NEED FOR QUANTITATIVE ASSESSMENT OF TRANSGLUTAMINASE AUTOANTIBODIES FOR CELIAC DISEASE IN SCREENING-IDENTIFIED CHILDREN

EDWIN LIU, MD, MARCELLA LI, MS, FEI BAO, MD, DONGMEI MIAO, MD, MARIAN J. REWERS, MD, PHD, GEORGE S. EISENBARTH, MD, PHD, AND EDWARD J. HOFFENBERG, MD

Objectives To assess several transglutaminase autoantibody (TGAA) assays in their ability to distinguish celiac disease (CD) in screening-identified children with abnormal intestine biopsy specimens from those with normal biopsy specimens.

Study design Children at risk for CD (n = 54) composed of type 1 diabetics, first-degree relatives of type 1 diabetics or CD, and HLA-DQ2+ individuals followed from birth received intestine biopsy. Sera obtained at the time of biopsy were tested for TGAA, using the radioimmunoassay and 5 other commercially available enzyme-linked immunosorbent assays.

Results False-positive rates ranged from 28% to 80%. The positive predictive value (PPV) of the tests ranged from 63% to 84% (lower than reported for symptomatic children). Setting a higher cutoff for each assay maximized PPV.

Conclusions There are significant quantitative differences among all TGAA assays that could affect interpretation of a positive test for CD. The overall false-positive rate for all assays was high in this population. Using the assay as a quantitative rather than qualitative tool by increasing the cutoff of positivity to indicate biopsy increases PPV. Multicenter workshops are needed to identify critical differences and to standardize TGAA assays among laboratories. *(J Pediatr 2005;146:494-9)*

New recommendations propose that children with a genetic risk for celiac disease (CD), such as type 1 diabetes, first-degree relatives, and Down syndrome, be screened periodically for CD with tissue transglutaminase autoantibodies (TGAA).[1] Asymptomatic children who are identified as having TGAA positivity are frequent referrals for intestine biopsy. The prevalence of CD in the Denver general population is estimated to be 1:104,[2] and in the United States, overall estimates are 1:133.[3] In individuals who are symptomatic or at a genetic risk for CD, the prevalence is much higher.[2-4] Our group has previously shown that the predictive value of a positive TGAA test in an asymptomatic genetically at-risk child is 70% to 83%,[5] much lower than that reported for symptomatic children (99%).[6,7] Thus, screening-identified children undergoing intestine biopsy are found more frequently to have normal small-intestine histology than those who are symptomatic. According to Bayes theorem, as the prevalence of a disease increases, the positive predictive value (PPV) increases and negative predictive value decreases. Surprisingly, this is not true in the case of symptomatic versus screening-identified at-risk children, since the reported prevalence of CD for both groups of children is equivalent (1:56 vs 1:30). Possible explanations include differing technical aspects of TGAA testing, different populations studied with regard to prevalence (children versus adults), variability in histologic interpretation, patchiness of duodenal lesions,[8] and intrinsic differences in disease characteristics between symptomatic and screening-identified children. The level of TGAA correlates in general with degree of intestine pathology.[9-11] However, some at-risk individuals express TGAA without evidence of histologic changes, with at least some demonstrating abnormal histology after months of persistent TGAA expression.[11,12] Since TGAA levels fluctuate over time, it is possible that

CD	Celiac disease	RIA	Radioimmunoassay
ELISA	Enzyme-linked immunoassay	TGAA	Transglutaminase autoantibody
PPV	Positive predictive value		

From Barbara Davis Center for Childhood Diabetes and the Section of Gastroenterology, Hepatology and Nutrition, the Departments of Preventive Medicine and Biometrics and the Department of Pediatrics and Immunology and the Pediatric General Clinical Research Center, University of Colorado Health Sciences Center and The Children's Hospital, University of Colorado School of Medicine, Denver, Colorado.

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Reprint requests: Edwin Liu, MD, 4200 East 9th Ave, B140, Denver, CO 80262.

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Table I. Characteristics of each TGAA assay studied

TGAA test	Substrate	Detection	Assay format	Positive cutoff
RIA	hrTG	^{35}S	RIA by 96-well plate	0.05 index
Inova Quanta Lite	hRBC TG	HRP/TMB	ELISA	20 U
Eurospital Eu-tTG	hrTG	HRP/TMB	ELISA	7 AU/mL
IMMCO	hrTG	Alk Phos/pNPP	ELISA	20 EU/mL
Biofons	hrTG	Alk Phos/pNPP	ELISA	15 AU
Binding Site	hrTG	HRP/TMB	ELISA	4 U/mL

Alk Phos, alkaline phosphatase; *hRBC TG,* human red blood cell transglutaminase; *HRP,* horseradish peroxidase; *hrTG,* human recombinant transglutaminase; *TMB,* tetramethylbenzidine; *AU,* arbitrary units; *EU,* ELISA units.

intestine histology may also reflect that change.[11] Thus, one may hypothesize a model of the intestine injury in CD characterized by relapse and remission along with tissue injury and healing, whether induced by dietary changes or intrinsic to the autoimmune process. Regardless, the much lower predictive value of a positive TGAA test in screening-identified children indicates that more stringent selection for intestine biopsy of children at genetic risk for CD is needed.

Deciding when to perform biopsy in an individual who is identified through screening with TGAA is a clinical dilemma. A normal intestine biopsy does not exclude the possibility of later development of CD and may reduce patient acceptance of future biopsies. The use of different forms of TGAA assays further confuses the picture. In our laboratory, we use the human recombinant TG radioimmunoassay (RIA), which has been suggested to be more sensitive and specific than traditional enzyme-linked immunosorbent assay (ELISA).[13] We found that quantitative adjustment of TGAA threshold for biopsy could improve test performance.[11] We hypothesized that similar cutoffs could be found for the ELISA assays and that using higher titers would minimize negative biopsies in screening-identified children. In this study, we analyzed a series of children at risk for CD who previously underwent serial testing for TGAA. Using stored sera obtained at the time of biopsy, we compared the RIA with several ELISA-based assays for TGAA levels.

METHODS

Subjects

Subjects were children under the age of 18 years, identified to be at risk for CD, based on 1 of the 3 criteria: (1) having type 1 diabetes, (2) being a first-degree relative of someone with type 1 diabetes or CD, or (3) participating in a follow-up study after being identified at birth as expressing HLA-DQ2. In all, a total of 54 children were included in this study: 27 subjects with type 1 diabetes, 14 subjects with a first-degree relative having type 1 diabetes[14,15] or CD,[16] and 13 children having HLA-DQ2. Of these, 34 had biopsy confirmation of CD and 20 had intestine biopsies without evidence of villus atrophy. Sera from 10 healthy donors were used as negative control subjects, as identified by RIA. Exclusion criteria were prior diagnosis of CD and gluten-free diet. Subjects and parents provided informed signed consent. This study was approved by the Colorado Multiple Institutional Review Board.

Screening

Follow-up for all groups included serologic screening tests for CD obtained by blood draws at 9, 15, and 24 months of age and yearly thereafter. Any positive test (TGAA index by RIA >0.05) was repeated in 3 to 6 months. A patient was referred for evaluation and intestine biopsy when 2 blood samples drawn on separate visits were TGAA positive or sooner, if requested by the family. At the time of intestinal biopsy, TGAA was again measured and additional serum was stored.

Small-Intestine Biopsy

Subjects continued their usual gluten-containing diet before undergoing biopsy. At upper gastrointestinal endoscopy, our intent was to obtain 4 samples from the duodenum: 2 from the proximal duodenum and 2 from the distal duodenum. In a few cases, we were only able to obtain 1 biopsy from each site. A single pathologist, who was unaware of clinical and laboratory findings, interpreted the sample according to the system described by Marsh.[17] Normal biopsy specimens were assigned a score of 0. A biopsy specimen with normal villous architecture (villous height to crypt depth ratio >2:1) but with increased numbers of intraepithelial lymphocytes was assigned a score of 1 and was considered indeterminate for CD. A biopsy specimen with crypt hyperplasia and increased numbers of intraepithelial lymphocytes was assigned a score of 2. A biopsy specimen with any degree of villous atrophy was assigned a score of 3. Subjects with inadequate specimens and biopsies interpreted as indeterminate were excluded. Marsh 2 and 3 scores were considered to be consistent with CD.

Autoantibody Assay

Initial screening for TGAA positivity was done by RIA, using in vitro transcribed and translated human recombinant transglutaminase, as previously described.[4] The full-length complementary DNA clone encoding transglutaminase was obtained from human umbilical vein endothelial cells and labeled with ^{35}S. Samples were measured in the fluid phase with