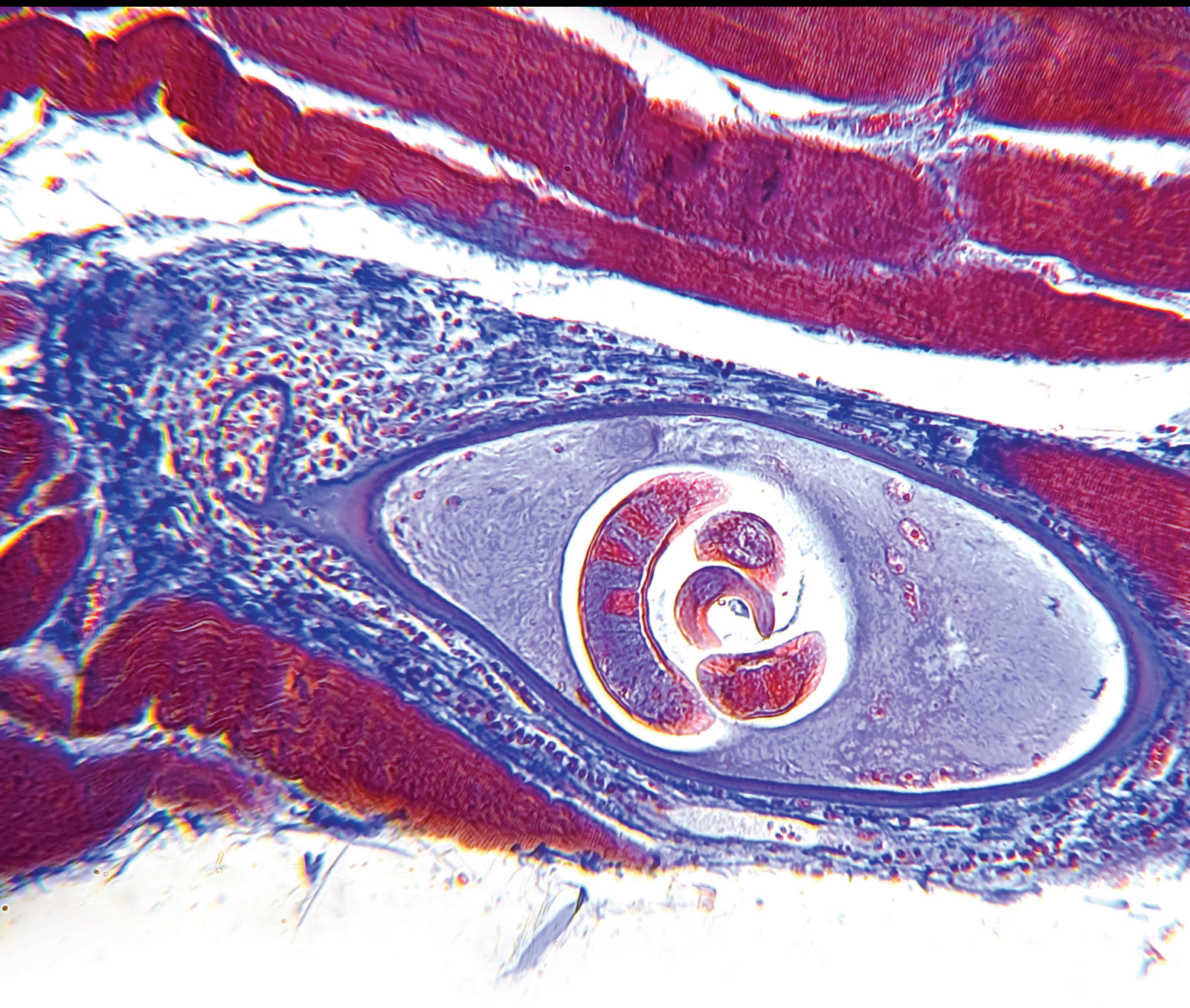
Gastroenterology Research and Practice

Hot Topics in Pediatric Celiac Disease

Lead Guest Editor: Francesco Valitutti
Guest Editors: Anna Rybak, Valentina Discepolo, and Kalle Kurppa



Hot Topics in Pediatric Celiac Disease

Gastroenterology Research and Practice

Hot Topics in Pediatric Celiac Disease

Lead Guest Editor: Francesco Valitutti
Guest Editors: Anna Rybak, Valentina Discepolo, and Kalle Kurppa

Contents

Hindawi
Gastroenterology Research and Practice
Volume 2020, Article ID 1670479, 8 pages
https://doi.org/10.1155/2020/1670479

Research Article

Fasting Neurotensin Levels in Pediatric Celiac Disease Compared with a Control Cohort

Donatella Iorfida,[1] **Monica Montuori**,[1] **Chiara Maria Trovato**,[1] **Claudio Tiberti**,[2] **Andrea Sansone**,[2,3] **Salvatore Cucchiara**,[1] **and Francesco Valitutti**[4,5]

[1]*Pediatric Gastroenterology and Liver Unit, Department of Maternal-Child Health and Urology, Sapienza University of Rome, Italy*
[2]*Department of Experimental Medicine, Sapienza University of Rome, Italy*
[3]*Center for Reproductive Medicine and Andrology, Universitätsklinikum Münster, Münster, Germany*
[4]*Pediatric Unit, AOU San Giovanni di Dio e Ruggi d'Aragona, Salerno, Italy*
[5]*EBRIS (European Biomedical Research Institute of Salerno), Salerno, Italy*

Correspondence should be addressed to Francesco Valitutti; francesco.valitutti@uniroma1.it

Received 30 June 2019; Revised 4 January 2020; Accepted 6 February 2020; Published 22 February 2020

Academic Editor: Andrew S. Day

Background and Aims. Neurotensin (NT) is a gut hormone secreted by specific endocrine cells scattered throughout the epithelial layer of the small intestine, which has been identified as an important mediator in several gastrointestinal functions and disease conditions. Its potential involvement in celiac disease (CD) has been investigated, but there are conflicting findings. The aim of this study was to evaluate serum NT levels in children with CD at diagnosis, compared to a control group, and to investigate whether NT correlated in CD patients with symptoms, antibody response, and intestinal mucosal damage. *Materials and Methods*. Children (1-16 years old) undergoing gastrointestinal endoscopy for CD or for other clinical reasons were included in this study. Patients with CD diagnosed according to the 2012 European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines without biopsy were also recruited. Fasting serum samples were analyzed for NT levels using ELISA. Logistic regression, Wilcoxon rank sum, and Spearman's rank tests were used for statistical analysis. *Results*. Thirty children (18 females, 2.2-15.9 years old) were enrolled. Of 25 patients who underwent endoscopy, 9 were CD patients, 13 were controls, and 3 were excluded due to nonspecific inflammation at duodenal biopsy. CD was diagnosed in 5 patients without biopsy. NT median was higher in CD patients compared to controls (13.25 (IQR 9.4-17.5) pg/ml vs. 7.8 (IQR 7.6-10) pg/ml; $p = 0.02$). No statistically significant association between NT and clinical, serological, or histological data of CD was observed in this CD cohort. *Conclusions*. To our knowledge, this is the first study that evaluates NT in CD children from Italy. Results show that NT is higher in the serum of CD children at diagnosis compared to controls. However, larger-scale studies are required to validate these findings. Whether serum NT levels can be an adjunctive marker for pediatric CD remains currently elusive.

1. Introduction

Neurotensin (NT) is a 13-amino acid peptide first isolated in 1973 from the bovine hypothalamus and digestive tract [1]. Its physiological functions are those of neurotransmitter in the central nervous system and hormone in the periphery.

Centrally, it affects sensory and motor functions, temperature regulation, neuroendocrine control of the pituitary, and control of blood flow and pressure [2].

In the gut, it is secreted by endocrine N cells scattered predominantly in the epithelial layer of the jejuno-ileum and released after a meal, particularly those containing high lipid levels [3]. It has a range of paracrine and endocrine functions regulating gastrointestinal secretion and motility under physiological conditions [2, 4].

Thus, NT has been shown to play an important role in the conduction of multiple physiologic processes in both the brain and the periphery. Disruption of these normal

mechanisms may contribute to the development of various diseases [5].

Previous studies have demonstrated that alterations of NT levels were associated with several neuropathological conditions [6] and have supported the role of NT in endocrine, autocrine, and paracrine growth stimulation of several types of cancer [5].

NT might also participate in various pathophysiological gastrointestinal processes, including the modulation of intestinal responses to stressful and inflammatory stimuli that share several common features such as mast cell and immune cell activation. By interacting with specific receptors, NT exerts direct and indirect effects on nerves, epithelial cells, and cells of the immune and inflammatory systems [4], an aspect that has captured scientific attention in the last three decades.

It would be fascinating to explore aspects that are still little known regarding the involvement of this peptide in gastrointestinal diseases, and experimental research towards this direction should be supported.

Among gastrointestinal diseases, celiac disease (CD) is in the foreground for its notable social burden [7] and its features of systemic condition affecting several organs [8]. CD presents a multifactorial etiopathogenesis, and despite many advances in terms of understanding the disease's mechanisms in recent years, many aspects remain to be defined.

CD is a systemic, immune-mediated disorder triggered by gluten and related prolamines in genetically susceptible individuals. It encompasses the presence of a variable combination of clinical manifestations, CD-specific antibodies, human leukocyte antigen- (HLA-) DQ2 or HLA-DQ8 haplotypes, and immune-mediated enteropathy [9].

Onset can occur at any age, and although the inflammatory process specifically targets the intestinal mucosa, patients may present with gastrointestinal signs or symptoms, extraintestinal signs or symptoms, or both [10]. Nevertheless, some patients display only minor clinical features or even no symptoms at diagnosis [11].

Antibodies against tissue transglutaminase (anti-tTG), an endogenous protein, are highly sensitive and used as specific markers for CD. However, in some pediatric cases and all adult cases, an intestinal biopsy is required to confirm the diagnosis [12].

The histological examination allows to identify different degrees of intestinal inflammation and villous atrophy, which correlates with levels of anti-tTG. In support of this, several studies confirmed that high concentration of anti-tTG in serum predicts villous atrophy better than low or borderline values [9, 13].

A potential involvement of NT in CD has been investigated, but there are conflicting findings.

In 1978, Bloom et al. showed that patients with untreated CD had an increase in postprandial NT levels compared to healthy controls and CD subjects on a gluten-free diet [14]. In that year, the same group of authors assessed the profile of intestinal hormones in CD patients, showing in these subjects an increase in fasting NT levels [15]. However, these data were not confirmed in a pediatric study a few years later [16]. In 2000, Bardella et al. identified increased fasting NT levels in CD patients paralleled by a reduced postprandial rebound compared to controls [17].

Recently, the goal of a pediatric study on CD patients has been to assess whether NT could be a spy for more important forms of intestinal inflammation [18]. In particular, Montén et al. have dosed the proneurotensin precursor fragment 1-117, referred to as proneurotensin (pro-NT), shown to be completely stable in human plasma and to be produced in equimolar amounts with respect to NT [19]. The correlation between pro-NT and severity of CD clinical picture was assessed with regard to antibody titers and histological damage. They found that plasma pro-NT levels were elevated in children with CD and in those with severe intestinal mucosal damage, hypothesizing that pro-NT could play a role in small intestinal inflammation.

Following the path of this latest research, the aim of our study was to evaluate serum NT levels in children with CD at diagnosis compared to a control group. A further aim was to investigate whether NT correlated in CD patients with symptoms, antibody response, and intestinal mucosal damage.

2. Materials and Methods

The study was conducted between November 2017 and May 2018 at the Pediatric Gastroenterology and Liver Unit of Policlinico Umberto I, Sapienza, University of Rome, Italy. The local ethical committee approved the study. All participants were informed about the aim of the study, and a parental written consent was obtained for each child.

2.1. Patient Selection. Children (1-16 years old) who underwent gastrointestinal endoscopy for CD or for other clinical indications (i.e., unexplained anemia, poor growth, dysphagia, heartburn, and epigastric pain) were included in the study. CD patients diagnosed according to the 2012 ESPGHAN guidelines [9] without biopsy were also recruited.

Exclusion criteria were gluten-free diet, neoplasia, immunosuppressive therapy, neurological/neuropsychiatric pathology, history of allergy/mastocytosis, and history of intestinal infection/inflammation (i.e., recent infectious gastroenteritis, recent fever episode, inflammatory bowel diseases, eosinophilic esophagitis, and eosinophilic gastroenteropathy).

2.2. Blood Sampling, Gastrointestinal Endoscopy, and Diagnostic Classification. All patients enrolled were fasting prior to blood sampling for at least six hours for toddlers (≤2 years) and at least nine hours for children older than 2. If endoscopy was performed, the blood sampling was taken at the same time as the procedure. All serum samples were analyzed for NT and screened for CD (anti-tTG IgA and total serum IgA). HLA typing and endomysial antibodies (EMA) were performed when required for diagnosis according to the 2012 ESPGHAN guidelines.

The same pathologist scored all biopsies according to Marsh-Oberhuber (MO) criteria, in a blinded way with regard to the results of CD screening and clinical data.

Children who resulted positive for anti-tTG and showing grading 2 or 3 according to MO classification were defined as

having CD. Anti-tTG-negative children with negative duodenal biopsy (i.e., without any kind of histopathological alterations and with less than 25 intraepithelial lymphocytes/100 enterocytes) were included as disease controls. Subjects with histopathologic signs of nonspecific inflammation or features attributable to other diseases in duodenal biopsies were excluded from the study.

Children positive for anti-tTG levels ≥ 10 times the upper limit of normal (ULN), EMA IgA, and HLA DQ2 and/or DQ8 with suggestive symptoms were defined as having CD without biopsy, according to the 2012 ESPGHAN guidelines.

2.3. CD Screening. Fasting serum samples were analyzed for anti-tTG levels using ELISA (Eurospital, Trieste, Italy). Values ≥ 16 UA/ml were considered positive results.

The dosage of serum total IgA, if not already available, was performed in each patient to rule out an IgA deficiency. The amount of serum IgA was measured by nephelometry.

When required for diagnosis, EMA were performed through indirect immunofluorescence (Eurospital, Trieste, Italy), whereas for HLA typing, a molecular biology system (Eu-Gen System, Eurospital, Trieste, Italy) was used.

2.4. Neurotensin Analysis. For quantitative determination of serum NT levels, the commercial Human Neurotensin ELISA was used (catalog number ABIN365746 on antibodies-online.com). Blood samples were collected using a serum separator tube and were centrifuged according to the manufacturer's instructions. Serum was removed, aliquoted, and stored at -80°C to avoid loss of bioactivity. After thawing the samples, serum NT levels were determined. The detection range of the kit was 15.6-1000 pg/ml. Samples were analyzed at the Department of Experimental Medicine, Sapienza, University of Rome.

2.5. Clinical Evaluation of Patients. For each child, body weight, height, and body mass index were recorded, and a detailed history was collected in order to define the presence of intestinal and/or extraintestinal symptoms/signs of CD.

2.6. Statistical Analysis. Logistic regression, Wilcoxon rank sum, and Spearman's rank tests were used for statistical analysis (R software). A p value < 0.05 was considered significant for all the tests performed. NT levels were log-transformed due to their skewed distribution.

3. Results

3.1. Patient Diagnosis. Thirty children (18 F; 2.2-15.9 years old) were enrolled in this study. CD was diagnosed in 14 patients (10 F; mean age 6.6 years), 13 were recruited as controls (8 F; mean age 12.1 years), and 3 patients were excluded due to nonspecific inflammation at duodenal biopsy. CD was diagnosed in 5 of 14 patients without biopsy, according to the 2012 ESPGHAN guidelines (Table 1).

Histology of all CD patients showed villous atrophy (MO grading: 3) (Table 1). None were found to have potential CD. Duodenum biopsies of all controls were negative, and only mild superficial chronic gastritis or mild reflux esophagitis was found at histology in this group (Table 2). These latter findings were observed also in some CD patients (2/9) (Table 1).

Among patients with CD, 7 had intestinal and extraintestinal symptoms of CD, 2 had no symptoms suggestive of CD, and in the remaining 5 patients, symptoms were purely intestinal or extraintestinal. As regards anti-tTG values, 7 of them had titers ≥ 10 times the ULN: among these, 2 had no symptoms suggestive of CD, so the no-biopsy option was not applied for diagnosis.

3.2. Neurotensin Results. NT median was higher in CD patients compared to controls (13.25 (IQR 9.4-17.5) pg/ml vs. 7.8 (IQR 7.6-10) pg/ml; $p = 0.02$) (Figure 1). Nevertheless, no statistically significant correlation of NT with clinical presentation of CD, anti-tTG titers, and mucosal damage graded according to MO criteria was observed in this cohort.

Moreover, no correlation was observed between NT and age within each of the two groups and among all the overall cohort of twenty-seven patients considered for NT analysis.

4. Discussion

In our study, we found that children with untreated CD had increased fasting serum NT levels compared to controls.

In line with our results, increased fasting NT levels were detected in two previous studies in adult CD patients: Besterman et al. have found in CD patients an increase in fasting NT levels [15], and the Italian group of Bardella et al. have reported similar evidence [17]. These data, however, were not confirmed in a pediatric study from 1987 [16].

Recently, Montén et al. have found elevated peripheral pro-NT levels to reflect more severe forms of active CD in children [18]. In this study, pro-NT levels were measured in plasma by a chemiluminometric sandwich immunoassay to detect a pro-NT precursor fragment, based on an assay described by Ernst et al. [19]. Fasting pro-NT levels were found higher in children with CD compared to the disease controls. An association was observed between the anti-tTG and pro-NT levels in plasma. Furthermore, plasma pro-NT levels in children with MO 3b and MO 3c histology were higher than those in children with MO 0 [18].

Released pro-NT ideally represents NT as they have been shown to circulate in equimolar amounts [19].

In our cohort, no statistically significant correlation of NT with serological or histological data of CD was observed. In particular, we have not found any statistically significant correlation of NT with anti-tTG levels or MO score, although it is likely that the small sample size influenced these results. None of the enrolled patients had a MO score < 3, so we could not unveil any correlation between NT and different degrees of intestinal mucosal damage.

In our study, we also looked for a potential relationship between NT and clinical presentation of CD.

The high rate of dyspepsia-like symptoms frequently reported in CD patients may be related to upper gastrointestinal tract motor abnormalities [20–23]. The pathophysiology of these motor abnormalities may involve gastrointestinal hormones, the secretion of which may be altered in CD patients as a consequence of intestinal mucosal damage

Table 1: This table illustrates clinical presentation, endoscopic, and histological findings for each enrolled CD patient. In the column "No-biopsy approach," CD patients diagnosed according to the 2012 ESPGHAN guidelines without biopsy are indicated.

CD patient	Gender (M/F)	Age (years)	Clinical presentation	Endoscopic findings	Histological findings	No-biopsy approach*
CD patient 1	M	6.1	Constipation, recurrent abdominal pain, fatigue, impaired growth, anemia	No-biopsy protocol has been adopted*	No-biopsy protocol has been adopted*	X
CD patient 2	F	3.6	Constipation	Irregular surface of duodenal folds	MO grading 3b. H. pylori-negative chronic superficial gastritis, mild reflux esophagitis	
CD patient 3	F	4.6	Recurrent abdominal pain, fatigue	Mild edema and hyperemia of the duodenal bulb and duodenum, reduced height and irregular surface of duodenal folds	MO grading 3b	
CD patient 4	M	3.1	Constipation, oral aphthosis, recurrent abdominal pain, astenia	No-biopsy protocol has been adopted*	No-biopsy protocol has been adopted*	X
CD patient 5	F	10.9	Meteorism, abdominal pain, tooth enamel defects, first-degree relative of the CD patient	No-biopsy protocol has been adopted*	No-biopsy protocol has been adopted*	X
CD patient 6	F	6.3	Impaired growth	Mild hyperemia of the duodenal bulb	MO grading 3a. Mild reflux esophagitis	
CD patient 7	M	2.2	Lack of appetite, fatigue, impaired growth	No-biopsy protocol has been adopted*	No-biopsy protocol has been adopted*	X
CD patient 8	F	7.7	No symptoms (screening)	Negative	MO grading 3b-3c	
CD patient 9	F	7.5	Recurrent abdominal pain	Negative	MO grading 3b	
CD patient 10	F	3.7	Lack of appetite, fatigue, impaired growth	Mild edema of the duodenal bulb	MO grading 3b-3c	
CD patient 11	F	13	Constipation, lack of appetite, recurrent abdominal pain, fatigue, anemia, tooth enamel defects	No-biopsy protocol has been adopted*	No-biopsy protocol has been adopted*	X
CD patient 12	F	6.5	No symptoms (screening)	Mild edema and hyperemia of the duodenal bulb, reduced height, and irregular surface of duodenal folds	MO grading 3b-3c	
CD patient 13	M	10.7	Dental enamel hypoplasia	Mild edema and hyperemia of the duodenal bulb, irregular surface of duodenal folds	MO grading 3c	
CD patient 14	F	6.6	Constipation, recurrent abdominal pain	Mild edema and hyperemia of duodenum, reduced height, and irregular surface of duodenal folds	MO grading 3c	

*According to the 2012 ESPGHAN guidelines: symptoms suggestive of CD, serum anti-tTG IgA levels ≥ 10 times the ULN, positive EMA-IgA, positive CD HLA risk alleles DQ2 and/or DQ8.

Table 2: This table illustrates, for each enrolled control, the clinical indication for endoscopy, endoscopic and histological findings, and related diagnosis.

Controls	Gender (M/F)	Age (years)	Clinical indication for endoscopy	Endoscopic findings	Histological findings	Diagnosis
Control 1	F	13.7	Vomiting blood	Antral nodularity	H. pylori-negative chronic superficial gastritis	H. pylori-negative chronic superficial gastritis
Control 2	F	15.9	Vomiting	Negative	Negative	Functional disorder
Control 3	F	13.6	Epigastric pain	Cardias incontinence	H. pylori-negative chronic superficial gastritis, mild reflux esophagitis	H. pylori-negative chronic superficial gastritis, mild reflux esophagitis
Control 4	F	14.9	Gastroesophageal reflux symptoms, abdominal pain, vomiting	Longitudinal striae in the distal esophagus	Mild reflux esophagitis	Mild reflux esophagitis
Control 5	F	12.7	Recurrent abdominal pain	Negative	Mild reflux esophagitis	Mild reflux esophagitis
Control 6	F	15.7	Epigastric pain, gastroesophageal reflux symptoms	Negative	Mild reflux esophagitis	Mild reflux esophagitis
Control 7	M	8.6	Chest pain, gastroesophageal reflux symptoms	White specks in the distal esophageal mucosa	H. pylori-negative chronic superficial gastritis	H. pylori-negative chronic superficial gastritis, esophagitis
Control 8	F	15.8	Epigastric pain	Negative	H. pylori-negative chronic superficial gastritis	H. pylori-negative chronic superficial gastritis
Control 9	M	11.4	Epigastric pain, vomiting	Mild hyperemia third distal esophagus, cardias incontinence	Mild reflux esophagitis	Mild reflux esophagitis
Control 10	M	14.7	Poor growth, lack of appetite	Negative	Negative	Poor growth
Control 11	F	2.7	Epigastric pain vomiting	Duodenal bulb nodularity	Mild reflux esophagitis	Mild reflux esophagitis
Control 12	M	8.1	Vomiting	Inlet patch	Inlet patch, mild reflux esophagitis	Inlet patch, mild reflux esophagitis
Control 13	M	8.4	Recurrent vomiting	Longitudinal striae in the distal esophagus	Mild reflux esophagitis	Mild reflux esophagitis

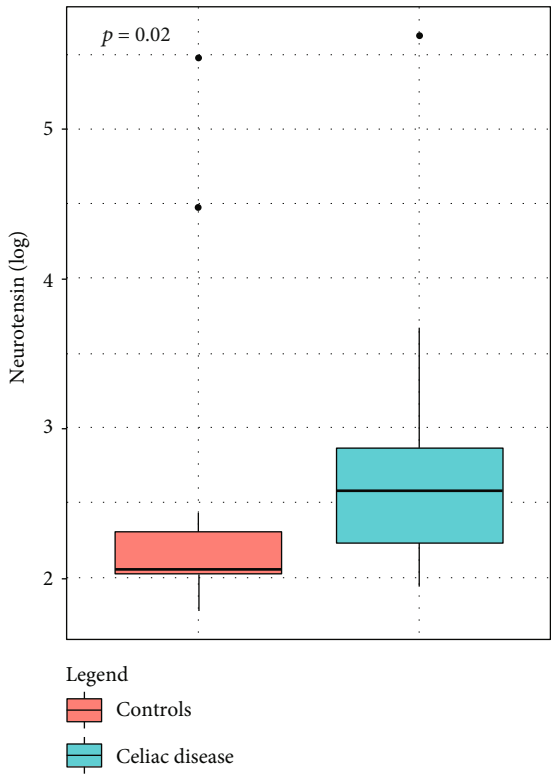


Figure 1: Fasting serum levels of neurotensin in CD patients and controls. The horizontal line indicates the medians (log-transformed scale) in a box and whisker plot ($p = 0.02$).

[15, 16, 24]. Regarding a potential role of NT, Bardella et al. [17] have found not only that untreated CD patients had significantly higher fasting plasma NT levels than controls but also that the baseline NT levels in both groups correlated significantly with the gastric emptying time, thus suggesting that NT may physiologically inhibit upper gastrointestinal motility.

Nevertheless, no association between NT levels and intestinal and/or extraintestinal symptoms was observed in our cohort, although a relationship with specifically dyspepsia-like symptoms was not investigated. It should be highlighted that NT is one of several hormones that modulate gastric emptying and intestinal motility.

With regard to CD symptoms, the frequent coexistence of irritable bowel syndrome (IBS) and CD [25] is also food for thought.

Some indirect data suggest the potential involvement of NT in the pathophysiology of IBS. Many factors related to the exacerbation of symptoms in IBS, such as psychiatric disorders, certain foods, and intestinal infections, influence the central or peripheral secretion of NT [26]. Moreover, mechanisms involved in this syndrome, like gut dysmotility, altered brain–gut axis, stress-related enteric responses, visceral hypersensitivity, microbial flora overgrowth, low-grade intestinal inflammation, and mast cell hyperreactivity, could be partially explained by dysfunction in NT pathway [26]. Furthermore, NT influences the molecular circuits of other peptides with a proven role in IBS [26].

It could be speculated that the frequent IBS-like syndrome observed in CD patients could be partially explained by a raised NT level, but further research to sustain this assumption is needed. However, this aspect was not investigated in our work, requiring obviously a larger-scale study.

It is notable that in our cohort, age was a confounder for CD, with increased frequency of this condition among young children: mean age of our CD patients was 6.6 years, whereas that of controls was 12.1 years. However, no correlation was observed between NT and age among patients enrolled, differently from the study of Montén et al. [18]. These authors have found an impact of age on pro-NT levels among controls with a tendency to lower levels among older children. Nevertheless, in their cohort, no correlation was noticed between age and pro-NT among children with CD, so the impact of age on pro-NT levels seemed irrelevant compared with the impact of age on CD diagnosis.

A limitation of our study is the small number of patients enrolled: this makes the chances of finding statistically significant associations very low. Moreover, the relatively large number of patients who have received biopsy-sparing CD diagnosis limits further the ability to correlate NT with the severity of histological damage.

Another limitation of our work could be that the disease controls were children investigated for various reasons. Access to reference levels for fasting NT levels in both young and older healthy children would be required, but it is not feasible from an ethical point of view.

The strength of this research was the enrollment of control subjects with clear absence of intestinal inflammation as shown by small bowel biopsy. Patients with history of intestinal infection/inflammation, with histopathologic signs of nonspecific inflammation, or features attributable to other diseases in duodenal biopsies were rigorously excluded from our study. The only histological abnormalities considered acceptable were mild superficial chronic gastritis or mild reflux esophagitis, outcomes that were also found in some CD patients (2/9). Moreover, in order to avoid any confounding factors, all subjects enrolled in this study were previously selected according to medical history to exclude any allergic, inflammatory, neoplastic, or neurologic conditions, in light of the possible involvement of NT in these diseases [27, 28].

Nevertheless, it is of note that for controls, no bulbar biopsy was obtained, differently from CD patients. Albeit this could theoretically be a bias, it is improbable that patients without serology and duodenal histology for CD host an inflammatory lesion in the duodenal bulb. However, a sufficient number of duodenal biopsies were taken altogether for controls (at least three in the second and third portions of the duodenum as per hospital endoscopy protocol). Thus, we are quite confident that, even though a small bias might be introduced to this regard, it is very unlikely that it undermines results and conclusions. Furthermore, there was no clinical indication for bulbar biopsy in control patients at the time of the procedure.

For discussion purposes, it is important to consider that NT and its receptors can be localized in both the central nervous system and along the length of the gastrointestinal tract.

As NT can directly activate immune, inflammatory, epithelial, and neuronal cells, the mechanisms by which this peptide triggers many diverse intestinal responses may be difficult to dissect. It is conceivable that conflicting findings regarding its involvement in intestinal conditions such as CD are to be partly attributed to this complex network [4]. The mechanisms governing the altered NT levels in CD patients are still unclear, although it has been suggested that this finding may be related to a compensatory increase in NT secretion from specific endocrine cells of the unaffected ileal mucosa [17]. Chronic small intestine mucosal inflammation leading to endocrine cell dysfunction and motor abnormalities in CD patients may also be implicated [21]. Thus, in order to clarify whether the increase in NT levels may represent an additional diagnostic marker for CD, further research is needed, especially with regard to underlying mechanisms that are poorly defined.

In addition, normal levels of NT have been reported by Besterman et al. [15] in CD patients on a gluten-free diet, and this data could suggest a potential role of NT also as a disease follow-up marker. Anyway, this evaluation was beyond the aim of our study, and for this purpose, a different study design would be appropriate.

An additional aspect to mention is that in recent years, pro-NT, the stable NT precursor fragment in human blood, has gained attention and its role has been investigated in different conditions [29]. A clinical study showed that plasma pro-NT is released in equimolar amounts similar to NT in circulation under physiological conditions [19] and it was found possibly converted into an active NT. However, there is no further evidence to support this point. In light of this consideration, although Montén et al. in a recent pediatric study [18] have suggested a potential role of pro-NT in CD, we aimed to evaluate a potential involvement of NT, echoing previous studies [14–17]. It is unquestionable that it would be interesting to evaluate both NT and pro-NT at the same time, albeit it was not feasible in our study.

5. Conclusions

To our knowledge, this is the first Italian study that evaluates NT in pediatric CD patients. These results have shown that NT is higher in the serum of CD children at diagnosis compared to controls. However, larger-scale studies are required to validate these findings and to allow further speculations on this issue. So far, whether serum NT levels can be an adjunctive marker for pediatric CD remains currently elusive.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

This study has been presented as conference abstract during the XXV Italian Society of Pediatric Gastroenterology, Hepatology and Nutrition (SIGENP) National Congress in October 2018 (Salerno, Italy) and during the 52nd Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in June 2019 (Glasgow, Scotland).

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

We thank all the patients and their family for the cooperation. This work was supported by "Sapienza" University of Rome starting grant for young investigators.

References

[1] R. Carraway and S. E. Leeman, "The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami," *Journal of Biological Chemistry*, vol. 248, no. 19, pp. 6854–6861, 1973.

[2] S. Qiu, G. Pellino, F. Fiorentino et al., "A review of the role of neurotensin and its receptors in colorectal cancer," *Gastroenterology Research and Practice*, vol. 2017, Article ID 6456257, 8 pages, 2017.

[3] C. F. Ferris, R. E. Carraway, R. A. Hammer, and S. E. Leeman, "Release and degradation of neurotensin during perfusion of rat small intestine with lipid," *Regulatory Peptides*, vol. 12, no. 2, pp. 101–111, 1985.

[4] D. Zhao and C. Pothoulakis, "Effects of NT on gastrointestinal motility and secretion, and role in intestinal inflammation," *Peptides*, vol. 27, no. 10, pp. 2434–2444, 2006.

[5] W. C. Mustain, P. G. Rychahou, and B. M. Evers, "The role of neurotensin in physiologic and pathologic processes," *Current Opinion in Endocrinology, Diabetes, and Obesity*, vol. 18, no. 1, pp. 75–82, 2011.

[6] N. Saiyasit, J. Sripetchwandee, N. Chattipakorn, and S. C. Chattipakorn, "Potential roles of neurotensin on cognition in conditions of obese-insulin resistance," *Neuropeptides*, vol. 72, pp. 12–22, 2018.

[7] F. Valitutti, C. M. Trovato, M. Montuori, and S. Cucchiara, "Pediatric celiac disease: follow-up in the spotlight," *Advances in Nutrition*, vol. 8, no. 2, pp. 356–361, 2017.

[8] G. Caio, R. De Giorgio, and U. Volta, "Coeliac disease and dermatitis herpetiformis," *The Lancet*, vol. 392, no. 10151, pp. 916-917, 2018.

[9] S. Husby, S. Koletzko, I. R. Korponay-Szabó et al., "European society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 54, no. 1, pp. 136–160, 2012.

[10] M. M. Leonard, A. Sapone, C. Catassi, and A. Fasano, "Celiac disease and nonceliac gluten sensitivity: a review," *Journal of the American Medical Association*, vol. 318, no. 7, pp. 647–656, 2017.

[11] C. M. Trovato, M. Montuori, C. Anania et al., "Are ESPGHAN "biopsy-sparing" guidelines for celiac disease also suitable for asymptomatic patients?," *The American Journal of Gastroenterology*, vol. 110, no. 10, pp. 1485–1489, 2015.

[12] S. Husby, S. Koletzko, I. Korponay-Szabó et al., "European Society Paediatric Gastroenterology, Hepatology and

Nutrition guidelines for diagnosing coeliac disease 2020," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 70, no. 1, pp. 141–156, 2020.

[13] F. Valitutti, G. Di Nardo, M. Barbato et al., "Mapping histologic patchiness of celiac disease by push enteroscopy," *Gastrointestinal Endoscopy*, vol. 79, no. 1, pp. 95–100, 2014.

[14] S. R. Bloom, H. S. Besterman, D. I. Sarson et al., "Hormone profile of coeliac disease," *Gastroenterology*, vol. 74, no. 5, p. 1011, 1978.

[15] H. S. Besterman, S. R. Bloom, D. L. Sarson et al., "Gut-hormone profile in coeliac disease," *The Lancet*, vol. 1, no. 8068, pp. 785–788, 1978.

[16] A. Hernanz, I. Polanco, R. Codoceo, R. Lama, and C. Vazquez, "Gastrointestinal peptide profile in children with celiac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 6, no. 3, pp. 341–345, 1987.

[17] M. T. Bardella, M. Fraquelli, M. Peracchi, B. M. Cesana, P. A. Bianchi, and D. Conte, "Gastric emptying and plasma neurotensin levels in untreated celiac patients," *Scandinavian Journal of Gastroenterology*, vol. 35, no. 3, pp. 269–273, 2000.

[18] C. Montén, Å. Torinsson Naluai, and D. Agardh, "Role of proneurotensin as marker of paediatric coeliac disease," *Clinical and Experimental Immunology*, vol. 186, no. 3, pp. 387–392, 2016.

[19] A. Ernst, S. Hellmich, and A. Bergmann, "Proneurotensin 1-117, a stable neurotensin precursor fragment identified in human circulation," *Peptides*, vol. 27, no. 7, pp. 1787–1793, 2006.

[20] S. Cucchiara, G. Bassotti, G. Castellucci et al., "Upper gastrointestinal motor abnormalities in children with active celiac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 21, no. 4, pp. 435–442, 1995.

[21] G. Bassotti, G. Castellucci, C. Betti et al., "Abnormal gastrointestinal motility in patients with celiac sprue," *Digestive Diseases and Sciences*, vol. 39, no. 9, pp. 1947–1954, 1994.

[22] G. Chiaroni, G. Bassotti, U. Germani, and I. Vantini, "Gluten-free diet normalizes mouth-to-cecum transit of a caloric meal in adult patients with celiac disease," *Digestive Diseases and Sciences*, vol. 42, pp. 2100–2105, 1997.

[23] P. Usai, P. Usai Satta, M. Lai et al., "Autonomic dysfunction and upper digestive functional disorders in untreated adult coeliac disease," *European Journal of Clinical Investigation*, vol. 27, no. 12, pp. 1009–1015, 1997.

[24] M. Fraquelli, M. T. Bardella, M. Peracchi, B. M. Cesana, P. A. Bianchi, and D. Conte, "Gallbladder emptying and somatostatin and cholecystokinin plasma levels in celiac disease," *The American Journal of Gastroenterology*, vol. 94, no. 7, pp. 1866–1870, 1999.

[25] U. Volta, G. Caio, V. Stanghellini, and R. de Giorgio, "The changing clinical profile of celiac disease: a 15-year experience (1998-2012) in an Italian referral center," *BMC Gastroenterology*, vol. 14, no. 1, p. 194, 2014.

[26] K. Kalafatakis and K. Triantafyllou, "Contribution of neurotensin in the immune and neuroendocrine modulation of normal and abnormal enteric function," *Regulatory Peptides*, vol. 170, no. 1-3, pp. 7–17, 2011.

[27] M. Vasiadi, A. P. Mondolfi, K. D. Alysandratos et al., "Neurotensin serum levels and skin gene expression are increased in atopic dermatitis," *The British Journal of Dermatology*, vol. 169, no. 3, pp. 695–699, 2013.

[28] A. Angelidou, K. Francis, M. Vasiadi et al., "Neurotensin is increased in serum of young children with autistic disorder," *Journal of Neuroinflammation*, vol. 7, no. 1, p. 48, 2010.

[29] O. Melander, A. S. Maisel, P. Almgren et al., "Plasma proneurotensin and incidence of diabetes, cardiovascular disease, breast cancer, and mortality," *Journal of the American Medical Association*, vol. 308, no. 14, pp. 1469–1475, 2012.

Hindawi
Gastroenterology Research and Practice
Volume 2019, Article ID 9370397, 6 pages
https://doi.org/10.1155/2019/9370397

Research Article

The Use of Biopsy and "No-Biopsy" Approach for Diagnosing Paediatric Coeliac Disease in the Central European Region

Petra Riznik,[1] Márta Balogh,[2] Piroska Bódi,[3] Luigina De Leo,[4] Jasmina Dolinsek,[5] Ildikó Guthy,[6] Judit Gyimesi,[7] Ágnes Horváth,[8] Ildikó Kis,[9] Martina Klemenak,[1] Berthold Koletzko,[10,11] Sibylle Koletzko,[11,12] Ilma Rita Korponay-Szabó,[7,13] Tomaz Krencnik,[1] Tarcisio Not,[4] Goran Palcevski,[14] Éva Pollák,[15] Daniele Sblattero,[16] István Tokodi,[17] Matej Vogrincic,[18] Katharina Julia Werkstetter,[11] and Jernej Dolinsek[1,19]

[1]*University Medical Centre Maribor, Department of Paediatrics, Gastroenterology, Hepatology and Nutrition Unit, Maribor, Slovenia*
[2]*Markusovszky Teaching Hospital, Szombathely, Hungary*
[3]*Pándy Kálmán Hospital, Gyula, Hungary*
[4]*IRCCS Burlo Garofolo Trieste, Institute for Maternal and Child Health, Trieste, Italy*
[5]*Municipality of Maribor, Project Office, Maribor, Slovenia*
[6]*Jósa András County Hospital, Nyíregyháza, Hungary*
[7]*Heim Pál National Paediatric Institute, Coeliac Disease Centre, Budapest, Hungary*
[8]*Csolnoky Ferenc County Hospital, Veszprém, Hungary*
[9]*St. Barbara County Hospital, Tatabánya, Hungary*
[10]*Stiftung Kindergesundheit (Child Health Foundation) at Dr. von Hauner Children's Hospital, LMU Munich, Munich, Germany*
[11]*Dr. von Hauner Children's Hospital, Clinical Medical Centre, LMU Munich, Munich, Germany*
[12]*Department of Pediatrics, Gastroenterology and Nutrition, School of Medicine Collegium Medicum University of Warmia and Mazury, Olsztyn, Poland*
[13]*University of Debrecen, Faculty of Medicine, Department of Paediatrics, Debrecen, Hungary*
[14]*University Hospital Rijeka, Department for Gastroenterology, Paediatric Clinic, Rijeka, Croatia*
[15]*Ajka County Hospital, Ajka, Hungary*
[16]*University of Trieste, Trieste, Italy*
[17]*St. George Fejér County University Teaching Hospital, Székesfehérvár, Hungary*
[18]*University Medical Centre Maribor, Department of Informatics, Maribor, Slovenia*
[19]*Medical Faculty, Department of Paediatrics, University of Maribor, Maribor, Slovenia*

Correspondence should be addressed to Jernej Dolinsek; jernej_dolinsek@hotmail.com

Received 16 June 2019; Revised 30 August 2019; Accepted 23 September 2019; Published 15 November 2019

Guest Editor: Anna Rybak

Objectives. The current European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) guidelines introduced the option to diagnose coeliac disease (CD) in children and adolescents without upper endoscopy if the defined criteria are met. The aim of our study was to evaluate how frequently paediatric gastroenterologists in Central Europe used the "no-biopsy" approach and how often the duodenal biopsy could have been omitted. *Methods*. Medical records of patients aged < 19 years diagnosed with CD in 2016 from five European countries were analysed, focusing on levels of transglutaminase antibodies (TGA) at the time of diagnosis and on whether the diagnosis was confirmed using duodenal biopsy or "no-biopsy" approach. Clinical presentation and delays until final diagnosis were analysed according to diagnostic approach. *Results*. Data from 653 children (63.9% female, median age: 7 years, range: 7 months-18.5 years) from Croatia, Hungary, Germany, Italy, and Slovenia were analysed. One fifth ($n = 134$) of included children were asymptomatic at diagnosis. Of 519 symptomatic children,

107 (20.6%) were diagnosed by the "no-biopsy" approach. Out of the remaining 412 children who underwent duodenal biopsies, 214 (51.9%) had TGA ≥ 10 times upper level of normal (ULN) and would have been eligible for the "no-biopsy" approach. Signs and symptoms of malabsorption were more frequent in children diagnosed without duodenal biopsies. There were no differences in diagnostic delays with respect to the diagnostic approach. *Conclusion*. In this cohort, about 60% of symptomatic CD patients could have been diagnosed without duodenal biopsies. The aim of the "no-biopsy" approach was to make the diagnostic procedure less challenging without compromising its reliability. However, this option was applied only in 20%, in spite of fewer burdens to the family and reduced costs. The reasons for this discrepancy are unknown. Physicians should be made more aware about the reliability of CD diagnosis without biopsies when the ESPGHAN guidelines for CD diagnosis are followed.

1. Introduction

Coeliac disease (CD) is a lifelong systemic autoimmune disorder, elicited by gluten and related prolamins in genetically susceptible individuals. Traditionally defined as gluten-related enteropathy, it is one of the most common chronic illnesses with very diverse clinical presentation, involving intestinal and extraintestinal manifestations [1]. Histological findings of villous atrophy and crypt hyperplasia with increased levels of intraepithelial T lymphocytes from duodenal biopsies, classified according to the Marsh-Oberhuber, have been regarded as the gold standard for diagnosing CD [2–4].

The first diagnostic criteria for CD were the Interlaken criteria, formalised in 1969 by the experts in the newly born European Society for Paediatric Gastroenterology, today known as ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology, and Nutrition). Three duodenal biopsies (initial on gluten, after treatment with a gluten-free diet, and after gluten challenge) were required for the confirmation of the diagnosis, and these criteria served worldwide as the accepted diagnostic standard for over 20 years [5]. In the revised ESPGHAN criteria, published in 1990, the need for gluten challenge for children over the age of 2 years was removed and serological tests were added to the diagnostic procedure [6, 7]. One duodenal biopsy was required for the confirmation of the diagnosis and with clinical and serological improvement after introduction of gluten-free diet; no further biopsies were needed [6].

In the current ESPGHAN guidelines, published in 2012, the initial diagnostic step is the determination of CD-specific IgA autoantibodies against type-2 (tissue) transglutaminase (TGA) together with total IgA in serum [1]. In case of low or undetectable total IgA, an IgG-based test should be used. Positive autoantibodies imply a high probability of mucosal atrophy, and to confirm the diagnosis, an upper endoscopy with multiple duodenal biopsies should be performed [1]. However, these guidelines are the first allowing paediatric gastroenterologists to diagnose the disease without intestinal biopsy if all of the following criteria are fulfilled: the child shows symptoms and signs suggestive of CD, has high levels of TGA antibodies above 10 times upper level of normal (ULN), a positive confirmatory EMA test in a 2nd blood sample, specific HLA DQ2 or DQ8 genes, and consent of the patient and caregiver for this "no-biopsy" diagnostic approach [1]. A year later, the so-called "no-biopsy" approach, proposed by ESPGHAN, was adopted by the British Society for Paediatric Gastroenterology, Hepatology and Nutrition (BSPGHAN) [8]. The only difference between the two guidelines is that the joint BSPGHAN and Coeliac UK guidelines allow the substitution of 2nd EMA test with 2nd strongly positive TGA test, where EMA test is not locally available. However, the serum of the patient should be saved for later EMA testing [8]. On the other hand, the guidelines by the North American Society for Paediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) recommend the intestinal biopsy for the confirmation of the diagnosis of CD in all cases, regardless of the value of TGA [9, 10].

Although the so-called "no-biopsy" approach could have been used for the past 6 years, to our knowledge, there is not much data on how often the diagnosis was confirmed without duodenal biopsy.

The aim of our study was to evaluate how frequently the "no-biopsy" approach was used to diagnose children with CD in Central Europe (CE) and how often the duodenal biopsy could have been omitted.

2. Materials and Methods

The study, conducted in the scope of the Focus IN CD project (CE 111) and co-financed by the Interreg CE Programme, was carried out between the end of March and the middle of August 2017. Twelve partners from five CE countries (Croatia, Germany, Hungary, Italy, and Slovenia) participate in the project. Paediatric gastroenterologists from the participating regions were asked by the regional project partners to complete a web-based survey, providing anonymized medical records of children and adolescents below 19 years of age who were diagnosed with CD in 2016. In Croatia, Hungary, and Slovenia, the majority of CD patients diagnosed by paediatric gastroenterologists during this year were included. The questionnaire (https://www.interreg-central.eu/Content.Node/surveys.html) was translated into the languages of all project partners and focused on clinical presentation, diagnostic methods used, and management of CD. We analysed medical records of all included CD patients, focusing on levels of TGA at the time of diagnosis and on whether the diagnosis was confirmed using duodenal biopsy showing Marsh 2-3 lesion or "no-biopsy" approach. We also compared diagnostic approach with clinical presentation of the disease (with or without signs and symptoms of malabsorption) and the diagnostic delays, calculated as the duration from the first symptoms to the confirmation of the diagnosis. Statistical analysis was performed using IBM SPSS Statistics 22.0 for Windows. One-way ANOVA, chi-square

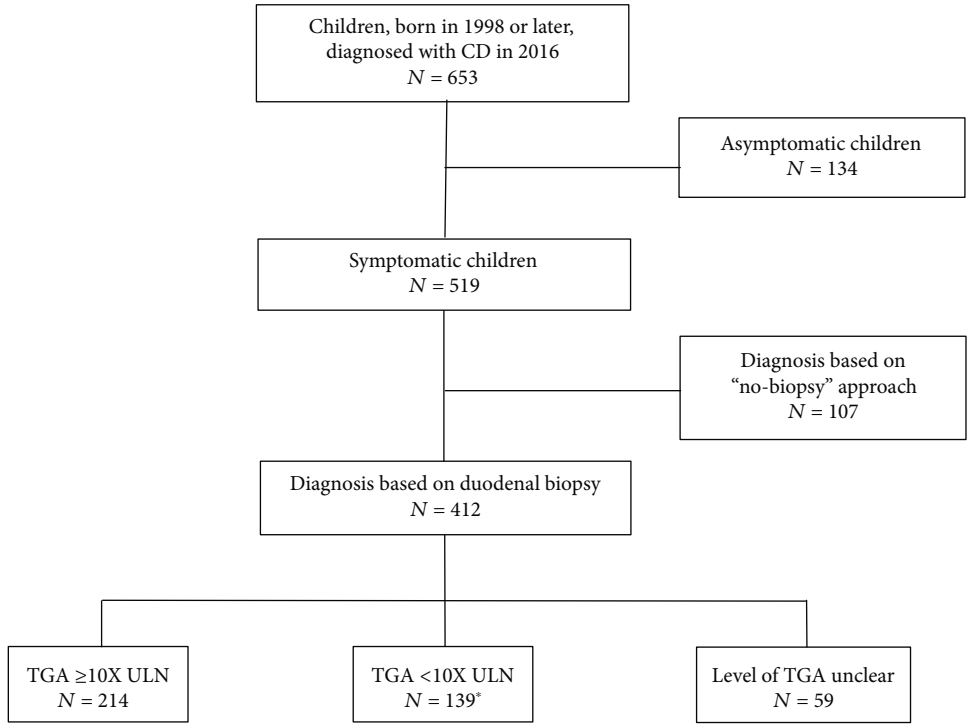


Figure 1: Diagnostic approach in children with CD in CE. *15 patients had IgA deficiency.

test, and Kruskal-Wallis H test with post hoc test were used for the analysis.

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (0120-383).

3. Results

Data from 653 children and adolescents from Croatia ($n = 66$), Germany ($n = 69$), Hungary ($n = 382$), Italy ($n = 82$), and Slovenia ($n = 54$) were available for the analysis. Median age of the children at the time of diagnosis was 7 years (range: 7 months-18.5 years), 63.9% were female. One fifth ($n = 134$) of included children were asymptomatic at the confirmation of the diagnosis (65.7% had TGA $\geq 10\times$ ULN). Analysis of the diagnostic procedure (Figure 1) showed that 20.6% ($n = 107$) of symptomatic children were diagnosed using "no-biopsy" approach. Out of 412 children who underwent duodenal biopsy, 51.9% ($n = 214$) had TGA $\geq 10\times$ ULN and could be considered as eligible for the "no-biopsy" approach (Table 1). Final diagnosis in this case should have been confirmed by positive genetic tests and positive EMA in the 2nd blood sample. However, since duodenal biopsy was chosen as confirmatory test, confirmatory serology and genetic tests were often not performed.

Of 519 symptomatic children, endoscopy with biopsies to confirm CD was performed in 412 (79.4%). Proportion of patients diagnosed using biopsy approach was highest in Croatia (93.1%) and was significantly higher compared to Slovenia (62.8%) ($p < 0.05$). No statistically significant differences between other countries were found.

Clinical presentation of patients diagnosed with or without biopsy was analysed separately (Table 2).

We also compared diagnostic delays between children diagnosed without biopsy and those who underwent duodenal biopsy. In order to be able to calculate diagnostic delays, we excluded symptomatic patients with unclear data about the time of the first symptoms or first visit to the paediatric gastroenterologist ($n = 126$). Data from 393 children were available for the analysis.

There were no differences between children diagnosed without duodenal biopsy and those diagnosed with biopsy who would have been eligible for the "no-biopsy" approach. Interval from the first visit at the paediatric gastroenterologist to the confirmation of the diagnosis was longer in the "no-biopsy" group compared to the group of children who underwent biopsy and were not eligible for the "no-biopsy" approach ($p < 0.05$), without differences in total delay (from symptoms to final diagnosis) (Table 3).

4. Discussion

The ESPGHAN guidelines for the diagnosis of CD for the last 6 years allow the possibility of using "no-biopsy" approach in children and adolescents if certain criteria are fulfilled. This diagnostic approach had been shown to be safe with a positive predictive value for enteropathy of >99% in a large prospective international study including 707 paediatric patients [11]. Our data provided by paediatric gastroenterologist for patients diagnosed with CD in 2016 shows that only about 20% of children in Central Europe were diagnosed without duodenal biopsy, although about 60% would have been eligible based on the level of TGA being higher than 10 times ULN. The highest proportion of children diagnosed with the "no-biopsy" approach was reported from Slovenia, where

Table 1: Data on serological testing in symptomatic patients diagnosed with CD who underwent duodenal biopsies in Central Europe.

	Croatia ($N = 58$)	Germany ($N = 53$)	Hungary ($N = 302$)	Italy ($N = 61$)	Slovenia ($N = 45$)	Central Europe ($N = 519$)
"No-biopsy" approach, n (% of all patients)	4 (6.9%)	12 (22.6%)	64 (21.2%)	10 (16.4%)	17 (37.8%)	**107 (20.6%)**
Duodenal biopsy, n (% of all patients)	54^{Π} (93.1%)	41 (77.4%)	238 (78.8%)	51 (83.6%)	28^{Π} (62.2%)	**412 (79.4%)**
TGA ≥ 10 times ULN						
Yes (% within a group)	16 (29.6%)	21 (51.2%)	139 (58.4%)	27 (52.9%)	11 (39.3%)	**214 (51.9%)**
No (% within a group)	23 (42.6%)	8 (19.5%)	72 (30.3%)	21 (41.2%)	15 (53.6%)	**139 (33.7%)**
Unclear (% within a group)	15 (27.8%)	12 (29.3%)	27 (11.3%)	3 (5.9%)	2 (7.1%)	**59 (14.3%)**

$^{\Pi}p < 0.05$.

Table 2: Clinical presentation (with or without symptoms and signs of malabsorption) and diagnostic approach of children with CD. In the group of patients who underwent duodenal biopsy, signs and symptoms of malabsorption were slightly more common in those who would have been eligible for the "no-biopsy" approach (67.8% vs 59.6%; NS). There were no significant differences in clinical presentation between children, diagnosed using "no-biopsy" approach and those who underwent duodenal biopsy but would have been eligible (by the TGA level ≥ 10 × ULN) for the "no-biopsy" approach (72.0% vs 67.8%; NS). However, signs and symptoms of malabsorption were significantly more common in patients who were diagnosed using "no-biopsy" approach in comparison to those that were not eligible for the "no-biopsy" approach (72.0% vs 59.6%; $p < 0.05$).

	"No-biopsy" approach	Duodenal biopsy	
		Eligible* for "no-biopsy"	Not eligible for "no-biopsy"
Malabsorptive (% within group)	$77^{\#}$ (72.0%)	145 (67.8%)	$118^{\#}$ (59.6%)
Non-malabsorptive (% within group)	30 (28.0%)	69 (32.2%)	80 (40.4%)
Number of patients	**107**	**214**	**198**

*Eligible by *TGA level* ≥ 10 × ULN. $^{\#}p < 0.05$ "no-biopsy" vs not eligible for the "no-biopsy" group.

Table 3: Diagnostic delays in children with CD with the respect to the diagnostic procedure.

	"No-biopsy" approach ($N = 78$)	Duodenal biopsy	
		Eligible** for "no-biopsy" approach ($N = 163$)	Not eligible for "no-biopsy" approach ($N = 152$)
Time from 1st symptom until 1st visit to PaedGI, median (Q1; Q3)	4.5 m (2 m; 9.5 m)	5 m (2 m; 11 m)	5 m (2 m; 12 m)
Time from 1st visit to PaedGI until diagnosis, median (Q1; Q3)	$1\ m^{\#}$ (0 m; 2 m)	1 m (0 m; 2 m)	$1\ m^{\#}$ (0 m; 3 m)
Time from symptoms to diagnosis (diagnostic delay), median (Q1; Q3)	6 m (3 m; 12 m)	6 m (3 m; 12 m)	7 m (4 m; 17 m)

*PaedGI: paediatric gastroenterologist; m: month. **Eligible by TGA level ≥ 10 × ULN. $^{\#}p < 0.05$ not eligible for "no-biopsy" vs "no-biopsy".

CD is diagnosed in only few centres and where several awareness-rising campaigns have been carried out during the last few years. However, further half of the CD children diagnosed with duodenal biopsy would also have been eligible for the "no-biopsy" approach since their TGA levels were very high (≥10× ULN). In the majority of these patients, genetic tests and confirmatory EMA were not performed, since duodenal biopsy was chosen as a confirmatory test. No information on how many of them had perhaps been additionally tested for EMA in a second sample was available, since this was not specifically asked for in patients who underwent duodenal biopsy. Altogether, at least 60% of children diagnosed in Central Europe could have been considered to be diagnosed without duodenal biopsy. In a substantial number of patients (14%), we were not able to define the eligibility for the "no-biopsy" approach because of the incomplete data on either TGA levels or the cut-off values of the used tests. This might be one of the reasons for uneven proportion of very high levels of TGA among countries, especially in Croatia, were the percentage of TGA ≥ 10 times ULN was the lowest.

The majority of all patients presented with at least one sign or symptom of malabsorption, with a significantly higher proportion in patients, who were diagnosed using "no-biopsy" approach compared to those who were not eligible (TGA levels < 10 times ULN) for the "no-biopsy" approach.

Our data show that duodenal biopsy is still performed in majority of children with CD, regardless of the possibility of the "no-biopsy" approach. The reasons for this might be a

higher trust in biopsy results compared to serology, possibly because the physicians want to avoid misdiagnosis of this lifelong disease where compliance with the diet is extremely important. Another possible reason for choosing biopsy pathway in children can be the existing belief that genetic tests and serological tests are expensive compared to duodenal biopsy where biopsy is made with existing equipment and existing personnel. Also, there could be a perception that using the "no-biopsy" approach would be more time consuming, since after an endoscopy, the child can be put on a diet immediately without a fear of influencing further tests that need to be done if a "no-biopsy" approach is chosen. It is also important to note that in many centres, endoscopy is readily available, and serological tests are performed outside the institution. This creates an impression that endoscopy is more accessible and is associated with lower risk of long diagnostic delays and false diagnosis. However, based on our study, no difference in diagnostic delays was found with the respect to different diagnostic procedure used.

Our results are similar to the study of Bishop et al. [12], where more than half of the included patients fulfilled ESPGHAN criteria for the "no-biopsy" approach but the guidelines were not adopted since different laboratory testing platforms that were used have not been sufficiently validated to completely trust the serological results [12]. Also, NASPGHAN guidelines for diagnosing CD do not include the possibility of "no-biopsy" approach since there is no standardisation of serological tests in the USA [10]. On the other hand, European studies clearly confirmed the reliability of serological tests in the "no-biopsy" approach [11, 13]. In the study of Werkstetter et al. [11], the current ESPGHAN guidelines regarding the "no-biopsy" approach were prospectively evaluated and it was confirmed that children could be safely diagnosed without biopsy, based on the reliable serological kits [11]. Moreover, recent prospective validation studies show that for the "no-biopsy" approach, HLA analysis is probably not necessary [11, 13].

Another possible reason for choosing duodenal biopsy in CD diagnosis is the potential risk of missing other diseases, which would have been detected if upper endoscopy was performed [14]; however, this has not been demonstrated in studies on children and adolescents with suspected CD [11, 15].

One of the concerns related to the "no-biopsy" approach might also be lack of implementation of existing ESPGHAN guidelines for this approach in general practice [14]. The awareness about CD and about existing guidelines is low among healthcare professionals [16–19] and this can rise a suspicion that proposed diagnostic standards brought by the guidelines are not fully met, leading to uncertain diagnosis, with either over- or underdiagnosis of CD [14].

When choosing to perform a duodenal biopsy, several pitfalls in the interpretation of duodenal biopsies regardless of them being a gold standard in diagnosing CD must be considered. Histological analysis has been reported to lack diagnostic accuracy owing to the high interobserver variability, differences between routine and more specialised pathology laboratories, low rates of correct orientation of biopsy samples, and low number of samples taken. These factors can lead to inadequate interpretation of mucosal changes [14, 20]. Adequate sampling by the endoscopist aware of the potential for patchy nature of the enteropathy includes at least four biopsies from the duodenum distal to the papilla of Vateri and at least one from the duodenal bulb during a gluten-containing diet [1, 4, 14, 20].

The major advantage of a "no-biopsy" approach in children and adolescents is the avoidance of upper endoscopy, which, in many centres, requires general anaesthesia or deep sedation, leading to higher costs in comparison to serological diagnosis [14]. Risk of multiple duodenal biopsies and risks of general anaesthesia or deep sedation and endoscopy itself must also be considered. Patients and parents/caregivers must be informed if they are eligible for the "no-biopsy" approach. They should be aware of the potential benefits and disadvantages of two different diagnostic pathways before they decide to undergo duodenal biopsy.

One of the limitations of our study is the retrospective nature of assessment of existing healthcare records and the uneven number of included patients between participating countries, with more patients in Hungary than in other countries. One possible limitation is also the use of different serological tests to determine the levels of TGA among countries, which might partly explain the different proportion of TGA ≥ 10 times ULN among countries [11]. Since we have not anticipated that "no-biopsy" approach is used so rarely, we had not included any question on the possible reasons for performing the biopsy in cases where it might not be needed. Therefore, our results can serve as a basis for further studies of this issue.

5. Conclusion

It has been shown that high levels of TGA accurately predict advanced histological changes of the type Marsh 2-3 in the duodenum, and no concern in misdiagnosing CD is justified. In our study, 60% of patients were eligible for the "no-biopsy" approach. Nevertheless, it is important that the diagnosis is confirmed in specialised gastroenterology services with standardised serological tests and not in the general practice.

The aim of the "no-biopsy" approach proposed by current ESPGHAN guidelines is to make the diagnostic procedure less challenging without compromising its reliability. It is therefore important to raise the awareness about CD and possible diagnostic approaches among physicians in order to increase compliance to the guidelines with respect to the "no-biopsy" approach.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors have no conflicts of interest to disclose.

Acknowledgments

The authors wish to thank all the participating physicians from Croatia, Germany, Hungary, Italy, and Slovenia, who uploaded their patients' medical records, thus enabling us to do the study. We are also grateful to all the project partners, who helped in designing the questionnaire and its translation to the partner languages as well as in distributing the questionnaire to the physicians from participating regions. The study was supported by the Interreg Central Europe (CE 111) Focus IN CD project. The collection of Hungarian data was also supported by the NKFI120392 and EFOP-3.6.1-16-2016-00022 grants and the collection of German data by Medical Faculty, Ludwig Maximilian University Munich, and Bavarian State Ministry for Education, Culture, Science and Arts. The authors acknowledge also the financial support from the Slovenian Research Agency (No. P3-0036).

References

[1] S. Husby, S. Koletzko, I. R. Korponay-Szabó et al., "European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the diagnosis of coeliac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 54, no. 1, pp. 136–160, 2012.

[2] B. C. Dickson, C. J. Streutker, and R. Chetty, "Coeliac disease: an update for pathologists," *Journal of Clinical Pathology*, vol. 59, pp. 1008–1016, 2006.

[3] N. M. Marsh, W. M. Johnson, and K. Rostami, "Mucosal histopathology in celiac disease: a rebuttal of Oberhuber's sub-division of Marsh III," *Gastroenterology and Hepatology from Bed to Bench*, vol. 8, no. 2, pp. 99–109, 2015.

[4] S. Husby and J. Murray, "Diagnosing coeliac disease and the potential for serological markers," *Nature Reviews Gastroenterology & Hepatology*, vol. 11, no. 11, pp. 655–663, 2014.

[5] A. McNeish, H. Harms, J. Rey, D. Shmerling, J. Visakorpi, and J. Walker-Smith, "The diagnosis of coeliac disease. A commentary on the current practices of members of the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN)," *Archives of Disease in Childhood*, vol. 54, no. 10, pp. 783–786, 1979.

[6] J. Walker-Smith, S. Guandalini, J. Schmitz, D. Shmerling, and J. Visakorpi, "Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition," *Archives of Disease in Childhood*, vol. 65, no. 8, pp. 909–911, 1990.

[7] S. Guandalini, A. Ventura, N. Ansaldi et al., "Diagnosis of coeliac disease: time for a change?," *Archives of Disease in Childhood*, vol. 64, no. 9, pp. 1320–1325, 1989.

[8] S. Murch, H. Jenkins, M. Auth et al., "Joint BSPGHAN and Coeliac UK guidelines for the diagnosis and management of coeliac disease in children," *Archives of Disease in Childhood*, vol. 98, no. 10, pp. 806–811, 2013.

[9] I. D. Hill, M. H. Dirks, G. S. Liptak et al., "Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 40, no. 1, pp. 1–19, 2005.

[10] I. D. Hill, A. Fasano, S. Guandalini et al., "NASPGHAN Clinical Report on the Diagnosis and Treatment of Gluten-related Disorders," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 63, no. 1, pp. 156–165, 2016.

[11] K. J. Werkstetter, I. R. Korponay-Szabó, A. Popp et al., "Accuracy in diagnosis of celiac disease without biopsies in clinical practice," *Gastroenterology*, vol. 153, no. 4, pp. 924–935, 2017.

[12] J. Bishop, P. Reed, P. Austin et al., "Prospective evaluation of the ESPGHAN guidelines for diagnosis of celiac disease in New Zealand children," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 67, no. 6, pp. 749–754, 2018.

[13] J. Wolf, D. Petroff, T. Richter et al., "Validation of antibody-based strategies for diagnosis of pediatric celiac disease without biopsy," *Gastroenterology*, vol. 153, no. 2, pp. 410–419, 2017.

[14] N. Reilly, S. Husby, D. Sanders, and P. Green, "Coeliac disease: to biopsy or not?," *Nature Reviews Gastroenterology & Hepatology*, vol. 15, no. 1, pp. 60–66, 2018.

[15] A. Lucendo, Á. Arias, and J. Tenias, "Systematic review: the association between eosinophilic oesophagitis and coeliac disease," *Alimentary Pharmacology and Therapeutics*, vol. 40, no. 5, pp. 422–434, 2014.

[16] A. Assiri, A. Saeed, E. Saeed et al., "Assessment of knowledge of celiac disease among health care professionals," *Saudi Medical Journal*, vol. 36, no. 6, pp. 751–753, 2015.

[17] S. Shergill and G. K. Makharia, "Awareness about celiac disease amongst physicians," *Indian Journal of Gastroenterology*, vol. 36, no. 4, pp. 327–329, 2017.

[18] R. D. Zipser, M. Farid, D. Baisch, B. Patel, and D. Patel, "Physician awareness of celiac disease," *Journal of General Internal Medicine*, vol. 20, no. 7, pp. 644-645, 2005.

[19] M. Jinga, A. Popp, D. Balaban, A. Dima, and C. Jurcut, "Physicians' attitude and perception regarding celiac disease: a questionnaire-based study," *The Turkish Journal of Gastroenterology*, vol. 29, pp. 419–426, 2018.

[20] J. Turner, "Diagnosis of celiac disease: taking a bite out of the controversy," *Digestive Diseases and Sciences*, vol. 63, no. 6, pp. 1384–1391, 2018.

Hindawi
Gastroenterology Research and Practice
Volume 2019, Article ID 8974751, 6 pages
https://doi.org/10.1155/2019/8974751

Review Article

The Challenge of Treatment in Potential Celiac Disease

Chiara Maria Trovato,[1] **Monica Montuori**,[1] **Francesco Valitutti**,[1,2] **Beatrice Leter**,[3] **Salvatore Cucchiara**,[1] **and Salvatore Oliva**[1]

[1]*Pediatric Gastroenterology and Liver Unit, Department of Pediatrics, Sapienza University of Rome, Rome, Italy*
[2]*European Biomedical Research Institute of Salerno, Salerno, Italy*
[3]*Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy*

Correspondence should be addressed to Chiara Maria Trovato; chiaramariatrovato@gmail.com

Received 28 May 2019; Accepted 19 August 2019; Published 20 October 2019

Academic Editor: Amosy M'Koma

Potential celiac disease (PCD) is defined by the presence of positive serum antibodies, HLA-DQ2/DQ8 haplotypes, and a normal small intestinal mucosa (Marsh grade 0-1). This condition occurs in one-fifth of celiac disease (CD) patients and usually represents a clinical challenge. We reviewed genetic, histologic, and clinical features of this specific condition by performing a systematic search on MEDLINE, Embase, and Scholar database. Accordingly, we identified different genetic features in patients with PCD compared to the classical forms. Frequently, signs of inflammation (deposits of immunoglobulin A (IgA) and/or increased number of intraepithelial lymphocytes) can be clearly identify in the mucosa of PCD patients after an accurate histological assessment. Finally, the main challenge is represented by the treatment: the gluten-free diet should be considered only in the presence of gluten-dependent symptoms in both children and adults. *What is known*: (i) potential celiac disease (PCD) occurs in one-fifth of all celiac diseases (CD), and (ii) despite the absence of classical lesions, clear signs of inflammation are often detectable. *What is new*: (i) patients with PCD show different genetic features, and (ii) the presence of gluten-dependent symptoms is the main determinant to initiate the gluten-free diet, after a complete diagnostic work-up.

1. Potential Celiac Disease

Celiac disease (CD) is a systemic disorder caused by gluten and characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes, and enteropathy [1]. Potential CD (PCD) is the condition related to people with a normal (Marsh grade 0) or minimally abnormal (Marsh grade 1) intestinal mucosa who are at increased risk of developing CD, as indicated by both positive serum endomysial (EmA) and tissue transglutaminase antibodies (tTGA2) and a positive histocompatibility leukocyte antigen (HLA-DQ2 or HLA-DQ8) genotype [2]. Symptoms and signs of the disease are not always clinically manifest, and even when present, they can range from mild to severe.

The term "potential CD" was first introduced by Ferguson in 1993 [3], and it has long been used interchangeably with "latent CD"; however, the latter has recently been discontinued, as suggested by the Oslo definition [2]. The diagnosis of PCD has significantly increased in the last years as a result of increased CD screening in the general population [4–6]. The number of patients with PCD is now sizeable, and this condition represents about one-fifth of total CD patients [7]. Compared with active classical CD, PCD is characterized by features including lower prevalence of DQ2 and higher prevalence of DQ8 [8]. Patients with PCD more frequently show low-to-moderate HLA-related risk; these cases bear half of the DQ2 heterodimer, either DQB1*02 or DQA1*05 only. Furthermore, six polymorphisms have been differently distributed in potential CD; these factors could be implicated with CD pathogenesis maybe with a "gene-dosage" effect as reported for HLA [9]. Establishing a certain diagnosis of PCD is of the utmost importance. False positive values of antibodies can be determined by analytical or random errors in the assay. Con-

versely, negative histological findings can be generated by a small number of biopsies due to "patchy" involvement of the bulb and duodenal mucosa [10–13], inappropriate biopsy orientation, the lack of the pathologists' expertise [14, 15], and an inadequate gluten intake before the endoscopy [16].

2. Histology Features and Prognostic Biomarkers

In PCD, despite the absence of severe mucosal damages, clear signs of inflammation are often present. There is a remarkable research activity to improve the diagnosis and identify initial mucosal changes in PCD: the four most important prognostic factors for villous atrophy are described in Figure 1. A short history of the most important findings concerning PCD is reported in Table 1, and results from these studies are here described more in detail.

Paparo et al., in 2005, showed immunohistochemical features of immune activation in the epithelium, lamina propria, and crypts in PCD: 70.8% of PCD patients presented an increased number of lamina propria CD25+ and/or enhanced expression of ICAM-1 and crypt HLA-DR [17]. It has been hypothesized that circulating antitissue transglutaminase 2 (tTGA2) may be the result of a "spillover" from the intestinal mucosal layer [18, 19]. Therefore, identifying anti-tTGA2 deposits in the mucosal layer can be a key factor in the histological assessment of CD: such deposits have been reported below the epithelial layer and around blood vessels in both pediatric and adult patients with overt CD [20, 21]. These features could also have a predictive role for villous atrophy, since they have been described in early-stage CD [22]. In 2006, Salmi et al. demonstrated that the detection of anti-tTGA2 deposits in the mucosa seems to be rather specific for CD and might be helpful in predicting the evolution to more severe histological damage [23]. The same data have been discussed in a recent review and, in the same way, have been considered as "markers of existing early disease" [24].

tTGA2 deposits were observed by Tosco et al. [25] following a patchy distribution with areas of clear positivity and areas with absent signal, as already described in mucosal damage of active CD [10, 13]; however, these deposits can also be found only in bulb duodenal biopsies [26]. In 2017, an Italian study demonstrated that in at-risk infants for CD, detection of mucosal deposits of anti-tTG2 IgA resulted in 88.3% positive predictive value [22]. The prevalence of $\gamma\delta$ T-cell has also been suggested as a histological biomarker of CD. In fact, an increase in intraepithelial lymphocytes at the villus tip and a high $\gamma\delta$+ intraepithelial cell count can be considered good predictors of CD in patient with PCD, as described by two different studies from Finland [27, 28]. Some authors suggest that high density of $\gamma\delta$ T-cell receptor-bearing intraepithelial lymphocytes (IELs) can be a prerequisite for developing CD in patients with no morphological abnormality, yet carrying the susceptibility genes; however, despite an increased density of $\gamma\delta$ T-cell in potential CD, these findings cannot be considered pathognomonic for celiac disease [29, 30]. It has been hypothesized that in PCD, the intestinal mucosa is maintained architecturally normal by an increased enterocyte proliferation, which will

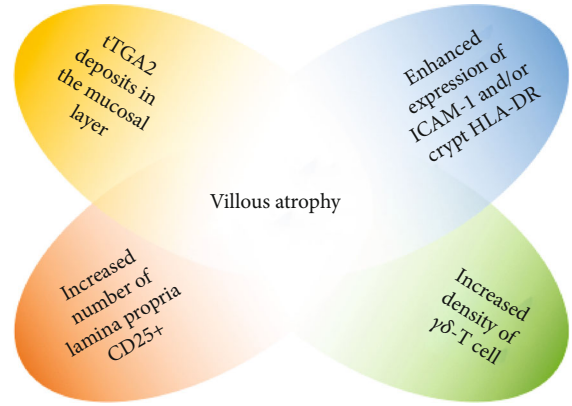


Figure 1: The four most important prognostic factors for villous atrophy in PCD.

end up in a reduced enterocyte maturity and will thus lead to reduced absorptive capacity of the small bowel [31]. In the same year, another study demonstrated how T-cells seem to be activated and differentiating toward a Th1 pattern, as suggested by high levels of interleukin-2 (IL-2), interferon-γ (IFN-γ), and TGF-β transcription factor. The same study showed an increased density of CD4+CD25+Foxp3+ T regulatory cells, which exert suppressive effect not impaired by IL-15 in potential CD [32]. A recent paper from Borrelli et al. in PCD patients showed reduced expression and increased upregulation in the presence of specific stimuli of interleukin-21 (IL-21), an important cytokine regulating innate and adaptive immune response, differently from active CD. In this study, PCD density of IL-21-producing cells in the lamina propria was found to correlate with serum titer of tTGA2, suggesting a lack of ability of IL-21 to enhance and maintain chronic inflammation in early phases of disease in active or potential CD [33]. In active CD, the overexpression of IL-21 is likely to play a crucial role in the activation of cytotoxic T-cells leading to epithelial cell death and mucosal destruction [34]. Aside from immunological controversies, an overlapping metabolomic signature was found for PCD and active disease, suggesting that common functional-biochemical stigmata might call for the same dietary treatment [35].

3. To Treat or Not to Treat?

The therapeutic management of PCD patients represents the main challenge. The only accepted treatment for CD is gluten-free diet (GFD), but the treatment for potential celiac disease still remains unclear. Likewise, there is no clear consensus in the PCD follow-up [36]. The natural history of PCD, both in adults [7] and children [25], is not sufficient to recommend GFD in any patient. Recently, Auricchio et al. [37] developed a model to predict the evolution to villous atrophy in PCD. They suggested GFD when symptoms of CD can be clearly detected, even without a mucosal damage. This approach aims at reducing symptoms and antibody titers (tTGA2 and EMA), as well as healing minimal alterations in intestinal mucosa [1]. Conversely, the use of GFD in asymptomatic patients is still debated. In 2009 and 2010, two studies from Finland showed that both adults [38] and

Table 1: A short history of the most important findings concerning PCD.

Study	Year	Conclusions
Holm et al. [29]	1992	A healthy person who initially has a normal biopsy, but who also has an increased density of $\gamma\delta$ T-cells, may later develop mucosal atrophy compatible with CD.
Iltanen et al. [30]	1999	39 of 79 (49%) children with normal jejunal mucosa had an increased density of intraepithelial $\gamma\delta$ T-cells.
Jarvinen et al. [28]	2003	An increase especially in $\gamma\delta$ T-cells strengthens the probability of CD.
Korponay-Szabo et al. [20]	2004	TG2-related IgA deposits in the morphologically normal jejunum were predictive of forthcoming overt coeliac disease with villous atrophy.
Jarvinen et al. [27]	2004	The villous tip intraepithelial lymphocyte count was statistically significantly higher in patients with early-stage coeliac disease than in nonceliac controls (sensitivity, 0.84; specificity, 0.88).
Paparo et al. [17]	2005	Increased number of lamina CD25+ and/or enhanced expression of ICAM 1 and crypt HLA DR.
Salmi et al. [23]	2006	Intestinal coeliac autoantibody deposit had a sensitivity and specificity of 93% and 93%, respectively, in detecting subsequent coeliac disease.
Koskinen et al. [21]	2010	Mucosal transglutaminase 2-specific autoantibody deposits proved to be accurate gluten-dependent markers of celiac disease.
Tosco et al. [25]	2011	In most positive cases a patchy distribution of the deposits was observed with areas of clear positivity and areas with absent signal.
Bernini et al. [35]	2011	Potential CD largely shares the metabolomic signature of overt CD. Results prove that metabolic alterations may precede the development of small intestinal villous atrophy.
Biagi et al. [31]	2013	In PCD, the intestinal mucosa is maintained architecturally normal thanks to an increased enterocytic proliferation.
Borrelli et al. [32]	2013	Potential CD patients show a low grade of inflammation that could likely be due to active regulatory mechanism preventing the progression toward a mucosal damage.
Borrelli et al. [33]	2016	In potential CD, IL-21 is less expressed than that in active CD.
Borrelli et al. [22]	2018	In CD, the intestinal deposits of anti-tTG2 are a constant presence and appear very early in the natural history of the disease.

TABLE 2: Results of available evidence in support or against GFD in PCD asymptomatic patients.

Study	About GFD	Study population	Conclusions	Limitations
Tosco et al. [25]	Against GFD	106 children	33% of incidence of villous atrophy after 3 years in with PCD	Unknown number of patients lost at follow-up
Lionetti et al. [44]	Against GFD	24 asymptomatic children	CD markers disappear in most young children with potential CD despite a regular diet	Small sample size
Silvester et al. [45]	Against GFD	*Review paper*	In the absence of symptoms or villous atrophy, treatment with a GFD does not appear to be necessary in most cases	N/A
Mandile et al. [41]	Against GFD	47 children	Association between CD and irritable bowel syndrome may be a significant confounding factor	Irritable bowel syndrome is overlapping with CD
Lionetti et al. [43]	Against GFD	23 asymptomatic children	Risk of progression to overt CD while on a gluten-containing diet is very low in the long-term.	Age of the study group and study design
Kurppa et al. [38]	Supports GFD	23 adults	Patients with endomysial antibodies benefit from a GFD regardless of the degree of enteropathy.	Marsh II included in study population
Kurppa et al. [39]	Supports GFD	17 children	Children benefit from early treatment despite normal mucosal structure	Small sample size

children [39] with PCD obtained a clinical response to GFD regardless of the presence of small-bowel lesions. According to these studies, the authors suggested to start the dietary treatment as early as possible since treatment would result in reduced risks of delayed puberty and gynecological issues, while avoiding effects on bone mineralization, dental enamel development, and growth. Conversely, in a recent review, Itzlinger et al. considered GFD as inappropriate treatment in asymptomatic patients with PCD [40].

Diverging results emerged from Mandile's work, in which only 54% of PCD symptomatic patients have a positive clinical response during the first 12 months of GFD. However, the authors speculated about irritable bowel syndrome as a significant confounding factor in these patients

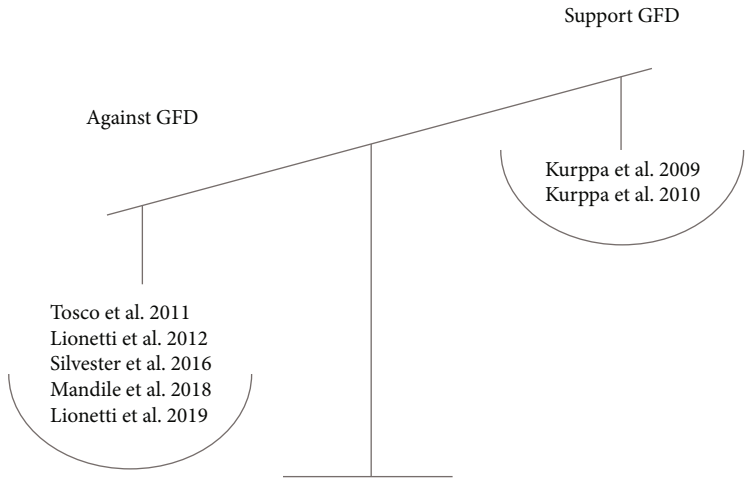


Figure 2: Results of available evidence in support or against GFD in PCD asymptomatic patients.

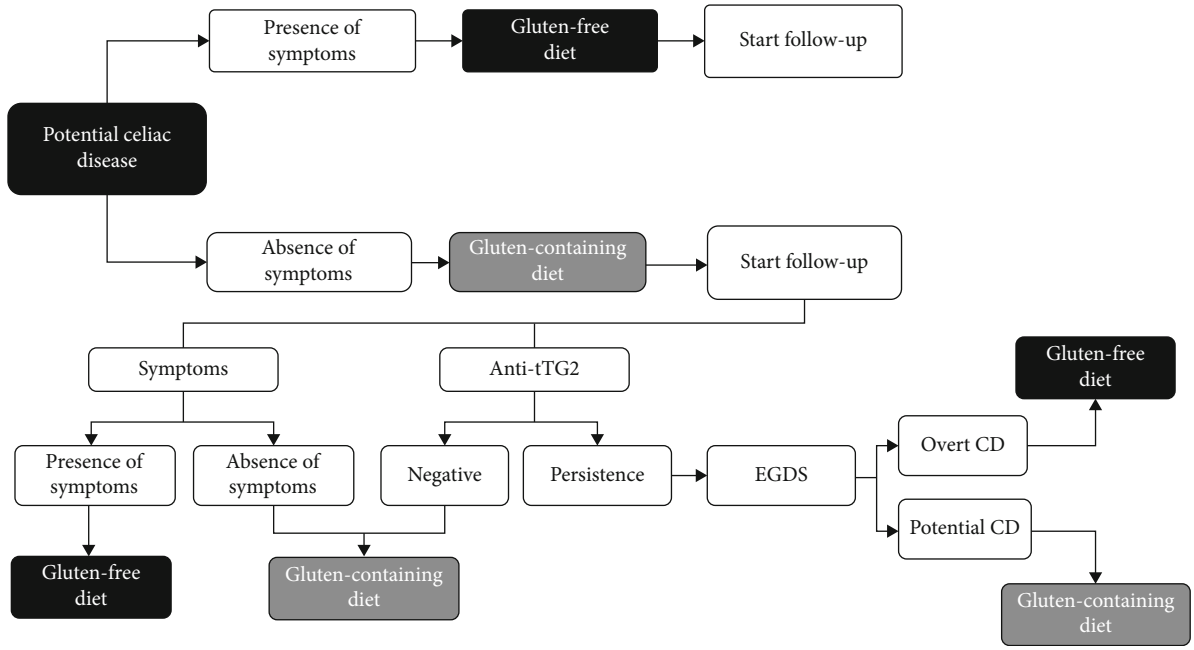


Figure 3: Diagnostic algorithm for PCD.

[41]. In 2014, Auricchio et al. demonstrated that a considerable proportion of PCD patients usually had a fluctuation or decrease of antibody levels, while in those with persistently positive anti-TG2 under a free diet, the mucosal damage was not detectable in 66% of cases until 9 years of follow-up [42]. In 2019, Lionetti et al. reached similar conclusions: in PCD children on free diet, the risk of progression to overt CD is trivial [43].

Previously, Tosco et al. demonstrated that approximately 33% of asymptomatic children with PCD would develop villous atrophy after 3 years without prescribing a GFD [25]. The authors suggested that most children with potential celiac disease remain healthy and for these reason only symptomatic children would start GFD.

In 2012, a decision tree for asymptomatic children with tTGA values lower than 11-fold the upper limit normal was proposed [44]. Symptomless children with a family history of CD and positive CD markers could initially remain on normal free diet, particularly in the case of modest tTGA titer increase. Biopsies should be recommended after a persistent antibody positivity for at least 3-6 months. In 2016, another group indicated that asymptomatic patients can be monitored for the development of new symptoms and/or substantial increase in serum tTGA2 antibodies [45]. These studies are summarized in Table 2 and Figure 2.

In conclusion, the presence of symptoms in both adults and children should be considered as the main determinant to prescribe a GFD in potential celiac disease. It is important to remember that all symptoms have to be considered important for the beginning of a GFD. There is no difference in the decision tree, in fact, if patient has gastrointestinal (diarrhea, constipation, abdominal pain) or extraintestinal

manifestation (anemia, osteoporosis, migraine), as suggested by Popp and Maki in a recent review too [24]. As the timing of flattening is totally unpredictable, asymptomatic patients with PCD should undergo a comprehensive follow-up in order to detect early symptoms and promptly start a GFD. A conclusive algorithm is proposed in Figure 3 with the aim to provide valuable information in the management of this challenging condition.

Further research is necessary in order to establish the optimal frequency of testing the antibodies and clinical evaluation for PCD patients (both adults and children) continuing after initial evaluations on gluten-containing diet. Dietary habits and gluten intake during clinical evaluation should be routinely checked during clinical evaluation, as following a diagnosis of PCD, the patient or his family could decrease the amount of gluten, resulting in false negative serology and fluctuating antibodies.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

[1] S. Husby et al., "European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 54, no. 1, pp. 136–160, 2012.

[2] J. F. Ludvigsson, D. A. Leffler, J. C. Bai et al., "The Oslo definitions for coeliac disease and related terms," *Gut*, vol. 62, no. 1, pp. 43–52, 2013.

[3] A. Ferguson, E. Arranz, and S. O'Mahony, "Clinical and pathological spectrum of coeliac disease–active, silent, latent, potential," *Gut*, vol. 34, no. 2, pp. 150-151, 1993.

[4] J. F. Ludvigsson et al., "Screening for celiac disease in the general population and in high-risk groups," *United European Gastroenterology Journal*, vol. 3, no. 2, pp. 106–120, 2015.

[5] M. Bonamico, R. Nenna, M. Montuori et al., "First salivary screening of celiac disease by detection of anti-transglutaminase autoantibody radioimmunoassay in 5000 Italian primary schoolchildren," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 52, no. 1, pp. 17–20, 2011.

[6] C. Catassi et al., "The coeliac iceberg in Italy. A multicentre antigliadin antibodies screening for coeliac disease in school-age subjects," *Acta Paediatrica. Supplement*, vol. 412, pp. 29–35, 1996.

[7] U. Volta et al., "Features and progression of potential celiac disease in adults," *Clin Gastroenterol Hepatol*, vol. 14, no. 5, pp. 686–93 e1, 2016.

[8] F. Biagi, P. I. Bianchi, C. Vattiato et al., "Influence of HLA-DQ2 and DQ8 on severity in celiac disease," *Journal of Clinical Gastroenterology*, vol. 46, no. 1, pp. 46–50, 2012.

[9] M. P. Sperandeo, A. Tosco, V. Izzo et al., "Potential celiac patients: a model of celiac disease pathogenesis," *PLoS One*, vol. 6, no. 7, article e21281, 2011.

[10] A. Ravelli, V. Villanacci, C. Monfredini, S. Martinazzi, V. Grassi, and S. Manenti, "How patchy is patchy villous atrophy?: distribution pattern of histological lesions in the duodenum of children with celiac disease," *The American Journal of Gastroenterology*, vol. 105, no. 9, pp. 2103–2110, 2010.

[11] M. Bonamico, P. Mariani, E. Thanasi et al., "Patchy villous atrophy of the duodenum in childhood celiac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 38, no. 2, pp. 204–207, 2004.

[12] M. Bonamico, E. Thanasi, P. Mariani et al., "Duodenal bulb biopsies in celiac disease: a multicenter study," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 47, no. 5, pp. 618–622, 2008.

[13] F. Valitutti, G. di Nardo, M. Barbato et al., "Mapping histologic patchiness of celiac disease by push enteroscopy," *Gastrointestinal Endoscopy*, vol. 79, no. 1, pp. 95–100, 2014.

[14] A. Ravelli and V. Villanacci, "Tricks of the trade: how to avoid histological pitfalls in celiac disease," *Pathology, Research and Practice*, vol. 208, no. 4, pp. 197–202, 2012.

[15] P. H. Green and B. Jabri, "Coeliac disease," *Lancet*, vol. 362, no. 9381, pp. 383–391, 2003.

[16] H. Andersson et al., "Influence of the amount of dietary gluten on gastrointestinal morphology and function in dermatitis herpetiformis," *Human Nutrition. Clinical Nutrition*, vol. 38, no. 4, pp. 279–285, 1984.

[17] F. Paparo, E. Petrone, A. Tosco et al., "Clinical, HLA, and small bowel immunohistochemical features of children with positive serum antiendomysium antibodies and architecturally normal small intestinal mucosa," *The American Journal of Gastroenterology*, vol. 100, no. 10, pp. 2294–2298, 2005.

[18] R. Marzari, D. Sblattero, F. Florian et al., "Molecular dissection of the tissue transglutaminase autoantibody response in celiac disease," *Journal of Immunology*, vol. 166, no. 6, pp. 4170–4176, 2001.

[19] K. Kaukinen, M. Peräaho, P. Collin et al., "Small-bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: a prospective and randomized clinical study," *Scandinavian Journal of Gastroenterology*, vol. 40, no. 5, pp. 564–572, 2005.

[20] I. R. Korponay-Szabo et al., "In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies," *Gut*, vol. 53, no. 5, pp. 641–648, 2004.

[21] O. Koskinen et al., "Usefulness of small-bowel mucosal transglutaminase-2 specific autoantibody deposits in the diagnosis and follow-up of celiac disease," *Journal of Clinical Gastroenterology*, vol. 44, no. 7, pp. 483–488, 2010.

[22] M. Borrelli, M. Maglio, I. R. Korponay-Szabó et al., "Intestinal anti-transglutaminase 2 immunoglobulin A deposits in children at risk for coeliac disease (CD): data from the prevent CD study," *Clinical and Experimental Immunology*, vol. 191, no. 3, pp. 311–317, 2018.

[23] T. T. Salmi et al., "Immunoglobulin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming coeliac disease," *Alimentary Pharmacology & Therapeutics*, vol. 24, no. 3, pp. 541–552, 2006.

[24] A. Popp and M. Maki, "Gluten-induced extra-intestinal manifestations in potential celiac disease-celiac trait," *Nutrients*, vol. 11, no. 2, p. 320, 2019.

[25] A. Tosco et al., "Natural history of potential celiac disease in children," *Clin Gastroenterol Hepatol*, vol. 9, no. 4, pp. 320–325, 2011, quiz e36.

[26] L. De Leo et al., "Immunohistologic analysis of the duodenal bulb: a new method for celiac disease diagnosis in children," *Gastrointestinal Endoscopy*, vol. 88, no. 3, pp. 521–526, 2018.

[27] T. T. Jarvinen et al., "Villous tip intraepithelial lymphocytes as markers of early-stage coeliac disease," *Scandinavian Journal of Gastroenterology*, vol. 39, no. 5, pp. 428–433, 2004.

[28] T. T. Jarvinen et al., "Intraepithelial lymphocytes in celiac disease," *The American Journal of Gastroenterology*, vol. 98, no. 6, pp. 1332–1337, 2003.

[29] K. Holm, M. Mäki, E. Savilahti, P. Laippala, V. Lipsanen, and S. Koskimies, "Intraepithelial gamma delta T-cell-receptor lymphocytes and genetic susceptibility to coeliac disease," *Lancet*, vol. 339, no. 8808, pp. 1500–1503, 1992.

[30] S. Iltanen, K. Holm, J. Partanen, P. Laippala, and M. Mauki, "Increased density of jejunal gammadelta+ T cells in patients having normal mucosa–marker of operative autoimmune mechanisms?," *Autoimmunity*, vol. 29, no. 3, pp. 179–187, 1999.

[31] F. Biagi et al., "Prevalence and natural history of potential celiac disease in adult patients," *Scandinavian Journal of Gastroenterology*, vol. 48, no. 5, pp. 537–542, 2013.

[32] M. Borrelli, V. M. Salvati, M. Maglio et al., "Immunoregulatory pathways are active in the small intestinal mucosa of patients with potential celiac disease," *The American Journal of Gastroenterology*, vol. 108, no. 11, pp. 1775–1784, 2013.

[33] M. Borrelli et al., "In the intestinal mucosa of children with potential celiac disease IL-21 and IL-17A are less expressed than in the active disease," *The American Journal of Gastroenterology*, vol. 111, no. 1, pp. 134–144, 2016.

[34] M. Strengell, S. Matikainen, J. Sirén et al., "IL-21 in synergy with IL-15 or IL-18 enhances IFN-gamma production in human NK and T cells," *Journal of Immunology*, vol. 170, no. 11, pp. 5464–5469, 2003.

[35] P. Bernini, I. Bertini, A. Calabrò et al., "Are patients with potential celiac disease really potential? The answer of metabonomics," *Journal of Proteome Research*, vol. 10, no. 2, pp. 714–721, 2011.

[36] F. Valitutti, C. M. Trovato, M. Montuori, and S. Cucchiara, "Pediatric celiac disease: follow-up in the spotlight," *Advances in Nutrition*, vol. 8, no. 2, pp. 356–361, 2017.

[37] R. Auricchio, R. Mandile, M. R. del Vecchio et al., "Progression of celiac disease in children with antibodies against tissue transglutaminase and normal duodenal architecture," *Gastroenterology*, vol. 157, no. 2, pp. 413–420.e3, 2019.

[38] K. Kurppa, P. Collin, M. Viljamaa et al., "Diagnosing mild enteropathy celiac disease: a randomized, controlled clinical study," *Gastroenterology*, vol. 136, no. 3, pp. 816–823, 2009.

[39] K. Kurppa et al., "Celiac disease without villous atrophy in children: a prospective study," *J Pediatr*, vol. 157, no. 3, pp. 373–380, 2010, 380 e1.

[40] A. Itzlinger, F. Branchi, L. Elli, and M. Schumann, "Gluten-free diet in celiac disease-forever and for all?," *Nutrients*, vol. 10, no. 11, p. 1796, 2018.

[41] R. Mandile, V. Discepolo, S. Scapaticci et al., "The effect of gluten-free diet on clinical symptoms and the intestinal mucosa of patients with potential celiac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 66, no. 4, pp. 654–656, 2018.

[42] R. Auricchio, A. Tosco, E. Piccolo et al., "Potential celiac children: 9-year follow-up on a gluten-containing diet," *The American Journal of Gastroenterology*, vol. 109, no. 6, pp. 913–921, 2014.

[43] E. Lionetti, S. Castellaneta, R. Francavilla et al., "Long-term outcome of potential celiac disease in genetically at-risk children: the prospective CELIPREV cohort study," *Journal of Clinical Medicine*, vol. 8, no. 2, p. 186, 2019.

[44] E. Lionetti, S. Castellaneta, A. Pulvirenti et al., "Prevalence and natural history of potential celiac disease in at-family-risk infants prospectively investigated from birth," *The Journal of Pediatrics*, vol. 161, no. 5, pp. 908–914.e2, 2012.

[45] J. A. Silvester and C. P. Kelly, "The potential for treatment of potential celiac disease," *Clinical Gastroenterology and Hepatology*, vol. 14, no. 5, pp. 694-695, 2016.

Hindawi
Gastroenterology Research and Practice
Volume 2019, Article ID 4504679, 5 pages
https://doi.org/10.1155/2019/4504679

Research Article

Is There a Role of Using a Rapid Finger Prick Antibody Test in Screening for Celiac Disease in Children?

Kristina Baraba Dekanić,[1] Ivona Butorac Ahel,[1] Lucija Ružman,[1] Jasmina Dolinšek,[2] Jernej Dolinšek,[3] and Goran Palčevski[1]

[1]*Department of Pediatrics, University Hospital Center Rijeka, Istarska 43, 51 000 Rijeka, Croatia*
[2]*Office of Project Development-Project Office, Municipality of Maribor, Ulica Heroja Staneta 1, Slovenia*
[3]*Department of Pediatrics, University Medical Center Maribor, Ljubljanska 5, 2 000 Maribor, Slovenia*

Correspondence should be addressed to Kristina Baraba Dekanić; k.baraba.dekanic@gmail.com

Received 8 May 2019; Accepted 3 September 2019; Published 7 October 2019

Guest Editor: Francesco Valitutti

Introduction. Celiac disease (CD) is an autoimmune disease triggered by gluten in genetically predisposed individuals. Despite the increasing prevalence of CD, many patients remain undiagnosed. Standard serology tests are expensive and invasive, so several point-of-care tests (POC) for CD have been developed. We aimed to determine the prevalence of CD in first-grade pupils in Primorje-Gorski Kotar County, Croatia, using a POC test. *Methods*. A Biocard celiac test that detects IgA antibodies to tissue transglutaminase in whole blood was used to screen for celiac disease in healthy first-grade children born in 2011 and 2012 who consumed gluten without restrictions. *Results*. 1478 children were tested, and none of them were tested positive with a rapid test. In 10 children (0,6%), IgA deficiency has been suspected; only 4 of them agreed to be tested further for total IgA, anti-tTG, and anti-DGP antibodies. IgA deficiency was confirmed in 3 patients, and in all 4 children, CD has been excluded. *Conclusion*. Our results have not confirmed the usefulness of the POC test in screening the general population of first-grade schoolchildren. Further research is needed to establish the true epidemiology of CD in Primorje-Gorski Kotar County and to confirm the value of the rapid test in comparison with standard antibody CD testing.

1. Introduction

Celiac disease (CD) is an autoimmune disease involving innate and adaptive immune responses triggered by the gluten ingestion in genetically predisposed individuals. In persons with HLA-DQ2 and/or DQ8 haplotypes, activated immune reaction results in small intestinal mucosal damage with villous atrophy, crypt hyperplasia, and an increased number of intraepithelial lymphocytes [1, 2]. Patients with CD may present with gastrointestinal symptoms; however, a substantial number of patients present with atypical extraintestinal symptoms of variable severity [2–4].

CD is a common disorder with the overall prevalence of 1%, with differences among countries (Germany 0.3%, Italy 0.7%, Finland 2.4%, and USA 1%) [1, 2, 5]. The prevalence substantially increased in the last 50 years [6]. However, the majority of patients are not identified; the data shows that almost 90% of patients, both children and adults, remain undiagnosed, possibly because of the high proportion of asymptomatic or oligosymptomatic patients [2, 3, 7]. After the last quarter of the 20th century, the dramatic shift from typical gastrointestinal manifestations to atypical and asymptomatic presentations has been noticed [2, 8].

There are few data about the incidence and prevalence of CD in Croatia. Only limited data from 10-year research from limited region exist; the cumulative incidence is 1.9 : 1000 life-births and prevalence 1 : 461 [9, 10].

Patients with CD have a modestly increased risk of malignancy and mortality [11]. Untreated illness is associated with numerous long-term complications, for example, delayed puberty, other autoimmune disorders (thyroid disease and diabetes mellitus), cerebellar ataxia, epilepsy, neuropsychiatric disorders, infertility, osteoporosis, small-for-date births, and malignancies (enteropathy-associated T cell lymphoma,

small intestinal adenocarcinoma) [3, 4, 8, 12]. There is a strong evidence that undiagnosed CD is associated with nearly 4-fold increase risk of death compared to people without it [6].

Strict adherence to gluten-free diet (GFD) reduces the rate of morbidity and mortality [8], emphasizing the importance of early detection of patients who benefit from GFD [4].

Specific subgroups of individuals have an increased risk for CD, among these are first-degree relatives of CD patients and people with other autoimmune diseases (type 1 diabetes mellitus, autoimmune thyroiditis, and autoimmune hepatitis) and specific genetic disorders (Down syndrome, Turner syndrome, Williams syndrome, and IgA deficiency) [2]. Current ESPGHAN guidelines recommend active search for CD among these subgroups [13].

Despite the low rate of diagnosis, there are still no general recommendations for screening in the general population [1]. Based on the research of Greco et al., the burden of unrecognized CD patients will grow substantially in the Mediterranean region with an estimated number of 5 million cases in 2020; the estimated medical costs caused by delayed CD diagnosis are about €4 billion during a 10-year period. This emphasizes the need for simplified diagnostic protocols that will be available not only in specialized centers but also in rural areas [14]. Highly sensitive and specific point-of-care tests (POCT) might be a solution to shorten diagnostic delays. Besides, the data clearly shows that the mass screening could be the best strategy for secondary CD prevention [8]. There are few studies that examined the role of CD screening in Europe based on the increased prevalence of the disease [15–17].

Conventional laboratory methods (anti-transglutaminase 2 (tTG) IgA and anti-endomysial (EMA) autoantibodies) are expensive, not easily available, and difficult to use for mass screening [3, 4]. Therefore, rapid methods of antibody detection using blood from finger pricks that can be performed at the point of care have already showed their efficacy [3, 4, 18, 19].

The aim of our study was to determine the frequency of CD among first-grade schoolchildren in Primorje-Gorski Kotar County, Croatia, using a rapid point-of-care test.

2. Materials and Methods

2.1. Subjects. We screened first-grade schoolchildren from elementary schools in Primorje-Gorski Kotar County, Croatia. All children attending the first grade born in 2011 and 2012 were eligible for the study. Children already diagnosed with CD on GFD and children without CD, but who do not consume gluten for other reasons (e.g., allergic to gluten or wheat and parents' decision for not eating gluten), were excluded from the study, as well as children who have already been tested for CD during the last year. The goal of the study and principles of testing were presented to parents in every school, and written informed consent was obtained. Only children with signed informed consent were included in the study.

The team consisting of three pediatricians and two trained nurses visited all schools. The screening period lasted for 6 months, from September 2018 to February 2019. The study was a part of the Focus IN CD project (CE-111) cofinanced by the EU Interreg Central Europe Program and was approved by the Ethics Committee of the University Hospital Center Rijeka and Croatian Ministry of Science, Croatia.

2.2. Screening Procedure. The Biocard Celiac Test, Ani Biotech, Vantaa, Finland, was used for screening. This test is based on endogenous tissue transglutaminase (tTG) found in the erythrocytes of patients. According to the manufacturer's instructions, 10 μl of whole blood is drawn and instilled into the 0,5 ml buffer, which causes hemolysis. As a consequence, tTG is released from erythrocytes. Three drops of the hemolyzed blood are added to the application field on the test. Persons with CD have circulating IgA anti-tTG specific antibodies that bind to released tTG. These complexes can bind to the solid surface coated with tTG-capturing proteins and anti-IgA antibodies labelled with a colloidal gold particle. As a result, in the case of CD, a visible test line is formed. A control line serves as a proof that the blood sample and the reagents moved over the test line. The results can be interpreted after 5 minutes, but no longer than 10 minutes; positive results can be seen already after 1-2 minutes. The test is negative if there is only line in the control area and positive if there are visible lines in both test and control areas, and in case of IgA deficiency, there are no lines in any of the areas. The sensitivity and specificity of the test were shown to be different in different age groups; in younger than 16 years, the sensitivity was 99% and specificity was 97%, while in older than 16 years, the sensitivity was 93% and specificity was 97% [19, 20].

Children with eventual positive results or the ones with suspected IgA deficiency were referred to Clinical Hospital Center Rijeka for total IgA measurements, IgA anti-tTG measurements (IDS, automated chemiluminescence immunoassay, CLIA) with a cutoff value of 7 U/ml, and IgG anti-DGP measurements (IDS, automated chemiluminescence immunoassay, CLIA) with a cutoff value of 7 U/ml.

3. Results

Primorje-Gorski Kotar County is located in the western part of Croatia, and Rijeka is the capital city. According to the census in 2011, it has 296195 inhabitants. There are 60 elementary schools in the county with a total of 2391 children in the first grade in the school year 2018/2019.

There was a total number of 1893 children whose parents attended parent meetings and agreed with the participation of their children in the study. According to inclusion criteria, children with known CD ($n = 2$) and children who already had CD testing within one year ($n = 35$) were excluded from the screening. Parents of 258 children refused to participate, and 120 children were absent from school on the day of the screening because of other reasons (e.g., illness).

We screened 1478 children (61.82% of all eligible children). There were 964 (65.22%) girls and 514 (34.78%) boys. There was no invalid test reported. We did not find any patients with a positive rapid test, and 10 children were suspected to have IgA deficiency. They were referred to the

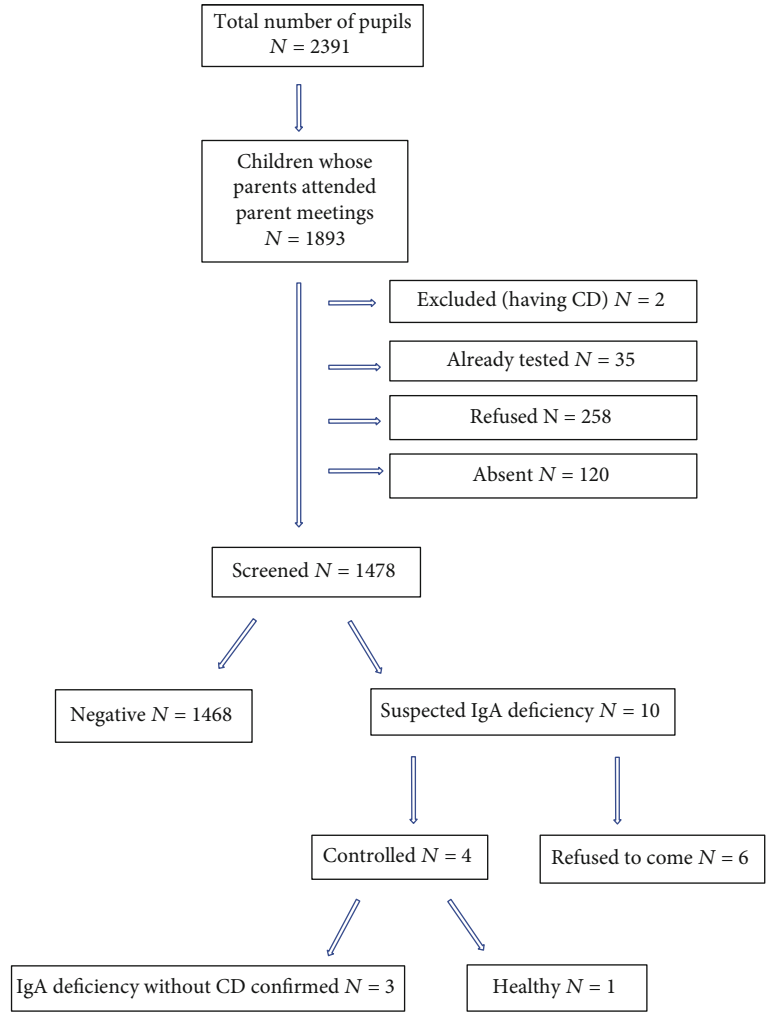


Figure 1: Results of the screening.

Department of Pediatrics in Clinical Hospital Center Rijeka for total IgA, anti-tTG, and anti-DGP antibody measurements. Out of 10 children, only 4 of them came for the testing. IgA deficiency was confirmed in 3 patients who all had low IgG anti-DGP antibodies, and in one child, the total IgA level was normal and CD-specific antibodies were low (Figure 1). The data with IgA and CD-specific antibody levels is listed in Table 1.

Table 1: Levels of total IgA, IgA anti-tTG, and IgG anti-DGP in children with suspected IgA deficiency after the rapid test.

Initials	Gender	Total IgA	IgA anti-tTG (U/ml)	IgG anti-DGP (U/ml)
LK	F	1.6	2	1.1
AB	M	<0.4	<0.8	<0.8
JT	M	0.3	1.1	1.6
MK	M	0.2	0.9	2.7

4. Discussion

CD is one of the most frequent genetically based diseases of humankind [21], and majority of patients are misdiagnosed or not diagnosed at all [3, 7, 21]. There is ongoing discussion whether to screen for CD and whom to screen [22]. The World Health Organization (WHO) provides criteria for mass screening [7, 23, 24]: the disease must be common; screening tests must be simple, fast, and accurate and acceptable in different cultures; early clinical detection should be difficult; treatment must be available; and undiagnosed disease can lead to severe complications. CD clearly fits majority of the criteria. There are some open questions that need further investigations, including the degree of the risk for severe complications in asymptomatic individuals [20], cost-benefit ratio of the screening, benefit and compliance of GFD in asymptomatic individuals, and the appropriate age when to perform the screening [7, 8, 24, 25]. Generally, screening must be performed early enough to prevent late complications of the disease, but since a proportion of patients develop the disease later in life, early screening could miss them [7, 8, 26].

Nowadays, there are still no recommendation for mass screening [8, 21, 24, 25, 27]. According to ESPGHAN guidelines, screening should be undertaken for high-risk groups [13].

Our study on 1478 first-grade children tried to establish the prevalence of CD among 7-year-old children, the age by which a significant proportion of CD should have developed, and the potential use of rapid CD testing in general population screening. If the screening method is simple, the cost-benefit balance could be favorable even though benefits are only moderate [3].

Rapid POCT is cheap and easy to perform in comparison to standard CD testing [3, 4]. Studies made with the Biocard celiac disease test kit showed sensitivity, specificity, and positive and negative predictive values comparable with a standard CD test (anti-tTG and EMA), all higher than 93% [3, 19]; it was successfully used in screening first-degree relatives of CD patients, but the study was conducted on a small number of subjects [4], and in determining prevalence of CD among school-aged children in Turkey [18]. Although Comba et al. [18] had a representative sample, the lack of their study was the possibility of missing IgA-deficient patients with CD.

We were not able to detect children with CD. There are several possible explanations. First, prevalence among countries differs significantly; it ranges from 0.3% in Germany to 0.7% in Italy 0.7%, 1% in USA, and 2.4% in Finland [1, 2, 5]. According to our best knowledge, there are no data on CD epidemiology in Croatia, so our data could reflect lower prevalence of CD in Croatia compared with other countries. Second, we found 10 children with possible IgA deficiency and higher risk for CD development. Only four children came to our hospital to control IgA, anti-tTG, and anti-DGP levels. They were all negative for CD-specific antibodies, but there is a possibility that among the other 6 children whose parents refused to come for the specific CD antibody testing there are ones with CD. Third, although we followed the producer's instructions completely, there is still possibility of unintentional mistake. The test is qualitative in its nature, so a slightly visible test line could be missed.

5. Conclusion

To conclude, based on our study results, the POC test was not shown to be a useful tool in mass screening. Further research is needed to establish the incidence and prevalence of CD in Croatia, with more data to confirm the value of rapid tests in comparison with standard antibody CD testing.

Data Availability

The patients' personal data used to support the findings of this study are restricted by the Ethics Committee of the University Hospital Center Rijeka in order to protect patients' privacy. The general data of results of rapid tests used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The study was a part of the Focus IN CD project (CE-111) cofinanced by the EU Interreg Central Europe Program.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

The authors thank the Association of Coeliac Patients Primorsko-Goranska County and Primorje-Gorski Kotar County for their help in visiting schools and collecting the data.

References

[1] P. H. R. Green, B. Lebwohl, and R. Greywoode, "Celiac disease," *The Journal of Allergy and Clinical Immunology*, vol. 135, no. 5, pp. 1099–1106, 2015.

[2] S. Guandalini, "The approach to celiac disease in children," *International Journal of Pediatrics and Adolescent Medicine*, vol. 4, no. 3, pp. 124–127, 2017.

[3] I. R. Korponay-Szabó, K. Szabados, J. Pusztai et al., "Population screening for coeliac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study," *BMJ*, vol. 335, no. 7632, pp. 1244–1247, 2007.

[4] A. Popp, M. Jinga, C. Jurcut et al., "Fingertip rapid point-of-care test in adult case-finding in coeliac disease," *BMC Gastroenterology*, vol. 13, no. 1, 2013.

[5] K. Mustalahti, C. Catassi, A. Reunanen et al., "The prevalence of celiac disease in Europe: results of a centralized international mass screening project," *Annals of Medicine*, vol. 42, no. 8, pp. 587–595, 2010.

[6] T.,. A. Rubio–, R. A. Kyle, E. L. Kaplan et al., "Increased prevalence and mortality in undiagnosed celiac disease," *Gastroenterology*, vol. 137, no. 1, pp. 88–93, 2009.

[7] M. Ravikumara, V. K. Nootigattu, and B. K. Sandhu, "Ninety percent of celiac disease is being missed," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 45, no. 4, pp. 497–499, 2007.

[8] C. Meijer, R. Shamir, H. Szajewska, and L. Mearin, "Celiac disease prevention," *Frontiers in Pediatrics*, vol. 6, p. 368, 2018.

[9] Z. Matek, M. Jungvirth-Hegedus, and S. Kolacek, "Epidemiology of coeliac disease in children in one Croatian county: the cumulative incidence over ten-year period and the way of clinical presentation (part I)," *Collegium Antropologicum*, vol. 23, no. 2, pp. 621–628, 1999.

[10] M. Hegedus-Jungvirth, S. Kolacek, and V. Zizic, "REzultati skrininga o prevalenciji celijakije u odrasloj populaciji u Međimurju," *Acta Medica Croatica*, vol. 55, Suppl 4, p. 139, 2001.

[11] J. West, R. F. A. Logan, C. J. Smith, R. B. Hubbard, and T. R. Card, "Malignancy and mortality in people with coeliac disease: population based cohort study," *BMJ*, vol. 329, no. 7468, pp. 716–719, 2004.

[12] P. H. R. Green, A. T. Fleischauer, G. Bhagat, R. Goyal, B. Jabri, and A. I. Neugut, "Risk of malignancy in patients with celiac disease," *The American Journal of Medicine*, vol. 115, no. 3, pp. 191–195, 2003.

[13] S. Husby, S. Koletzko, I. R. Korponay-Szabó et al., "European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 54, no. 1, pp. 136–160, 2012.

[14] L. Greco, L. Timpone, A. Abkari et al., "Burden of celiac disease in the Mediterranean area," *World Journal of Gastroenterology*, vol. 17, no. 45, pp. 4971–4978, 2011.

[15] M. Bonamico, R. Nenna, M. Montuori et al., "First Salivary Screening of Celiac Disease by Detection of Anti-transglutaminase Autoantibody Radioimmunoassay in 5000 Italian Primary School children," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 52, no. 1, pp. 17–20, 2011.

[16] S. Gatti, E. Lionetti, L. Balanzoni et al., "Increased prevalence of celiac disease in school-age children in Italy," *Clinical Gastroenterology and Hepatology*, 2019.

[17] R. Nenna, C. Tiberti, L. Petrarca et al., "The celiac iceberg: characterization of the disease in primary schoolchildren," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 56, no. 4, pp. 416–421, 2013.

[18] A. Comba, Department of Pediatrics, Hitit University School of Medicine Training et al., "Prevalence of celiac disease among school-age children in Çorum, Turkey," *The Turkish Journal of Gastroenterology*, vol. 29, no. 5, pp. 595–600, 2018.

[19] T. Raivio, K. Kaukinen, E. Nemes et al., "Self transglutaminase-based rapid coeliac disease antibody detection by a lateral flow method," *Alimentary Pharmacology & Therapeutics*, vol. 24, no. 1, pp. 147–154, 2006.

[20] S. Costa, L. Astarita, M. Ben-Hariz et al., "A point-of-care test for facing the burden of undiagnosed celiac disease in the Mediterranean area: a pragmatic design study," *BMC Gastroenterology*, vol. 14, no. 1, p. 219, 2014.

[21] A. Fasano, "European and North American populations should be screened for coeliac disease," *Gut*, vol. 52, no. 2, pp. 168-169, 2003.

[22] M. L. Mearin, A. Ivarsson, and W. Dickey, "Coeliac disease: is it time for mass screening?," *Best Practice & Research. Clinical Gastroenterology*, vol. 19, no. 3, pp. 441–452, 2005.

[23] *WHO Mass Screening Recommendations*http: //http://www.who.int/en/; approached in March 2019.

[24] J. F. Ludvigsson, T. R. Card, K. Kaukinen et al., "Screening for celiac disease in the general population and in high-risk groups," *United European Gastroenterology Journal*, vol. 3, no. 2, pp. 106–120, 2015.

[25] C. Catassi, S. Gatti, and A. Fasano, "The new epidemiology of celiac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 59, Suppl 1, pp. S7–S9, 2014.

[26] J. C. Gomez, G. S. Selvaggio, M. Viola et al., "Prevalence of celiac disease in Argentina: screening of an adult population in the La Plata area," *The American Journal of Gastroenterology*, vol. 96, no. 9, pp. 2700–2704, 2001.

[27] US Preventive Services Task Force, K. Bibbins-Domingo, D. C. Grossman et al., "Screening for celiac disease: US Preventive Services Task Force recommendation statement," *JAMA*, vol. 317, no. 12, p. 1252, 2017.

Hindawi
Gastroenterology Research and Practice
Volume 2019, Article ID 2916024, 10 pages
https://doi.org/10.1155/2019/2916024

Review Article

Diagnosing Celiac Disease: Towards Wide-Scale Screening and Serology-Based Criteria?

Alina Popp,[1,2] Laura Kivelä,[1,3] Valma Fuchs,[4] and Kalle Kurppa[1,5]

[1]*Center for Child Health Research, Tampere University and Department of Paediatrics, Tampere University Hospital, Tampere, Finland*
[2]*Institute for Mother and Child Health Bucharest, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania*
[3]*University of Helsinki and Helsinki University Hospital, Children's Hospital and Pediatric Research Center, Helsinki, Finland*
[4]*Celiac Disease Research Center, Tampere University, Tampere, Finland*
[5]*The University Consortium of Seinäjoki, Seinäjoki, Finland*

Correspondence should be addressed to Laura Kivelä; laura.kivela@fimnet.fi

Received 18 May 2019; Accepted 16 July 2019; Published 6 August 2019

Academic Editor: Walter Fries

Celiac disease is one of the most common food-related chronic disorders in children. Unfortunately, this multifaceted disease is challenging to recognize and remains markedly underdiagnosed. Screening of either known at-risk groups or even the whole population could increase the suboptimal diagnostic yield substantially. Many recent guidelines recommend screening of at least selected risk groups, but more wide-scale screening remains controversial. The increasing prevalence of celiac disease and the development of autoantibody assays have also led to a gradual shift in the diagnostics towards less invasive serology-based criteria in a subgroup of symptomatic children. The main open questions concern whether these criteria are applicable to all countries and clinical settings, as well as to adult patients. On the other hand, widening screening and the mistaken practice of initiating a gluten-free diet before the appropriate exclusion of celiac disease increase the number of borderline seropositive cases, which may also challenge the classical histopathological diagnostics. Sophisticated diagnostic methods and a deeper understanding of the natural history of early developing celiac disease may prove useful in these circumstances.

1. Introduction

With a prevalence of up to 1–3%, celiac disease is one of the most common chronic gastrointestinal diseases [1–3]. It is evident that the diagnostics of such a frequent condition should be effective and practical. Unfortunately, the heterogeneous clinical presentation makes the disease difficult to recognize, and currently the great majority of affected individuals remain undiagnosed, leaving them vulnerable to long-term complications [3, 4]. The most effective means of improving the diagnostic yield would be to screen known at-risk groups or even the whole population. The development of advanced serological tests has made screening rather straightforward, but the overall benefits of this approach remain a matter of debate [5]. Particularly controversial issues are the treatment of asymptomatic screen-detected individuals, the optimal age for rescreening, the optimal rescreening frequency, and the utilization of genetic testing to further delineate the susceptible cohort.

Traditionally, the diagnosis of celiac disease has been based on the demonstration of mucosal injury in duodenal biopsy. This invasive approach has been considered necessary to ensure the diagnosis before starting a demanding gluten-free diet. However, the high specificity of modern serological tests and the desire to reduce the need for invasive investigations led to the release of new criteria by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) in 2012, which allow for the first time a noninvasive approach to diagnosis in a subgroup of children [6]. Although a huge leap forward, these guidelines paradoxically created new challenges, as they are currently not accepted in all countries and were not drawn

Table 1: True prevalence of celiac disease based on screening studies and the proportion of clinically unrecognized patients.

Reference and year	Country	Diagnostic criteria	Prevalence (%)	Unrecognized (%)
Children				
Mäki et al., 2003 [122]	Finland	Biopsy	1.1	75.9
Tommasini et al., 2004 [123]	Italy	Biopsy	1.1	94.5
Myléus et al., 2009 [2]	Sweden	Biopsy	2.9	69.3
Mustalahti et al., 2010 [124]	UK	Seropositivity[a] or biopsy	0.9	94.4
Laass et al., 2015 [125]	Germany	Seropositivity[a]	0.8	91.7
Adults				
West et al., 2003 [126]	UK	Seropositivity[a]	1.2	95.7
Lohi et al., 2007 [1]	Finland	Seropositivity[a]	2.0	74.9
Mustalahti et al., 2010 [124]	Germany	Seropositivity[a] or biopsy	0.3	93.3
Mustalahti et al., 2010 [124]	Italy	Seropositivity[a] or biopsy	0.7	97.1
Rubio-Tapia et al., 2012 [127]	USA	Seropositivity[a]	0.7	90.1
Fukunaga et al., 2018 [128]	Japan	Biopsy	0.1	100

[a]Positive tissue transglutaminase and/or endomysial antibodies.

up for adults [7, 8]. Furthermore, even if the novel approach was adopted more widely, biopsy would still be needed in individuals with low positive serology, which are often diagnostically the most problematic cases. In fact, the number of such individuals is likely increasing due to more active screening.

In this review, we provide an overview of the current concepts of the diagnostics of celiac disease in children and adults. The main topics discussed are the possibilities for improving the suboptimal diagnostic yield and efforts to provide more unified diagnostic guidelines in the light of the most recent scientific evidence. Furthermore, we discuss the future directions in diagnostics, particularly concerning early developing celiac disease with minor or no histopathological changes and otherwise challenging cases.

2. Diagnostic Approach: From Case Finding towards Screening

The phenotype of celiac disease extends from varying gastrointestinal and extraintestinal complaints to an apparent lack of symptoms [9]. This variation makes recognition of the disease challenging, and currently the majority of affected children and adults remain undiagnosed (Table 1). The main approaches to detect untreated celiac disease are active case finding based on clinical symptoms and signs and targeted screening of at-risk groups, such as the relatives of celiac disease patients and subjects with certain other autoimmune diseases. However, there are major differences in the diagnostic approach between and even within countries, and this is also reflected in the inconsistencies between the true population-based prevalence of celiac disease and the number of actually diagnosed patients (Table 1).

2.1. Case Finding. Case finding is, in theory, an effective approach to find at least those patients with a characteristic clinical presentation. However, only those who seek medical help because of their symptoms or other clinical signs can be found, which requires activity from the patients themselves. Furthermore, medical practitioners should be alert to the possibility of celiac disease behind the various complaints they encounter in daily practice. Unfortunately, this seems to be very challenging in the case of celiac disease. It has been observed that up to 85% of patients eventually found by screening have suffered from unrecognized symptoms for some time—even for several years—before the diagnosis [10–14]. The situation is further complicated by the low predictive value of even "typical" gastrointestinal symptoms for celiac disease [15, 16].

2.2. Screening: Current Approaches and Open Questions. There is a clear need for more effective diagnostic approaches rather than relying on ineffective case finding. The development of practical serological tests in recent decades has enabled easier noninvasive screening, but the matter of who should be screened is all but clear [5, 17]. Celiac disease fulfills most of the World Health Organization's general criteria for screening, but further studies are needed, particularly regarding the cost-effectiveness of screening and the natural history of clinically unrecognized patients [17]. The main issue is whether the benefits of an early diagnosis overcome the costs, laboriousness, and social burden of a gluten-free diet [5].

One argument against screening is the low risk for complications in unrecognized celiac disease patients. However, as already mentioned, many screening-detected subjects actually suffer from unrecognized symptoms. Moreover, even truly asymptomatic patients might be at risk for ill-health and long-term complications if left untreated [14, 18–22]. Particularly in children, many complications—such as dental enamel defects, poor height gain, and reduced bone accrual—may remain permanent if not detected early enough [23–25]. Furthermore, if the disease remains untreated until adulthood, there is an increased risk, e.g., for infertility, refractory celiac disease, and even small-bowel lymphoma [26, 27]. Delayed diagnosis may also predispose to reduced quality of life, the incremental use of medicines, and persistent symptoms even on a gluten-free diet [28, 29].

Table 2: Recommendations on screening for celiac disease according to the most recent diagnostic guidelines.

Reference	Organization	Age group	Screening recommendation
Downey et al., 2015 [66]	NICE	Children and adults	T1D, autoimmune thyroidal disease, and family risk
Ludvigsson et al., 2014 [8]	BSG	Adults	T1D, irritable bowel syndrome, Down syndrome, and family risk
Rubio-Tapia et al., 2013 [69]	ACG	Children and adults	Symptomatic T1D and family risk
Husby et al., 2012 [6]	ESPGHAN	Children	T1D, autoimmune thyroidal and liver diseases, IgA deficiency, family risk, and Down, Turner, and Williams syndromes
Hill et al., 2005 [7]	NASPGHAN	Children	T1D, autoimmune thyroidal and liver diseases, family risk, and Down, Turner, and Williams syndromes

ACG: American College of Gastroenterology; BSG: British Society of Gastroenterology; ESPGHAN: European Society for Paediatric Gastroenterology, Hepatology, and Nutrition; NASPGHAN: North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition; NICE: The National Institute for Health and Care Excellence; T1D: type 1 diabetes.

A critical aspect regarding the rationale of screening is the patient's willingness to adhere to a life-long treatment. There has been concern that asymptomatic patients in particular would have poor motivation to maintain the diet with no apparent clinical benefits. Reported dietary adherence in screen-detected patients has varied from 23% to 93% [12, 14, 30–36]. In more recent studies, the general tendency has been better adherence, possibly reflecting the increasing knowledge and better availability of gluten-free products in grocery stores and restaurants [31, 37–41]. In fact, nowadays even asymptomatic screen-detected patients may show excellent dietary adherence [14, 42].

The most recent guidelines recommend screening for celiac disease in selected at-risk individuals (Table 2), although the actual implementation of the screening in clinical practice varies [43]. However, even with targeted screening, a substantial percentage of affected individuals will remain unrecognized [44]. The only option to find almost all celiac disease patients would be to screen the entire population or, alternatively, subjects with a genetic predisposition. This approach would still leave the question of the frequency of repeated screening after negative results, as celiac disease may develop at any age [2, 45]. Furthermore, wide-scale screening would likely reveal a significant number of seropositive subjects who do not fulfill the current diagnostic criteria. At present, the prognosis and benefits of the early diagnosis and treatment of such cases are poorly known [46–51].

3. Changing Guidelines and Development of Diagnostic Tools

3.1. Histology and Serological Tests. During the past 70 years, the diagnostics of celiac disease has evolved from symptom-based deduction to the use of sophisticated serological and histological methods. The development of biopsy techniques, followed by the description of duodenal injury, have been critical milestones [52, 53]. Before the 1970s, histology was the only diagnostic method in all age groups [54]. A less invasive approach for case finding could be attained by using serum antibodies, the first of which were antigliadin antibodies with moderate sensitivity and specificity [55, 56]. More specific autoantibodies to reticulin and—particularly from the 1980s—endomysial antibodies (EmA) proved to be valuable tools for initial screening [57–59]. The identification of transglutaminase 2 as the autoantigen recognized by EmA [60] enabled practical ELISA tests for the detection of transglutaminase 2 antibodies (TG2ab) [61].

There have also been improvements in the histopathological assessment. The original biopsy capsule was gradually replaced by endoscopic duodenal sampling. In 1992, Marsh introduced the now widely used grouped classification for histological injury [62], and a modified version of this grading was later advocated by Oberhuber [63]. For these classifications, the histological injury is divided for practical purposes into three classes: infiltrative (Marsh 1), hyperplastic (Marsh 2), and atrophic (Marsh 3) lesions. In the Oberhuber classification, stage 3 is further divided into subclasses 3a, 3b, and 3c. The more quantitative assessment of the mucosal damage using villous-height crypt depth measurement was introduced in the early 1980s and later further improved [64, 65]. At present, however, this methodology is used mostly in research settings.

3.2. Evolving Guidelines towards a Less Invasive Diagnostic Approach. With some modifications, the ESPGHAN 1990 criteria for celiac disease remained the basis of practically all pediatric and adult diagnostic guidelines until 2012 [6–8, 66–70]. Demonstration of the characteristic histological lesion, followed by the resolution of symptoms on a gluten-free diet, allowed the establishment of the diagnosis, with positive serology giving further support to the diagnosis [67]. In the early 2000s, the testing of TG2ab came to the forefront in initial case screenings in both children and adults, although histological confirmation was still required [7, 71]. Groups at risk for celiac disease were also increasingly recognized, and their low-threshold screening was recommended.

In 2012, the new EPSGHAN diagnostic criteria were launched [6]. The main driving forces for the revision were the necessity for general anesthesia for invasive endoscopy in children and the excellent positive predictive value of modern serological tests, particularly high tTGab values (Table 3) and positive EmA. For the first time, the revolutionary guidelines allowed diagnosis without biopsy in specific circumstances; i.e., for symptomatic children with tTGab values $> 10\times$ the upper limit of normal (ULN), positive EmA, and the presence of the at-risk human leucocyte antigen (HLA) DQ2/DQ8 haplotype [6]. Recent prospective studies have provided strong support for the accuracy of

Table 3: Studies assessing the positive predictive value (PPV) of high tissue transglutaminase antibody (tTGab) values in the diagnosis of celiac disease.

Reference	Cohort	Country	tTGab threshold	Number of tested assays	PPV (%)
Children					
Paul et al., 2018 [76]	157	UK	10x ULN	1[b]	100
Werkstetter et al., 2017 [72]	707	Multicenter	10x ULN	8	99.6–100[c]
Wolf et al., 2017 [73]	898	Germany	10x ULN	1	98.8
Smarrazzo et al., 2017 [129]	1,974	Multicenter	10x ULN	8	96.1
Elitsur et al., 2017 [88]	240	USA	10x ULN	1[b]	87.7
Trovato et al., 2015 [75]	286	Italy	10x ULN	1	91.0–92.5
Gidrewicz et al., 2015 [130]	17,505	Canada	10x ULN	1	92.8
Adults					
Efthymakis et al., 2017 [93]	234	Italy	10x ULN	2	97.6
Ganji et al., 2016 [131]	299[a]	Iran	10x ULN	1	100
Tortora et al., 2014 [101]	310	Italy	8.9x ULN	1	100

[a]Adults and adolescents. [b]tTGab assay was not specified. [c]Lowest obtained specificity when testing different diagnostic scenarios and excluding inconclusive patients. ULN: upper limit of normal; ND: no data.

these criteria [72, 73]. There is growing evidence that the nonbiopsy diagnostic approach could be applied reliably also for asymptomatic children [74–76] and without mandatory genetic testing [72]. In adults, histological evaluation has remained the cornerstone of the diagnosis, excluding the recently published Finnish guidelines that allow a nonbiopsy approach in some patients, regardless of their age [68].

4. Challenges with the Diagnostic Criteria and Future Directions

4.1. Technical Challenges. A major challenge in the current diagnostics is the lack of standardization in tTGab kits. This is particularly problematic for the nonbiopsy criteria, as the resulting incomparability between the assays may even predispose to misinterpretations [77, 78]. In order to err on the side of safety, ESPGHAN recommends using only tTGab tests with an appropriate calibration curve [6]. Furthermore, the rather high ULN cutoff value and the requirement of HLA and EmA testing were included partly to control the assay variation. As mentioned, it might be possible to omit HLA testing in the future [72], and the role of EmA could also be questioned. Although EmA is highly specific, the required immunofluorescence method is laborious and not universally available. By applying well-validated tTGab assays, it might be possible to abandon EmA and also lower the diagnostic ULN threshold [72, 79].

Histopathology might not be as good a diagnostic reference standard as previously thought. The mucosal lesion can be patchy, the quality of the biopsies is often inadequate, and duodenal injury is not fully specific to celiac disease [80, 81]. In order to improve the diagnostic yield, the current recommendation is to take at least four biopsies from the distal duodenum and one from the bulb [6, 66, 82, 83]. However, due to the lower specificity, the added value of the duodenal bulb biopsy is controversial, and caution is needed when a diagnosis of celiac disease is based solely on bulb samples [84]. In addition, even if representative biopsies are obtained, their correct handling and orientation are often challenging and prone to mistakes [65]. Accordingly, several studies have shown poor intra- and interobserver agreement between pathologists when applying a grouped histological classification [85–87].

4.2. Lack of Unified Guidelines. One of the main challenges with the current diagnostic criteria is their age- and country-related variation [6, 8, 69]. Despite the aforementioned problems, duodenal histopathology as the gold standard used to be the unifying feature of all the guidelines [67]. This changed radically when the ESPGHAN criteria introduced the possibility of omitting endoscopy for some European children [6], while the biopsy remains mandatory, e.g., in the USA [7]. These discrepancies might be explained to some extent by the different health care systems [88]. In addition, most of the studies on this issue have been made in Europe and, for unclear reasons, studies from North America have reported the inferior accuracy of tTGab tests (Table 3). As it is unlikely that children differ significantly between the continents, and since joint guidelines exist for many other diseases [89, 90], unified criteria for celiac disease would seem reasonable.

Another issue is the acceptance of serology-based diagnoses of celiac disease in adults by physicians. Only one of the current guidelines makes a clear statement on this issue; it does not support the taking of routine duodenal biopsies to reconfirm the diagnosis in adults when the diagnosis has been set strictly according to the ESPGHAN criteria [91]. This issue is particularly important in the transitional period from childhood to adulthood, when some young patients and/or their physicians may question the initial diagnosis [92]. To avoid confusion and the unnecessary repetition of diagnostic procedures, general acceptance—or preferably unified adult and pediatric criteria—is important.

Recent studies have given evidence that the nonbiopsy criteria would apply also to adults [79, 93, 94], but many experts remain cautious [95, 96]. One fear is the misuse of

the criteria by general practitioners [96–98]. However, there is evidence that accurate diagnostics can be achieved by education and close collaboration with primary care [94, 99]. Another feared consequence of omitting endoscopies is missing a coexisting disease or complication, such as refractory celiac disease or malignancy [93, 96]. In practice, however, this does not seem to be a major problem, although more evidence is called for [79, 93, 100, 101]. In general, the new guidelines do not aim to ban biopsies, but rather to offer the option for diagnosis without endoscopy in definite cases [6]. Endoscopy would still be preferable if red flag symptoms such as bloody stools, dysphagia, or severe weight loss appear, or if there is incomplete clinical recovery [6, 79, 101].

4.3. Challenging Diagnostic Scenarios. Despite the tendency towards less invasive approaches, duodenal biopsy will likely remain a part of celiac disease diagnostics for quite some time. The main problem with serology is that the specificity decreases with lower antibody values [72, 79]. Unfortunately, such patients are usually also histologically the most problematic cases, as they may present only with mild or patchy duodenal changes [46, 80]. In these circumstances, it is important to confirm that all stages of duodenal sampling and histological analysis have been done correctly [65, 102]. The more quantitative measurement of architectural changes, e.g., by applying validated duodenal histomorphometry, might also prove useful [65].

The widening use of screening can be expected to increase the number of patients detected with early stage celiac disease and morphologically normal villi [46, 49, 103]. There is evidence that seropositive individuals may suffer from symptoms and signs already at this point and benefit from a gluten-free diet [46, 49, 50, 104], indicating that the whole definition of celiac disease might require reevaluation. Nevertheless, many such individuals are asymptomatic and do not develop duodenal lesions even during a long-term follow-up [49, 105–107]. It is essential to learn more about the natural history of early developing celiac disease in order to discern cases that would truly benefit from early diagnosis [108].

Another challenge in the differential diagnosis of patients with borderline or negative serology is brought by the now common practice of initiating a gluten-free diet before appropriate diagnostic investigations [109, 110]. It still might be possible to establish the diagnosis using sophisticated techniques, e.g., determination of small-bowel mucosal $\gamma\delta$+intraepithelial lymphocytes and celiac disease-specific tTG-targeted IgA deposits [46, 111–114]. Genetic testing and recently introduced innovative methods, such as HLA-DQ-gluten tetramer-based assays, might further help to exclude or confirm the presence of celiac disease [115].

4.4. Prevention of Celiac Disease? In the future, it might even be possible to proceed a step further, as several ongoing prospective birth cohort studies are steadily providing a deeper understanding of the early development of celiac disease [116–120]. Increasing information about the disturbed balance of genetics and environmental factors in celiac disease might offer possibilities for the early detection of high-risk children, and perhaps even provide means for primary prevention [116–119, 121].

5. Conclusions

Owing to the high prevalence of celiac disease, even minor changes in the diagnostic approach may have substantial effects on health care and society. It is evident that the only effective way to improve the currently unsatisfactory diagnostic yield is more widespread screening. Such an approach could be expected to prevent ill-health and severe complications in the long run, but it must be backed up with high-quality scientific evidence. Effective implementation of intensified case finding and screening also requires close collaboration with primary care and general practitioners, who are responsible for the first-line diagnostics.

Simultaneously with the increasing prevalence, the diagnostic criteria of celiac disease are currently undergoing revolutionary changes. At present, the serology-based diagnosis is limited to a minority of patients—i.e., mainly to symptomatic European children. This may cause problems, e.g., in the acceptance of the diagnosis in different countries and after the transition from pediatric to adult care. Since there is no apparent biological reason for the age- and site-related differences in the criteria, it would be desirable for more unified evidence-based global guidelines for celiac disease to be formed.

Notwithstanding the increasing tendency towards non-invasive diagnostics, biopsy will likely play an important role also in the future, particularly in individuals with low and/or borderline positive serology. In fact, the number of these cases will likely increase significantly concurrently with the widening screening and earlier testing. Novel sophisticated diagnostic tools may offer better possibilities for differential diagnosis in these often challenging situations. Open questions and issues remain concerning the natural history of these often asymptomatic individuals, particularly whether they should be diagnosed and treated with a gluten-free diet.

Abbreviations

EmA: Endomysial antibodies
ESPGHAN: European Society for Paediatric Gastroenterology, Hepatology, and Nutrition
HLA: Human leucocyte antigen
TG2ab: Transglutaminase 2 antibodies
ULN: Upper limit of normal.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was supported by the Competitive State Research Financing of Tampere University Hospital and the

Foundation for Paediatric Research, the Maire Rossi Foundation, the Maud Kuistila Foundation, the Mary and Georg Ehrnrooth Foundation, the Paolo Foundation, and the Emil Aaltonen Foundation.

References

[1] S. Lohi, K. Mustalahti, K. Kaukinen et al., "Increasing prevalence of coeliac disease over time," *Alimentary Pharmacology & Therapeutics*, vol. 26, no. 9, pp. 1217–1225, 2007.

[2] A. Myléus, A. Ivarsson, C. Webb et al., "Celiac disease revealed in 3% of Swedish 12-year-olds born during an epidemic," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 49, no. 2, pp. 170–176, 2009.

[3] P. Singh, A. Arora, T. A. Strand et al., "Global prevalence of celiac disease: systematic review and meta-analysis," *Clinical Gastroenterology and Hepatology*, vol. 16, no. 6, pp. 823–836.e2, 2018.

[4] P. Laurikka, S. Nurminen, L. Kivelä, and K. Kurppa, "Extraintestinal manifestations of celiac disease: early detection for better long-term outcomes," *Nutrients*, vol. 10, no. 8, p. 1015, 2018.

[5] R. Chou, C. Bougatsos, I. Blazina, K. Mackey, S. Grusing, and S. Selph, "Screening for celiac disease: evidence report and systematic review for the US Preventive Services Task Force," *Journal of the American Medical Association*, vol. 317, no. 12, pp. 1258–1268, 2017.

[6] S. Husby, S. Koletzko, I. R. Korponay-Szabó et al., "European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 54, no. 1, pp. 136–160, 2012.

[7] I. D. Hill, M. H. Dirks, G. S. Liptak et al., "Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 40, no. 1, pp. 1–19, 2005.

[8] J. F. Ludvigsson, J. C. Bai, F. Biagi et al., "Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology," *Gut*, vol. 63, no. 8, pp. 1210–1228, 2014.

[9] L. Kivelä, K. Kaukinen, M.-L. Lähdeaho et al., "Presentation of celiac disease in Finnish children is no longer changing: a 50-year perspective," *The Journal of Pediatrics*, vol. 167, no. 5, pp. 1109–1115.e1, 2015.

[10] D. Hansen, B. Brock-Jacobsen, E. Lund et al., "Clinical benefit of a gluten-free diet in type 1 diabetic children with screening-detected celiac disease: a population-based screening study with 2 years' follow-up," *Diabetes Care*, vol. 29, no. 11, pp. 2452–2456, 2006.

[11] E. J. van Koppen, J. J. Schweizer, C. G. D. S. Csizmadia et al., "Long-term health and quality-of-life consequences of mass screening for childhood celiac disease: a 10-year follow-up study," *Pediatrics*, vol. 123, no. 4, pp. e582–e588, 2009.

[12] S. Kinos, K. Kurppa, A. Ukkola et al., "Burden of illness in screen-detected children with celiac disease and their families," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 55, no. 4, pp. 412–416, 2012.

[13] M. Jansen, M. van Zelm, M. Groeneweg et al., "The identification of celiac disease in asymptomatic children: the Generation R Study," *Journal of Gastroenterology*, vol. 53, no. 3, pp. 377–386, 2017.

[14] L. Kivelä, K. Kaukinen, H. Huhtala, M.-L. Lähdeaho, M. Mäki, and K. Kurppa, "At-risk screened children with celiac disease are comparable in disease severity and dietary adherence to those found because of clinical suspicion: a large cohort study," *The Journal of Pediatrics*, vol. 183, pp. 115–121.e2, 2017.

[15] K. D. Katz, S. Rashtak, B. D. Lahr et al., "Screening for celiac disease in a North American population: sequential serology and gastrointestinal symptoms," *The American Journal of Gastroenterology*, vol. 106, no. 7, pp. 1333–1339, 2011.

[16] A. Rosén, O. Sandström, A. Carlsson et al., "Usefulness of symptoms to screen for celiac disease," *Pediatrics*, vol. 133, no. 2, pp. 211–218, 2014.

[17] J. F. Ludvigsson, T. R. Card, K. Kaukinen et al., "Screening for celiac disease in the general population and in high-risk groups," *United European Gastroenterology Journal*, vol. 3, no. 2, pp. 106–120, 2015.

[18] M. A. Verkasalo, O. T. Raitakari, J. Viikari, J. Marniemi, and E. Savilahti, "Undiagnosed silent coeliac disease: a risk for underachievement?," *Scandinavian Journal of Gastroenterology*, vol. 40, no. 12, pp. 1407–1412, 2005.

[19] I. R. Korponay-Szabó, K. Szabados, J. Pusztai et al., "Population screening for coeliac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study," *BMJ*, vol. 335, no. 7632, pp. 1244–1247, 2007.

[20] J. Turner, G. Pellerin, and D. Mager, "Prevalence of metabolic bone disease in children with celiac disease is independent of symptoms at diagnosis," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 49, no. 5, pp. 589–593, 2009.

[21] S. Nurminen, L. Kivelä, J. Taavela et al., "Factors associated with growth disturbance at celiac disease diagnosis in children: a retrospective cohort study," *BMC Gastroenterology*, vol. 15, no. 1, p. 125, 2015.

[22] S. Björck, C. Brundin, M. Karlsson, and D. Agardh, "Reduced bone mineral density in children with screening-detected celiac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 65, no. 5, pp. 526–532, 2017.

[23] A. G. Kalayci, A. Kansu, N. Girgin, O. Kucuk, and G. Aras, "Bone mineral density and importance of a gluten-free diet in patients with celiac disease in childhood," *Pediatrics*, vol. 108, no. 5, article E89, 2001.

[24] B. Weiss, Y. Skourikhin, D. Modan-Moses, E. Broide, A. Fradkin, and Y. Bujanover, "Is adult height of patients with celiac disease influenced by delayed diagnosis," *The American Journal of Gastroenterology*, vol. 103, no. 7, pp. 1770–1774, 2008.

[25] J. Cheng, T. Malahias, P. Brar, M. T. Minaya, and P. H. R. Green, "The association between celiac disease, dental enamel defects, and aphthous ulcers in a United States cohort," *Journal of Clinical Gastroenterology*, vol. 44, no. 3, pp. 191–194, 2010.

[26] G. Bona, D. Marinello, and G. Oderda, "Mechanisms of abnormal puberty in coeliac disease," *Hormone Research in Pædiatrics*, vol. 57, no. 2, pp. 63–65, 2002.

[27] T. Ilus, K. Kaukinen, L. J. Virta, E. Pukkala, and P. Collin, "Incidence of malignancies in diagnosed celiac patients: a population-based estimate," *The American Journal of Gastroenterology*, vol. 109, no. 9, pp. 1471–1477, 2014.

[28] P. Paarlahti, K. Kurppa, A. Ukkola et al., "Predictors of persistent symptoms and reduced quality of life in treated coeliac disease patients: a large cross-sectional study," *BMC Gastroenterology*, vol. 13, no. 1, p. 75, 2013.

[29] V. Fuchs, K. Kurppa, H. Huhtala, P. Collin, M. Mäki, and K. Kaukinen, "Factors associated with long diagnostic delay in celiac disease," *Scandinavian Journal of Gastroenterology*, vol. 49, no. 11, pp. 1304–1310, 2014.

[30] E. Fabiani, L. Taccari, I.-M. Rätsch, S. Di Giuseppe, G. Coppa, and C. Catassi, "Compliance with gluten-free diet in adolescents with screening-detected celiac disease: a 5-year follow-up study," *The Journal of Pediatrics*, vol. 136, no. 6, pp. 841–843, 2000.

[31] E. Roma, A. Roubani, E. Kolia, J. Panayiotou, A. Zellos, and V. P. Syriopoulou, "Dietary compliance and life style of children with coeliac disease," *Journal of Human Nutrition and Dietetics*, vol. 23, no. 2, pp. 176–182, 2010.

[32] A. Paavola, K. Kurppa, A. Ukkola et al., "Gastrointestinal symptoms and quality of life in screen-detected celiac disease," *Digestive and Liver Disease*, vol. 44, no. 10, pp. 814–818, 2012.

[33] C. Webb, A. Myléus, F. Norström et al., "High adherence to a gluten-free diet in adolescents with screening-detected celiac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 60, no. 1, pp. 54–59, 2015.

[34] E. Altobelli, R. Paduano, T. Gentile et al., "Health-related quality of life in children and adolescents with celiac disease: survey of a population from Central Italy," *Health and Quality of Life Outcomes*, vol. 11, no. 1, p. 204, 2013.

[35] S. Mahadev, R. Gardner, S. K. Lewis, B. Lebwohl, and P. H. Green, "Quality of life in screen-detected celiac disease patients in the United States," *Journal of Clinical Gastroenterology*, vol. 50, no. 5, pp. 1–397, 2016.

[36] J. B. Torres, E. Román, M. Cilleruelo, M. Márquez, M. Mearin, and C. Fernández, "Health-related quality of life in Spanish children with coeliac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 62, no. 4, pp. 603–608, 2016.

[37] O. Jadrešin, Z. Mišak, K. Sanja, Z. Sonicki, and V. Žižić, "Compliance with gluten-free diet in children with coeliac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 47, no. 3, pp. 344–348, 2008.

[38] G. Wagner, G. Berger, U. Sinnreich et al., "Quality of life in adolescents with treated coeliac disease: influence of compliance and age at diagnosis," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 47, no. 5, pp. 555–561, 2008.

[39] S. Errichiello, O. Esposito, R. Di Mase et al., "Celiac disease: predictors of compliance with a gluten-free diet in adolescents and young adults," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 50, no. 1, pp. 54–60, 2010.

[40] K. Kurppa, O. Lauronen, P. Collin et al., "Factors associated with dietary adherence in celiac disease: a nationwide study," *Digestion*, vol. 86, no. 4, pp. 309–314, 2012.

[41] K. MacCulloch and M. Rashid, "Factors affecting adherence to a gluten-free diet in children with celiac disease," *Paediatrics & Child Health*, vol. 19, no. 6, pp. 305–309, 2014.

[42] K. Kurppa, A. Paavola, P. Collin et al., "Benefits of a gluten-free diet for asymptomatic patients with serologic markers of celiac disease," *Gastroenterology*, vol. 147, no. 3, pp. 610–617.e1, 2014.

[43] A. Pham-Short, K. C. Donaghue, G. Ambler, H. Phelan, S. Twigg, and M. E. Craig, "Screening for celiac disease in type 1 diabetes: a systematic review," *Pediatrics*, vol. 136, no. 1, pp. e170–e176, 2015.

[44] C. Catassi and A. Fasano, "Coeliac disease. The debate on coeliac disease screening—are we there yet?," *Nature Reviews Gastroenterology & Hepatology*, vol. 11, no. 8, pp. 457-458, 2014.

[45] A. Vilppula, K. Kaukinen, L. Luostarinen et al., "Increasing prevalence and high incidence of celiac disease in elderly people: a population-based study," *BMC Gastroenterology*, vol. 9, no. 1, article 49, 2009.

[46] K. Kurppa, P. Collin, M. Viljamaa et al., "Diagnosing mild enteropathy celiac disease: a randomized, controlled clinical study," *Gastroenterology*, vol. 136, no. 3, pp. 816–823, 2009.

[47] R. Auricchio, A. Tosco, E. Piccolo et al., "Potential celiac children: 9-year follow-up on a gluten-containing diet," *American Journal of Gastroenterology*, vol. 109, no. 6, pp. 913–921, 2014.

[48] S. L. Vriezinga, R. Auricchio, E. Bravi et al., "Randomized feeding intervention in infants at high risk for celiac disease," *The New England Journal of Medicine*, vol. 371, no. 14, pp. 1304–1315, 2014.

[49] U. Volta, G. Caio, F. Giancola et al., "Features and progression of potential celiac disease in adults," *Clinical Gastroenterology and Hepatology*, vol. 14, no. 5, pp. 686–693.e1, 2016.

[50] R. Mandile, V. Discepolo, S. Scapaticci et al., "The effect of gluten-free diet on clinical symptoms and the intestinal mucosa of patients with potential celiac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 66, no. 4, pp. 654–656, 2018.

[51] K. Kurppa, T. Räsänen, P. Collin et al., "Endomysial antibodies predict celiac disease irrespective of the titers or clinical presentation," *World Journal of Gastroenterology*, vol. 18, no. 20, pp. 2511–2516, 2012.

[52] M. Shiner, "Duodenal biopsy," *The Lancet*, vol. 267, no. 6906, pp. 17–19, 1956.

[53] J. Sakula and M. Shiner, "Coeliac disease with atrophy of the small-intestine mucosa," *The Lancet*, vol. 270, no. 7001, pp. 876-877, 1957.

[54] H. A. Weijers, B. Lindquist, C. H. M. Anderson et al., "Diagnostic criteria in coeliac disease," *Acta Paediatrica Scandinavica*, vol. 59, p. 461, 1970.

[55] U. Volta, G. R. Corazza, M. Frisoni et al., "IgA antigliadin antibodies and persistence of jejunal lesions in adult coeliac disease," *Digestion*, vol. 47, no. 2, pp. 111–114, 1990.

[56] M. Mäki, O. Hällstrom, and A. Marttinen, "Reaction of human non-collagenous polypeptides with coeliac disease autoantibodies," *The Lancet*, vol. 338, no. 8769, pp. 724-725, 1991.

[57] P. P. Seah, L. Fry, M. A. Rossiter, A. V. Hoffbrand, and E. J. Holborow, "Anti-reticulin antibodies in childhood cœliac disease," *The Lancet*, vol. 2, no. 7726, pp. 681-682, 1971.

[58] T. P. Chorzelski, E. H. Beutner, J. Sulej et al., "IgA anti-endomysium antibody. A new immunological marker of dermatitis herpetiformis and coeliac disease," *British Journal of Dermatology*, vol. 111, no. 4, pp. 395–402, 1984.

[59] B. Ladinser, E. Rossipal, and K. Pittschieler, "Endomysium antibodies in coeliac disease: an improved method," *Gut*, vol. 35, no. 6, pp. 776–778, 1994.

[60] W. Dieterich, T. Ehnis, M. Bauer et al., "Identification of tissue transglutaminase as the autoantigen of celiac disease," *Nature Medicine*, vol. 3, no. 7, pp. 797–801, 1997.

[61] S. Sulkanen, T. Halttunen, K. Laurila et al., "Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease," *Gastroenterology*, vol. 115, no. 6, pp. 1322–1328, 1998.

[62] M. N. Marsh, "Gluten, major histocompatibility complex, and the small intestine: a molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue')," *Gastroenterology*, vol. 102, no. 1, pp. 330–354, 1992.

[63] G. Oberhuber, G. Granditsch, and H. Vogelsang, "The histopathology of coeliac disease: time for a standardized report scheme for pathologists," *European Journal of Gastroenterology & Hepatology*, vol. 11, no. 10, pp. 1185–1194, 1999.

[64] P. Kuitunen, I. Kosnai, and E. Savilahti, "Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 1, no. 4, pp. 525–532, 1982.

[65] J. Taavela, O. Koskinen, H. Huhtala et al., "Validation of morphometric analyses of small-intestinal biopsy readouts in celiac disease," *PLoS One*, vol. 8, no. 10, article e76163, 2013.

[66] L. Downey, R. Houten, S. Murch, and D. Longson, "Recognition, assessment, and management of coeliac disease: summary of updated NICE guidance," *BMJ*, vol. 351, no. article h4513, 2015.

[67] J. A. Walker-Smith, S. Guandalini, J. Schmitz, D. H. Shmerling, and J. K. Visakorpi, "Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition," *Archives of Disease in Childhood*, vol. 65, no. 8, pp. 909–911, 1990.

[68] Working group set up by the Finnish Medical Society Duodecim and the Finnish Gastroenterology Society, "Celiac disease. Current Care Guidelines, 2018," March 2019, http://www.kaypahoito.fi/web/kh/suositukset/suositus?id=hoi08001#K1.

[69] A. Rubio-Tapia, I. D. Hill, C. P. Kelly, A. H. Calderwood, J. A. Murray, and American College of Gastroenterology, "ACG clinical guidelines: diagnosis and management of celiac disease," *American Journal of Gastroenterology*, vol. 108, no. 5, pp. 656–676, 2013.

[70] J. Bai and C. Ciacci, "World Gastroenterology Organisation global guidelines: celiac disease February 2017," *Journal of Clinical Gastroenterology*, vol. 51, no. 9, pp. 755–768, 2017.

[71] A. Rostom, J. A. Murray, and M. F. Kagnoff, "American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease," *Gastroenterology*, vol. 131, no. 6, pp. 1981–2002, 2006.

[72] K. J. Werkstetter, I. R. Korponay-Szabó, A. Popp et al., "Accuracy in diagnosis of celiac disease without biopsies in clinical practice," *Gastroenterology*, vol. 153, no. 4, pp. 924–935, 2017.

[73] J. Wolf, D. Petroff, T. Richter et al., "Validation of antibody-based strategies for diagnosis of pediatric celiac disease without biopsy," *Gastroenterology*, vol. 153, no. 2, pp. 410–419.e17, 2017.

[74] A. Popp, M. Mihu, M. Munteanu et al., "Prospective antibody case finding of coeliac disease in type 1 diabetes children: need of biopsy revisited," *Acta Paediatrica*, vol. 102, no. 3, pp. e102–e106, 2013.

[75] C. M. Trovato, M. Montuori, C. Anania et al., "Are ESPGHAN "biopsy-sparing" guidelines for celiac disease also suitable for asymptomatic patients?," *The American Journal of Gastroenterology*, vol. 110, no. 10, pp. 1485–1489, 2015.

[76] S. P. Paul, B. K. Sandhu, C. H. Spray, D. Basude, and P. Ramani, "Evidence supporting serology-based pathway for diagnosing celiac disease in asymptomatic children from high-risk groups," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 66, no. 4, pp. 641–644, 2018.

[77] W. Egner, A. Shrimpton, R. Sargur, D. Patel, and K. Swallow, "ESPGHAN guidance on coeliac disease 2012: multiples of ULN for decision making do not harmonise assay performance across centres," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 55, no. 6, pp. 733–735, 2012.

[78] L. Beltran, M. Koenig, W. Egner et al., "High-titre circulating tissue transglutaminase-2 antibodies predict small bowel villous atrophy, but decision cut-off limits must be locally validated," *Clinical and Experimental Immunology*, vol. 176, no. 2, pp. 190–198, 2014.

[79] V. Fuchs, K. Kurppa, H. Huhtala et al., "Serology-based criteria for adult coeliac disease have excellent accuracy across the range of pre-test probabilities," *Alimentary Pharmacology & Therapeutics*, vol. 49, no. 3, pp. 277–284, 2019.

[80] A. Ravelli, V. Villanacci, C. Monfredini, S. Martinazzi, V. Grassi, and S. Manenti, "How patchy is patchy villous atrophy? Distribution pattern of histological lesions in the duodenum of children with celiac disease," *American Journal of Gastroenterology*, vol. 105, no. 9, pp. 2103–2110, 2010.

[81] L. Elli, F. Branchi, R. Sidhu et al., "Small bowel villous atrophy: celiac disease and beyond," *Expert Review of Gastroenterology & Hepatology*, vol. 11, no. 2, pp. 125–138, 2017.

[82] I. D. Hill, "The role of biopsies in childhood celiac disease—do we need less or more?," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 64, no. 2, pp. 173-174, 2017.

[83] S. Husby, J. A. Murray, and D. A. Katzka, "AGA clinical practice update on diagnosis and monitoring of celiac disease—changing utility of serology and histologic measures: expert review," *Gastroenterology*, vol. 156, no. 4, pp. 885–889, 2019.

[84] J. Taavela, A. Popp, I. R. Korponay-Szabo et al., "A prospective study on the usefulness of duodenal bulb biopsies in celiac disease diagnosis in children: urging caution," *American Journal of Gastroenterology*, vol. 111, no. 1, pp. 124–133, 2016.

[85] C. Arguelles-Grande, C. A. Tennyson, S. K. Lewis, P. H. R. Green, and G. Bhagat, "Variability in small bowel histopathology reporting between different pathology practice settings: impact on the diagnosis of coeliac disease," *Journal of Clinical Pathology*, vol. 65, no. 3, pp. 242–247, 2012.

[86] A. Picarelli, R. Borghini, G. Donato et al., "Weaknesses of histological analysis in celiac disease diagnosis: new possible scenarios," *Scandinavian Journal of Gastroenterology*, vol. 49, no. 11, pp. 1318–1324, 2014.

[87] C. Montén, K. Bjelkenkrantz, A. H. Gudjonsdottir et al., "Validity of histology for the diagnosis of paediatric coeliac disease: a Swedish multicentre study," *Scandinavian Journal of Gastroenterology*, vol. 51, no. 4, pp. 427–433, 2016.

[88] Y. Elitsur, T. Sigman, R. Watkins et al., "Tissue transglutaminase levels are not sufficient to diagnose celiac disease in North American practices without intestinal biopsies," *Digestive Diseases and Sciences*, vol. 62, no. 1, pp. 175–179, 2017.

[89] M. Jones, S. Koletzko, K. Goodman et al., "Joint ESPGHAN/-NASPGHAN guidelines for the management of *Helicobacter*

pylori in children and adolescents (update 2016)," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 64, no. 6, pp. 991–1003, 2017.

[90] M. M. Tabbers, C. DiLorenzo, M. Y. Berger et al., "Evaluation and treatment of functional constipation in infants and children," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 58, no. 2, pp. 265–281, 2014.

[91] J. F. Ludvigsson, L. Agreus, C. Ciacci et al., "Transition from childhood to adulthood in coeliac disease: the Prague consensus report," *Gut*, vol. 65, no. 8, pp. 1242–1251, 2016.

[92] C. O'Leary, P. Wieneke, M. Healy, C. Cronin, P. O'Regan, and F. Shanahan, "Celiac disease and the transition from childhood to adulthood: a 28-year follow-up," *The American Journal of Gastroenterology*, vol. 99, no. 12, pp. 2437–2441, 2004.

[93] K. Efthymakis, M. Serio, A. Milano et al., "Application of the biopsy-sparing ESPGHAN guidelines for celiac disease diagnosis in adults: a real-life study," *Digestive Diseases and Sciences*, vol. 62, no. 9, pp. 2433–2439, 2017.

[94] G. K. T. Holmes, J. M. Forsyth, S. Knowles, H. Seddon, P. G. Hill, and A. S. Austin, "Coeliac disease: further evidence that biopsy is not always necessary for diagnosis," *European Journal of Gastroenterology & Hepatology*, vol. 29, no. 6, pp. 640–645, 2017.

[95] M. Kurien, J. F. Ludvigsson, D. S. Sanders, and authors of the BSG guidelines, "A no biopsy strategy for adult patients with suspected coeliac disease: making the world gluten-free," *Gut*, vol. 64, no. 6, pp. 1003-1004, 2015.

[96] L. J. Marks, M. Kurien, and D. S. Sanders, "The serological diagnosis of adult coeliac disease—a cautious step forward?," *Gastroenterology and Hepatology From Bed to Bench*, vol. 11, no. 3, pp. 175–177, 2018.

[97] F. Biagi, P. I. Bianchi, J. Campanella, G. Zanellati, and G. R. Corazza, "The impact of misdiagnosing celiac disease at a referral centre," *Canadian Journal of Gastroenterology*, vol. 23, no. 8, pp. 543–545, 2009.

[98] M. Jinga, A. Popp, D. V. Balaban, C. Jurcut, and C. Jurcut, "Physicians' attitude and perception regarding celiac disease: a questionnaire-based study," *The Turkish Journal of Gastroenterology*, vol. 29, no. 4, pp. 419–426, 2018.

[99] H. Hin, G. Bird, P. Fisher, N. Mahy, and D. Jewell, "Coeliac disease in primary care: case finding study," *BMJ*, vol. 318, no. 7177, pp. 164–167, 1999.

[100] M. Salo, P. Collin, S. Kyrönpalo, M. Rasmussen, H. Huhtala, and K. Kaukinen, "Age, symptoms and upper gastrointestinal malignancy in primary care endoscopy," *Scandinavian Journal of Gastroenterology*, vol. 43, no. 1, pp. 122–127, 2008.

[101] R. Tortora, N. Imperatore, P. Capone et al., "The presence of anti-endomysial antibodies and the level of anti-tissue transglutaminases can be used to diagnose adult coeliac disease without duodenal biopsy," *Alimentary Pharmacology & Therapeutics*, vol. 40, no. 10, pp. 1223–1229, 2014.

[102] H. J. Freeman, "Pearls and pitfalls in the diagnosis of adult celiac disease," *Canadian Journal of Gastroenterology*, vol. 22, no. 3, pp. 273–280, 2008.

[103] K. Kurppa, M. Ashorn, S. Iltanen et al., "Celiac disease without villous atrophy in children: a prospective study," *The Journal of Pediatrics*, vol. 157, no. 3, pp. 373–380.e1, 2010.

[104] K. Kurppa, P. Collin, H. Sievänen, H. Huhtala, M. Mäki, and K. Kaukinen, "Gastrointestinal symptoms, quality of life and bone mineral density in mild enteropathic coeliac disease: a prospective clinical trial," *Scandinavian Journal of Gastroenterology*, vol. 45, no. 3, pp. 305–314, 2010.

[105] A. Tosco, V. M. Salvati, R. Auricchio et al., "Natural history of potential celiac disease in children," *Clinical Gastroenterology and Hepatology*, vol. 9, no. 4, pp. 320–325, 2011.

[106] E. Lionetti, S. Castellaneta, R. Francavilla et al., "Long-term outcome of potential celiac disease in genetically at-risk children: the prospective CELIPREV cohort study," *Journal of Clinical Medicine*, vol. 8, no. 2, p. 186, 2019.

[107] R. Auricchio, R. Mandile, M. R. Del Vecchio et al., "Progression of celiac disease in children with antibodies against tissue transglutaminase and normal duodenal architecture," *Gastroenterology*, vol. 157, no. 2, pp. 413–420.e3, 2019.

[108] M. Borrelli, C. Gianfrani, G. Lania et al., "In the intestinal mucosa of children with potential celiac disease IL-21 and IL-17A are less expressed than in the active disease," *American Journal of Gastroenterology*, vol. 111, no. 1, pp. 134–144, 2016.

[109] A. Sapone, J. C. Bai, C. Ciacci et al., "Spectrum of gluten-related disorders: consensus on new nomenclature and classification," *BMC Medicine*, vol. 10, no. 1, p. 13, 2012.

[110] B. Palmieri, M. Vadala', and C. Laurino, "A review of the gluten-free diet in non-celiac patients: beliefs, truths, advantages and disadvantages," *Minerva Gastroenterologica e Dietologica*, vol. 65, no. 2, 2018.

[111] I. R. Korponay-Szabó, T. Halttunen, Z. Szalai et al., "In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies," *Gut*, vol. 53, no. 5, pp. 641–648, 2004.

[112] T. T. Salmi, P. Collin, T. Reunala, M. Mäki, and K. Kaukinen, "Diagnostic methods beyond conventional histology in coeliac disease diagnosis," *Digestive and Liver Disease*, vol. 42, no. 1, pp. 28–32, 2010.

[113] O. Koskinen, P. Collin, I. Korponay-Szabo et al., "Gluten-dependent small bowel mucosal transglutaminase 2-specific IgA deposits in overt and mild enteropathy coeliac disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 47, no. 5, pp. 436–442, 2008.

[114] M. Borrelli, M. Maglio, I. R. Korponay-Szabó et al., "Intestinal anti-transglutaminase 2 immunoglobulin A deposits in children at risk for coeliac disease (CD): data from the PreventCD study," *Clinical and Experimental Immunology*, vol. 191, no. 3, pp. 311–317, 2018.

[115] V. K. Sarna, K. E. A. Lundin, L. Mørkrid, S.-W. Qiao, L. M. Sollid, and A. Christophersen, "HLA-DQ-gluten tetramer blood test accurately identifies patients with and without celiac disease in absence of gluten consumption," *Gastroenterology*, vol. 154, no. 4, pp. 886–896.e6, 2018.

[116] E. Liu, H.-S. Lee, C. A. Aronsson et al., "Risk of pediatric celiac disease according to HLA haplotype and country," *The New England Journal of Medicine*, vol. 371, no. 1, pp. 42–49, 2014.

[117] C. A. Aronsson, H.-S. Lee, S. Koletzko et al., "Effects of gluten intake on risk of celiac disease: a case-control study on a Swedish birth cohort," *Clinical Gastroenterology and Hepatology*, vol. 14, no. 3, pp. 403–409.e3, 2016.

[118] M. Barroso, S. A. Beth, T. Voortman et al., "Dietary patterns after the weaning and lactation period are associated with celiac disease autoimmunity in children," *Gastroenterology*, vol. 154, no. 8, pp. 2087–2096.e7, 2018.

[119] K. Størdal, R. A. White, and M. Eggesbø, "Early feeding and risk of celiac disease in a prospective birth cohort," *Pediatrics*, vol. 132, no. 5, pp. e1202–e1209, 2013.

[120] E. Liu, F. Dong, A. E. Barón et al., "High incidence of celiac disease in a long-term study of adolescents with susceptibility genotypes," *Gastroenterology*, vol. 152, no. 6, pp. 1329–1336.e1, 2017.

[121] C. Meijer, R. Shamir, H. Szajewska, and L. Mearin, "Celiac disease prevention," *Frontiers in Pediatrics*, vol. 6, p. 368, 2018.

[122] M. Mäki, K. Mustalahti, J. Kokkonen et al., "Prevalence of celiac disease among children in Finland," *The New England Journal of Medicine*, vol. 348, no. 25, pp. 2517–2524, 2003.

[123] A. Tommasini, T. Not, V. Kiren et al., "Mass screening for coeliac disease using antihuman transglutaminase antibody assay," *Archives of Disease in Childhood*, vol. 89, no. 6, pp. 512–515, 2004.

[124] K. Mustalahti, C. Catassi, A. Reunanen et al., "The prevalence of celiac disease in Europe: results of a centralized, international mass screening project," *Annals of Medicine*, vol. 42, no. 8, pp. 587–595, 2010.

[125] M. Laass, R. Schmitz, H. Uhlig, K. P. Zimmer, M. Thamm, and S. Koletzko, "The prevalence of celiac disease in children and adolescents in Germany," *Deutsches Ärzteblatt International*, vol. 112, no. 33-34, pp. 553–560, 2015.

[126] J. West, K. Fleming, L. J. Tata, T. R. Card, and C. J. Crooks, "Incidence and prevalence of celiac disease and dermatitis herpetiformis in the UK over two decades: population-based study," *American Journal of Gastroenterology*, vol. 109, no. 5, pp. 757–768, 2014.

[127] A. Rubio-Tapia, J. Ludvigsson, T. Brantner, J. A. Murray, and J. E. Everhart, "The prevalence of celiac disease in the United States," *The American Journal of Gastroenterology*, vol. 107, no. 10, pp. 1538–1544, 2012.

[128] M. Fukunaga, N. Ishimura, C. Fukuyama et al., "Celiac disease in non-clinical populations of Japan," *Journal of Gastroenterology*, vol. 53, no. 2, pp. 208–214, 2018.

[129] A. Smarrazzo, G. Magazzù, M. Ben-Hariz et al., "Variability of anti-human transglutaminase testing in celiac disease across Mediterranean countries," *World Journal of Gastroenterology*, vol. 23, no. 24, pp. 4437–4443, 2017.

[130] D. Gidrewicz, K. Potter, C. L. Trevenen, M. Lyon, and D. J. Butzner, "Evaluation of the ESPGHAN celiac guidelines in a North American pediatric population," *The American Journal of Gastroenterology*, vol. 110, no. 5, pp. 760–767, 2015.

[131] A. Ganji, A. Esmaeilzadeh, A. Bahari et al., "Correlation between cut-off level of tissue transglutaminase antibody and Marsh classification," *Middle East Journal of Digestive Diseases*, vol. 8, no. 4, pp. 318–322, 2016.

Hindawi
Gastroenterology Research and Practice
Volume 2019, Article ID 7369014, 7 pages
https://doi.org/10.1155/2019/7369014

Research Article

HLA Haplotype Association with Celiac Disease in Albanian Pediatric Patients from Kosovo

Atifete Ramosaj-Morina,[1] **Marija Burek Kamenaric**,[2] **Mehmedali Azemi**,[1] **Lidvana Spahiu**,[1] **Zorana Grubic**,[2] **and Renata Zunec**[2]

[1]*Pediatric Clinic, University Clinical Centre of Kosovo, Rrethi i Spitalit p.n., 10000 Prishtina, Kosovo*
[2]*Tissue Typing Centre, Clinical Department of Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb, Kispaticeva 12, HR-10000 Zagreb, Croatia*

Correspondence should be addressed to Renata Zunec; rzunec@kbc-zagreb.hr

Received 7 March 2019; Accepted 19 May 2019; Published 10 June 2019

Guest Editor: Francesco Valitutti

Genetic predisposition to celiac disease (CD) is strongly associated with the presence of HLA alleles in the individual genotype encoding HLA-DQ2 and/or HLA-DQ8 heterodimers. The main aim of this study was to analyze the HLA-A, -B, -DRB1, and -DQ allele and five-locus haplotype frequencies in 60 Albanian pediatric CD patients and 124 non-CD children from Kosovo. The most prevalent haplotype in patients was the ancestral AH 8.1 haplotype present in 22.5% of the cases compared to 2.8% of the controls ($P < 0.0001$). Additionally, two other haplotypes were also overrepresented in patients (HLA-A*02~B*50~DRB1*07~DQA1*02:01~DQB1*02:02 and HLA-A*68~B*44~DRB1*07~DQA1*02:01~DQB1*02:02). Analysis showed that 95.0% of CD patients and 43.3% of controls were carriers of HLA-DQ2 and/or HLA-DQ8 heterodimers. The most frequent CD-predisposing HLA-DQ haplotypes in patients were HLA-DQ2.5 (46.7%) and HLA-DQ2.2 (11.6%), while the most prevalent genotypes were HLA-DQ2.5/DQX (58.3%) and HLA-DQ2.5/DQ2.2 (20.0%). The frequency of the HLA-DQ8 heterodimer among CD patients (4.2%) compared to the control group (8.1%) was without statistical significance. The given data demonstrate differences in the distribution of HLA haplotypes among Albanian CD patients from Kosovo in comparison to other European and non-European populations, as well as provide additional population data to supplement the thus far undisputed importance of the role of HLA-DQ2 and HLA-DQ8 heterodimers in the development of CD.

1. Introduction

Celiac disease (CD) is an autoimmune systemic disorder characterized by chronic inflammation of the small intestinal mucosa triggered by gluten and related prolamine uptake that occurs in genetically susceptible individuals [1]. Europe is historically considered a geographical area with a high incidence of CD, with a prevalence of 1%, which may be higher in Northern European countries [2, 3]. Some studies from Scandinavian countries and the United Kingdom population tended to show a higher prevalence of CD of approximately 1.0-1.5% [4]. Although it was thought that some countries, including the United States, were exempt from this disease, it has recently been shown that it has a similar prevalence as in Europe, 0.5-1.0% of the general population [5].

Susceptibility to CD and its activation and perpetuation involve a combination of environmental and genetic factors through some immunological mechanisms [6]. The involvement of Human Leukocyte Antigens (HLA) in CD pathogenesis was first described in the 1970s [7]. Over the subsequent years, the specific alleles that underlie the described associations became clear: CD is strongly associated with HLA-DQ, which encodes HLA-DQ2 and HLA-DQ8 heterodimers.

At least 90% of the patients with CD are positive for the HLA-DQ2 heterodimer in *cis* formation (HLA-DQ2*cis*) encoded by HLA-DQA1*05:01 (α-chain) and DQB1*02:01 (β-chain) alleles on a HLA-DRB1*03 haplotype, although the HLA-DQ2 heterodimer may also be encoded in *trans* position (HLA-DQ2*trans*) with the HLA-DQA1*05:05 allele, usually on HLA-DRB1*11, DRB1*12, DRB1*13:03, and

DRB1*13:05 haplotypes, and the HLA-DQB1*02:02 allele, usually on a HLA-DRB1*07 haplotype [8, 9], but in some populations on HLA-DRB1*04 haplotypes as well. The majority of the remaining CD patients carry the HLA-DQ8 heterodimer formed by one α-chain and one β-chain encoded with HLA-DQA1*03:01 and HLA-DQB1*03:02 alleles on the HLA-DRB1*04 haplotypes [10] and sporadically on HLA-DRB1*08 haplotypes.

Most of the studies exploring the linkage of HLA genes with CD are focused on HLA class II genes encoding HLA-DQ heterodimers, while very few studies investigate extended HLA haplotypes [11]. In contrast to a relatively conserved HLA-DR3 haplotype, the linkage between HLA loci forming the HLA-DR7 and HLA-DR11 haplotypes are not so strong, and since they can form the HLA-DQ2.5*trans* heterodimer, we wanted to explore if there is a difference in HLA haplotype distribution among CD patients in comparison to healthy subjects.

The importance of genetic factors in the pathogenesis of CD is well documented by many studies. It is clear that CD rarely develops in the absence of HLA-DQ2 and/or HLA-DQ8 heterodimers, and that the predisposing HLA-DQ2 and HLA-DQ8 subtypes are necessary, but not sufficient for causing the disease [12]. Anderson et al. suggested that a combination of HLA typing and confirmatory serology could reduce the number of unnecessary endoscopies as well as the number of false negatives and/or positive diagnoses [13]. In 2012, the role of HLA in the diagnosis of CD was firmly recognized, which resulted in important changes in diagnostic criteria and the inclusion of HLA typing in CD diagnostic guidelines [1].

As far as we know, no previous study has focused on the frequency of CD-predisposing HLA genotypes in affected and nonaffected individuals in Kosovar Albanian children. Thus, the main aim of this research was to analyze the HLA-A~B~DRB1~DQA1~DQB1 haplotype distribution as well as the frequency of CD-predisposing HLA-DQ genotypes in Albanian pediatric CD and non-CD subjects from Kosovo.

2. Materials and Methods

2.1. Patients and Control Group. Sixty pediatric patients with CD (40 females and 20 males) at the University Clinical Centre of Kosovo (UCCK) and 124 unrelated age- and gender-matched children (64 females and 60 males) without a history of autoimmune diseases were included in this study. The patients' age at time of diagnosis ranged from 17 months to 18 years, with a mean age of 5.5 years (SD ± 3.31). The control group age at the time of sample collection ranged from 1 to 18 years with the mean age of 8.7 years (SD ± 5.8). The CD diagnosis was achieved according to the criteria of the European Society of Pediatric Gastroenterology and Nutrition [14] and the revised guidelines of the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition [1]. The study was approved by the Ethics Committee of the UCCK (approved February 12th, 2013) and by the Ethics Committee of the University Hospital Centre (UHC) Zagreb (approved March 28th, 2013) and is in accordance with the Helsinki Declaration. Written informed consent was obtained from parents or caregivers of all children prior to their enrollment in the study.

2.2. HLA Typing. Three milliliters of EDTA blood samples were collected from each child included in the study. Blood samples were stored frozen at -30°C until all samples were collected. DNA isolation and HLA typing were performed in the Tissue Typing Centre, Department of Transfusion Medicine and Transplantation Biology, UHC Zagreb. Genomic DNA was isolated from whole blood with the MagNA Pure Compact Instrument using the corresponding commercially available MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics GmbH, Mannheim, Germany). The final DNA concentration was adjusted to 50 ng/μl. All CD patients and controls were typed for HLA-A, -B, -DRB1, -DQA1, and -DQB1 applying the standard polymerase chain reaction–sequence-specific oligonucleotide probing (PCR-SSOP) method using the commercially available Immucor LIFECODES HLA-SSO typing kit (Immucor Transplant Diagnostics Inc., Stamford, USA), based on the hybridization of a labeled single-stranded PCR product to SSO probes [15]. After hybridization, the samples were processed and quantified on the Luminex 200 flow analyzer (Luminex Corporation, Austin, USA) and the resulting data were analyzed with Match It! DNA Software, version 1.2.4. The HLA-DQA1 and -DQB1 alleles determined at medium resolution level were assigned to the most common corresponding four-digit HLA allele which was additionally confirmed when necessary by the standard polymerase chain reaction–sequence-specific primer (PCR-SSP) high-resolution protocol (CareDx, Olerup SSP AB, Sweden).

2.3. Statistical Analysis. The observed HLA-A, -B, -DRB1, -DQA1, and -DQB1 frequencies in both research groups and five-locus haplotype estimates were calculated using the PyPop (Python for Population genetics, version 0.7.0; http://www.pypop.org) program [16].

Regarding the HLA-DQ profile of the subjects, they were categorized according to the presence of susceptible haplotypes (DQ2.5*cis*, DQ2.5*trans*, and DQ8), fractional susceptible haplotypes (DQ2.2, DQ2.3, and DQ7), or nonsusceptible haplotypes (DQ4, DQ5, DQ6, and DQ9). Additionally, based on the copy number of the CD-predisposing HLA-DQ2 and/or HLA-DQ8 alleles in each individual, dual-dosage susceptible genotypes (DQ2/DQ2, DQ2/DQ8, and DQ8/DQ8), sole-dosage susceptible genotypes (DQ2/DQX or DQ8/DQX), and nonsusceptible (DQ2 and/or DQ8 negative) genotypes were defined. Fisher's exact test was applied for comparisons between the HLA allelic groups, haplotypes, and genotypes of CD patients and those of the control group, and calculated differences were considered statistically significant if the P value was less than 0.05.

3. Results

3.1. HLA Allele Frequencies. The observed HLA allele frequencies of HLA-A, -B, -DRB1, -DQA1, and -DQB1 loci

with a statistically significant difference between the Albanian pediatric CD patients and the control group from Kosovo are shown in Table 1 and Table 2.

A total of 15 HLA-A alleles were found in the CD patient group, among which the most frequent were HLA-A*01 (26.7%), -A*02 (21.7%), and -A*03 (10.0%). A statistically significant difference was observed for HLA-A*01 ($P < 0.0001$), present with a higher frequency among CD patients compared to the control group, while the HLA-A*02 allele had a lower frequency among CD patients ($P = 0.0312$).

At the most polymorphic HLA-B loci with 21 different alleles detected in the CD patient group, seven alleles showed a frequency > 5.0%: HLA-B*07 (8.3%), -B*08 (29.2%), -B*18 (6.7%), -B*38 (5.0%), -B*44 (8.3%), -B*50 (5.8%), and -B*51 (10.8%). A significantly higher frequency of HLA-B*08 ($P < 0.0001$) and HLA-B*50 ($P = 0.0012$) as well as a significantly lower frequency of HLA-B*35 ($P = 0.0093$) was detected among CD patients in comparison to the control group.

Out of the 12 different alleles observed at the HLA-DRB1 locus among CD patients, the three most frequent were HLA-DRB1*03 (38.3%), -DRB1*07 (17.5%), and -DRB1*11 (13.3%). Additionally, HLA-DRB1*03 and -DRB1*07 are alleles with a much higher frequency among CD patients than in the control group ($P < 0.0001$ and $P = 0.0023$, respectively), as opposed to HLA-DRB1*11, -DRB1*13, and -DRB1*16 with a significantly lower frequency among CD patients ($P = 0.0460$, $P = 0.0102$, and $P = 0.0289$, respectively).

At the HLA-DQ locus, 10 different HLA-DQA1 and 12 different HLA-DQB1 alleles were observed in CD subjects. The most frequent HLA-DQA1 alleles were HLA-DQA1*01:02 (13.3%), -DQA1*02:01 (17.5%), -DQA1*05:01 (40.0%), and -DQA1*05:05 (13.3%), with a statistically significant difference and higher frequency for HLA-DQA1*02:01 ($P = 0.0023$) and -DQA1*05:01 ($P < 0.0001$) in comparison to the control group. On the other hand, the frequency of the HLA-DQA1*01:02 ($P = 0.0049$) and -DQA1*05:05 ($P = 0.0115$) alleles is lower among CD patients. HLA-DQB1*02:01 (40.0%), -DQB1*02:02 (18.3%), and -DQB1*03:01 (14.2%) were the most frequent alleles of the HLA-DQB1 locus in the CD patient group, and a significantly higher frequency in the CD patient group in comparison to controls was observed just for HLA-DQB1*02:01 ($P < 0.0001$) and -DQB1*02:02 ($P = 0.0020$). On the other hand, the frequencies of HLA-DQB1*03:01 and -DQB1*05:02 alleles were significantly lower among CD patients ($P = 0.0084$ and $P = 0.0341$, respectively) in comparison to the control group.

3.2. HLA-A~B~DRB1~DQA1~DQB1 Haplotype Frequencies. A total of 71 different five-locus haplotypes (HLA-A~B~DRB1~DQA1~DQB1) were detected in the CD patient group with a total of 55 haplotypes that occurred only once. The most prevalent haplotype observed was HLA-A*01~B*08~DRB1*03~DQA1*05:01~DQB1*02:01 (22.5%) followed by HLA-A*02~B*50~DRB1*07~DQA1*02:01~DQB1*02:02 and HLA-A*02~B*51~DRB1*11~DQA1*05:05~DQB1*03:01 with a frequency of 4.1%, each. When comparing the CD patients' haplotype frequency results to the frequencies of 156 different five-locus haplotypes observed in the control group (Table 3), a statistically significant difference was calculated for the HLA-A*01~B*08~DRB1*03~DQA1*05:01~DQB1*02:01 ($P < 0.0001$) and HLA-A*02~B*50~DRB1*07~DQA1*02:01~DQB1*02:02 ($P = 0.0311$) haplotypes, both more frequent among CD patients. Also, the HLA-A*68~B*44~DRB1*07~DQA1*02:01~DQB1*02:02 haplotype was observed with a high frequency (3.3%) among the CD patient group, which resulted in a significant

Table 1: The HLA-A, -B, and -DRB1 allele frequencies observed in the Albanian pediatric celiac disease patients from Kosovo ($N = 60$) with statistically significant differences in comparison to healthy individuals in the control group ($N = 124$).

	Patients ($N = 60$)		Control group ($N = 124$)		
	n	AF	n	AF	P
HLA-A*					
01	32	0.2667	26	0.1048	**<0.0001**
02	26	0.2167	81	0.3266	**0.0312**
HLA-B*					
08	35	0.2917	14	0.0565	**<0.0001**
35	4	0.0333	31	0.1250	**0.0093**
50	7	0.0583	1	0.0040	**0.0012**
HLA-DRB1*					
03	46	0.3833	18	0.0726	**<0.0001**
07	21	0.1750	17	0.0685	**0.0023**
11	16	0.1333	55	0.2218	**0.0460**
13	6	0.0500	36	0.1452	**0.0102**
16	7	0.0583	34	0.1371	**0.0289**

Legend (alphabetic order): AF = allele frequency; N = number of tested individuals; n = number of allelic group occurrence.

Table 2: The HLA-DQA1 and -DQB1 allele frequencies observed in the Albanian pediatric celiac disease patients from Kosovo ($N = 60$) with statistically significant differences in comparison to healthy individuals in the control group ($N = 124$).

	Patients ($N = 60$)		Control group ($N = 124$)		
	n	AF	n	AF	P
HLA-DQA1*					
01:02	16	0.1333	66	0.2661	**0.0049**
02:01	21	0.1750	17	0.0685	**0.0023**
05:01	48	0.4000	19	0.0766	**<0.0001**
05:05	16	0.1333	62	0.2500	**0.0115**
HLA-DQB1*					
02:01	48	0.4000	18	0.0726	**<0.0001**
02:02	22	0.1833	19	0.0766	**0.0020**
03:01	17	0.1417	66	0.2661	**0.0084**
05:02	8	0.0667	36	0.1452	**0.0341**

Legend (alphabetic order): AF = allele frequency; N = number of tested individuals; n = number of allele occurrence.

Table 3: The HLA-A~B~DRB1~DQA1~DQB1 haplotypes with ≥2 number of haplotype copies in the Albanian pediatric celiac disease patients from Kosovo ($N = 60$) and compared with the frequencies of those haplotypes in the control group ($N = 124$).

Haplotype HLA	Patients ($N = 60$)		Control group ($N = 124$)		
A*~B*~DRB1*~DQA1*~DQB1*	n	HF	n	HF	P
01~08~03~05:01~02:01	27.0	0.2250	7.0	0.0282	**<0.0001**
02~50~07~02:01~02:02	5.0	0.0416	1.0	0.0041	**0.0311**
02~51~11~05:05~03:01	5.0	0.0416	6.0	0.0242	0.3619
68~44~07~02:01~02:02	4.0	0.0334	0	0	**0.0049**
24~07~15~01:02~06:02	3.0	0.0250	3.0	0.0121	0.3699
23~18~11~05:05~03:01	3.0	0.0250	0	0	0.0755
68~40~03~05:01~02:01	2.0	0.0167	1.0	0.0041	0.2443
02~13~07~02:01~02:02	2.0	0.0167	1.0	0.0041	0.2443
02~08~03~05:01~02:01	2.0	0.0167	1.0	0.0041	0.2443
03~44~07~02:01~02:02	2.0	0.0167	1.0	0.0041	0.2443
24~08~03~05:01~02:01	2.0	0.0167	0	0	0.1303
32~08~03~05:01~02:01	2.0	0.0167	1.0	0.0041	0.2443
03~38~13~01:03~06:03	2.0	0.0167	3.0	0.0121	0.7237
29~27~11~05:01~02:01	2.0	0.0167	1.0	0.0041	0.2443
23~44~07~02:01~02:02	2.0	0.0167	6.0	0.0242	0.6444
03~07~03~05:01~02:01	2.0	0.0167	0	0	0.1303

Legend (alphabetic order): HF = haplotype frequency; N = number of tested individuals; n = number of observed haplotypes.

difference ($P = 0.0049$) due to a 0% frequency of this haplotype in the control group.

3.3. Frequencies of CD-Predisposing HLA-DQ Haplotypes and Genotypes. The presence of the HLA-DQ2 and/or HLA-DQ8 heterodimers was detected in 57/60 (95.0%) CD patients and in 50/124 (40.3%) control subjects. The detailed CD-predisposing HLA-DQ haplotype and genotype distribution among CD patients and a comparison with the frequency of those haplotypes/genotypes in the control group is presented in Table 4. The most frequent CD-predisposing HLA-DQ haplotypes found in patients were HLA-DQ2.5 (*cis* and *trans* conformation) and HLA-DQ2.2 with a frequency of 46.7% and 11.6%, respectively. Consequently, the most prevalent genotype among CD patients was HLA-DQ2.5/DQX with a frequency of 58.3%, followed by the HLA-DQ2.5/DQ2.2 genotype with a frequency of 20.0%. When compared with the control group, both genotypes show a statistically significantly higher frequency among CD patients ($P < 0.0001$ and $P = 0.0005$, respectively). Three patients were homozygous for HLA-DQ2.5 (having a genotype positive for two copies of the HLA-DQ2.5 heterodimer), with no presence of this genotype in controls. The frequency of the HLA-DQ8 haplotype among CD patients is low (4.2%), and there is no significant difference compared to the control group (8.1%). Looking at the HLA-DQ8-related genotypes, 3 (5.0%) CD patients and 1 (0.8%) individual from the control group carried HLA-DQ8/DQ2.5, while 2 (3.3%) CD patients, but as many as 17 (13.7%) individuals from the control group, carried the HLA-DQ8/DQX genotype ($P = 0.0459$). Only one individual in the control group had the HLA-DQ8 genotype alone (DQ8/DQ8). Three CD patients who are not carriers of any of the susceptible variants (HLA-DQ2.5, HLA-DQ2.2, or HLA-DQ8) had the HLA-DQ7/DQ7, HLA-DQ7/DQ5, and HLA-DQ5/DQ6 genotypes.

4. Discussion

A strong association between the CD and HLA-DQ allelic groups encoding for HLA-DQ2 and HLA-DQ8 heterodimers is well-described, and these specific HLA heterodimers are usually seen in more than 90.0% of the patients with CD [17]. However, due to the huge polymorphism of the HLA system and the different HLA risk levels of CD among populations [18], it is important to perform the analysis in each specific population separately. This is the first analysis of the HLA-A, -B, -DRB1, -DQA1, and -DQB1 allele frequencies and the HLA-DQ2 and HLA-DQ8 heterodimer frequencies in 60 Albanian children from Kosovo diagnosed with CD. The limitation of the study is the small sample size, although the post hoc sample size calculation showed that the number of 60 CD patients was enough for the obtained results with a confidence interval of 85%.

The analysis of the HLA-A, -B, -DRB1, and -DQ frequencies among CD patients and compared to the control group revealed a pronounced increase of the HLA-A*01, -B*08, -B*50, -DRB1*03, -DRB1*07, -DQA1*02:01, -DQA1*05:01, -DQB1*02:01, and DQB1*02:02 alleles. On the other hand, HLA-A*02, -B*35, -DRB1*11, -DRB1*13, -DRB1*16, -DQA1*01, DQB1*05, and -DQB1*03:01 were significantly less present among CD patients than in controls. The higher frequency of these HLA alleles among CD patients is also reflected in the haplotype frequency. The top-ranked haplotype observed with a high frequency of 22.5% was the ancestral AH 8.1 haplotype (HLA-A*01~B*08~DRB1*03~DQA1*05:01~DQB1*02:01), which was by contrast present in 2.8% of controls. These results are in concordance with

Table 4: The frequency of celiac disease-predisposing HLA-DQ haplotypes and genotypes detected among Albanian pediatric celiac disease patients from Kosovo ($N = 60$) and the control group ($N = 124$).

	Patients ($N = 60$)	Control group ($N = 124$)	
HLA-DQ haplotypes	*n* (%)	*n* (%)	*P*
Susceptible haplotypes			
DQ2.5*cis*	48 (40.01)	19 (7.66)	**<0.0001**
DQ2.5*trans*	8 (6.66)	3 (1.20)	**0.0088**
DQ8	5 (4.17)	20 (8.06)	0.1712
Fractional susceptible haplotypes			
DQ2.2	14 (11.66)	14 (6.45)	0.0631
DQ2.3	0	1 (0.41)	0.8170
DQ7	17 (14.16)	67 (27.01)	**0.0068**
Nonsusceptible haplotypes			
DQ4	1 (0.83)	6 (2.41)	0.3191
DQ5	15 (12.50)	68 (27.41)	**0.0017**
DQ6	12 (10.00)	48 (19.35)	**0.0252**
DQ9	0	2 (0.80)	0.5650
HLA-DQ genotypes			
DQ2 and/or DQ8 positive	57 (95.00)	50 (40.32)	**<0.0001**
Dual-dosage susceptible genotypes			
DQ2.5/DQ2.2	12 (20.00)	2 (1.61)	**0.0005**
DQ2.5/DQ2.5	3 (5.00)	0	0.0738
DQ2.2/DQ2.2	0	2 (1.61)	0.5616
DQ8/DQ2.5	3 (5.00)	1 (0.81)	0.1091
DQ8/DQ2.2	0	0	n/a
DQ8/DQ8	0	1 (0.81)	0.8144
Sole-dosage susceptible genotypes			
DQ2.5/DQX	35 (58.33)	19 (15.32)	**<0.0001**
DQ2.2/DQX	2 (3.33)	8 (6.45)	0.3902
DQ8/DQX	2 (3.33)	17 (13.71)	**0.0459**
DQ2 and/or DQ8 negative	3 (5.00)	74 (59.68)	**<0.0001**

Legend (alphabetic order): DQ2.5 = HLA-DQA1*05~DQB1*02 (HLA-DRB1*03); DQ8 = HLA-DQA1*03~DQB1*03:02 (HLA-DRB1*04); DQ2.2 = HLA-DQA1*02~DQB1*02 (HLA-DRB1*07); DQ2.3 = HLA-DQA1*03~DQB1*02 (HLA-DRB1*04/*09/*11); DQ7 = HLA-DQB1*03:01 (HLA-DRB1*11/*12/X); DQ4, DQ5, DQ6, and DQ9 were assigned if HLA-DQB1*04, HLA-DQB1*05, HLA-DQB1*06, and HLA-DQB1*03:03 alleles were present; DQX = presence of any other HLA-DQB1 allele than HLA-DQ2 and/or HLA-DQ8; N = number of tested individuals; n = number of HLA-DQ haplotype/genotype occurrence; n/a = not applicable (due to $n = 0$).

results showing that DQ2 is an absolute requirement for the development of CD, but the presence of the well-known ancestral haplotype AH 8.1 and additional genetic factors in the HLA class I region induce an increased risk of CD [19–22].

What is also interesting are the two HLA alleles with a surprisingly high frequency in the CD patient group: HLA-B*50 and HLA-DRB1*07, with frequencies that were more than four times higher and three times higher in comparison to controls, respectively. Consequently, the second most frequent five-locus haplotype observed in our CD patients was HLA-A*02~B*50~DRB1*07~DQA1*02:01~DQB1*02:02. This haplotype has not been previously reported as a haplotype associated with CD, and it is mostly associated with a probable Euro-Asiatic origin, having been reported in Mongolians (HF: 3.2%), in the Chaouya population from Morocco (HF: 2.9%), Turks and Kurds (HF: 1.3%), Spaniards (HF: 1.2%), and Italians (HF: 0.5%) [23–25]. The second uncommon and unexpectedly frequent haplotype, ranked fourth among our CD patients, was HLA-A*68~B*44~DRB1*07~DQA1*02:01~DQB1*02:02. This haplotype was not observed in the control group, and this five-locus haplotype has not been reported in any European population so far, but only in the Sri Lanka Colombo population with a frequency of 7.0% [26].

These results raise new questions: what is the distribution of this five-locus HLA haplotype in other neighboring populations as well as in other populations of European origin? Furthermore, is the presence of this haplotype specific just for Albanian CD patients from Kosovo, or is this haplotype present among CD patients from other countries?.

The results of this research revealed that 95.0% of the Albanian pediatric CD patients from Kosovo were HLA-DQ2- and/or HLA-DQ8-positive compared to the 40.3% of positive individuals in the control group. The given results are similar to those reported from other studies, and at the

same time they confirm the variability of HLA-DQ2 and HLA-DQ8 heterodimer frequencies among CD patients from different populations. One large European collaborative study comprising 1008 CD patients from Finland, France, Italy, Norway, Sweden, and the UK showed that HLA-DQ2 and HLA-DQ8 heterodimer frequencies were higher in the Northern European population (Finland—96.0%, Norway + Sweden—96.6%, UK—95.7%) than in the Southern European population (France—93.4% and Italy—89.5%) [27]. Since Kosovo is situated in Southeastern Europe, our result of 95.0% DQ2- and DQ8-positive patients is in concordance with this observation. Furthermore, our results are very similar to results from Greece where a single centre study found that 95.8% of pediatric CD patients were HLA-DQ2- and/or HLA-DQ8-positive in comparison to 32.5% of healthy individuals [28]. On the other hand, a study from Croatia reports a much higher frequency (98.0%) of patients with CD who were carriers of HLA-DQ2 and HLA-DQ8 heterodimers [29].

Epidemiological HLA studies have shown that the HLA-DQ gene dose has a strong quantitative effect on the magnitude of gluten-specific T cell responses, and these individuals have the highest risk of developing CD [10]. In the present study, 18 (30.0%) patients had a dual dosage of HLA-DQ-susceptible genotypes; 12 were HLA-DQ2.5/2.2 heterozygous, 3 HLA-DQ2.5 homozygous, and 3 were HLA-DQ2/DQ8 heterozygous. The majority of HLA-DQ2-positive patients were found to be homozygous or heterozygous for the HLA-DRB1*03~DQA1*05:01~DQB1*02:01 haplotype (HLA-DR3~DQ2), so the likelihood of HLA-DQ2 positivity (91.6%) is in line with reports from the European populations (90.0-95.0%) [18, 23]. Interestingly, the incidence of the HLA-DQ8 heterodimer (4.2%) alone, in double dose or in combination with other heterodimers, was lower in our CD cases than in controls, but with no statistically significant difference. Those result are in line with the studies from different European populations reporting the incidence of the HLA-DQ8 heterodimer among CD patients from 2.8% to 7.9% [27, 29, 30], although there are also studies reporting much higher HLA-DQ8 heterodimer frequencies, even up to 25.0% [28, 31].

In our cohort, 5.0% (3/60) of CD patients were HLA-DQ2- and/or HLA-DQ8-negative, which is comparable to the data originating from different countries reporting the percentage of HLA-DQ2/DQ8-negative CD patients between 0 and 10.0% [27, 29, 30, 32]. Two out of three HLA-DQ2/DQ8-negative CD patients were positive for the DQA1 part of the DQ2 heterodimer, carrying the HLA-DRB1*11~DQA1*05:05~DQB1*03:01 haplotype. The third patient was negative for all CD-predisposing DQ alleles (HLA haplotypes: HLA-DRB1*13~DQA1*01:03~DQB1*06:03 and HLA-DRB1*15~DQA1*01:02~DQB1*05:02), with positive serology and partial villous atrophy who responded to a gluten-free diet.

In conclusion, this study supports the idea that possibly, in addition to the well-known association of CD with HLA class II alleles, extended HLA haplotypes might be considered as a potential genetic risk factor. The given results also provide population data of Albanian CD patients from Kosovo and support the importance of HLA-DQ2 and HLA-DQ8 heterodimers in the development of CD.

Data Availability

The HLA data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The abstract of this paper was presented at the 33rd European Immunogenetics and Histocompatibility Conference as a poster presentation with interim findings. The poster's abstract was published in "Special Issue: Abstracts for the 33rd European Immunogenetics and Histocompatibility Conference, Lisbon, Portugal, May 8–11, 2019" in HLA Immune Response Genetics, 93(5): 249-413. https://onlinelibrary.wiley.com/doi/full/10.1111/tan.13518.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Authors' Contributions

Atifete Ramosaj-Morina and Marija Burek Kamenaric contributed equally to this manuscript.

Acknowledgments

A special acknowledgment goes to the Tissue Typing Centre, Clinical Department of Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb for their help with HLA typing for patients from Kosovo. We also want to thank the nurses of the Department of Gastroenterology, Pediatric Clinic, University Clinical Centre of Kosovo and Main Family Medical Centre, Prishtina, Kosovo, for their help during the blood sample collection for patients and controls. This work was supported in part by the Ministry of Education, Science, and Technology, Republic of Kosovo (grant number 2347-04/12/2014).

References

[1] S. Husby, S. Koletzko, I. R. Korponay-Szabó et al., "European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 54, no. 1, pp. 136–160, 2012.

[2] K. Mustalahti, C. Catassi, A. Reunanen et al., "The prevalence of celiac disease in Europe: results of a centralized, international mass screening project," *Annals of Medicine*, vol. 42, no. 8, pp. 587–595, 2010.

[3] M. Leja, Z. Shums, L. Nikitina-Zake et al., "Prevalence estimation of celiac disease in the general adult population of Latvia using serology and HLA genotyping," *United European Gastroenterology Journal*, vol. 3, no. 2, pp. 190–199, 2015.

[4] C. Dubé, A. Rostom, R. Sy et al., "The prevalence of celiac disease in average-risk and at-risk Western European populations: a systematic review," *Gastroenterology*, vol. 128, no. 4, pp. S57–S67, 2005.

[5] A. Fasano, I. Berti, T. Gerarduzzi et al., "Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study," *Archives of Internal Medicine*, vol. 163, no. 3, pp. 286–292, 2003.

[6] M. F. Kagnoff, "Celiac disease: pathogenesis of a model immunogenetic disease," *Journal of Clinical Investigation*, vol. 117, no. 1, pp. 41–49, 2007.

[7] P. L. Stokes, G. K. T. Holmes, P. Asquith, P. Mackintosh, and W. T. Cooke, "Histocompatibility antigens associated with adult coeliac disease," *The Lancet*, vol. 300, no. 7769, pp. 162–164, 1972.

[8] J. Ek, D. Albrechtsen, B. G. Solheim, and E. Thorsby, "Strong association between the HLA-Dw3-related B cell alloantigen -DRw3 and coeliac disease," *Scandinavian Journal of Gastroenterology*, vol. 13, no. 2, pp. 229–233, 2010.

[9] S. Trabace, A. Giunta, M. Rosso et al., "HLA-ABC and DR antigens in celiac disease. A study in a pediatric Italian population," *Vox Sanguinis*, vol. 46, no. 2, pp. 102–106, 1984.

[10] A. S. Louka, S. Nilsson, M. Olsson et al., "HLA in coeliac disease families: a novel test of risk modification by the "other" haplotype when at least one DQA1*05-DQB1*02 haplotype is carried," *Tissue Antigens*, vol. 60, no. 2, pp. 147–154, 2002.

[11] N. Bratanic, D. Smigoc Schweiger, A. Mendez, N. Bratina, T. Battelino, and B. Vidan-Jeras, "An influence of HLA-A, B, DR, DQ, and MICA on the occurrence of celiac disease in patients with type 1 diabetes," *Tissue Antigens*, vol. 76, no. 3, pp. 208–215, 2010.

[12] G. Trynka, K. A. Hunt, N. A. Bockett et al., "Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease," *Nature Genetics*, vol. 43, no. 12, pp. 1193–1201, 2011.

[13] R. P. Anderson, M. J. Henry, R. Taylor et al., "A novel serogenetic approach determines the community prevalence of celiac disease and informs improved diagnostic pathways," *BMC Medicine*, vol. 11, no. 1, 2013.

[14] "Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition," *Archives of Disease in Childhood*, vol. 65, no. 8, pp. 909–911, 1990.

[15] K. Dalva and M. Beksac, "HLA typing with sequence-specific oligonucleotide primed PCR (PCR-SSO) and use of the Luminex™ technology," *Methods in Molecular Biology*, vol. 1109, pp. 87–99, 2014.

[16] A. K. Lancaster, R. M. Single, O. D. Solberg, M. P. Nelson, and G. Thomson, "PyPop update—a software pipeline for large-scale multilocus population genomics," *Tissue Antigens*, vol. 69, pp. 192–197, 2007.

[17] M. P. Horan, S. Y. Chai, N. Munusamy et al., "High rates of variation in HLA-DQ2/DQ8 testing for coeliac disease: results from an RCPAQAP pilot program," *Journal of Clinical Pathology*, vol. 71, no. 10, pp. 900–905, 2018.

[18] P. Margaritte-Jeannin, M. C. Babron, M. Bourgey et al., "HLA-DQ relative risks for coeliac disease in European populations: a study of the European Genetics Cluster on Coeliac Disease," *Tissue Antigens*, vol. 63, no. 6, pp. 562–567, 2004.

[19] K. Karell, P. Holopainen, K. Mustalahti, P. Collin, M. Mäki, and J. Partanen, "Not all HLA DR3 DQ2 haplotypes confer equal susceptibility to coeliac disease: transmission analysis in families," *Scandinavian Journal of Gastroenterology*, vol. 37, no. 1, pp. 56–61, 2009.

[20] E. Bolognesi, K. Karell, S. Percopo et al., "Additional factor in some HLA DR3/DQ2 haplotypes confers a fourfold increased genetic risk of celiac disease," *Tissue Antigens*, vol. 61, no. 4, pp. 308–316, 2003.

[21] L. M. Medrano, B. Dema, A. López-Larios et al., "HLA and celiac disease susceptibility: new genetic factors bring open questions about the HLA influence and gene-dosage effects," *PLoS One*, vol. 7, no. 10, article e48403, 2012.

[22] J. Gutierrez-Achury, A. Zhernakova, S. L. Pulit et al., "Fine mapping in the MHC region accounts for 18% additional genetic risk for celiac disease," *Nature Genetics*, vol. 47, no. 6, pp. 577-578, 2015.

[23] T. Imanishi, T. Akaza, A. Kimura, K. Tokunaga, and T. Gojobori, "Allele and haplotype frequencies for HLA and complement loci in various ethnic groups," in *Proceedings of the Eleventh International Histocompatibility Workshop and Conference*, K. Tsuji, M. Aizawa, and T. Sasazuki, Eds., vol. 1, pp. 1065–1220, Oxford University Press, Yokohama, Japan, 1992.

[24] A. Arnaiz-Villena, M. Karin, N. Bendikuze et al., "HLA alleles and haplotypes in the Turkish population: relatedness to Kurds, Armenians and other Mediterraneans," *Tissue Antigens*, vol. 57, no. 4, pp. 308–317, 2001.

[25] A. Canossi, D. Piancatelli, A. Aureli et al., "Correlation between genetic HLA class I and II polymorphisms and anthropological aspects in the Chaouya population from Morocco (Arabic speaking)," *Tissue Antigens*, vol. 76, no. 3, pp. 177–193, 2010.

[26] F. F. González-Galarza, L. Y. C. Takeshita, E. J. M. Santos et al., "Allele frequency net 2015 update: new features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations," *Nucleic Acids Research*, vol. 43, no. D1, pp. D784–D788, 2015.

[27] K. Karell, A. S. Louka, S. J. Moodie et al., "HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease," *Human Immunology*, vol. 64, no. 4, pp. 469–477, 2003.

[28] M. Krini, G. Chouliaras, M. Kanariou et al., "HLA class II high-resolution genotyping in Greek children with celiac disease and impact on disease susceptibility," *Pediatric Research*, vol. 72, no. 6, pp. 625–630, 2012.

[29] R. Žunec, Z. Grubić, Z. Jurčić, M. Peršić, A. Kaštelan, and V. Kerhin-Brkljačić, "HLA-DQ2 heterodimer in the diagnosis of celiac disease," *Biochemia Medica*, vol. 14, pp. 119–124, 2004.

[30] L. Koskinen, J. Romanos, K. Kaukinen et al., "Cost-effective HLA typing with tagging SNPs predicts celiac disease risk haplotypes in the Finnish, Hungarian, and Italian populations," *Immunogenetics*, vol. 61, no. 4, pp. 247–256, 2009.

[31] A. Basturk, R. Artan, and A. Yilmaz, "The incidence of HLA-DQ2/DQ8 in Turkish children with celiac disease and a comparison of the geographical distribution of HLA-DQ," *Gastroenterology Review*, vol. 4, no. 4, pp. 256–261, 2017.

[32] N. Selleski, L. M. Almeida, F. C. d. Almeida, C. B. Pratesi, Y. K. d. M. Nóbrega, and L. Gandolfi, "Prevalence of celiac disease predisposing genotypes, including HLA-DQ2.2 variant, in Brazilian children," *Arquivos de Gastroenterologia*, vol. 55, no. 1, pp. 82–85, 2018.