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RESEARCH ARTICLE

A new algorithm for the diagnosis of celiac disease

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Celiac disease (CD) affects at least 1% of the Western population but remains largely unrecognized. In our laboratory, we utilize a novel algorithm to diagnose pediatric CD that offers both high sensitivity and high specificity for diagnosis in an outpatient setting. The aim of the present study was to challenge this algorithm and to test its performance in children and adults suspected of having CD. Using a three-assay algorithm, screening with the most sensitive tissue transglutaminase (tTG) complexed with deamidated gliadin peptide neoepitope immunoglobulin A (IgA)+IgG assay and confirming with the two specific tTG IgA and tTG IgA+IgG assays, we examined the serological results from 112 children aged 0–17 years old and 60 adults in comparison to their respective biopsy results. The algorithm performance was calculated by statistical analysis. The use of the new algorithm enabled us to diagnose CD with 98% sensitivity, 93% specificity and 95% accuracy in the pediatric group and 94% sensitivity, 92% specificity and 93% accuracy in the total population studied. The false-negative cases in the adult group were attributed to previous adherence to a gluten-free diet, and the single false-negative result in a young child became a true positive after 6 months. We have also monitored three celiac patients before and after diagnosis and found that the algorithm may be suitable for disease monitoring. The newly proposed three-assay algorithm for celiac detection is very reliable in both children and adults. Due to the high performance of this assay, the further need for confirmatory intestinal biopsies will be reassessed.
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INTRODUCTION

Celiac disease (CD) is an autoimmune-mediated enteropathy caused by permanent gluten intolerance in susceptible individuals.[1,2] The prevalence of CD in the Western world has been increasing steadily, and it is now recognized as a common disorder.[3] Its incidence has increased due to recognition of atypical CD forms[4] and the identification of silent cases.[5] The increased ability to identify silent cases along with classical celiac patients is mainly attributable to the increasing number of serological tests available, providing noninvasive tools along with improved performance. According to the latest consensus report on CD, small bowel biopsies are considered to be the gold standard and are used to confirm diagnosis.[6] The accepted screening tool for CD is the enzyme-linked immunosorbent assay (ELISA) test for immunoglobulin A (IgA) against the tissue transglutaminase (tTG) antigen.[7,8] However, the combined detection of tTG IgA antibodies along with deamidated gliadin peptide antibodies increases both the sensitivity and the specificity of CD diagnosis. Our laboratory uses a novel algorithm, starting with a sensitive screening assay that uses human recombinant tTG crosslinked with gliadin-specific peptides as antigens, which allows detection of both IgG and IgA antibodies (Aesku Diagnostics, Wendelsheim, Germany). Positive samples were further confirmed with two assays that we find to be more specific, the tTG IgA (DiaSorin S.p.A., Saluggia, Italy) and tTG IgA+IgG (ORGENTEC Diagnostika GmbH, Mainz, Germany) assays. A negative result with the first screening test was a final negative result; however, a positive result with the screening test was further confirmed with a tTG IgA assay. Samples positive for both tests were regarded as positive for CD screening. Samples positive in the first screening test and negative with the tTG IgA test were further tested with the tTG IgA+IgG assay. A negative result in the last confirmatory test (thus positive in the screening test only) rendered a final negative result. Samples with positive results on both tests, the screening and the tTG IgA+IgG assay, were regarded as samples that were serologically positive for CD. This algorithm enabled our laboratory to detect CD with 100% sensitivity, 96.2% specificity and 98.1% accuracy according to preliminary results when tested against pediatric samples. In the present study, we challenged the performance of this algorithm to empower its diagnostic yield in biopsy-proven samples from children, as well as from adults.

MATERIAL AND METHODS

Study population

Patients who had undergone serological tests and intestinal biopsy for CD during 2009–2010 were retrospectively identified by their gastroenterologist. The cohort included 112 children and 60 adults as detailed in Table 1. Diagnosis of CD was proved by intestinal histology. Serological testing was performed as part of the routine algorithm in our laboratory.

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Table 1 Study population characteristics

		Children			Adults		
		All	Controls	Celiac patients	All	Controls	Celiac patients
No.		112	72	40	60	31	29
Age	AVG	7	7	7	36	38	35
	SD	5	5	4	16	16	16
	Range	1–17	1–17	1–17	18–79	18–79	18–65
	Median	6	7	6[a]	32	35	31[b]
Gender	Males	57	37	20	15	9	6
	Females	55	35	20	45	22	23
Marsh	I/II			5			
	III/IV			35			

Abbreviations: AVG, average; SD, standard deviation.
[a] No significant difference between controls and patients, $P<0.8$.
[b] No significant difference between controls and patients, $P<0.5$.

ELISA assays

Three ELISA assays are included in our algorithm. The assay characteristics are detailed in Table 2. The Aesku CeliCheck Neoepitope assay was tested on the Triturus analyzer (Grifols S.A., Barcelona, Spain). The DiaSorin tTG IgA assay was tested on the Liaison (DiaSorin S.p.A.), and the Orgentec tTG IgA+IgG assay was tested on the ETI-MAX 3000 analyzer (DiaSorin S.p.A.). NEQAS (United Kingdom National External Quality Assessment Service) is routinely used as an external quality control program.

Endoscopy and intestinal histology

All patients underwent esophago-gastro-duodenoscopy using a GIF-xp 20 endoscope (Pentax, Tokyo, Japan). At least five biopsy samples were obtained from each patient: four from the second part of the duodenum for the diagnosis or exclusion of CD and one from the antrum.

The biopsies were immediately fixed in buffered formalin and embedded on edge in paraffin. Sections were stained with hematoxylin eosin and Giemsa and were analyzed by a trained pathologist and graded according to Marsh criteria as previously described.[9] The local ethics committee approved the study.

Statistical analysis

Receiver-operating characteristic curve analysis (including receiver-operating characteristic cutoff), area under the curve, *P* values to test significance and sensitivities and specificities were calculated using the MedCalc statistical software (Mariakerke, Belgium, Brussels). A *P* value of $\leqslant 0.05$ was considered significant.

RESULTS

The diagnostic sensitivity and specificity of the three-assay algorithm were calculated according to the recommended manufacturer cutoffs in comparison to biopsy results (Table 3). Borderline results were regarded as positive. The sensitivity and specificity of the algorithm tested against the pediatric samples were 98 and 80%, respectively (Table 3), with one false-negative result and 14 false-positive results. Sensitivity and specificity of the algorithm tested against the adult samples were 90 and 81%, respectively (Table 3), with three false-negative results and six false-positive results. The number of samples tested against each of the assays was different according to the algorithm employed, because negative results with the screening test are no longer tested against the other assays, but all samples with positive results using the neoepitope assay were further tested with the tTG IgA assay. The samples that were positive with both assays were no longer tested with the tTG IgA+IgG assay. Positivity with the neoepitope assay only accompanied by negative results with the two other assays was regarded as a negative final result. To improve the performance of the algorithm, we performed receiver-operating characteristic curve analysis. The cutoff value of the neoepitope assay was only slightly increased from a 16–24 U/ml borderline to a 23–27 U/ml borderline; the cutoff of the tTG assay was raised from a 7.2–8.8 AU/ml borderline to a 12.5–20 AU/ml borderline; and the tTG IgA+IgG assay cutoff was raised from 15 to 20 PGL U/ml. The change in cutoffs improved the specificity of the total study population by 12% (Table 3), and the number of false-positive results decreased from 20 cases to eight (five in the pediatric group and three in the adult group). Among these eight cases, one was a child with high levels of total IgA, one was an adult with type I diabetes and one was an adult positive for antinuclear antibodies. Three of the celiac patient samples (two children and one adult) belonged to IgA-deficient patients. All three samples were detected as positive samples with the assays detecting IgG antibodies: the neoepitope and the tTG IgA+IgG assays. Out of the entire study population, four false-negative results were detected: the only false-negative case in a child belonged to a young girl who, at the age of 9 months, had a negative result for the neoepitope antibodies. A week later, her neoepitope antibody level was 58.2 U/ml, and at the age of 10

Table 2 Characteristics of the assays in the algorithm

Kit name, antigen	Short name	Manufacturer	Antibodies	Manufacturer cutoff (borderline range)
Aesku CeliCheck, neoepitopes (human recombinant tTG crosslinked with gliadin specific peptides)	Neoepitope	Aesku Diagnostics, Wendelsheim, Germany	IgA, IgG	20.0 (±20%) U/ml
Liaison tTG IgA	tTG IgA	DiaSorin S.p.A., Saluggia, Italy (Liaison)	IgA	8.0 (±10%) AU/ml
Anti-tTG screen, human recombinant tTG	tTG IgA+IgG	ORGENTEC Diagnostika GmbH, Mainz, Germany	IgA, IgG	15 PGL U/ml

Abbreviations: tTG, tissue transglutaminase; U, units.

Table 3 Overall performance of the three-assay algorithm[a]

	Parameters calculated according to manufacturer cutoffs				Parameters calculated according to ROC CutOffs			
Group	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
Children	0.98	0.80	0.74	0.98	0.98	0.93	0.89	0.99
Adults	0.90	0.81	0.81	0.89	0.90	0.93	0.90	0.90
Children+adults	0.94	0.80	0.77	0.95	0.94	0.92	0.89	0.96

Abbreviations: PPV, positive predictive value; NPV, negative predictive value.
[a] The statistical parameters were calculated with the MedCalc statistical software. Borderline results were regarded as positive results.

months, her neoepitope antibody level was above 300 U/ml. At the age of 12 months, her biopsy result was diagnostic for CD, and at the age of 15 months, the positive result for neoepitope antibodies was accompanied by a positive result using the tTG IgA assay. In adults with suspected CD, serological tests for celiac diagnosis are routinely performed after positive biopsy results are obtained, usually after the patients are already on a gluten-free diet. The three false-negative results in adults are attributed to adherence to the gluten-free diet.

Three celiac-diagnosed children were followed before and after diagnosis: all of them had detectable responses and were monitored by the neoepitope and tTG IgA assays. At diagnosis, each of these children were reactive against the neoepitope and had tTG IgA antibody levels of 300 and 67, 300 and 669, and 300 and 211, respectively. Two months after biopsy spent on a gluten-free diet, the antibody levels for the neoepitope and the tTG IgA declined to 11 and 0, 56 and 13, and 90 and 42, respectively.

DISCUSSION

In the present study, we have shown that, by challenging our unique three-assay algorithm with 173 biopsy-proven samples from children as well as adults, we were able to reach a performance of 94% sensitivity, 92% specificity and 93% accuracy for CD diagnosis. However, when analyzing the pediatric data alone (113 samples), the capability of our algorithm to diagnose CD was much higher (98% sensitivity, 93% specificity and 95% accuracy). The difference in the performance of this algorithm when comparing children and adults may stem from the differential timing of the serological tests; all adults underwent biopsy prior to the relevant serological tests, whereas the children underwent serological tests first and only underwent biopsy on suspicion of disease. In the adult patients, the CD diagnosis was performed according to the results of the histopathological test and thus the serological tests were performed when the patients were on a gluten-free diet, explaining the relatively low specificity of our three-assay algorithm in the adult samples. The elevation of the cutoffs of the assays significantly increased the specificity in both populations. A single false-negative result was found in the pediatric group. This sample belonged to a 9-month-old girl who was probably gradually exposed to gluten and developed antibodies developed with the time, because at the age of 10 months only the neoepitope assay was positive (and the entire algorithm was negative), and 5 months later, her serological results were positive according to our algorithm. It has been previously suggested that pediatric CD patients (<2 years old) may have normal tTG IgA levels.[10] tTG can both deamidate gliadin peptides and transamidate them,[11] with transamidation being the major reaction (75% versus 25% for deamidation). This crosslinking also occurs outside the active site of tTG and results in permanently and covalently linked deamidated gliadin peptide/tTG complexes. The neoepitope screening assay detects antibodies to tTG, deamidated gliadin peptide and to the neoepitope and is thus the first assay used in our algorithm. This assay was able to provide earlier detection than the other tTG ELISA kits;[12,13] however, such cases may be interpreted as false-positive cases by the first line screen. Applying the second-step screen with the tTG IgA and tTG IgA+IgG assays eliminated the false-positive results in many cases. Taking the iceberg model, where the CD diagnosed/undiagnosed ratio is 1:9, into account, the present screening algorithm seems reasonable. Monitoring false-positive cases should be a reasonable approach. One of the false-positive cases belonged to a child with high levels of total IgA, which could have caused the rise in tTG IgA as well. In two of the three false-positive adult cases, celiac antibodies were associated with other autoimmune antibodies: one patient was a type I diabetic patient and the other was positive for antinuclear antibodies. It is well known that there are shared genetic risk factors for type I diabetes and CD,[14] and around 10% of the diabetic type I patients will develop CD over time.[15] Anti-tTG antibodies were rarely found in SLE (systemic lupus erythematosus) patients and generally did not indicate the presence of CD.[16] When using the tTG IgA assay for CD diagnosis, testing for total IgA antibodies was necessary to compensate for the higher prevalence of IgA deficiency in CD patients.[17] However, as the screening neoepitope assay and the tTG IgA+IgG assay both include IgG antibodies, the determination of total IgA in the new celiac algorithm was unnecessary. This was shown for the three IgA-deficient patients in our study, who were correctly diagnosed with the algorithm.

Small bowel biopsies are considered to be the gold standard for diagnosis of CD,[6] although biopsy results are sometimes misleading. Duodenal histology is frequently unclear and may show variable severity even within a single biopsy fragment.[18] It has been suggested that the duodenal bulb mucosa might be the only area to show histological changes, and therefore biopsies should include distal duodenum and also bulb as biopsy sites to improve the diagnostic yield.[19] Moreover, measurement of fewer than 25 intraepithelial lymphocytes (IEL) per 100 enterocytes correlates with serologic indicators of CD and a higher IEL threshold could miss 50% of cases.[20] In view of the recent trend towards changing the diagnostic criteria for CD, skipping the routine intestinal histology and replacing it with reliable serologic and genetic markers,[21,22] and using different algorithms in symptomatic and asymptomatic subjects (Celiac Disease Working Group ESPGHAN Meeting, Istanbul, June 2010, unpublished), this three-assay algorithm is a step forward. It seems that CD is on the verge of changing with respect to pathophysiology, disease understanding, diagnostic criteria and therapeutic strategies.[23] The present study describes the performance of a unique algorithm for CD diagnosis. This algorithm showed excellent performance in children and very good performance

in adults under gluten restriction. The performance of this algorithm should be further examined for adequacy for disease monitoring and has proven to be suitable for routine implementation.

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