac disease with high accuracy, we sought to establish whether the test also identifies coeliac cases when applied at the point of care, i.e. at doctor's consultation. Rate of POCT positivity was 58.7% (27 of 46) in the high clinical suspicion group, 13.1% (11 of 84) among at-risk subjects and one patient (2.8%) tested positive from the 35 primary care patients. Altogether 120 patients (all 39 with positive and 81 of the patients with negative POCT results) underwent small intestinal biopsy on clinical grounds and only these were used for the calculation of sensitivity and specificity (Table 1). Antibody-negative low risk people who did not have gastrointestinal symptoms were not eligible to biopsy.

Thirty-seven of the 39 patients with positive POCT results had small intestinal villous atrophy confirming coeliac disease (Table 1). One coeliac patient was negative in POCT for both the test and the IgA control line and was later shown to have IgA deficiency. Thus, POCT found 97.4% of coeliac patients with 97.6% specificity. However, if the IgA-deficient patient correctly picked out by the IgA control line and not having IgA autoantibodies is excluded from the calculations, the sensitivity of the test was 100%. This patient was found to have IgG class EMA and anti-TG2 antibodies in her serum. From the prospectively evaluated cohort nine patients were diagnosed with Crohn's disease and all had negative POCT results.

κ-statistics indicated excellent agreement of POCT results with either serum EMA or TG2 antibody detection ($\kappa = 0.96$, 95% CI: 0.91–1.0). The results obtained in laboratory vs. onsite testing did not differ statistically (Table 1), there was no difference between the results of children ($n = 184$) and adults ($n = 100$); the overall sensitivity of the rapid test was 97.1% and the specificity 97.3% in the 137 untreated coeliac disease patients and 147 biopsied controls having different gastrointestinal diseases (Table 1). Interobserver agreement between POC evaluators and laboratory personnel was 96.7% ($\kappa = 0.90$, 95% CI: 0.72–1.0).

Use of POCT to monitor dietary compliance

During the evaluation of known coeliac patients on diet, POCT identified all nine IgA-deficient blood samples by the absence of the IgA-positive control line. These nine patients thus yielded invalid POCT results and were excluded from the evaluation of dietary compliance. In the case of the 254 IgA-competent patients, coeliac autoantibodies were detected in 52 patients by POCT (20.5%), 50 by the EMA test (19.7%) and 47 by measuring serum anti-TG2 antibodies (18.5%).

All three tests gave either negative or positive results in 91% of all diet samples ($n = 263$). The POCT results agreed with EMA in 93.9% ($\kappa = 0.81$, 95% CI: 0.71–0.90), and with serum TG2 antibody in 92.0% ($\kappa = 0.74$, 95% CI: 0.63–0.85) of the samples, and the EMA and serum TG2 antibody results were concordant in 95.8% ($\kappa = 0.86$, 95% CI: 0.78–0.94). POCT was similarly sensitive as EMA in finding samples with low TG2 antibody positivity and around the cut-off of ELISA (Table 3).

Table 3. Comparison of positive point-of-care (POCT) test results and positive endomysial antibody (EMA) results at low serum transglutaminase (TG2) antibody levels in coeliac patients on diet

TG2 antibody level (U/mL)	Number of samples	POCT+ (%)	EMA+ (%)
>8	31	28 (90.3)	31 (100)
5–8*	15	11 (73.3)	11 (73.3)
3–5†	24	8 (33.3)	8 (33.3)
<3	184	5 (2.7)	0
Total	254	52	50

* Equivocal range according to the manufacturer.
† Cut-off of positivity suggested by the manufacturer: 5 U/mL.

The rates for coeliac antibody positivity declined in all three tests with time on diet, and after 6 months on diet, 95% of compliant patients were antibody-negative (Table 4). However, both POCT and serum antibody tests detected serological activity indicating gluten consumption in a high percentage of patients with clinically suspected or admitted dietary transgressions (Table 4).

POCT and serum antibody results after intensified dietary advice

Of the long-term treated patients receiving intensified dietary instructions onsite after a positive POCT result, 16 were evaluated by POCT a second time, 3–6 months after the initial testing. In 12 of these patients (75%), POCT and EMA became negative and the whole group showed a significant reduction ($P < 0.001$) in serum TG2 antibody levels (Figure 2a). There was also a clear improvement in weight gain and iron status (data not shown). In contrast, out of a control group of 14 EMA-positive coeliac patients who did not participate in POCT and received only written advice to improve the diet, only four (28.6%) reverted to negative EMA results on a second examination after 3–6 months, and there was no significant change in TG2 antibody levels ($P = 0.57$; Figure 2b).

DISCUSSION

Increasing application of serology, i.e. the use of coeliac disease-specific autoantibody tests, has in recent years substantially contributed to our current understanding

Table 4. Positivity in point-of-care testing by self-transglutaminase-based whole blood rapid test, serum IgA endomysial antibody (EMA) and serum IgA transglutaminase antibody (TG2-Ab) tests in treated coeliac patients according to clinically estimated compliance with a gluten-free diet (IgA-deficient patients were excluded)

	Number of patients	Rapid test+ (%)	EMA+ (%)	TG2-Ab ELISA+ (%)
Admitted diet transgressions*	17	15 (88.2, 72.5–100)	16 (94.1, 82.6–100)	16 (94.1, 82.6–100)
Clinically suspected but not admitted diet transgressions†	20	14 (70.0, 49.4–90.6)	11 (55.0, 32.6–77.4)	9 (45.0, 22.6–67.4)
Strict gluten-free diet for ≤ 6 months	31	12 (38.7, 21.3–56.1)	14 (45.2, 27.4–63.0)	12 (38.7, 21.3–56.1)
Strict gluten-free diet for >6 months	186	11 (5.9‡, 3.4–9.3)	9 (4.8‡, 1.7–7.9)	10 (5.4‡, 2.1–8.6)
Total	254	52 (20.5, 15.5–25.4)	50 (19.7, 14.8–24.6)	47 (18.5, 13.7–23.3)

Percentages with 95% confidence intervals are shown in parentheses.
* Median time on diet 6.3 years (range: 1–13).
† Median time on diet 4 years (range: 0.8–12).
‡ $P < 0.001$ vs. all other diet groups by Fisher's exact test.
IgA, immunoglobulin A; ELISA, enzyme-linked immunosorbent assay.

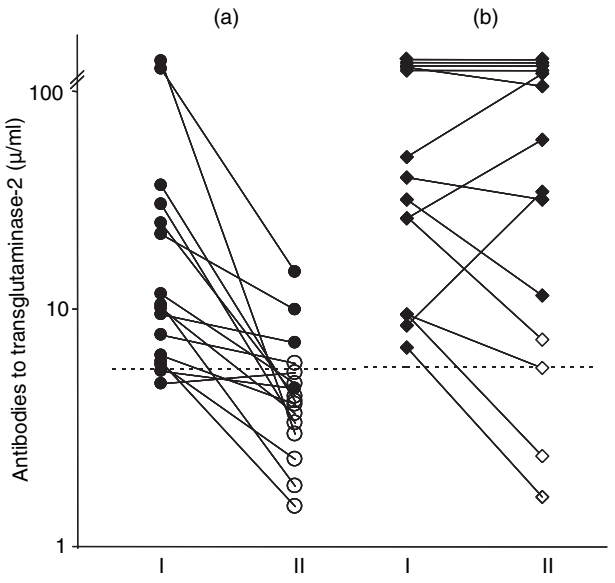

Figure 2. Serum immunoglobulin A transglutaminase antibody (TG2 Ab) concentrations at two consecutive evaluations in treated coeliac patients. (a) Patients ($n = 16$) receiving intensified dietary advice on site upon a positive rapid whole blood point-of-care test. (b) Patients ($n = 14$) receiving dietary advice by ordinary mail upon proving serum antibody-positive. I, initial testing; and II, follow-up evaluation after 3–6 months. Filled circles indicate positive, open circles negative results in the rapid point-of-care test. Filled diamonds indicate positive, open diamonds negative results in the serum endomysial antibody test. The dotted line represents the cut-off for serum TG2 Ab positivity (5 U/mL).

that the disorder may present with a variety of symptoms and organ manifestations which make recognition of patients on solely clinical grounds difficult.[1, 2, 17] These serological tests have acquired a central position in algorithms for diagnosing coeliac disease.[17, 18] We have now developed a novel, easy-to-perform and rapid detection method for coeliac disease case finding, a whole blood test with the same sensitivity and specificity in detecting the disease as the laborious traditional serum-based EMA and TG2 antibody tests. We further evaluated this new test prospectively in a POC setting and showed POCT to be a valid approach to detect coeliac disease-related antibodies directly by doctors or staff in office or ward. In addition, dietary intervention in cases tested positive while on diet improved dietary compliance.

The new test, based on the novel principle[11] whereby an autoantigen, the patients' own TG2 liberated by haemolysis from red blood cells, complexes itself with the autoantibody present in the same whole blood sample and is then captured to enable the detection of the bound antibody, is the first of its kind in medicine. This is a simple POC procedure yielding immediate information on coeliac antibody status applicable in the selection of patients for more invasive diagnostic tests. The present academically developed 'proof-of-concept' test, showing 97% sensitivity and specificity in both laboratory and POC settings for biopsy-proven coeliac disease might be used as such in doctors' offices or further developed by the industry to furnish even more rapid and user-friendly test kits. We wish to emphasize that even if this onsite rapid whole blood test is highly accurate and did not show positivity in other disease groups, such as e.g. Crohn's disease, the diagnosis of coeliac disease today relies on histological demonstration of villous atrophy, and a small intestinal biopsy therefore remains a requisite.[17, 19] Screening studies based on serum anti-TG2 antibody measurements have shown that the disease affects nearly 1% of the population in European countries,[6, 7] and similar figures are now available also from the USA.[8, 20] Given the high prevalence and diverse clinical problems, general practitioners and doctors in many other fields have thus a key role in case finding and referral to the appropriate specialists.[5, 6] Two TG2-based immunochromatographic rapid assays and one dot-blot assay have hitherto been published, and these tests use laboratory serum samples.[21–23] The present study is to our knowledge the first to report on the clinical application of a rapid coeliac test in a real POC setting.

Although POCT for coeliac disease has certain limitations, such as observer-dependency, the same holds for coeliac antibody detections in general.[17, 19] The results of EMA test are highly influenced by expert reading, whereas the simple rapid test used here yielded high agreement between untrained and trained personnel. In addition, the antibodies may be negative if the subject has adopted a gluten-free diet before testing. Furthermore, the serum autoantibody tests commonly used detect only IgA class antibodies and IgA-deficient coeliac patients may thus be missed.[24] This problem is vital for rapid tests, where results are interpreted immediately, precluding the use of laboratory serum IgA measurements. As information on both coeliac antibodies and IgA status is required for decision in the diagnostic algorithms most commonly recommended,[18] we chose total plasma IgA detection as the positive test control in our test kit. This strategy indeed enabled us to pick out IgA-deficient samples.

The currently available, serum-based coeliac antibody diagnostic kits use purified or human recombinant TG2 antigens and are reliable only in a controlled laboratory environment.[9] TG2 is a protein particularly sensitive to heat, storage and oxidation, which may influence its antigenic properties.[10, 25] Use of fresh self-TG2 antigen from the patient's own blood may overcome kit storage problems in warm climates or developing countries and makes testing economical, as neither industrially purified TG2 nor serum separation is required.

In the present study, our novel rapid test found coeliac patients both in high risk and low risk patient groups including primary care, and worked similarly to EMA even in cases when the antibody levels were low. The gelatine-coated test surface binds blood TG2 via blood fibronectin in the same and oriented fashion as TG2 epitopes are exposed in natural tissue sections used in the EMA test.[4] Unlike to ELISA, where the plate-coated TG2 antigen may be exposed in distorted or non-physiological ways and may also attract some non-specific antibodies,[26] the EMA reaction is coeliac-specific[3, 4, 7, 9, 16] and basically on-off. The similar antigenic orientation in the rapid test ensured specificity and enabled us to adjust the plus/minus colour development to high sensitivity. The EMA test has already proved to be reliable also in low prevalence situations, e.g. population screening,[7] thus the POCT test equipped with the IgA control line could be equally efficient and even more convenient for such applications.

Based on the low interobserver variability and the experiments presented here, the POCT kit can satisfy quality assurance requirements. For quality assurance regarding the policy of POCT for finding coeliac disease, further education of all health care professionals will be important that after a positive POCT result a confirmatory biopsy is still needed and cases with severe gastrointestinal symptoms may require a referral to gastroenterologist despite a negative POCT result.

The setting where POCT, in addition to new case finding, may have a special role is the long-term surveillance of coeliac patients after adopting a gluten-free diet. In the present study, both POCT and serum antibody measurements detected coeliac antibody positivity in the majority of patients who admitted dietary transgressions, but also in a sizeable proportion of those who could not be identified by history alone. Being a test with positive/negative results, the rapid test is not suitable to show initial decrease in the antibody titres, but in the present study, it was able to correctly demonstrate negative seroconversion after a diet for 6 months or longer. Although occasional dietary lapses may not lead to measurable seropositivity,[27, 28] sustained detectability of coeliac autoantibodies is consistent with ongoing gluten consumption, in most cases accompanied by damaged villous structure.[28] Such patients are at risk of late complications, including osteoporosis and malignancy, even if they are currently clinically asymptomatic.[1, 2, 17] The long-term success of coeliac disease care is largely dependent on a good doctor–patient relationship reinforcing the diet,[29] and readily available antibody results may help to target non-compliant individuals already during the consultation. In the present study, better dietary adherence was achieved following this type of intervention.

In conclusion, we have shown that subjects with undetected coeliac disease and known patients with dietary failure can be picked out by POCT using a simple self-TG2-based rapid technique. This test can be applied already in its present form or might be further developed into commercial user-friendly kits.

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