CME

Antibodies to Deamidated Gliadin Peptide in Diagnosis of Celiac Disease in Children

*Anne Lammi, †Pekka Arikoski, ‡Satu Simell, *Tuure Kinnunen, ‡Ville Simell, ‡Sari Paavanen-Huhtala, §Ari Hinkkanen, ||Riitta Veijola, ¶Mikael Knip, ‡Jorma Toppari, #Outi Vaarala, ‡Olli Simell, and *Jorma Ilonen

ABSTRACT

Objectives: Determination of antibodies to synthetic deamidated gliadin peptides (anti-DGPs) may work as an alternative or complement the commonly used test for tissue transglutaminase antibodies (TGA) in the diagnosis of celiac disease (CD). We analyzed the performance of a time-resolved immunofluorometric anti-DGP assay (TR-IFMA) in the diagnosis of CD in children and also retrospectively analyzed the appearance of anti-DGP antibodies before TGA seroconversion.

Methods: The study included 92 children with biopsy-confirmed CD. Serum samples were taken at the time or just before the clinical diagnosis. The control group comprised 82 TGA-negative children who were positive for human leucocyte antigen-DQ2 or -DQ8.

Results: Based on receiver operating characteristic curves, the optimal cutoff value for immunoglobulin (Ig) A anti-DGP positivity was 153 arbitrary units (AUs) with a sensitivity of 92.4% and specificity of 97.6% and that for IgG anti-DGP 119 AU, with a sensitivity of 97.8% and specificity of 97.6%. All 92 children with CD were either IgA or IgG anti-DGP positive at the time of diagnosis. Sera from 48 children with CD were also analyzed retrospectively before the diagnosis. Anti-DGP antibodies preceded TGA positivity in 35 of the 48 children with CD and appeared a median of 1 year earlier.

Conclusions: The TR-IFMA assay for detecting anti-DGP antibodies shows high sensitivity and specificity for the diagnosis of CD in children. In a majority of our study population, anti-DGP seropositivity preceded TGA positivity, indicating that earlier detection of CD may be possible by monitoring anti-DGP antibodies frequently in genetically susceptible children.

Key Words: celiac disease, children, deamidated gliadin peptide antibodies

(*JPGN* 2015;60: 626–631)

What Is Known

- Determination of deamidated gliadin peptides (anti-DGPs) antibodies performs as an alternative or complements the tissue transglutaminase (TGA) testing in the diagnosis of celiac disease (CD).
- Parallel testing of immunoglobulin G (IgG) anti-DGP and TGA antibodies has the best specificity in screening for CD in pediatric patients

.

What Is New

- IgG anti-DGP testing alone has been shown to be at least as useful as TGA for detecting CD in children.
- The anti-DGP time-resolved immunofluorometric anti-DGP assay performed high sensitivity and specificity in the pediatric diagnosis of CD. Anti-DGP antibodies, especially of the IgG class, preceded TGA in most children, and thus may allow earlier detection of CD in genetically susceptible children.

Since the identification of tissue transglutaminase antibodies (TGA) as the endomysial autoantigen in celiac disease (CD), serological screening of the disease has been mainly based on measuring immunoglobulin (Ig) A tissue TGA (1,2). Although the sensitivity and specificity of TGA have been reported to be >95%, it may perform at lower sensitivity and specificity, especially in young children (3–6). Antiendomysial antibody testing also has good sensitivity and specificity and is often regarded as the criterion standard for the diagnosis of CD, although it measures the same antigen as the TGA assay (7,8). The final clinical diagnosis of CD has been based on the presence of villous atrophy and crypt hyperplasia in small intestinal biopsy samples. The new criteria proposed by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), however, largely omit the

Received August 31, 2014; accepted December 3, 2014.

From the *Department of Clinical Microbiology, and the †Department of Pediatrics, University of Eastern Finland, the ‡Department of Pediatrics and Adolescent Medicine, University of Turku, Turku, the §A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, the ||Department of Pediatrics, University of Oulu and Oulu University Hospital, Oulu, the ¶Children's Hospital, University of Helsinki, Helsinki, and the #Institute of Clinical Medicine, University of Helsinki, Helsinki, Finland.

Address correspondence and reprint requests to Anne Lammi, MD, Department of Clinical Microbiology, Institute of Clinical Medicine, University of Eastern Finland, PO Box 1627, FI-70211 Kuopio, Finland (e-mail: anne.lammi@uef.fi).

This article has been developed as a Journal CME Activity by NASPGHAN. Visit *http://www.naspghan.org/content/59/en/Continuing-Medical-Education-CME* to view instructions, documentation, and the complete necessary steps to receive CME credit for reading this article.

The study was supported by grants from the Finnish Coeliac Society, Finnish-Norwegian Medical Foundation, Finnish Cultural Foundation, Oscar Öflund Foundation, Finland, Juvenile Diabetes Research Foundation International, Sigrid Juselius Foundation, Finland, Päivikki and Sakari Sohlberg Foundation, Finland, and Special Research Funds for University Hospitals in Finland.

The authors report no conflicts of interest.

DOI: 10.1097/MPG.0000000000000666

use of biopsy in symptomatic subjects and emphasize the significance of serological testing in the diagnosis of CD (2).

Assays for the detection of antibodies to deamidated gliadin peptides (anti-DGP) have been developed and have largely replaced the conventional gliadin antibody tests with known poor specificity (9,10). Studies have indicated that parallel testing for both IgG anti-DGP and TGA antibodies has the best specificity in screening for CD in both adults and children (11,12). Moreover, IgG anti-DGP testing has been reported to be at least as useful as TGA for detecting CD in young children (4,13). It was shown that anti-DGP antibodies even preceded the appearance of TGA in some children with a genetic risk for CD (11). Anti-DGP assays have also been demonstrated to be useful in monitoring dietary compliance to gluten-free diet in children (4,14,6). In all, these studies demonstrate that anti-DGP antibodies may have an important role in monitoring celiac autoimmunity.

We have earlier described a time-resolved immunofluorometric anti-DGP assay (TR-IFMA), which is able to detect IgA- and IgG-class antibodies in a single well using 2 different lanthanide labels (15). The fluorescence of the lanthanides is long-lived and allows for differentiation of the short-lived background fluorescence of biological material, plastics, and optics (16). It also has a superior range of linearity compared with enzyme-based immunoassays. Performing the assay is essentially similar to a routine enzyme-linked immunosorbent assay (ELISA), and the specific reagents needed, such as streptavidin plates and lanthanide-labeled antibodies, labeling kits, and labeling services, are widely available and their costs are reasonable. Multilabel counters able to detect TR fluorescence are routine equipment in many clinical laboratories. In the present study, we analyzed the performance of this assay using sera from children at the time of diagnosis of CD. We further analyzed whether anti-DGP antibodies precede TGA positivity and thus may allow earlier detection of CD development.

METHODS

Study Population

The study included 92 Finnish children with CD confirmed by a duodenal biopsy based on the histological changes of crypt hyperplasia and villous atrophy of the intestinal mucosa (Table 1) (17). Although the Marsh classification was not used routinely for the evaluation of biopsy results, namely, the number of intraepithelial lymphocytes was not recorded in the pathology report, based on villous atrophy, it is clear that all of our patients belong to Marsh classes 3A–C. Forty-four of the children were analyzed for TGA positivity because of a clinical suspicion of CD, and the duodenal biopsy was taken with a Watson capsule at the Kuopio University Hospital (23 boys and 21 girls). Forty-eight of the children were participating in the Finnish Type 1 Diabetes Prediction and Prevention study (DIPP) at the Turku and Tampere university hospitals (31 boys and 17 girls). Children in the DIPP follow-up study have been selected based on the HLA class II–conferred genetic risk for type 1 diabetes (T1D) and are monitored prospectively for the development of T1D-associated autoantibodies. A subset of children positive for DQA1*05 and DQB1*02 alleles encoding the CD risk–associated DQ2 molecule or DQB1*03:02 encoding the DQ8 molecule were also monitored for the development of TGA positivity, and if TGA was found positive in 2 consecutive samples, they were biopsied to verify the diagnosis of CD. Serum samples from children in the DIPP study were collected every 3 to 6 months from birth until 2 years of age and every 6 to 12 months thereafter. The serum sample used for the analysis of anti-DGP assay sensitivity and specificity was taken either at the time of biopsy (n = 60) or at the last available follow-up visit before the biopsy (n = 32, median 31 days, range 1–252 days). The median age at the time of CD diagnosis was 5.6 years (range 1.2–15.0 years). There were altogether 54 boys and 38 girls in the CD study group. Fifty-one of the 89 patients genotyped (57.3%) were positive for HLA-DQ2 (DQA1*05 and DQB1*02), 15 (16.9%) were positive for both HLA-DQ2 and -DQ8 (DQB1*03:02), and the remaining 23 of them (25.8%) were positive for HLA-DQ8. HLA-typing was not performed in 3 patients because of a missing sample. Because of the original screening for genetic risk for T1D in the DIPP study, the proportion of HLA-DQ8 positive children with CD in this study is higher than in clinical studies.

TABLE 1. Distribution of histology findings in the clinical and DIPP cohorts

Histology	Clinical cohort (n = 44)	DIPP cohort (n = 48)
Villous atrophy, partial	14 (31.8%)	15 (31.3%)
Villous atrophy, subtotal	5 (11.4%)	16 (33.3%)
Villous atrophy, total	22 (50.0%)	12 (25.0%)
Villous atrophy, stage not determined	3 (6.8%)	5 (10.4%)

DIPP = Type 1 Diabetes Prediction and Prevention Study.

The control group comprised 82 age- and sex-matched healthy children (49 boys) carrying the CD risk–associated HLA-DQ2 or -DQ8 molecules. The control samples were obtained independently from the Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (FINDIA) follow-up study in which otherwise healthy children have been screened for T1D HLA risk alleles (18). The median age of the control children was 6.0 years (range 3.0–6.8 years) at the time of sampling. Twenty-seven (32.9%) of the controls were HLA-DQ2 positive, 8 (9.8%) were positive for both HLA-DQ2 and -DQ8, and 47 (57.3%) were HLA-DQ8 positive. All of the control children were negative for TGA at the time of sampling.

Frozen serum samples from 48 children with confirmed CD from the DIPP study were also analyzed retrospectively in order to monitor the development of anti-DGP antibodies before the TGA positivity.

The study protocol was approved by the ethical committees of the participating university hospitals. Written informed consent was obtained from parents/guardians and from children >7 years of age.

Anti-DGP Antibody Assay

All of the serum samples were kept frozen at −80°C until analyzed for TGA and anti-DGP antibody reactivity. IgA- and IgG-class anti-DGP antibodies to a synthetic peptide comprising amino acids 86 to 103 of γ gliadin of wheat were measured by a solid-phase lanthanide–based, TR-IFMA, as previously described (15). Total IgA was not separately tested in our study population. The TR-IFMA anti-DGP assay, however, allows simultaneous determination of IgA and IgG antibodies, and extremely low background reactivity in the IgA assay indicates the possibility of IgA deficiency.

Tissue TGA Autoantibody Assay

Commercial recombinant human Celikey kit was used to measure IgA tissue TGA antibodies in patients and controls (Phadia, Freiburg, Germany). Values >8 U/mL were considered positive, as suggested by the manufacturer.

HLA Typing

The presence of HLA-DQA1 and -DQB1 alleles in all of the samples were analyzed using sequence-specific oligonucleotide hybridization reactions, as described earlier (19,20).

Statistical Analysis

All of the statistical analyses were performed by GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA). Mann-Whitney *U* test was used to compare distributions of anti-DGP antibodies between patients and controls and median ages of children with CD and controls. The cutoff values for IgA and IgG anti-DGP antibody reactivity with the highest sensitivity and specificity were determined by using receiver operating characteristic (ROC) curves. Fisher exact test was used to compare the sensitivity and specificity of anti-DGP IgA and IgG tests. Spearman correlation test was used to assess the correlation between antibody levels of IgA and IgG and severity of villous atrophy of the intestinal mucosa. Wilcoxon matched-pairs signed-rank test was used to compare the antibody levels of anti-DGP in the first and last positive serum samples. P values <0.05 were considered significant.

RESULTS

Comparison of Antibody Levels in Patients With CD and Controls and Determination of the Cutoff Values for the Anti-DGP Antibody Assay

In order to evaluate the performance of our anti-DGP assay, we analyzed serum samples from children with a clinical diagnosis of CD. We used either a serum sample taken at the time of the diagnostic biopsy or the latest available serum sample before the diagnosis. Serum samples from healthy age- and sex-matched control children positive for CD-associated HLA-DQ2 or -DQ8 molecules were used as controls. All of the serum samples from patients with CD were positive for TGA and those from healthy controls were negative.

The median value of IgA anti-DGP in children with CD was 943 arbitrary units (AUs) (range 27–7607 AU) and that of IgG anti-DGP was 718 AU (range 72–5138 AU). In controls, the median value of IgA anti-DGP was 22 (range 4–227 AU) and IgG anti-DGP 16 (1–225 AU). The levels of both IgA and IgG anti-DGP antibodies were significantly higher in patients than in controls ($P < 0.0001$, Mann-Whitney *U* test) (Fig. 1A). Moreover, the levels of both IgA and IgG anti-DGP antibodies correlated with the severity of villous atrophy in small intestinal biopsies (Fig. 1B).

ROC curves were used to analyze the sensitivity and specificity of the assays and for determining the optimal cutoff values for seropositivity. The area under the curve was 0.99 (95% CI 0.98–1.00) for IgA anti-DGP and 0.99 (95% CI 0.99–1.00) for IgG anti-DGP assay (Fig. 2). Based on the ROC curves, the optimal cutoff value for IgA anti-DGP positivity was 153 AU with a sensitivity of 92.4%, specificity of 97.6%, and likelihood ratio (LR) of 37.9. The optimal cutoff value for IgG anti-DGP positivity was 119 AU, with a sensitivity of 97.8%, specificity of 97.6%, and LR of 40.1. The sensitivity of anti-DGP IgG was significantly higher than that of anti-DGP IgA assay ($P = 0.04$, Fisher exact test). There was no statistical difference when comparing the specificity of anti-DGP IgA and IgG assays ($P = 0.38$, Fisher exact test). In conclusion, the anti-DGP assay used showed high sensitivity and specificity for both IgA- and IgG-class antibodies.

Anti-DGP Seropositivity at Diagnosis

According to the cutoff values for the anti-DGP assay, all of the children with CD were either IgA or IgG anti-DGP positive at the time of diagnosis or the latest sampling before the diagnosis. Eighty-five of the 92 children with CD (92.4%) were IgA anti-DGP positive, and 91 of the 92 children (98.9%) were IgG anti-DGP positive, and 84 of the 92 children (91.3%) were both IgA and IgG anti-DGP positive. Instead, only 2 of the 82 control children (2.4%)

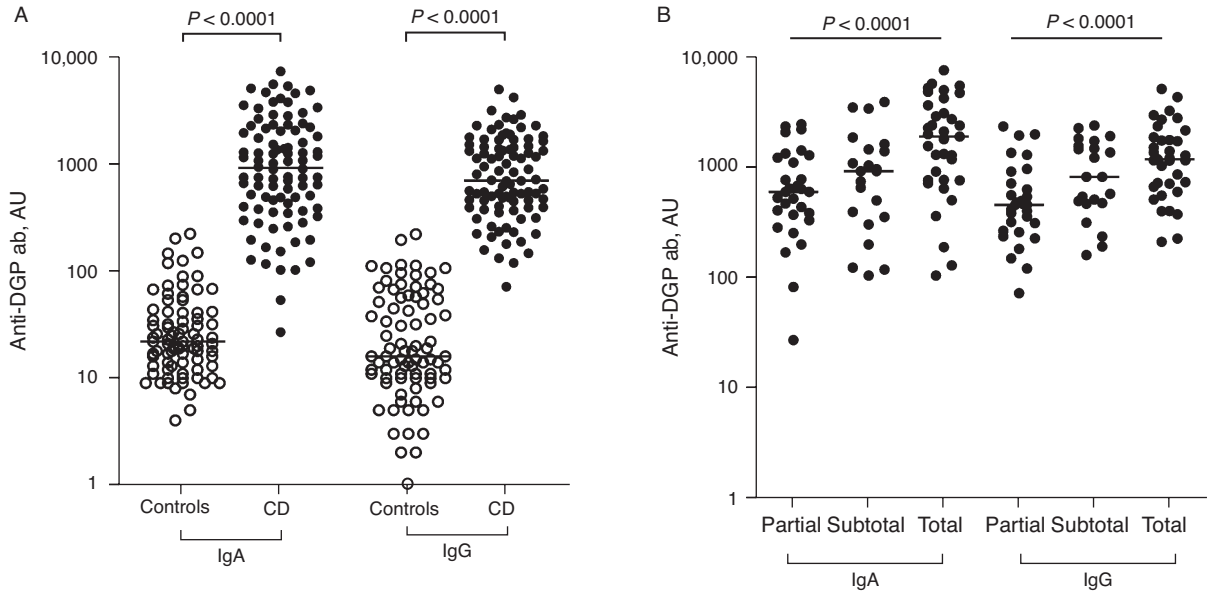

FIGURE 1. A, Median levels of both IgA and IgG anti-DGP are significantly higher in children with CD than in healthy controls at or just before CD diagnosis (Mann-Whitney *U* test). B, IgA and IgG anti-DGP levels correlate with the severity of villous atrophy in small intestinal biopsies (Spearman correlation). ab = antibodies; AU = arbitrary unit; CD = celiac disease; DGP = deamidated gliadin peptide; Ig = immunoglobulin.

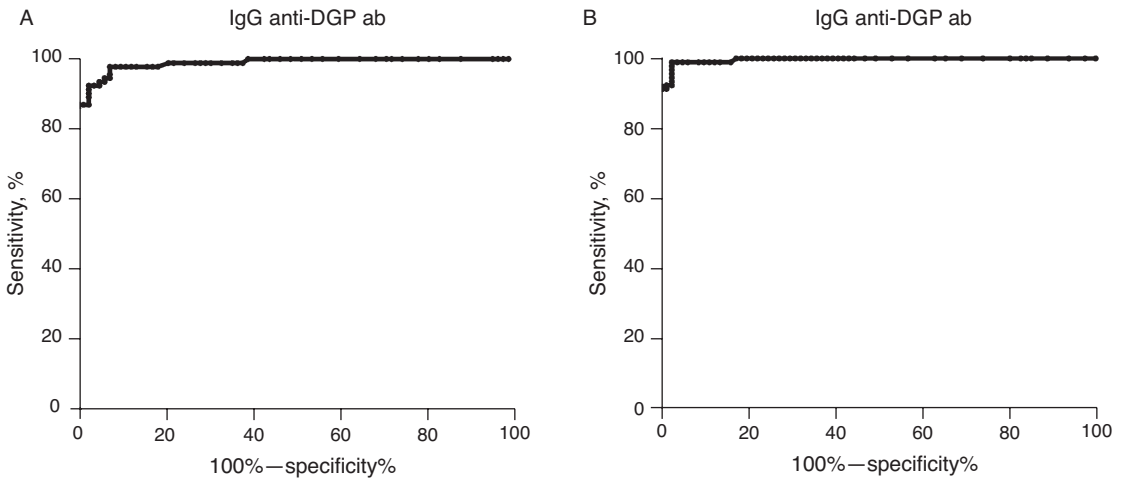

FIGURE 2. ROC of IgA (A) or IgG (B) anti-DGP in all of the children at or just before CD diagnosis. The area under the curve is 0.99 (95% CI 0.98–1.00) for IgA and 0.99 (95% CI 0.99–1.00) for IgG, respectively. ab = antibodies; CD = celiac disease; DGP = deamidated gliadin peptide; Ig = immunoglobulin; ROC = receiver operating characteristic.

were positive for IgA anti-DGP and also 2 of the 82 controls (2.4%) were IgG anti-DGP positive. None of the controls were positive for both IgA and IgG anti-DGP.

IgG-Class Anti-DGP Positivity Precedes TGA Positivity in Patients With CD

Forty-eight of the children who developed CD were participating in the follow-up program of the Finnish DIPP study in which serum samples were collected at regular intervals from birth. In this cohort, frozen serum samples taken before the TGA positivity and diagnosis of CD were analyzed retrospectively also for the presence of IgA and IgG anti-DGP antibodies (Table 2). Using the criteria for positivity described above, anti-DGP preceded the TGA positivity in 35 of the 48 (72.9%) children with CD. In almost half of the cases (21 of 48, 43.8%) only IgG anti-DGP was positive before TGA. Thirteen of the children were both IgA and IgG anti-DGP positive before TGA positivity, and only 1 of the children was IgA anti-DGP positive preceding TGA positivity. IgA anti-DGP positivity was first detected a median 0.9 years earlier (range 0.3–6.6 years) and IgG anti-DGP a median 1.0 year earlier (range 0.2–7.6 years) than TGA positivity. In 16 of these 35 children (33.3%), anti-DGP was positive in at least 2 time points before TGA positivity. Nineteen of the children with CD (39.6%) had anti-DGP positivity once before the TGA positivity, and 13 children (27.1%) converted to anti-DGP positive at the same time as the TGA was positive for the first time.

In general, the levels of both anti-DGP IgA and IgG antibodies increased during the follow-up after their first appearance. The levels of both IgA and IgG anti-DGP antibodies were significantly higher in the last positive serum sample before CD diagnosis than at the first positive serum sample ($P = 0.0013$ and $P = 0.0004$, respectively, Wilcoxon matched-pairs signed-rank test) (Fig. 3A and B).

DISCUSSION

In this study, we analyzed the performance of a TR-IFMA in the diagnosis of CD in children. For this purpose, we first used serum samples from children taken just at the time of diagnosis of CD or the latest sample available before the diagnosis. An ROC curve analysis demonstrated that the optimal cutoff value for IgA anti-DGP was 153 AU and for IgG anti-DGP 119 AU. All 92 children with CD were either IgA or IgG anti-DGP positive. The cutoff values derived in this study are actually similar to the 150 AU for both Ig classes defined by adult serum samples in an earlier study (15). Only 1 of the children with CD was not IgG anti-DGP positive and 7 children were IgA anti-DGP negative, indicating sensitivities of 97.8% and 92.4%, respectively. The sensitivity of our TR-IFMA assay appears to be remarkably high compared with findings observed in pediatric subjects using commercial ELISA tests. Hope et al (21) reported that only 35 of the 79 (44.3%) children with CD were IgA or IgG DGP positive by using the Inova's combined IgA and IgG anti-DGP assay. In contrast, Olen et al (22) reported a 91% sensitivity by using the GAF3X (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany) and QUANTA Lite Celiac DGP Screen ELISA tests (Inova Diagnostics, San Diego, CA). The ESPGHAN working group has published a systematic review and meta-analysis on serological tests used in CD diagnostics (5). The sensitivity of IgA anti-DGP ranged between 80.7% and 95.1% and that of IgG anti-DGP between 80.1% and 98.6%. Taken together, the poor performance of some tests may give a spurious picture on the performance characteristics of the anti-DPG tests as a whole.

Several studies have demonstrated that IgG anti-DGP has high specificity for CD in both adult and pediatric patients (4,6,23,24). Volta et al (6) reported a 98.9% specificity for IgG anti-DGP in adult patients with CD by using 2 commercial ELISA tests (Quanta Lite Gliadin II IgG, Inova Diagnostics, and Eurospital DGP-AGA IgG, Trieste, Italy). Basso et al (4) observed even a

TABLE 2. Appearance of anti-DGP antibody positivity before TGA positivity

		Time before TGA positivity			
		≥3 mo	≥12 mo	≥24 mo	≥36 mo
IgA anti-DGP ab	n positive	16/48	9/48	5/40	3/33
	% positive	33.3%	18.8%	12.5%	9.1%
IgG anti-DGP ab	n positive	38/48	18/48	13/40	9/33
	% positive	79.2%	37.5%	32.5%	27.3%

ab = antibodies; DGP = deamidated gliadin peptide; Ig = immunoglobulin; TGA = transglutaminase.

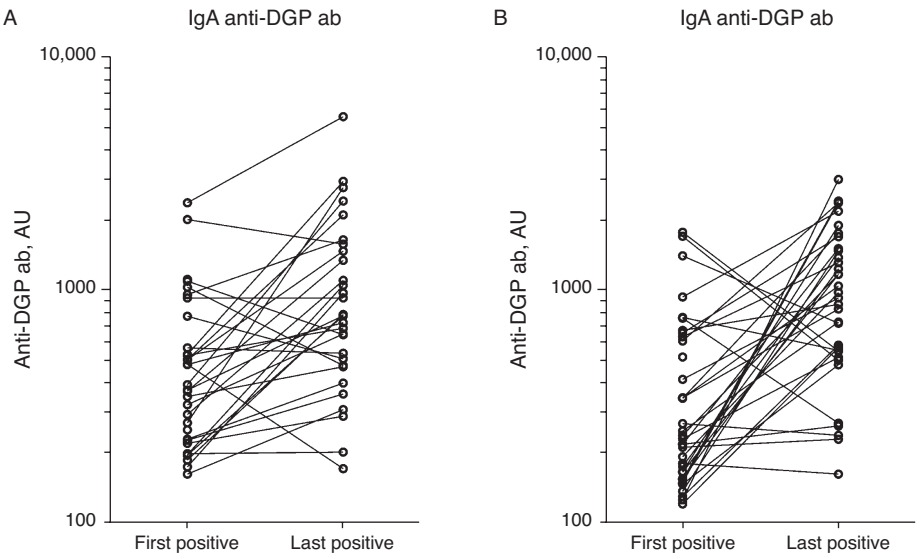

FIGURE 3. The levels of both IgA (A) and IgG (B) anti-DGP antibodies are significantly higher in the last positive serum sample taken at the time of diagnosis or just before diagnosis than in the first positive serum sample analyzed retrospectively (Wilcoxon test, paired sample *t* test, $P = 0.0013$, and $P = 0.0004$, respectively). ab = antibodies; DGP = deamidated gliadin peptide; Ig = immunoglobulin.

100% specificity for CD in children <2 years of age using commercial ELISA test for IgG anti-DGP (ELISA Quanta Lite Gliadin). We also demonstrated that both IgA- and IgG-class antibodies performed with high specificity in our hands. The specificity of both IgA and IgG anti-DGP assays was 97.6%.

Our control group consisted of healthy age-matched children carrying the CD-associated HLA-DQ2 or -DQ8 risk alleles. In contrast, previous studies (4,6,25,26) assessing the specificity of anti-DGP antibodies for CD diagnosis have used symptomatic individuals with normal histology findings in duodenal biopsies as controls. Our present approach can be criticized as healthy children may have lower levels of anti-DGP antibodies than symptomatic subjects with other gastrointestinal or autoimmune diseases than CD, leading to overestimation of the specificity of the assay. On the contrary, previous studies have not stratified their controls based on the HLA background, and therefore it is likely that in those studies the frequency of HLA-DQ2 and -DQ8–positive subjects is drastically different in CD and control groups, a factor that may also influence the evaluation of anti-DGP specificity. Importantly, because both in our study and in the previous studies the specificity values of both IgA and especially IgG anti-DGP antibodies are largely similar, the choice of control group most likely does not affect the interpretation of the results dramatically.

In this study, we did not follow our control children, and although these children were TGA negative at the time of sampling, we cannot exclude the possibility of some of these children developing CD later on, because they carry the high-risk HLA alleles. Nevertheless, even the few positive samples in the control group had relatively low levels of IgA or IgG anti-DGP reactivity, emphasizing the importance of high levels of anti-DGP antibodies in the diagnosis, as similarly suggested for TGA by the ESPGHAN criteria. Based on our results, it is apparent that the levels of anti-DGP antibodies usually both increase toward the clinical diagnosis of CD (Fig. 3) and also correlate with the severity of villous atrophy in small intestinal biopsies (Fig. 1B), which indicates that follow-up of serological reactivity may be informative.

In several studies, it has been suggested that the best sensitivity and specificity for diagnosis of CD, especially in young children, can be achieved by combining assays detecting IgA TGA and IgG anti-DGP (12,5,23–25). In the present study, we further analyzed whether anti-DGP antibodies appear before TGA positivity and demonstrated that in up to 72.9% of children with CD, anti-DGP antibodies preceded TGA. We demonstrated that in particular, IgG-class anti-DGP antibodies were detectable earlier than TGA. Of the children with CD and IgG anti-DGP antibody reactivity preceding TGA reactivity 13 had also IgA-class antibodies to DGP. Only 1 of the children examined had IgA, but not IgG anti-DGP antibodies preceding TGA positivity. The first IgA or IgG anti-DGP seropositivity could be detected a median 0.9 or 1.0 year earlier than TGA seropositivity, respectively. In the only prospective study previously published, anti-DGP positivity preceded the appearance of TGA in only 19% of the subjects studied (11). Although both this and our present study demonstrate an earlier appearance of anti-DGP antibodies, the percentage of anti-DGP positivity before TGA positivity was much higher in our study. More important, in the previous study, a considerable number of TGA-positive children with CD were negative for anti-DGP antibodies at diagnosis, suggesting a lower sensitivity for the anti-DGP assay they have used (11).

Transient TGA seropositivity has been reported in young children in a large prospective Finnish study (27). Similarly, it is possible that seropositivity for anti-DGP may be a transient phenomenon in young children and does not necessarily predict the clinical onset of CD in a similar way as transient seropositivity for TGA (11). Based on our present data, however, we assume that this is a rare phenomenon because there were only 4 anti-DGP positive children in the control group. It is also notable that the level of the DGP antibodies in these control children was significantly lower than the median level of IgA and IgG anti-DGP in patients with CD, even at their first appearance (Figs. 1 and 3).

In line with previous studies (4,6,26,28), we demonstrate here that the sensitivity of IgG anti-DGP antibodies in the diagnosis of CD is higher than that of IgA anti-DGP. Moreover, both at the time of the CD diagnosis (Fig. 1) and in retrospective samples preceding TGA positivity (Table 2), IgA anti-DGP positivity without IgG anti-DGP positivity appears to be extremely rare. Because the specificity of IgA anti-DGP has also been reported to be inferior to IgG anti-DGP (4,6,24), it remains questionable

whether IgA anti-DGP positivity without concurrent IgG anti-DGP positivity provides additional benefit in the diagnosis of CD.

It is well known that the T-cell–mediated inflammation and villous atrophy of small intestine have already begun when CD-associated antibody positivity in serum samples develops. It has been hypothesized that immunization to deamidated gliadin peptides initiates the process, and antibodies to tissue TGA are generated later on (29). The earlier appearance of anti-DGP may offer an important tool for research purposes in addition to enhancing clinical diagnostics. It is apparent that there are environmental factors affecting the development of CD in genetically susceptible subjects, and in order to identify these it is important to detect the CD-related immune process as early as possible (30–32).

In conclusion, we have shown that the TR-IFMA assay for detecting antibodies to a synthetic deamidated gliadin peptide performs with a high sensitivity and specificity in the diagnosis of CD in pediatric patients. The TR-IFMA is also cost-effective because it allows the simultaneous detection of IgA- and IgG-class anti-DGP antibodies. Moreover, we demonstrated retrospectively that anti-DGP antibodies, in particular those of the IgG class, appear earlier than IgA antibodies to tissue TGA in the majority of young children. This indicates that earlier detection of CD may be possible by monitoring anti-DGP antibodies frequently in genetically susceptible children.

Acknowledgments: The authors thank Terttu Laurén for excellent technical assistance.

REFERENCES

1. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997;3:797–801.
2. Husby S, Koletzko S, Korponay-Szabo IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012;54:136–60.
3. Leffler DA, Schuppan D. Update on serologic testing in celiac disease. *Am J Gastroenterol* 2010;105:2520–4.
4. Basso D, Guariso G, Fogar P, et al. Antibodies against synthetic deamidated gliadin peptides for celiac disease diagnosis and follow-up in children. *Clin Chem* 2009;55:150–7.
5. Giersiepen K, Lelgemann M, Stuhldreher N, et al. Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. *J Pediatr Gastroenterol Nutr* 2012;54:229–41.
6. Volta U, Granito A, Parisi C, et al. Deamidated gliadin peptide antibodies as a routine test for celiac disease: a prospective analysis. *J Clin Gastroenterol* 2010;44:186–90.
7. Chorzelski TP, Beutner EH, Sulej J, et al. IgA anti-endomysium antibody. A new immunological marker of dermatitis herpetiformis and coeliac disease. *Br J Dermatol* 1984;111:395–402.
8. Ladinser B, Rossipal E, Pittschieler K. Endomysium antibodies in coeliac disease: an improved method. *Gut* 1994;35:776–8.
9. Aleanzi M, Demonte AM, Esper C, et al. Celiac disease: antibody recognition against native and selectively deamidated gliadin peptides. *Clin Chem* 2001;47:2023–8.
10. Schwertz E, Kahlenberg F, Sack U, et al. Serologic assay based on gliadin-related nonapeptides as a highly sensitive and specific diagnostic aid in celiac disease. *Clin Chem* 2004;50:2370–5.
11. Liu E, Li M, Emery L, et al. Natural history of antibodies to deamidated gliadin peptides and transglutaminase in early childhood celiac disease. *J Pediatr Gastroenterol Nutr* 2007;45:293–300.
12. Villalta D, Tonutti E, Prause C, et al. IgG antibodies against deamidated gliadin peptides for diagnosis of celiac disease in patients with IgA deficiency. *Clin Chem* 2010;56:464–8.
13. Mozo L, Gomez J, Escanlar E, et al. Diagnostic value of anti-deamidated gliadin peptide IgG antibodies for celiac disease in children and IgA-deficient patients. *J Pediatr Gastroenterol Nutr* 2012;55:50–5.
14. Monzani A, Rapa A, Fonio P, et al. Use of deamidated gliadin peptide antibodies to monitor diet compliance in childhood celiac disease. *J Pediatr Gastroenterol Nutr* 2011;53:55–60.
15. Ankelo M, Kleimola V, Simell S, et al. Antibody responses to deamidated gliadin peptide show high specificity and parallel antibodies to tissue transglutaminase in developing coeliac disease. *Clin Exp Immunol* 2007;150:285–93.
16. Soini E, Kojola H. Time-resolved fluorometer for lanthanide chelates—a new generation of nonisotopic immunoassays. *Clin Chem* 1983; 29:65–8.
17. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). *Gastroenterology* 1992;102:330–54.
18. Vaarala O, Ilonen J, Ruohtula T, et al. Removal of bovine insulin from cow's milk formula and early initiation of beta-cell autoimmunity in the FINDIA pilot study. *Arch Pediatr Adolesc Med* 2012;166:608–14.
19. Kiviniemi M, Nurmi J, Lövgren T, et al. Locked nucleic acid (LNA) probes in high-throughput genetic analysis: application to an assay for type 1 diabetes-related HLA-DQB1 alleles. *Clin Biochem* 2005;38: 1015–22.
20. Nejentsev S, Sjöroos M, Soukka T, et al. Population-based genetic screening for the estimation of type 1 diabetes mellitus risk in Finland: selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA-DRB1 loci. *Diabet Med* 1999;16:985–92.
21. Hope BC, Ameratunga R, Austin PM, et al. The diagnostic utility of modified gliadin peptide antibody assays in New Zealand children. *J Pediatr Gastroenterol Nutr* 2013;57:43–8.
22. Olen O, Gudjonsdottir AH, Browaldh L, et al. Antibodies against deamidated gliadin peptides and tissue transglutaminase for diagnosis of pediatric celiac disease. *J Pediatr Gastroenterol Nutr* 2012;55:695–700.
23. Sugai E, Vazquez H, Nachman F, et al. Accuracy of testing for antibodies to synthetic gliadin-related peptides in celiac disease. *Clin Gastroenterol Hepatol* 2006;4:1112–7.
24. Prause C, Ritter M, Probst C, et al. Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease. *J Pediatr Gastroenterol Nutr* 2009;49:52–8.
25. Wolf J, Hasenclever D, Petroff D, et al. Antibodies in the diagnosis of coeliac disease: a biopsy-controlled, international, multicentre study of 376 children with coeliac disease and 695 controls. *PLoS One* 2014; 9:e978523.
26. Prause C, Richter T, Koletzko S, et al. New developments in serodiagnosis of childhood celiac disease: assay of antibodies against deamidated gliadin. *Ann N Y Acad Sci* 2009;1173:28–35.
27. Simell S, Hoppu S, Hekkala A, et al. Fate of five celiac disease-associated antibodies during normal diet in genetically at-risk children observed from birth in a natural history study. *Am J Gastroenterol* 2007;102:2026–35.
28. Agardh D. Antibodies against synthetic deamidated gliadin peptides and tissue transglutaminase for the identification of childhood celiac disease. *Clin Gastroenterol Hepatol* 2007;5:1276–81.
29. Lionetti E, Catassi C. New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment. *Int Rev Immunol* 2011;30:219–31.
30. Ivarsson A. The Swedish epidemic of coeliac disease explored using an epidemiological approach—some lessons to be learnt. *Best Pract Res Clin Gastroenterol* 2005;19:425–40.
31. Norris JM, Barriga K, Hoffenberg EJ, et al. Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease. *JAMA* 2005;293:2343–51.
32. Stene LC, Honeyman MC, Hoffenberg EJ, et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. *Am J Gastroenterol* 2006;101:2333–40.