A Biopsy Is Not Always Necessary to Diagnose Celiac Disease

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ABSTRACT

Objectives: Small intestinal histology is the criterion standard for the diagnosis of celiac disease (CD). However, results of serological tests such as anti-endomysium antibodies and anti-tissue transglutaminase antibodies (tTGA) are becoming increasingly reliable. This raises the question of whether a small intestinal biopsy is always necessary. The aim of the present study was, therefore, to investigate whether a small intestinal biopsy can be avoided in a selected group of patients.

Patients and Methods: Serology and histological slides obtained from 283 pediatric patients suspected of having CD were examined retrospectively. The response to a gluten-free diet (GFD) in patients with a tTGA level $\geq$100 U/mL was investigated.

Results: A tTGA level $\geq$100 U/mL was found in 128 of the 283 patients. Upon microscopic examination of the small intestinal epithelium, villous atrophy was found in 124 of these patients, confirming the presence of CD. Three patients had crypt hyperplasia or an increased number of intraepithelial lymphocytes. In 1 patient no histological abnormalities were found. This patient did not respond to a GFD.

Conclusions: Pediatric patients with a tTGA level $\geq$100 U/mL in whom symptoms improve upon consuming a GFD may not need a small intestinal biopsy to confirm CD.

Key Words: anti-endomysium antibodies, anti-tissue transglutaminase antibodies, celiac disease, diagnosis, serology

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Celiac disease (CD) is a gluten-sensitive enteropathy characterized by small intestinal damage with loss of absorptive villi, classically leading to malabsorption, diarrhea, and failure to thrive (1). The disease occurs in genetically susceptible individuals upon dietary ingestion of gluten, a storage protein in wheat, barley, and rye, and usually resolves upon its withdrawal (1,2). Although the prevalence of CD may be as high as 0.5% to 1%, it is frequently not diagnosed because symptoms may be minimal or aspecific (3,4).

In symptomatic patients serological tests are performed by measuring circulating disease-associated antibodies, particularly immunoglobulin A (IgA) auto-antibodies against endomysium, that is, anti-endomysium antibodies (EMA), and tissue transglutaminase (tTG), that is, anti-tissue transglutaminase antibodies (tTGA) (5–7). tTG, a calcium-dependent thiol enzyme, has been identified as the main, if not sole, autoantigen for both antibodies and is thought to play a major role in the pathogenesis of CD (8–11).

To date, a small intestinal biopsy, which typically shows villous atrophy, increased intraepithelial lymphocytes, and hyperplastic crypts in patients with CD on a gluten-containing diet, is the criterion standard for the diagnosis of CD (6). Considering the inconvenience and high costs associated with a biopsy, and because CD is a disease with a high prevalence, there is a growing call for less invasive tests to diagnose CD. Because both the sensitivity and specificity of the serological tests have increased to nearly perfect values, it is increasingly questioned whether these tests alone may be sufficient to confirm the diagnosis and thereby avoid the requirement for a biopsy in specific cases (12–18). Nevertheless, the positive predictive value (PPV) of the serological tests, reflecting the probability that a patient with a positive test indeed has the disease, is far from ideal, especially in the general population (12,19–21).

Consequently, if a biopsy is not performed in the workup of CD, a number of patients with falsely raised serological markers would unnecessarily follow a gluten-free diet (GFD), hitherto the only treatment available for CD. The aim of the present study was, therefore, to investigate whether specific factors may optimize the PPV and thus determine whether a small intestinal biopsy can be avoided in a selected group of patients.

PATIENTS AND METHODS

Study Population

The data of all of the patients who were referred between 1998 and 2009 to the Wilhelmina Children's Hospital in Utrecht, the Netherlands, suspected of having CD, were examined retrospectively according to the guidelines of the medical ethics board of the University Medical Center Utrecht, the Netherlands. Patients were referred to us because of symptoms that are associated with CD or because they belonged to a group at risk for CD.

All of the patients who had both a small intestinal biopsy and serological testing, including total serum IgA levels, were included in the study. Serological testing had been performed between 3 months before and 1 week after the initial small intestinal biopsy, whereas patients were on a gluten-containing diet; patients in whom the biopsy or serological testing was obtained during a GFD or a gluten challenge were excluded from the study. Patients with an IgA deficiency and patients with giardiasis were also excluded. For the patients with a tTGA level $\geq$100 U/mL, we retrospectively collected data regarding clinical presentation and responsiveness to the GFD.

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TABLE 1. Results of small intestinal biopsy, EMA, and tTGA with a cutoff value of >10 and ≥100 U/mL

	Biopsy data		
	Patients n = 283	Patients with CD n = 163 (57.6%)	Patients with normal histology n = 120 (42.4%)
IgA EMA			
Negative (%)	85 (30.0)	6 (3.7)	79 (65.8)
Positive (%)	198 (70.0)	157 (96.3)	41 (34.2)
IgA tTGA >10			
Negative (%)	106 (37.5)	7 (4.3)	99 (82.5)
Positive (%)	177 (62.5)	156 (95.7)	21 (17.5)
IgA tTGA ≥100			
Negative (%)	155 (54.8)	39 (23.9)	116 (96.7)
Positive (%)	128 (45.2)	124 (76.1)	4 (3.3)

CD = celiac disease; EMA = anti-endomysium antibodies; IgA = immunoglobulin A; tTGA = anti-tissue transglutaminase antibodies.

Serological Assessment

IgA EMA were detected by means of indirect immunofluorescence using sections of distal monkey esophagus mounted on glass slides (IMMCO Diagnostics Inc, Buffalo, NY). Serum IgA tTGA were measured using the ELiA Celikey IgA kit (Phadia AB, Uppsala, Sweden). As recommended by the manufacturer, the serum samples containing an antibody titer of >10 U/mL were considered positive. Total IgA was measured in all of the patients, and a serum IgA concentration <0.07 g/L was regarded as IgA deficiency.

All of the blood samples that were obtained between 1998 and 2009 had been stored at −80°C. To maximize experimental consistency, we retested all of the blood samples that had been investigated using other test versions than the ones described here. Because serological testing for tTGA was not available in our hospital before 2002, the serum tTGA of all of the patients who underwent biopsy before 2002 were measured using the stored blood samples.

Histological Evaluation

A minimum of 2 biopsies were taken from the distal duodenum by upper gastrointestinal endoscopy. Histological diagnosis for all of the patients was made by a single experienced pathologist using the Marsh classification. The pathologist had no knowledge of the serological results or the clinical presentation of the patients. An increased number of intraepithelial lymphocytes (Marsh I) and crypt hyperplasia (Marsh II), without villous atrophy, was considered insufficient for the diagnosis of CD. Only patients who had villous atrophy in addition to crypt hyperplasia and an increased number of intraepithelial lymphocytes (Marsh III) upon microscopic examination were diagnosed as having CD.

Data Analysis

The sensitivity, specificity, PPV, and negative predictive value (NPV) of the screening tests, along with their 95% confidence intervals (CI), were calculated using the histological evaluation as the criterion standard. Subsequently we determined whether a tTGA level ≥100 U/mL or dual positivity for tTGA and EMA could improve the PPV. Finally, in patients with a tTGA level ≥100 U/mL descriptive analysis of presenting symptoms and responsiveness to the GFD was performed.

RESULTS

In 301 patients both a small intestinal biopsy and serological testing were performed. Four patients with giardiasis and 14 patients with an IgA deficiency were excluded, leaving 283 patients for analysis. Of those, 130 (45.9%) were boys and 153 (54.1%) were girls with an age range between 0.7 and 17.8 years and a mean age of 6 years.

A total of 163 (57.6%) patients had a biopsy diagnostic for CD (Marsh III), whereas a normal histology was found in 120 patients (42.4%) (Table 1). False-positive EMA were found in 41 patients (34.2%) and false-positive tTGA in 21 patients (17.5%). Twenty of these patients also had positive EMA. The clinical characteristics of these patients did not differ from the total study population (data not shown).

False-negative EMA were found in 6 patients (3.7%) and false-negative tTGA in 7 patients (4.3%). In 4 patients with CD, EMA and tTGA were both undetectable. Three of these patients were younger than 2 years.

The resulting sensitivity was equally high for EMA and tTGA (96%) (Table 2); however, the specificity of EMA was as low as 66%, whereas specificity for tTGA was 83%. PPV for EMA was 79% and for tTGA 88%. Dual positivity for EMA and tTGA did not lead to a significant improvement in the diagnostic accuracy

TABLE 2. Sensitivity, specificity, PPV, and NPV of different screening tests for CD

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IgA EMA	96 (0.92–0.98)	66 (0.57–0.74)	79 (0.73–0.85)	93 (0.85–0.97)
IgA tTGA >10	96 (0.91–0.98)	83 (0.74–0.89)	88 (0.82–0.92)	93 (0.86–0.97)
IgA tTGA ≥100	76 (0.69–0.82)	97 (0.91–0.99)	97 (0.92–0.99)	75 (0.67–0.81)

The 95% confidence intervals are given in parentheses. CD = celiac disease; EMA = anti-endomysium antibodies; IgA = immunoglobulin A; NPV = negative predictive value; PPV = positive predictive value; tTGA = anti-tissue transglutaminase antibodies.

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TABLE 3. Symptoms in 128 patients with tTGA ≥100 U/mL and response to the GFD

Symptoms*	Response to the GFD
Growth failure (n = 72)	Responsive (n = 111)
Abdominal pain (n = 49)	Not responsive (n = 3)
Diarrhea (n = 46)	No follow-up data (n = 6)
Fatigue (n = 36)	Did not start or adhere to the diet (n = 4)
Bloating (n = 34)	Asymptomatic (n = 4)
Constipation (n = 32)	
Anorexia (n = 20)	
Vomiting (n = 17)	
Behavioral changes (n = 10)	
Asymptomatic (n = 5)	
Nausea (n = 3)	
Tooth enamel defects (n = 2)	
Dermatitis herpetiformis (n = 1)	

GFD = gluten-free diet.
* Most patients had more than 1 symptom.

because PPV was 89% (CI 0.83–0.93) instead of 88% for tTGA alone. Combining negative EMA and tTGA to exclude CD resulted in a NPV of 95% (CI 0.87–0.98).

A total of 49 patients (17.3%) had a tTGA level between 10 and 100 U/mL. Of those, 32 (65.3%) had CD, whereas the diagnosis could be histologically excluded in 17 (34.7%) patients. By contrast, of the 128 patients with a tTGA level ≥100 U/mL only 4 patients, all positive for EMA, did not have villous atrophy, and consequently did not have CD (Table 1). The corresponding PPV was 97% (Table 2).

More important, of these 4 patients 3 had histological changes that are compatible with but not diagnostic for CD: 2 had crypt hyperplasia and 1 had an increased number of intraepithelial lymphocytes (Marsh I). Only 1 of the patients with a tTGA level ≥100 U/mL did not have any histological abnormality.

Presenting symptoms in the 128 patients with tTGA ≥100 U/mL included growth failure, diarrhea, and abdominal pain, as well as various other symptoms, with most patients having more than 1 symptom (Table 3). Response to the GFD could be judged in 114 because 6 patients were lost to follow-up, 4 patients did not start or adhere to the GFD, and 4 patients were asymptomatic at diagnosis.

One of them was diagnosed during routine screening in Down syndrome and the remaining 3 were identified during family screening after a sibling or parent was diagnosed as having CD. In all of the symptomatic patients clinical symptoms improved after the GFD started, with the exception of 3. One of these patients turned out to have an irritable bowel syndrome, which may explain her persisting symptoms of abdominal pain and constipation. The second was an 11.5-year-old girl presenting with short stature, who did not exhibit catch-up growth in the 3 years after diagnosis. The third and final patient, who did not respond, was the patient with a tTGA level ≥100 U/mL and a normal histology of the small intestinal mucosa upon microscopic examination.

DISCUSSION

Small intestinal histology is considered the criterion standard for the diagnosis of CD. However, with the advent of reliable serological tests, it is being questioned whether a biopsy is really necessary in all cases. Indeed, in patients with tTGA ≥100 U/mL there is increasing evidence that serology may be sufficient to diagnose CD (22–24). Barker et al (22) showed that 48 of 49 pediatric patients with a tTGA level ≥100 U/mL had at least Marsh II duodenal histology. A subsequent study, also in a pediatric population, showed that 38 of the 38 patients with tTGA ≥100 U/mL had Marsh III duodenal histology (23). More recently, in a study conducted in a mixed adult/pediatric population, it was shown that tTGA ≥100 U/mL almost exclusively occurs in the setting of Marsh III (73/76 patients) and that the 3 cases without villous atrophy did have minimal histological changes (Marsh I and II) suggestive of early CD (24). By contrast, Freeman reported that 3 of 14 adult patients with tTGA ≥100 U/mL did not have CD (25).

In the present study, 124 of 128 patients with a tTGA level ≥100 U/mL were shown to have a Marsh III lesion upon histological examination. Four patients with tTGA levels ≥100 U/mL did not match the classical diagnostic criteria for CD. However, only 1 of them had a normal biopsy. This patient also did not respond to a GFD. The remaining 3 patients had histological abnormalities, that is, crypt hyperplasia (Marsh II) or an increased number of intraepithelial lymphocytes (Marsh I), which suggests that these patients had in fact an early stage of CD. They are reminiscent of the patients who have abnormal serology but insufficient histological evidence of CD, but who later on develop more pronounced histological lesions, after which CD can be diagnosed (26–32). In fact the diagnostic criteria, as recently issued by the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition, state that Marsh I and II along with positive tTGA is compatible with CD (33). In addition, histological lesions in CD can be patchy and can therefore be missed sometimes; this may have been the case in at least some of the false-positive patients in the present study, for example the patient with Marsh 0 but a tTGA level ≥100 and positive EMA (34). Similarly, CD may have been missed in some of these patients because duodenal bulb biopsies were not obtained routinely, whereas has been described recently, this is sometimes the only site affected (35). Finally, the patients with a false-positive serology may have potential CD and may thus develop CD at some point in their lives. Unfortunately, our study did not include follow-up of these patients to verify in how many patients CD eventually developed.

Even with the current caveats, our data imply that tTGA ≥100 U/mL is highly suggestive for CD because only 1 patient would have been misdiagnosed if a biopsy would not have been performed. Remarkably, this patient did not respond to the GFD, whereas almost all of the other symptomatic patients with tTGA ≥100 U/mL showed an excellent clinical response if they were compliant with the diet. Therefore, it can be considered to start a GFD in all symptomatic patients with a tTGA level ≥100 U/mL. If symptoms disappear the diagnosis is final, without a biopsy being required. The present study suggests that by applying this strategy, no patients will be misdiagnosed as having CD, whereas the number of biopsies performed can be reduced significantly: of the 114 patients with a tTGA level ≥100 U/mL in whom the response to a GFD could be judged only 3 would have needed a biopsy.

By contrast, of the 49 patients with a positive tTGA yet with <100 U/mL, 17 patients would have been misdiagnosed if a biopsy would not have been performed. Obviously, in these patients a biopsy is still necessary to confirm the diagnosis. Likewise, positivity for both EMA and tTGA did not guarantee the presence of CD. With a PPV of 89% this combination was only slightly more reliable than using tTGA alone (PPV 88%). When both EMA and tTGA are negative, CD is unlikely. However, a small intestinal biopsy should still be performed if CD is highly suspected on clinical grounds because 4 patients in the present study were negative for both EMA and tTGA (NPV 95%), but still had CD. This presence of seronegative patients with CD has been reported before, especially in children younger than age 2 years (14,36–39). Indeed, in our study 3 of the 4 patients with false-negative results were younger than age 2 years.

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The present study was performed retrospectively and patients with a positive serology were obviously more likely to be referred to our hospital for a small intestinal biopsy. This may have negatively affected the reliability of the serology in the present study. In addition, the specificity of EMA was substantially lower than the values that are generally reported (12). This could be due to the routine clinical setting in which the study was performed; comparable low values for EMA have been reported from a similar setting (40). Under these circumstances interobserver variability in judging the results of the semiquantative EMA immunofluorescence method is difficult to avoid, especially if a study is performed over a long period, such as ours.

In summary, no serological test was found to be 100% pathognomonic for CD. Histological confirmation is still needed in most cases. Nevertheless, in the present study all of the symptomatic patients with a tTGA level $\geq$100 U/mL in whom symptoms improved on a GFD had histological lesions compatible with CD. It can therefore be considered to omit a biopsy in this specific subgroup.

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REFERENCES

1. Mäki M, Collin P. Coeliac disease. *Lancet* 1997;349:1755–9.
2. Wolters VM, Wijmenga C. Genetic background of celiac disease and its clinical implications. *Am J Gastroenterol* 2008;103:190–5.
3. Catassi C, Fabiani E, Rätsch IM, et al. The coeliac iceberg in Italy. A multicentre antigliadin antibodies screening for coeliac disease in school-age subjects. *Acta Paediatr Suppl* 1996;412:29–35.
4. Hoffenberg EJ, Mackenzie T, Barrica KJ, et al. A prospective study of the incidence of childhood celiac disease. *J Pediatr* 2003;143:308–14.
5. Abdulkarim AS, Murray JA. Review article: the diagnosis of coeliac disease. *Aliment Pharmacol Ther* 2003;17:987–95.
6. Chan AW, Butzner JD, McKenna R, et al. Tissue transglutaminase enzyme-linked immunosorbent assay as a screening test for celiac disease in pediatric patients. *Pediatrics* 2001;107:E8.
7. Bürgin-Wolff A, Dahlbom I, Hadziselimovic F, et al. Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease. *Scand J Gastroenterol* 2002;37:685–91.
8. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997;3:797–801.
9. Brusco G, Muzi P, Ciccocioppo R, et al. Transglutaminase and coeliac disease: endomysial reactivity and small bowel expression. *Clin Exp Immunol* 1999;118:371–5.
10. Sjöström H, Lundin KE, Molberg O, et al. Identification of a gliadin T-cell epitope in coeliac disease: general importance of gliadin deamidation for intestinal T-cell recognition. *Scand J Immunol* 1998;48:111–5.
11. Molberg O, Mcadam SN, Körner R, et al. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat Med* 1998;4:713–7.
12. Rostom A, Dubé C, Cranney A, et al. The diagnostic accuracy of serologic tests for celiac disease: a systematic review. *Gastroenterology* 2005;128:S38–46.
13. Reeves GE, Squance ML, Duggan AE, et al. Diagnostic accuracy of coeliac serological tests: a prospective study. *Eur J Gastroenterol Hepatol* 2006;18:493–501.
14. Baudon JJ, Johanet C, Absalon YB, et al. Diagnosing celiac disease: a comparison of human tissue transglutaminase antibodies with antigliadin and antiendomysium antibodies. *Arch Pediatr Adolesc Med* 2004; 158:584–8.
15. Collin P, Kaukinen K, Vogelsang H, et al. Antiendomysial and antihuman recombinant tissue transglutaminase antibodies in the diagnosis of coeliac disease: a biopsy-proven European multicentre study. *Eur J Gastroenterol Hepatol* 2005;17:85–91.
16. Fabiani E, Peruzzi E, Mandolesi A, et al. Anti-human versus anti-guinea pig tissue transglutaminase antibodies as the first-level serological screening test for coeliac disease in the general population. *Dig Liver Dis* 2004;36:671–6.
17. Hadithi M, von Blomberg BM, Crusius JB, et al. Accuracy of serologic tests and HLA-DQ typing for diagnosing celiac disease. *Ann Intern Med* 2007;147:294–302.
18. Hill PG, Forsyth JM, Semeraro D, et al. IgA antibodies to human tissue transglutaminase: audit of routine practice confirms high diagnostic accuracy. *Scand J Gastroenterol* 2004;39:1078–82.
19. Hopper AD, Hadjivassiliou M, Hurlstone DP, et al. What is the role of serologic testing in celiac disease? A prospective, biopsy-confirmed study with economic analysis. *Clin Gastroenterol Hepatol* 2008;6:314–20.
20. Bardella MT, Trovato C, Cesana BM, et al. Serological markers for coeliac disease: is it time to change? *Dig Liver Dis* 2001;33:426–31.
21. Lock RJ, Stevens S, Pitcher MC, et al. Is immunoglobulin A anti-tissue transglutaminase antibody a reliable serological marker of coeliac disease? *Eur J Gastroenterol Hepatol* 2004;16:467–70.
22. Barker CC, Mitton C, Jevon G, et al. Can tissue transglutaminase antibody titers replace smallbowel biopsy to diagnose celiac disease in select pediatric populations? *Pediatrics* 2005;115:1341–6.
23. Donaldson MR, Firth SD, Wimpee H, et al. Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. *Clin Gastroentrol Hepatol* 2007;5:567–73.
24. Donaldson MR, Book LS, Leiferman KM, et al. Strongly positive tissue transglutaminase antibodies are associated with Marsh 3 histopathology in adult and pediatric celiac disease. *J Clin Gastroenterol* 2008; 42:256–60.
25. Freeman HJ. Strongly positive tissue transglutaminase antibody assays without celiac disease. *Can J Gastroenterol* 2004;18:25–8.
26. Collin P, Helin H, Maki M, et al. Follow-up of patients positive in reticulin and gliadin antibody tests with normal small-bowl biopsy findings. *Scan J Gastroenerol* 1993;28:595–8.
27. Corazza GR, Andreani ML, Biagi F, et al. Clinical, pathological, and antibody pattern of latent celiac disease; report of three adult cases. *Am J Gastroenterol* 1996;91:2203–7.
28. Dickey W, Hughes DF, McMillan SA. Patients with serum IgA endomysial antibodies and intact duodenal villi: clinical characteristics and management options. *Scand J Gastroenterol* 2005;40:1240–3.
29. Mohamed BM, Feighrey C, Coates C, et al. The absence of a mucosal lesion on standard histological examination does not exclude diagnosis of celiac disease. *Dig Dis Sci* 2008;53:52–61.
30. Paparo F, Petrone E, Tosco A, et al. Clinical, HLA, and small bowl immunohistochemical features of children with positive serum antiendomysium antibodies and architecturally normal small intestinal mucosa. *Am J Gastroenterol* 2005;100:2294–8.
31. Salmi TT, Collin P, Jarvinen O, et al. Immunoglobulin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease. *Aliment Pharmacol Ther* 2006;24:541–52.
32. Westerlund A, Ankelo M, Simell S, et al. Affinity maturation of immunoglobulin A anti-tissue transglutaminase autoantibodies during development of coeliac disease. *Clin Exp Immunol* 2007;148:230–40.
33. Hill ID, Dirks MH, Liptak GS, et al. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology. *J Pediatr Gastroenterol Nutr* 2005;40:1–19.
34. Bonamico M, Mariani P, Thanasi E, et al. Patchy villous atrophy of the duodenum in childhood celiac disease. *J Pediatr Gastroenterol Nutr* 2004;38:204–7.
35. Bonamico M, Thanasi E, Mariani P, et al. Duodenal bulb biopsies in celiac disease: a multicenter study. *J Pediatr Gastroenterol Nutr* 2008;47:618–22.
36. Dickey W, McMillan SA, Hughes DF. Sensitivity of serum tissue transglutaminase antibodies for endomysial antibody positive and negative coeliac disease. *Scand J Gastroenterol* 2001;36:511–4.
37. Bürgin-Wolff A, Gaze H, Hadziselimovic F, et al. Antigliadin and antiendomysium antibody determination for coeliac disease. *Arch Dis Child* 1991;66:941–7.
38. Lagerqvist C, Dahlbom I, Hansson T, et al. Antigliadin immunoglobulin A best in finding celiac disease in children younger than 18 months of age. *J Pediatr Gastroenterol Nutr* 2008;47:428–35.
39. Maglio M, Tosco A, Paparo F, et al. Serum and intestinal celiac disease-associated antibodies in children with celiac disease younger than 2 years of age. *J Pediatr Gastroenterol Nutr* 2010;50:43–8.
40. Parizade M, Bujanover Y, Weiss B, et al. Performance of serology assays for diagnosing celiac disease in a clinical setting. *Clin Vaccine Immunol* 2009;16:1576–82.

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