Utility of the New ESPGHAN Criteria for the Diagnosis of Celiac Disease in At-risk Groups

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See ''Nonbiopsy Diagnosis of Celiac Disease: Are We Nearly There Yet?'' by Hill and Horvath on page 310.

ABSTRACT

Objective: Demonstration of small-bowel mucosal damage has been the basis of celiac disease diagnosis, but the diagnostic approach is undergoing changes. The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition recently stated that in a subgroup of children, high positive transglutaminase 2 antibody (TG2ab) values may be sufficient for the diagnosis. The utility of these new criteria was evaluated by applying the human red blood cell TG2 antibody test (RBC-TG2ab) to a large cohort of children and adults belonging to at-risk groups.

Methods: RBC-TG2ab and endomysial antibodies (EmA) were measured in 3031 family members or other relatives of patients with celiac disease. The RBC-TG2ab values were classified as weak (20–29 U), moderate (30–99 U), and strong (≥100 U) positive. Seropositive subjects were further tested by human recombinant TG2ab (Hr-TG2ab) and for the presence of celiac disease–associated human leukocyte antigen-DQ alleles. Gastroscopy was recommended for all with positive RBC-TG2ab, EmA, or Hr-TG2ab, or weak positive RBC-TG2ab and symptoms.

Results: Strong positive RBC-TG2ab has good correlation with EmA and Hr-TG2ab and positivity of DQ2/8, and the diagnosis was established in 94% of both children and adults. In contrast, moderately positive (≥30 U) RBC-TG2ab showed poor correlation with the other tests, and celiac disease was diagnosed in 69% of children and 86% of adults. Most participants with weak positive RBC-TG2ab were negative for EmA and Hr-TG2ab.

Conclusions: In accordance with the new European Society for Pediatric Gastroenterology, Hepatology, and Nutrition criteria, strong positive RBC-TG2ab showed good accuracy and excellent correlation with the other antibodies and celiac-type human leukocyte antigen. In contrast, low or moderately positive RBC-TG2ab values were of unsatisfactory prognostic value for a subsequent diagnosis.

Key Words: celiac disease, criteria, diagnosis, European Society for Pediatric Gastroenterology, Hepatology, and Nutrition

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For the past few decades, the classical picture of celiac disease as a childhood diarrheal disease has been replaced by the conception of an autoimmune-mediated disorder with a strong genetic predisposition. It has become evident that the disease may appear at any age and with heterogeneous clinical presentation (1). Contemporaneously, the prevalence has increased up to 1% to 2% (2,3), and diagnostic facilities have improved (4,5). Until now, the demonstration of small-bowel mucosal damage has been the basis for diagnosis (1,6), but because of the aforementioned changes and obvious problems in the interpretation of the histology, particularly in borderline cases, this standard approach has been questioned (7–10). Recently, new diagnostic criteria were published by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (11). It was stated that in a subgroup of children with high serum transglutaminase 2 antibody (TG2ab) values, celiac disease could be established without histological confirmation. It was evident that the feasibility of such an essential change should be tested in different study designs. One of the problems is that there are different TG2ab tests, and studies referring to the new ESPGHAN criteria have been performed mainly in tertiary centers in subjects with classical gastrointestinal disease (11). It is also important to know whether the new criteria are applicable to adults.

The human red blood cell TG2 antibody test (RBC-TG2ab) is approved by the US Food and Drug Administration as a serological assay for celiac disease. We investigated the validity of the new ESPGHAN criteria (11) by applying RBC-TG2ab in a large at-risk cohort of apparently asymptomatic children and adults. The results were compared with 2 other frequently applied serological tests and with the presence of celiac disease–associated human leukocyte antigen (HLA) DQ2 and DQ8 alleles.

METHODS

Study Design

The study was carried out at the Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital, and at the Pediatric Research Center, Tampere University Hospital and University of Tampere, Tampere, Finland. The study cohort was collected by recruiting first a total of 730 previously diagnosed

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patients with celiac disease (index cases) in a nationwide search using leaflets and via celiac disease societies (Fig. 1). All of the diagnoses were confirmed from the hospital patient records. Next, the family members and first- or second-degree relatives of the index patients were invited to undergo an evaluation of serum celiac antibodies and celiac disease–related genetics. The utility of the new ESPGHAN criteria was evaluated by dividing the participants into different subgroups on the basis of their baseline RBC-TG2ab values and offering the possibility of further endoscopic studies, as specified below. Subjects who had a previous celiac disease or dermatitis herpetiformis diagnosis, or were receiving a gluten-free diet for another reason, were excluded (Fig. 1).

The study procedure was approved by the ethical committee of Tampere University Hospital. All of the study participants, or in the case of children, their parents, gave written informed consent.

Serological Tests

Serum immunoglobulin A (IgA) class RBC-TG2ab values were measured in all of the study participants according to the manufacturers' instructions (Quanta Lite h-tTG IgA enzyme-linked immunosorbent assay [ELISA] kit; INOVA diagnostics, San Diego, CA). The specific cutoff limits recommended by the manufacturer were values <20 U negative, 20 to 29 U weak positive, and all of the values ≥30 U from moderate to strong positive. In the present study, values ≥100 U, that is 5 times the upper limit of normal (ULN), were considered strong positive (11). Serum immunoglobulin A-class endomysium antibodies (EmA) were determined by an indirect immunofluorescence method using human umbilical cord as substrate, and a dilution 1:≥5 was considered positive (12). The positive samples were further diluted 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000, and 1:4000. All of the serum samples positive for either RBC-TG2ab or EmA were further tested using the human recombinant TG2 antibody test (Hr-TG2ab) according to the manufacturer's instructions (Celikey ELISA kit; Phadia GmbH, Freiburg, Germany). Serum Hr-TG2ab values of ≥3.0 U were considered positive (9,11).

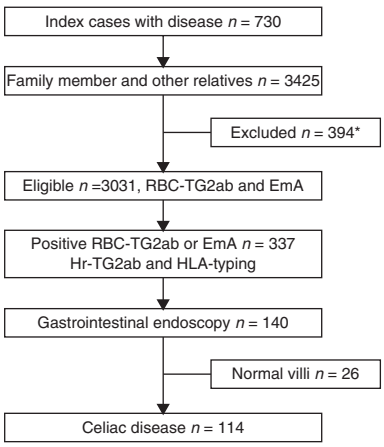

FIGURE 1. Flowchart of the study. EmA = serum endomysial antibodies; RBC-TG2ab = human red blood cell transglutaminase 2 antibodies; Hr-TG2ab = human recombinant transglutaminase 2 antibodies. *Previous celiac disease or dermatitis herpetiformis diagnosis, or a gluten-free diet for another reason.

Upper Gastrointestinal Endoscopy and Small-bowel Mucosal Biopsies

An upper gastrointestinal endoscopy with small-bowel mucosal biopsies was offered to all of the participants with moderate to strong positive RBC-TG2ab, positive EmA, or positive Hr-TG2ab. In the case of weak positive human RBC-TG2ab, further investigations were recommended only upon the request of the patient or if there were signs of celiac disease–associated clinical symptoms or complications. The endoscopies and histological evaluation of the mucosal specimens were carried out at local health care units according to our standard procedures (13). The diagnosis of celiac disease was based on the demonstration of small-bowel mucosal villous atrophy and crypt hyperplasia (Marsh III) (6).

Genotyping

The presence of celiac disease–associated HLA-DQ2 and -DQ8 alleles was genotyped from the RBC-TG2ab- and EmA-positive subjects with the tagging single-nucleotide polymorphism method detecting the DQ2.5, DQ2.2, DQ7, and DQ8 haplotypes (14,15) or with the Olerup SSP DQ low-resolution kit detecting the DQB1∗02 (DQ2, either DQ2.5 or DQ2.2), DQB1∗0302 (DQ8,) and DQB1∗0301 (DQ7) among the other DQB1 main allele groups (Olerup SSP AB, Stockholm, Sweden).

Statistical Analysis

Statistical data were presented as number of subjects and percentages or as median and ranges.

RESULTS

A total of 3031 nonceliac subjects were eligible and participated in the serological testing (Fig. 1). In total, 25% of the participants were younger than 18 years. The subjects' characteristics are shown in Table 1. The majority were first-degree relatives, particularly siblings and parents, who also had the highest percentage of new celiac disease diagnoses. In contrast, the percentage was significantly lower in second-degree relatives. None of the nonrelative spouses were found to have celiac disease.

There was an excellent relation between the strong positive (≥5 times ULN) RBC-TG2ab values and positivity for EmA and Hr-TG2ab (Table 2). In addition, the celiac disease–associated HLA-DQ2, -DQ8, or half DQ2 was found in all of those in whom it was evaluated (Table 3). In total, 91% of those with strong positive RBC-TG2ab underwent gastroscopy and celiac disease was diagnosed in 94% of children and 94% of adults (Table 2).

In contrast, the association between moderately positive (30–99 U) RBC-TG2ab values and positivity for EmA and Hr-TG2ab was poor (Table 2). A substantial proportion of such cases did not have celiac-type HLA (Table 3). Celiac disease was detected in 35% of children, 74% of adults, and, in total, 64% of those who had RBC-TG2ab 30 to 99 U and were biopsied. By applying an overall cutoff value ≥30 U, the corresponding figures were 69%, 86%, and 82%, respectively (Table 2).

The majority of participants with weak positive (20–29 U) RBC-TG2ab were negative for EmA and Hr-TG2ab (Table 2), and up to 16% also lacked the HLA-DQ2– or -DQ8–encoding alleles (Table 3). Only 4 adults and 1 child with weak positive RBC-TG2ab underwent gastroscopy because of significant clinical symptoms, and celiac disease was confirmed in 4 of them.

TABLE 1. Demographic data on the study cohort and the number of subjects with a new celiac disease diagnosis, and their relation to the celiac disease index patient

	Cases		Female patients		Age, y		Celiac disease*	
	n	%	n	%	Median	Range	n	%
Parent	540	18	317	59	64	23–91	20	4
Sibling	862	28	479	56	48	1–87	50	6
Child	941	31	519	55	23	1–77	29	3
Other†	635	21	349	55	20	1–96	15	2
Spouse	53	2	21	40	63	30–80	0	0
Total	3031	100	1682	55	37	1–96	114	4

* Biopsy-proven new diagnosis.
† Second-degree relatives.

There was 1 RBC-TG2ab–positive EmA-negative patient and 1 RBC-TG2ab–negative (value 19 U) EmA-positive patient who were found to have celiac disease. Conversely, 11% of those (3 children and 10 adults) with a new diagnosis were false-negative for Hr-TG2ab (median value 1.8 U, range 0.0–2.8 U) and would have been missed by applying this test only.

DISCUSSION

Apart from improvements in serological methods, several problems were found in the present histology-based criteria for celiac disease, which further emphasize the need for their revision. Endoscopies are expensive and burdensome, particularly in children in whom general anesthesia is usually required. The mucosal lesion can be patchy, and inaccurate slicing and an inappropriate orientation of the biopsy samples are much more common than is generally assumed (16). In addition, the interpretation of biopsy results is subjective, and even if correctly identified, small-bowel mucosal villous atrophy is not pathognomonic for celiac disease (1). The new diagnostic algorithm issued by ESPGHAN is based primarily on TG2ab, in view of its known accuracy and amenability to measurement by practical ELISA. The specificity of the quantitative method is thought to increase in parallel with the serum values, and depending on the test, TG2ab values 2.4 to 13.6 times ULN were offered in the new criteria as a cutoff value that could replace gastroscopy (11).

In the present study, we used the recommended 5 times ULN for RBC-TG2ab to challenge the ESPGHAN criteria. The results were for the most part in accord with these criteria because strong positive RBC-TG2ab values showed good accuracy for subsequent celiac disease in both children and adults. It was also shown that the criteria are applicable to a wide range of celiac patients, including apparently asymptomatic subjects detected by screening in at-risk groups. The positive predictive value was not 100%, but there was an extremely high correlation between the ≥5 times ULN RBC-TG2ab, and the presence of positive EmA, Hr-TG2ab, and celiac disease–associated HLA alleles in these individuals, which makes a false-positive result unlikely. We again emphasize that the positive predictive value of a biopsy is far less than 100%, not only because of difficulties in the interpretation of samples; the development of the small-bowel mucosal damage is a gradual process, and we and others have shown that positivity for EmA is a strong predictor of subsequent celiac disease even in subjects with normal villi (17–20). There is also evidence that these patients benefit from early dietary treatment even before the villous atrophy develops (19,20). The cost–benefit of a gluten-free diet in such cases is a subject for further study, but it must be realized that if the diagnosis is not obtained, symptomatic seropositive individuals must undergo repeated endoscopies until the mucosal damage develops. One can assume that the prevalence of early histological damage is increased in screen-detected populations such as that in our study group (21).

In an earlier study, Wong et al (22) observed 100% specificity for equal RBC-TG2ab test by applying a cutoff value 5 times ULN in adults. In addition, a small number of other studies have evaluated corresponding but guinea pig– or Hr-TG2ab–based

TABLE 2. Positivity of serum EmA and Hr-TG2ab and the number of new celiac disease diagnoses, divided according to human RBC-TG2ab values

		Antibodies					Endoscopy			
		EmA+		Hr-TG2+			Diagnosis		Normal*	
RBC-TG2ab, U	n	n	%	n	%	n†	n	%	n	%
≥100	88	87	99	87	99	80	75	94	5	6
≥30	212	141	67	131	62	133	109	82	24	18
≥20	336	146	43	133	40	138	113	82	25	18
<20	2695	1	0.04	ND	ND	1	1	100	0	0
Total	3031	147	5	ND	ND	139	114	82	25	18

EmA = serum endomysial antibodies; Hr-TG2 = human recombinant transglutaminase 2; ND = no data; RBC-TG2ab = human red blood cell transglutaminase 2 antibody.
* Celiac disease excluded by normal small-bowel mucosal villous morphology.
† Not all of the antibody-positive patients were endoscopied.

TABLE 3. Presence of celiac disease–associated HLA-type in EmA–, Hr-TG2ab–, and RBC-TG2ab–positive study participants*

Celiac-type HLA (%)	EmA+ 1: ≥5 n = 140	Hr-TG2ab+ ≥ 3.0 U n = 125	RBC-TG2+ 20–29 U n = 116	RBC-TG2+ 30–99 U n = 116	RBC-TG2+ ≥ 100 U n = 85
Negative	0	0	16.2	6.9	0
DQ2.2 or DQ7	1.4	0.9	9.4	6.0	2.4
DQ2 or DQ8	98.6	99.1	74.4	87.1	97.6
Total celiac-type HLA	100.0	100.0	83.8	93.1	100.0

EmA = endomysial antibody; HLA = human leukocyte antigen; Hr-TG2ab = human recombinant transglutaminase 2 antibody; RBC-TG2ab = red blood cell transglutaminase 2 antibody.
* Genetics samples of 20 subjects were not available.

TG2-ab tests. Donaldson et al (7) investigated 28 children and 48 adults and identified celiac disease in 96% (93% and 98%, respectively) of those with TG2ab ≥5 times ULN. In a study by Barker et al (8), 48 of 49 children received a diagnosis when an equivalent cutoff value for TG2ab was applied. In contrast, Freeman (23) observed normal villi in 3 of 14 subjects with TG2ab ≥5 times ULN and concluded that the small-bowel biopsy is still mandatory. These discrepancies suggest that variables such as age of patient, histological methods used, and pretest probability in the population in question may affect results. Nonetheless, it appears that a combination of strong positive TG2ab values and celiac disease–associated HLA alleles implies an extremely low risk of false-positive diagnosis. Further prospective studies are required to establish whether the new criteria are equally valid in countries and populations in which the disease prevalence and pretest probability are different.

In contrast to the strong positive RBC-TG2ab values, moderate or low positive values had rather poor specificity for the subsequent small-bowel mucosal villous atrophy and celiac disease diagnosis. Moreover, the low correlation with the other serological tests and the absence of celiac-type HLA alleles in several subjects suggest that they were truly false-positive. Previously, Barker et al (8) obtained similar results, because in their study celiac disease was confirmed in only 44% of the children with moderately positive TG2ab values. Conversely, Naiyer et al (24) achieved 95% specificity for RBC-TG2ab by applying a cutoff value ≥20 U in adults. Once again, these differences show that selection of the study population is of particular importance. In any case, it is evident that a more comprehensive diagnostic procedure, including histological examinations, is required before a final celiac disease diagnosis can be established in individuals with low positive RBC-TG2ab.

There were certain limitations to the present study. First, the endoscopies were not carried out in a single center or in all of the seropositive participants; however, the diagnostic procedure is well standardized in Finland (13), and the primary aim was to evaluate subjects with strong positive RBC-TG2ab values, of whom 94% underwent biopsy. Second, it was impossible to perform endoscopy in all of the participants and thus measure the overall sensitivities of the serological tests. A further limitation was that, even though it was an exclusion criterion, inadvertently reduced dietary gluten in relatives may have underestimated the true prevalence of celiac disease. Nevertheless, any significant effect on the overall results would appear to be minimal (25). Finally, our study group comprised primarily relatives of patients, and the results cannot be directly generalized to other groups at risk for celiac disease.

Our results were compatible with the new ESPGHAN criteria and showed that high positive RBC-TG2ab values had good predictive value for subsequent celiac disease. Some patients yielded negative biopsy results, but the strong association with the other serological tests and celiac-type HLA alleles indicates that they were not true false-positives. In addition, the results indicated that the new ESPGHAN criteria could be extended to the adult population. In the future, prospective studies should be carried out to confirm the validity of the new criteria in clinical practice.

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