Diagnosis of Coeliac Disease in Children Younger Than 2 Years

Zrinjka Mišak, Iva Hojsak, Oleg Jadrešin, Alemka Jaklin Kekez, Slaven Abdović, and Sanja Kolaček

ABSTRACT

Background and Aim: To diagnose coeliac disease (CD) in children younger than 2 years, the old ESPGHAN criteria based on 3 small bowel biopsies were recommended until recently. The aim of the present study was to investigate the applicability of only 1 small intestinal biopsy plus positive serology for the diagnosis of CD in children younger than 2 years.

Methods: A prospective cohort study included 81 patients younger than 2 years with symptoms suggestive of CD, who all completed the diagnostic procedure based on 3 small bowel biopsies. According to the finding of the third biopsy, patients were divided into group A—CD confirmed (N = 44), and group B—CD not confirmed, after the gluten challenge (N = 37).

Results: At the time of the first biopsy, total villous atrophy (Marsh IIIc) was found more often in group A than in group B (77% vs 27%, $P < 0.01$). Also, all of the studied antibodies were more frequently positive in group A than in group B ($P < 0.01$ for all of the tested antibodies). Positive anti-endomysial antibodies and Marsh IIIc finding were the best discriminators between the group A and the group B and considerably contributed to the prediction of CD.

Conclusions: The second and the third biopsies (before and after the gluten challenge) may also be avoided when diagnosing CD in children younger than 2 years provided that the child, at the time of presentation, has positive anti-endomysial antibodies and Marsh IIIc on the small bowel biopsy. A gluten challenge should be still considered in all other children younger than 2 years.

Key Words: children, coeliac disease, diagnostic criteria

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Coeliac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals. It is characterised by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, human leukocyte antigen (HLA)-DQ2 or -DQ8 haplotypes and enteropathy (1). The ingestion of gluten, a constituent of wheat, barley, and rye, in these susceptible individuals causes morphologic changes in small intestinal mucosa characterised by villous atrophy, leading ultimately to malabsorption (2,3). Population studies have shown that CD is affecting 0.5% to 1% of general population, although the majority of affected individuals are still undiagnosed (4). Early diagnosis and strict gluten-free diet (GFD) act protectively against complications and decrease mortality rates in patients with CD, and therefore, making a definitive diagnosis of CD is of great importance (5,6).

The first diagnostic criteria for CD in children were proposed by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) in 1969. According to these original criteria, the diagnosis of CD was made upon the sequence of 3 biopsies if there were structurally abnormal jejunal mucosa when taking a diet containing gluten; clear improvement of villous structure when taking a GFD; deterioration of the mucosa during the gluten challenge (7). This sequence of small bowel biopsies was meant to help differentiate CD from other transient causes of abnormal small intestinal mucosa. In 1990, ESPGHAN revised the original diagnostic criteria to omit the second and the third biopsies for children younger than 2 years at the time of the first biopsy. According to these, the diagnosis of CD is based on the typical histological finding of hyperplastic villous atrophy while the patient is eating adequate amounts of gluten; and unequivocal and full clinical remission after withdrawal of gluten from the diet (8); however, the gluten challenge was still recommended: when there were doubts about the initial diagnosis (eg, initial biopsy not performed, the biopsy specimen inadequate or uncharacteristic, lack of clinical response on GFD) (8,9). Also, in children younger than 2 years at presentation (as they can have enteropathy because of other causes such as cow's-milk–sensitive enteropathy, postenteritic syndrome, and giardiasis), a gluten challenge was advised, preceded, and followed by a small bowel biopsy (8,10); however, the new, just recently published, ESPGHAN guidelines for the diagnosis of CD state that in the presence of high antibody levels the diagnosis of CD may be based on a combination of symptoms, antibodies, and HLA, thus omitting the duodenal biopsy. The diagnosis is confirmed by an antibody decline and preferably a clinical response to a GFD, whereas gluten challenge and repetitive biopsies are only necessary in selected, unclear cases (1).

Conversely, children younger than 2 years represent a diagnostic problem and original protocol based on 3 biopsies is long-lasting, invasive, and particularly demanding for the patients and health care system in general. In Croatia, unlike the most western countries, a considerable proportion of paediatric patients with CD still presents with classic symptoms before the age of 2 years (11). That provided us with the opportunity to investigate the applicability of just 1 small intestinal biopsy and positive serology for the diagnosis of CD in children younger than 2 years and that was the aim of the present study.

METHODS

This is a prospective cohort study carried out at the Children's Hospital Zagreb. All of the consecutive patients younger

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than 2 years (N = 173) investigated for CD at our department during the period from 1995 to 2005 who underwent small bowel biopsy and had at least Marsh I type lesion were prospectively recruited into the study, following the same protocol (Fig. 1). Seventeen children did not reach 6 years of age before the end observational period and therefore were not challenged with gluten. Of the remaining 156 patients, during the observational period, 81 patients fulfilled the old ESPGHAN criteria based on 3 biopsies. The reasons for not performing 3 biopsies in other patients are presented in Figure 1.

According to the finding of the third small bowel biopsy, patients were divided into 2 groups: group A—patients in whom CD was confirmed after the gluten challenge, and group B—patients in whom CD was not confirmed after at least 6 months of gluten challenge.

Biopsies were performed with either Crosby's suction capsule or during the endoscopy. All of the biopsy specimens were analysed by 1 of 2 pathologists and were classified according to Marsh-Oberhuber criteria (type I–IV) (12).

All of the serologic tests were performed at the same laboratory under the supervision of 1 person. Serum anti-gliadin antibodies (AGA IgA and IgG) were determined quantitatively by fluoro-enzyme-immuno method using the kit from Eurospital, Trieste, Italy. The results are expressed as indexes and for AGA IgA, values >7 were considered positive, while for AGA IgG positive were values >15. Anti-endomysial antibodies (EMA of IgA class) were detected by the method of indirect fluorescence using sections of distal monkey oesophagus (''Antiendomysium test,'' Eurospital, Trieste, Italy) (13).

HLA DQ alleles encoding HLA-DQ2 and -DQ8 were investigated at the Tissue Typing Laboratory of the University Centre Zagreb. Patients were typed for HLA class II alleles by a high-resolution polymerase chain reaction sequence–specific oligonucleotide probes method (14).

Statistical Analysis

Quantitative data were expressed as medians and ranges or means and 95% confidence intervals (CIs). Statistical differences between study groups were evaluated using Student t test and χ^2 test, as appropriate. $P < 0.05$ was considered statistically significant. Receiver operating characteristic (ROC) curves and area under the curve (AUC) were created to determine the diagnostic value of observed diagnostic methods (small intestinal biopsy finding and serologic tests) and to determine optimal threshold values. Binary logistic regression analysis was used to investigate observed diagnostic tests in combination, and a stepwise method was used to find the best test combination.

Ethical Considerations

The study was approved by the ethical committee of the University of Zagreb School of Medicine, as well as by the ethical committee at our hospital. Informed consent was obtained for all of the patients included in the study.

RESULTS

In total, 81 patients younger than 2 years who fulfilled the old ESPGHAN criteria based on 3 small intestinal biopsies were included in this prospective study (Fig. 1). The diagnosis of CD was confirmed by the biopsy finding after the gluten challenge in 44 patients (group A), whereas in 37 patients who had normal small bowel biopsy finding after the gluten challenge, CD was not confirmed (group B).

In group A, there were 14 boys (32%) and 30 girls (68%) of mean age 12.8 months (range 6–24 months), whereas in group B, there were 15 boys (40%) and 22 girls (55%) of mean age 13.6 months (range 6–23 months). Groups did not differ in regard to sex and age.

Most common presenting symptom in group A was failure to thrive (in 93% of children) followed by diarrhoea (79%), abdominal distension (72%), anaemia (54%), vomiting (39%), and abdominal pain (11%). In group B, 76% of children presented with failure to thrive, whereas diarrhoea was present in 68%, abdominal distension in 54%, anaemia in 43%, vomiting in 22%, and abdominal pain in 19% of children (Fig. 2). Groups A and B did not differ in regard to any presenting symptom except for the failure to thrive, which was

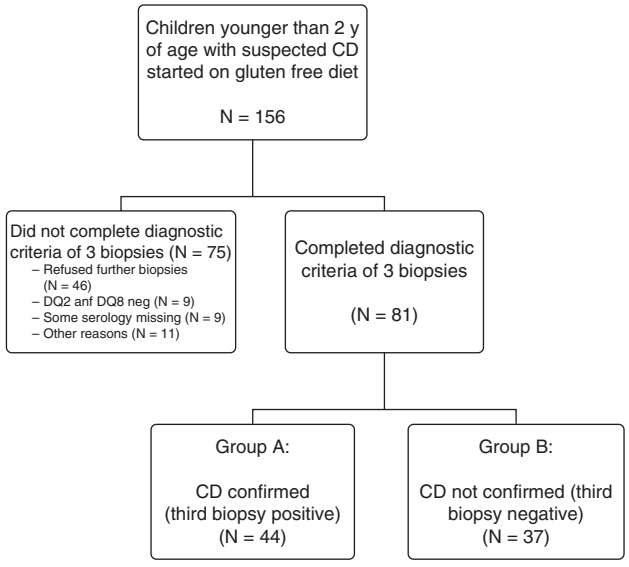

FIGURE 1. Flowchart of patients included in the study. CD = coeliac disease.

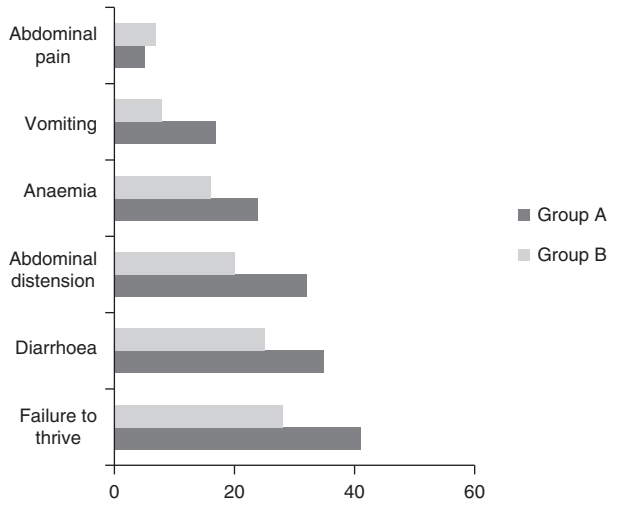

FIGURE 2. Clinical symptoms based on which the suspicion of coeliac disease (CD) was made and the first biopsy performed. Failure to thrive was the only symptom that was significantly more frequent in group A (with later proven CD) vs group B (CD later not confirmed).

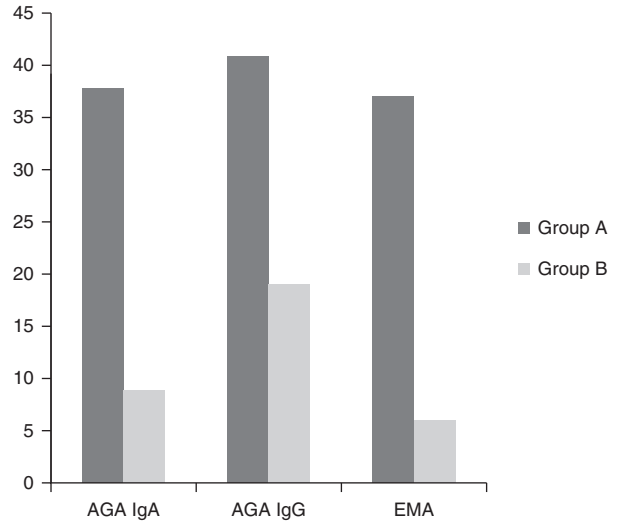

FIGURE 3. The proportion of positive serology testing in children of both groups (with confirmed and not confirmed CD) at the time of presentation. AGA = anti-gliadin antibodies; EMA = anti-endomysial antibodies; IgA = immunoglobulin A; IgG = immunoglobulin G.

more common in patients with finally proven CD (group A), $P < 0.05$.

Concerning serology, all of the children from group A were positive for AGA IgG, whereas 93% were positive for IgA AGA and 85% for EMA. In group B, 59% of children were positive for AGA IgG, 26% for AGA IgA, and 16% for EMA (Fig. 3). When compared, all of the studied antibodies were significantly more frequently positive in group A than in group B ($P < 0.01$ for all tested antibodies). Six patients from group A (with confirmed CD) were EMA negative, 1 patient had IgA deficiency and was only AGA IgG positive. The remaining 5 children (age 9–17 months) were IgA competent and all had both IgA- and IgG AGA-positive antibodies. Specificity and sensitivity of tested antibodies at manufacturer cutoff values are presented in Table 1.

At the time of the first biopsy, 84% of children with confirmed CD had villous atrophy (Marsh IIIa-c) that was significantly more common in group A than in group B (84% vs 59% $P = 0.01$). The same was true for total villous atrophy (Marsh IIIc 77% vs 27%, $P < 0.01$). Marsh I and II type of lesions were, however, more common in patients of group B (5% and 11% vs 11% and 30%, respectively) (Fig. 4).

Of those 10 patients from group B who had Marsh IIIc lesion on the small intestinal biopsy, 4 patients were also EMA positive; however, all 4 of them after the gluten challenge that lasted from 10 months to 5 years had normal small bowel biopsy. Because they also have HLA status compatible with CD, they are followed up as possible late relapsers.

Results of logistic regression analysis are presented in Table 2. Tests with good discriminatory power show a combination of a high odds ratio together with a low P value (ie, a significant difference between the groups). With the diagnosis of CD being a dependent variable, it was shown that EMA and AGA IgG were the tests that best differentiated between the group A and the group B. If AGA were excluded from the analysis (because AGA is not recommended anymore in the detection of CD) (1), variables that considerably contributed to the prediction of CD were EMA and the Marsh IIIc biopsy finding. Furthermore, if the final diagnosis was based only on the Marsh IIIc finding of the first small bowel biopsy and positive EMA (instead of 3 biopsies), the diagnosis of CD would be correct in 83.95% of our patients and this approach would not result in any patient with CD being missed.

TABLE 1. Sensitivity, specificity, and predictive values of performed serology testing (%)

	AGA IgA	AGA IgG	EMA
Sensitivity	92.50	100.00	84.09
Specificity	59.09	35.71	83.78
Positive predictive value	80.43	68.97	86.05
Negative predictive value	81.25	100.00	81.58

AGA = anti-gliadin antibodies; EMA = anti-endomysial antibodies; IgA = immunoglobulin A; IgG = immunoglobulin G.

DISCUSSION

In the present study, carried out to evaluate the possibility of simplifying the diagnostic algorithm for CD in children younger than 2 years, we showed that the CD can be diagnosed based on only 1 small bowel biopsy obtained at presentation, provided that there is a histology finding of Marsh IIIc and that EMA is positive.

Until recently, to diagnose CD in children younger than 2 years, the old ESPGHAN criteria of 3 biopsies were recommended. This was justified with some other conditions, causing in this age group similar histological changes, and presenting with similar clinical picture (8,10,15). Whether such an invasive and long-lasting procedure is truly required was explored in several studies (4,15–23). Based on the results obtained, the new ESPGHAN guidelines for the diagnosis of CD in children and adolescents were recently published. According to these, in case of extremely high tissue transglutaminase antibody (anti-TG2) titres, there is an option to diagnose CD without duodenal biopsies, but by applying a strict protocol with further laboratory tests (1); however, the gluten challenge with pre- and post-challenge biopsies is indicated in cases when there is a doubt about the diagnosis, when the first biopsy was not performed, or the biopsy specimen was not sufficient for the analysis (1,24,25).

Even before the publication of the new ESPGHAN criteria, many centres did not adhere to the 3-biopsy protocol in children younger than 2 years (26–28). For example, in Sweden, only 27% of paediatric clinics use the recommended 3 biopsies procedure

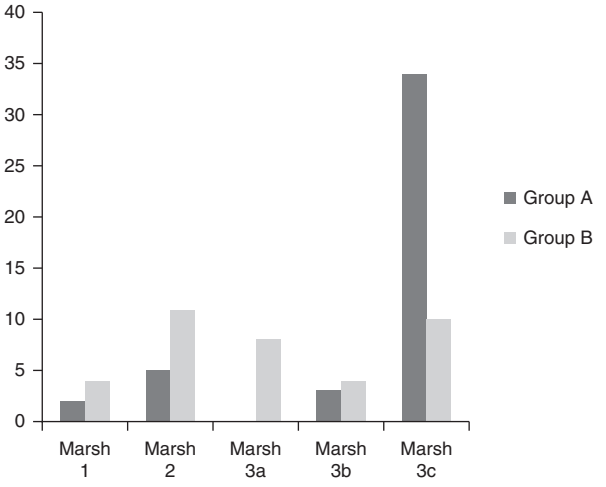

FIGURE 4. Results of the first small bowel biopsy.

TABLE 2. Logistic regression analysis

Test	*P*	Odds ratio	95% CI
Dependent variable: CD diagnosis			
Independent variables: EMA, IgG AGA, IgA AGA, IELs, Marsh IIIc			
EMA	0.0028	12.6592	2.3998–66.7783
IgG AGA	0.0008	1.0441	1.0180–1.0709
Dependent variable: CD diagnosis			
Independent variables: EMA, IELs, Marsh IIIc			
EMA	0.0001	17.0924	4.9010–59.6106
Marsh 3c	0.0135	4.8428	1.3847–16.9374

AGA = anti-gliadin antibodies; EMA = anti-endomysial antibodies; IEL = intraepithelial lymphocyte; IgA = immunoglobulin A; IgG = immunoglobulin G.

(10), whereas in Italy, >80% of centres do not perform gluten challenge in children younger than 2 years (27). Even in our study, a considerable proportion of patients younger than 2 years who had symptoms suggestive of CD (48%) did not complete the whole 3-biopsy procedure; however, in 81 patients younger than 2 years at disease presentation, who completed the study, the diagnosis of CD could have been correctly established at the time of the first biopsy in 84% of them, provided that the result of the biopsy was Marsh IIIc, and that EMA was positive. Thus, 29 children (66%) from the group A could have been spared from the second and the third biopsies. Therefore, we propose that gluten challenge with pre- and postchallenge biopsies is necessary in patients with Marsh I-IIIb histology finding and/or EMA negative, both taken at the time of disease presentation. This approach would not result in any patient with CD being missed. Similarly was shown in a study done by Wolters et al (28) (although that was a retrospective study and on a smaller proportion of patients who did not relapse after the gluten challenge) who suggested that routine gluten challenge in young children is not necessary when patients have villous atrophy in combination with positive EMA; however, for more detailed discussion and for further follow-up are 4 children from the group B, who would have also got the diagnosis of CD, although their third biopsy was negative. Whether they would be falsely diagnosed or they will ultimately present as late relapsers is the question that can be answered only after a long-term follow-up.

Serology plays an important role in diagnosing CD and distinguishing villous atrophy caused by CD from other conditions. Paediatric studies have shown the specificity of EMA to be nearly 100%, with a sensitivity ranging from 90% to 100% (9,29). IgA anti-TG2 has also a high sensitivity and specificity of 90% to 99%, but because of false-positives in up to 10% of cases, its positivity must be confirmed by EMA (29–32); however, despite being a highly sensitive and specific markers of CD, both EMA and anti-TG2 alone are not good markers in younger children (<2 years) (30,33). Burgin-Wolf et al (34) reported in children younger than than 2 years of age that the specificity of EMA was 98%, but the sensitivity was significantly lower than in older children (80% vs 97%, $P < 0.008$). This low sensitivity of EMA was also confirmed in our patients (84%), whereas specificity was also 84%. On the contrary, some authors did not observe any lack of antibody sensitivity in this age group (20,35,36). It seems that IgG antibodies against deamidated forms of gliadin peptides is a good tool for identifying CD in children younger than 2 years and in patients with IgA deficiency (1,33). Unfortunately, at the beginning of our study, we could not determine either anti-TG2 or deamidated forms of gliadin peptides. Instead, we used AGA, and there is some evidence that its sensitivity may be higher in children younger than 2 years in comparison with EMA and anti-TG2 tests (1,29,37); however, the new guidelines stated that the specificity of AGA tests is extremely low in this age group, making it not helpful for clinical practice (1,38,39). In our study, the sensitivity of AGA IgA was 92% and of AGA IgG 100%, whereas the specificity was extremely low (AGA IgA 59% and AGA IgG 36%). Even higher sensitivity of IgA was shown in the Lagerqvist et al (37) study on children younger than 18 months (97%) being also more sensitive than IgA anti-TG2 and IgA EMA (both 83%, $P < 0.0001$). Moreover, as many as 17% of the children with CD in this age group would have remained undiagnosed if anti-TG2 IgA was used alone.

Furthermore, patients with intermediate histopathologic changes, including partial villous atrophy, are occasionally anti-TG2 and/or EMA negative and the question is whether those really suffer from CD (18,40,41). In our study, 2 patients had mucosal lesions other than IIIc (one had Marsh I and the other Marsh II) and were EMA negative (although both were AGA positive), but after the gluten challenge, CD was confirmed. Therefore, if intermediate histopathologic mucosal changes are found even in children negative for CD-specific antibodies (but younger than 2 years and HLA-DQ2 or -DQ8-positive), later gluten challenge should be performed to confirm CD as a cause of the enteropathy.

We are aware of the several limitations to our study: there was a high dropout (48% of our patients younger than 2 years did not complete the whole procedure of 3 biopsies); at the time of the first biopsy, anti-TG2 and/or DGP could not have been performed; and the possibility that some of the patients from group B will be late relapsers cannot be excluded.

Despite these limitations, this is a unique prospective study that comprised children younger than 2 years who completed the classic diagnostic procedure of 3 biopsies, providing us with the opportunity to draw valid conclusion that the second and the third biopsies (before and after the gluten challenge) may be avoided in children younger than 2 years provided that the child, at disease presentation, has positive EMA and Marsh IIIc on the small bowel biopsy.

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