Chapter 9

Clinical Manifestations of Celiac Disease and Diagnostic Criteria: Differences Among Children, Adolescents and Adults

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Abstract

Celiac disease (CD) was originally considered a pediatric disorder characterized by malabsorption and steathorrhea. Subsequently it was recognized that CD could affect adults at any age. Currently, in some centers, the greatest number of diagnosis of CD is performed in adults between 30 and 50 years. An overall decrease in the prevalence of diarrheal presentations over the past 2 decades, accompanied by an increase in "non-classical" manifestations of the disease, has been well described in both children and adults. Among children, clinical presentation is affected especially by the age. Very young children (< 3 years old) present more often with diarrhea, abdominal distension, and failure to thrive, whereas older children and adolescents are more likely to present with other gastrointestinal symptoms (recurrent abdominal pain, vomiting, or constipation) or extraintestinal symptoms. In adults, the major mode of presentation is diarrhea, although this presentation occurs in fewer than 50% of patients, and non-specific gastrointestinal symptoms, which bear a large degree of overlap with functional dyspepsia, irritable bowel syndrome or functional diarrhea. Extraintestinal symptoms such as iron-deficiency anemia, osteoporosis, dermatitis herpetiformis, recurrent apthous stomatitis, hipertransaminasemia, or neuropsychiatric manifestations are not infrequent. With the objective of improve the recognition and diagnosis of CD several guides to clinical practice have been published in both children and adults. In general, these guidelines recommend offering serologic testing for CD in patients with symptoms or conditions associated with CD. The confirmation of a diagnosis of CD should be based on a combination of findings from the clinical presentation, CD-specific antibodies, duodenal biopsies, HLA-DQ2/DQ8 genotyping, and

the response to a gluten free diet. Duodenal biopsies may not be mandatory for CD diagnosis in HLA-DQ2 and/or -DQ8 symptomatic patients with anti-transglutaminase antibodies over 10 times the upper limit of normal and positive endomysial antibodies.

Keywords

Celiac disease, diarrhea, gastrointestinal symptoms, extraintestinal symptoms, anti-transglutaminase antibodies, HLA-DQ2/DQ8, duodenal biopsies, gluten free diet.

1. Introduction

Celiac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals and characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or -DQ8 haplotypes, and enteropathy[1]. In genetically predisposed individuals, CD is precipitated by the ingestion of gluten, which are storage proteins in wheat (gliadin), rye (secalin) and barley (hordein). CD is a chronic, multi-organ disease in which small intestinal mucosal damage may lead to malabsorption of nutrients. The treatment of CD, adherence to a gluten free diet, was discovered by the Dutch pediatrician Willem-Karel Dicke (1905-1962)[2].

Genetic, immunology and environmental factors are important in the development of CD. The disease has a strong genetic component and the principal determinants are the class II HLA-DQ2 and -DQ8 genes[3]. CD is primarily a T cell–mediated immune disorder and in the small intestinal mucosa of individuals with CD, CD4+ T cells recognize gluten peptides selectively in the context of HLA-DQ2 or -DQ8 molecules[4]. The enzyme transglutaminase 2 (TG2) deaminates the positive charged gluten peptides, enhancing their binding to HLA-DQ2 and -DQ8 molecules. Both gluten-specific CD4+ T cells and cytotoxic intraepithelial T lymphocytes (IELs) play a key role in the development of CD, as defined by the presence of anti-TG2 antibodies and villous atrophy. The most important environmental factor related to CD is gluten, but other factors, such as infections, dysbiosis and drug exposure have been implicated[5,6].

CD is a common but frequently unrecognized disease, in part because of its variable clinical presentation and symptoms[7]. Screening studies have shown that CD is severely underdiagnosed, with of about 1%-3% among the European population, both in adults as in children[8-11]. Because CD can be very effectively treated with a gluten-free diet (GFD) it is important to identify people with the undiagnosed disease so as to provide satisfactory individual treatment. To improve the recognition of CD and to increase the

number of people diagnosed with the condition, a significant number of diagnosis clinical guidelines has been published over the last years[1,12-15]. Besides, the Oslo and London Consensuses recommendations tried to reached agreement on the definition of terms related to CD and/or gluten sensitivity to improve communication among researchers, clinicians and the general public (Table 1)[16,17].

Table 1. Classification of the main modes of clinical presentation according to the Oslo definitions for CD and related terms[17] and to ESPGHAN guideline[1].

OSLO Consensus	ESPGHAN guideline
Asymptomatic CD Absence of symptoms even in response to direct questioning at initial diagnosis. These patients are often diagnosed through testing of populations enrolled in screening programmes	**Silent CD** Presence of positive CD-specific antibodies, HLA, and small-bowel biopsy findings that are compatible with CD but without sufficient symptoms and signs to warrant clinical suspicion of CD.
Classical CD Presents with signs and symptoms of malabsorption. Diarrhea, steathorrhea, weight loss or growth failure is required	**Gastrointestinal symptoms and signs** Because atypical symptoms may be considerably more common than classic symptoms, the ESPGHAN working group decided to use the following nomenclature: gastrointestinal symptoms and signs (eg, chronic diarrhea)
Non-classical CD Presents without signs and symptoms of malabsorption. Patients with monosymptomatic disease (other than diarrhea or steathorrhea) usually have non-classical CD	**Extraintestinal symptoms and signs** eg, anemia, neuropathy, decreased bone density, increased risk of fractures
Subclinical CD Disease that is below the threshold of clinical detection without signs or symptoms sufficient to trigger CD testing in routine practice.	Not used. See Silent

OSLO Consensus	ESPGHAN guideline
Symptomatic CD Characterized by clinically evident gastrointestinal and/or extraintestinal symptoms attributable to gluten intake	See above gastrointestinal and extraintestinal symptoms
Potential CD Relates to people with a normal small intestinal mucosa who are at increased risk of developing CD as indicated by positive CD serology	Presence of CD-specific antibodies and compatible HLA but without histological abnormalities in duodenal biopsies. The patient may or may not have symptoms and signs and may or may not develop a gluten-dependent enteropathy later.
Not used	**Latent CD** Presence of compatible HLA but without enteropathy in a patient who has had a gluten-dependent enteropathy at some point in his or her life. The patient may or may not have symptoms and may or may not have CD-specific antibodies.
Refractory CD Persistent or recurrent malabsorptive symptoms and signs with villous atrophy despite a strict GFD for more than 12 months	
CD autoimmunity Relates to increased anti-TG2 or EMA on at least two occasions when status of the biopsy is not known. If the biopsy is positive, then this is CD, if the biopsy is negative than this is potential CD	
Genetically at risk of CD Family members of patients with CD that test positive for HLA-DQ2/DQ8	
Non-celiac gluten sensitivity Relates to one or more of a variety of immunological, morphological or symptomatic manifestations that are precipitated by the ingestion of gluten in people in whom CD has been excluded	

OSLO Consensus	ESPGHAN guideline
Gluten ataxia Idiopathic sporadic ataxia and positive serum antigliadin antibodies even in the absence of duodenal enteropathy	
Dermatitis herpetiformis Cutaneous manifestation of small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten. It is characterized by herpetiform clusters of pruritic urticated papules and vesicles on the skin, especially on the elbows, buttocks and knees, and IgA deposits in the dermal papillae. DH responds to a GFD	
Terms to avoid Typical CD; Atypical CD; Silent CD; Overt CD; Latent CD	Typical CD; Atypical CD; Classical CD; non-classical CD

2. Clinical Manifestations

CD was originally considered a pediatric disorder characterized by malabsorption and steathorrhea. Subsequently it was recognized that CD could affect adults at any age. Currently, in some centers, the greatest number of diagnoses of CD is performed in adults between 30 and 50 years[18]. Most children and adults with CD diagnosed before 1980 presented with diarrhea. With the advent of serologic tests in the 1980s, the wide spectrum of clinical manifestations became apparent. An overall decrease in the prevalence of diarrheal presentations over the past 2 decades, accompanied by an increase in "non-classical" manifestations of the disease, has been well described in both children and adults[7,19,20]. Table 2 summarizes clinical signs, symptoms and types of presentation or conditions associated with CD in both children and adults.

Table 2. Signs, symptoms, and associated conditions, that should prompt consideration of celiac disease in children and adults, according to the NICE guideline[13].

Signs and symptoms
• Chronic or intermittent diarrhea • Persistent or unexplained gastrointestinal symptoms including nausea and vomiting • Recurrent abdominal pain, cramping or distension • Growth failure or short stature • Prolonged fatigue (“tired all the time”) • Sudden or unexpected weight loss • Unexplained iron-deficiency anemia, or other unspecified anemia • Premature reduced bone mineral density • Elevated serum aminotransferase levels when no other etiology is found • Oral apthous ulcers or dental enamel defects
Conditions
• Dermatitis herpetiformis • Irritable bowel syndrome • Autoimmune thyroid disease • Type 1 diabetes • Autoimmune liver conditions • Ataxia • Peripheral neuropathy • Down’s, William’s and Turner’s syndromes. • First-degree relatives (parents, siblings or children) with celiac disease
Other signs, symptoms and conditions to consider offering serological testing
• Other gastrointestinal disorders: – Persistent or unexplained constipation – Microscopic colitis – Lymphocytic gastritis • Neuropsychiatric manifestations: – Depression or bipolar disorder; irritability; dysthymia – Headache – Epilepsy • Gynecological: – Amenorrhea – Recurrent miscarriage – Unexplained infertility

- Immunological/autoimmune disease:
  - IgA deficiency
  - IgA nepropathy
  - Addison's disease
  - Chronic thrombocytopenia purpura
  - Autoinmune myocarditis
  - Sarcoidosis
  - Sjogren syndrome
  - Rheumatoid arthritis
  - Systemic lupus erythematosus
- Malignancy
  - Lymphoma
  - Small bowel adenocarcinoma

2.1. Children

Among children, CD has a varied clinical presentation, and is affected especially by the age at presentation. Very young children (< 3 years old) present more often with "classic" CD, characterized by diarrhea, abdominal distension, and failure to thrive, whereas older children and adolescents are more likely to present with other gastrointestinal symptoms such as recurrent abdominal pain, vomiting, or constipation. In addition, extraintestinal symptoms such as arthritis, neurologic symptoms and anemia are not infrequent, as are asymptomatic cases[21]. A Canadian study[20] evaluated the incidence and clinical presentation of CD in patients <18 years and compared the results according to the time of diagnosis, before (pretesting group) or after (testing group) the introduction of serological testing. The frequency of classic CD presentations decreased from 67% (pretesting group) to 19% (testing group). The frequency of Marsh 3c lesions decreased from 64% (pretesting group) to 44% (testing group). In the testing group, classic CD remained predominant (67%) in young children (<3 years), whereas atypical gastrointestinal and silent presentations predominated in older children. The primary symptoms, signs or associated conditions that led to intestinal biopsy are presented in Table 3.

Table 3. Age and primary symptoms, signs, or indication leading to intestinal biopsy to diagnose CD in children, according to the time of diagnosis before (pretesting) or after (testing) the introduction of serological testing[20].

	Pretesting (n = 36)(%)	**Testing (n = 199)(%)**	**p**
Age at diagnosis, median (95% CI)	2 (2-4)	9 (8-10)	<0.01
Classic presentation	24 (67)	39 (19)	<0.01
Gastrointestinal symptoms	7 (19)	76 (38)	0.048
Abdominal pain plus other symptoms	5 (14)	34 (17)	
Abdominal pain only	0	18 (9)	
Endoscopy for other reason	0	8 (4)	
Chronic diarrhea	1 (2.7)	7 (3.5)	
Constipation	0	5 (2.5)	
Vomiting	1 (2.7)	2 (1)	
Food allergy	0	1 (0.5)	
Abdominal distention	0	1 (0.5)	
Extraintestinal symptoms	5 (14)	29 (15)	0.9
Failure to thrive			
Iron deficiency, with or without	2 (5.5)	13 (6.5)	
anemia	2 (5.5)	6 (3)	
Short stature	0	6 (3)	
Dermatitis herpetiformis	0	2 (1)	
Elevated transaminase levels	0	1 (0.5)	
Dental enamel defects	0	1 (0.5)	
Hypoalbuminemia	1 (2.7)	0	
Silent	0	55 (28)	<0.01
Family history	0	35 (17.6)	
Type 1 diabetes mellitus	0	14 (7)	
Trisomy 21	0	5 (2.5)	
Hypothyroidism	0	1 (0.5)	

A recent study from the Netherlands revealed that CD was more frequently represented in a cohort of children with chronic constipation fulfilling Rome III criteria for irritable bowel syndrome (IBS)[22]. Overweight and obese children and adolescents with CD are now frequently identified. A North American study in children showed that nearly 19% of patients had an elevated body mass index at diagnosis (12.6% overweight, 6% obese) and 74.5% presented with a normal body mass index[23]. Conditions associated with CD apart from type 1 diabetes mellitus are autoimmune liver disease (13.5%), Williams syndrome (9.5%), Turner syndrome (6.5%), Down syndrome (5.5%), immunoglobulin A (IgA) nephropathy (4%), IgA deficiency (3%), autoimmune thyroid disease (3%) and juvenile chronic arthritis (2.5%)(Table 2)[1].

In the last years, several studies have suggested a protective role of breast feeding and/or the timing and quantity of gluten introduction in the subsequent development of CD in children[24]. Especially, the data from the Swedish epidemic of symptomatic CD during the mid-1980s suggested that prolonged breast feeding during the introduction of gluten-containing feeding was associated with a reduced risk of developing CD in infancy[25]. However, recently two multicenter, randomized, double-blind, placebo-controlled dietary-intervention studies have reported that neither the delayed introduction of gluten nor breast-feeding modified the risk of celiac disease among at-risk infants[26,27].

2.2. Adults

In adults, the mean age of CD presentation is 44 years (range 1-81 years), with a clear female predominance (1: 3), that has also been shown in young children[26]. Approximately 15-25% of cases are diagnosed at an age equal to or greater than 65 years[18]. In some cases, a history of growth failure or other symptoms suggestive of unrecognized childhood CD is discovered. The classic presentation of the disease with malabsorption, diarrhea, weight loss and abdominal distension is less common than in children[19]. The major mode of presentation is diarrhea, although this presentation occurs in fewer than 50% of patients, and non-specific gastrointestinal symptoms, which bear a large

degree of overlap with functional dyspepsia, irritable bowel syndrome (IBS) or functional diarrhea[28,29].

Dyspepsia is a common symptom in CD patients, which may be present in 40-60% of the cases at the time of diagnosis[30,31]. The prevalence of CD in patients with dyspepsia is also increased. A meta-analysis and systematic review of these studies also shows a higher frequency of positive celiac serology (7.9% vs 3.9%) as well as of CD diagnosed by duodenal biopsy (3.2% vs 1.3%) in dyspepsia patients compared to the control population, although these differences were not statistically significant[32]. If we consider the whole spectrum of histological CD lesions, including forms of mild enteropathy, this prevalence could be even higher. A retrospective study in Spain in patients with dysmotility-like dyspepsia (postprandial distress) and normal upper endoscopy showed that 19.7% of these patients had enteropathy and gluten-dependent symptoms[33].

CD can frequently present with symptoms that are also characteristic of IBS, including abdominal pain (77%), bloating (73%), diarrhea (52%), constipation (7%) and/or an alternating bowel pattern (24%)[31,34]. This means that IBS often constitutes the initial diagnosis in many patients before the discovery of CD many years later. A systematic review and meta-analysis including 2278 patients with IBS diagnostic criteria, showed in these patients a higher prevalence of IgA anti-gliadin antibodies (AGA) (4%; CI 95% 1.7-7.2), endomysial antibodies (EMA) or anti-TG2 antibodies (1.6%, CI 95% 0.7-3) as well as CD demonstrated by duodenal biopsy (4.1%, CI 95% 1.9-7)[35]. A prospective Spanish study in patients with chronic watery diarrhea and Rome II criteria for functional diarrhea or IBS-diarrhea diagnosis showed that 16.1% of these patients had enteropathy and gluten-sensitive diarrhea[36].

The presence of gastroesophageal reflux disease-related symptoms (GERD-rs) refractory to antisecretory drugs should encourage considering CD in the differential diagnosis. An Argentinean study which evaluated GERD-rs at diagnosis of CD in adults' patients found a significantly higher reflux symptom mean score than healthy controls. At baseline, 30.1% of CD patients had moderate to severe GERD-rs compared with 5.7% of controls[37]. A case

control study in patients with CD and GERD-rs showed that gluten free diet improved symptoms and it was a useful approach in the prevention of recurrence[38].

The prevalence of extraintestinal manifestations is very high among adult patients, especially if a specific search is performed. Anemia, mainly caused by iron deficiency, osteoporosis, dermatitis herpetiformis, recurrent apthous stomatitis, hipertransaminasemia, as well as a variety of neuropsychiatric conditions, can be a common mode of presentation of CD in adults (Table 2)[13,39].

Finally, serologic screening of high-risk groups, especially relatives of patients with CD, has increased detection of the disease both in children as in adults, some of whom are asymptomatic or present with mild and unspecific symptoms[21].

3. Diagnostic Criteria

3.1. Children

Strict criteria for CD diagnosis in children were first established by the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) in 1969[40]. The so-called 3 biopsies rule recommended performing at least three small bowel biopsies (SBB): the first one at clinical suspicion and while the child was on a gluten containing diet, the second after a period of gluten-free diet, and the third after gluten reintroduction, i.e. after performing a gluten challenge (GC). Characteristic histological lesions in the first SBB lead to CD suspicion, but a definite diagnosis of CD was finally confirmed only after in the 3rd biopsy histological relapse related to GC was verified. This strict diagnostic protocol aimed at demonstrating that gluten sensitivity was a permanent condition and to avoid misdiagnosis of transient gluten intolerance associated to other conditions especially in young infants[40].

After 20 years experience in large series of children it was shown that GC could probably be avoided in 95% of the cases[41]; accordingly diagnostic criteria were modified in 1990 and GC was restricted to infants younger than 2 years at the first biopsy to exclude other causes of enteropathy or whenever the initial diagnosis is uncertain; this latter covers different special circumstances such as gluten exclusion prior to or without a biopsy or uncharacteristic histological lesions for CD at diagnosis[42]. Moreover, the new criteria had for the first time a disease marker to rely on, i.e. the antigliadin antibodies (AGA), who had recently been found to be associated to active CD[43-45]; so it was considered that the presence of AGA in serum at disease onset, followed by antibody vanishing after gluten withdrawal, added support to the diagnosis[42]. However, further development of antiendomysial antibodies (EMA) in the late 80's[45-48], followed by TG2 being recognized as the autoantigen of CD in the 90´s, represented a true revolution in the field of CD diagnosis[49]. It was indeed shown that both EMA and anti-TG2 recognize the same autoantigen and overall display a sensitivity and specificity for CD diagnosis higher than 95%[49-51]. A new serological tests for antibodies against deaminated gliadin peptides (DGP)[52] has more recently turned out to display a higher sensitivity and specificity than conventional AGA, thus replacing the later ones for diagnostic purposes.

Although pediatric series are shorter as compared to adults´ ones, correlation between duodenal histopathology and anti-TG2 levels in pediatric patients with CD has been reported, higher levels being associated with villous atrophy[53-55]. Thus, it has recently been suggested that strongly positive anti-TG2 antibodies levels might be considered sufficient for CD diagnosis in children and replace the SBB in the diagnostic work up[53].

Moreover the strong association of CD with genetic markers HLA-DQ2 and -DQ8, which combined reach a sensitivity of 96%, implies a negative result of HLA-DQ2 and/or -DQ8 renders CD diagnosis unlikely[56-58].

Additionally, a very high relapse rate after GC in children younger than 2 years with positive EMA and villous atrophy at diagnosis has been

demonstrated, supporting the view that routine GC should not be mandatory in these cases[59].

Not surprising a recent survey conducted among ESPGHAN members revealed that about 90 % of responders requested a revision and modification of the 1990 diagnostic criteria; 44% wanted to omit the first SBB in specific circumstances, the majority of them declaring that no first biopsy should be required for symptomatic cases with positive IgA anti-TG2 or EMA in HLA-DQ2/DQ8 positive individuals. Additionally about half of the respondents believed that GC should not be mandatory for all children diagnosed (1st biopsy) before the age of 2[60].

Thus within ESPGHAN a working group performed a revision of scientific and technical developments in an evidence-based approach, producing a detailed evidence report on antibody testing in CD[61] which served as the basis for new guidelines for CD diagnosis recently published[1]. Additionally the working group developed a new and broader definition of CD as a systemic disorder with different degrees of mucosal lesions not restricted to villous atrophy therefore the diagnosis cannot rely on one single parameter, but on a combination of clinical symptoms, CD-specific antibodies, histology and genetics[1]. In summary, the new guidelines state that SBB may not be mandatory for CD diagnosis in HLA-DQ2 and/or -DQ8 symptomatic patients with anti-TG2 over 10 times the upper limit of normal (ULN) and positive EMA. As for GC they establish that GC is no longer obligatory in all cases that underwent SBB before the age of 2, but only in unclear cases. These guidelines have been validated by a recently published retrospective study[62] and a prospective international multicenter one (PROCEDE, www.procede2011.jimdo.com) is currently on-going.

3.1.1. Who to Test for CD?

According to the new 2012 ESPGHAN guidelines for CD diagnosis in children and adolescents, beside patients with the classic clinical picture, i.e. malabsorption syndrome with chronic diarrhea, weight loss, abdominal distension and anorexia, children with a wide spectrum of other

gastrointestinal and extraintestinal symptoms - most of them also reported in adults - should be tested for CD; these are shown in Table 4. Failure to thrive, short stature and pubertal delay are CD features specific for the pediatric age range and should thus prompt serological testing as well. Also asymptomatic individuals pertaining to the so called high risk groups and specially those with a first degree relative with confirmed CD should be screened for CD (Table 4)[1].

Table 4. Who should be tested for CD according to the new 2012 ESPGHAN guidelines for CD Diagnosis in children and adolescents[1].

Children and adolescents with the otherwise unexplained symptoms and signs of:
• Chronic or intermittent diarrhea • Failure to thrive, weight loss, stunted growth • Delayed puberty, amenorrhea • Iron-deficiency anemia • Nausea or vomiting • Chronic abdominal pain, cramping or distension • Chronic constipation • Chronic fatigue, recurrent apthous stomatitis (mouth ulcers) • Dermatitis herpetiformis–like rash • Fracture with inadequate traumas/osteopenia/osteoporosis • Abnormal liver biochemistry
Asymptomatic children and adolescents with an increased risk for CD such as:
• Type 1 diabetes mellitus (T1DM) • Down syndrome • Autoimmune thyroid disease • Turner syndrome • Williams syndrome • Selective immunoglobulin A (IgA) deficiency • Autoimmune liver disease • First-degree relatives with CD

3.1.2. How to Test for CD?

CD specific antibodies detection in serum, EMA by immunofluorescence or anti-TG2 by various immunoassays (enzyme-linked immunosorbent assay, radioimmunoassay, or others) is the preferred initial approach to find CD[1]. Immunofluorescent tests such as EMA are subjected to interobserver variability. Despite these limitations, the specificity of EMA is 98% to 100% in expert laboratories[51,63] and is thus considered the reference standard for CD-specific antibody.

The performance of a particular antibody test depends on patient characteristics (age, genetic predisposition, IgA deficiency), on pretest probability, on the used commercial kit and last but not least the expertise of the laboratory is also relevant[51].

However in children serological tests display a much higher efficiency as compared to adults , partially because usually more severe histological lesion are found in the pediatric age range. So in the 2012 ESPGHAN guidelines it is stated that in absence of CD specific antibodies (anti-TG2 and EMA) the diagnosis of CD is unlikely[1].

According to the ESPGHAN evidence report on CD serology[61], EMA display the best positive and negative likelihood ratios, followed by anti-TG2. Furthermore, EMA results were more homogeneous than results obtained with other CD antibody tests and had a high diagnostic odds ratio (OR = 553.6). Thus CD is likely if the EMA test is positive. Moreover EMA positivity also is associated with the later development of villous atrophy in the few reported cases who initially had normal small-intestinal architecture[64,65].

High concentrations of anti-TG2 in serum predict villous atrophy better than low or borderline values[54,55,66] and these studies suggest that high anti-TG2 antibody levels can be defined as those exceeding 10 times the upper limit of normal (ULN) depending on the cut off of each test (concentration-dependent antibody tests based on calibration curves)[55,66,67].

Anti-DGP antibodies performed favorably and much better than antibodies against native gliadin, however their performance is inferior compared with

anti-TG2 or EMA assays[55,61]; In addition, their role in the diagnosis of children younger than 2 to 3 years requires further assessment.

Anti-TG2 antibody detection also can be done from the blood at the point of contact using rapid test kits (POC test)[68,69], but although they may achieve a high accuracy for CD diagnosis (pooled sensitivity of 96.4%, pooled specificity of 97.7%)[61], performance of these tests needs to be confirmed not only in high prevalence populations as current published studies, but also in less selected populations and/or when handled by laypeople or untrained medical staff. Also anti -DGP based POC have lately been made available, although only very few studies have been reported up to now, effectiveness seems to be similar to the previous ones (personal observation).

3.1.3. Diagnostic Confirmation

In the last few years the leading role of histology for the diagnosis of CD has been questioned[53,55,63,65]. One of the mean reasons is that histological findings are not specific for CD, especially low grade lesions; these can be found in other entities, such as cow's milk or soy protein hypersensitivity, intractable diarrhea of infancy, infestation with *Giardia lamblia*, immunodeficiencies, tropical sprue, and bacterial overgrowth (Table 5). Another issue is that lesions may be patchy[70], they can occur in the duodenal bulb only[71], but the most important matter of concern is that interpretation depends on preparation of the mucosa sample and above all that a high interobserver variability has been acknowledged[72].

Not withstanding current evidence recommend that histological assessment should be omitted only in very specific situation, namely in symptomatic patients who have high IgA anti-TG2 levels 10 times above ULN, verified by EMA positivity, and are HLA-DQ2 and/or -DQ8 heterodimer positive. In all other circumstances histological evaluation is mandatory for a definite diagnosis[1]. This is mainly due to the fact that high levels of anti-TG2 (10 times ULN) correlates better with lesion severity than low values; borderline

or low levels may be found in non-CD conditions, specially autoimmune diseases and are not related to histological lesion[55,67,73].

Table 5. Other causes of enteropathy.

Villous atrophy	Lymphocytic enteropathy
• Tropical sprue • Small-bowell bacterial overgrowth • Autoinmune enteropathy • Hypogammaglobulinemic sprue • Drug-associated enteropathy (e.g., olmesartan) • Whipple disease • Collagenous sprue • Crohn's disease • Eosinophilic enteritis • Intestinal lymphoma • Intestinal tuberculosis • Infectious enteritis (e.g. giardasis) • Graft versus host disease • Malnutrition • Adquired immune deficiency syndrome enteropathy	• *H.pylori* infection • Small-bowell bacterial overgrowth • Drugs (e.g., NSAIDs) • Intolerance to non-gluten proteins (e.g., Cow's milk, eggs) • Infectious enteritis (e.g. giardasis) • IgA deficiency • Common variable immunodeficiency • Eosinophilic enteritis • Crohn's disease

The histological features of the small-intestine enteropathy in CD have a variable severity. The spectrum of histological findings ranges from lymphocytic infiltration of the epithelium (Marsh 1) to villous atrophy (Marsh 3)[74]. The description of the lesions according to Marsh -Obberhuber classification are described in Table 6[75,76]. Marsh 2-3 lesions are considered consistent with CD[1]. If histology is normal (Marsh 0) or only increased IELs counts are observed (Marsh 1), the diagnosis of CD can not be firmly established. Further work up is necessary at the mucosal level specially immunohistochemical analysis of biopsies looking for high $\gamma\gamma\delta\delta$ cells count or $\gamma\delta$/CD3 ratio[77] or the presence of IgA anti-TG2 deposits in the mucosa[78,79]. These deposits in the mucosa seem to be specific for CD and to predict the

evolution to more severe histological patterns[80]. Counting villous tip IELs also increases the specificity for CD[81].

Table 6. Histological classifications used for celiac disease[88].

Marsh modified (Oberhuber)	**Histologic criterion**			**Corazza**
	Increased IELs*	Crypt hyperplasia	Villous atrophy	
Type 0	No	No	No	None
Type 1	Yes	No	No	Grade A
Type 2	Yes	Yes	No	
Type 3a	Yes	Yes	Yes (partial)	Grade B1
Type 3b	Yes	Yes	Yes (subtotal)	
Type 3c	Yes	Yes	Yes (total)	Grade B2

*IELs: Intraepithelial lymphocytes per 100 enterocytes; > 40 for Marsh modified; > 25 for Corazza.

3.1.4. Role of HLA-DQ2/DQ8 Genotyping in Celiac Disease

HLA-DQ2 and -DQ8 testing is valuable because CD is unlikely if both haplotypes are negative[1,57,58]. Thus its main utility is to discard patients at risk for CD and accordingly HLA testing is useful to select asymptomatic persons with CD-associated conditions or pertaining to high risk groups for further CD-specific antibody testing[1]. In clinical practice it is noteworthy to stress the relevance of HLA typing of siblings or the offspring of CD patients as it will establish those at risk in which periodic testing for CD markers may be recommended, especially during the pediatric age range.

Moreover HLA testing should be performed when the diagnosis of CD is unclear, for example, in patients with negative CD-specific antibodies and

mild histological lesion. In children with a strong clinical suspicion of CD and high specific CD antibodies, if no SBB is going to be performed HLA-DQ2/DQ8 typing is strongly recommended to add strength to the diagnosis[1].

3.1.5. Special Situations

In subjects with humoral IgA deficiency, corresponding IgG class CD-specific antibodies should be measured, preferably IgG anti-TG2, but alternatively EMA-IgG, IgG anti-DGP or blended kits for both IgA and IgG antibodies[1,48]. Thus it is important to exclude IgA deficiency by measuring serum total IgA levels moreover considering IgA deficiency is more prevalent in CD as compared to the general population.

Children, mainly infants, presenting with a severe malabsorption syndrome and malnutrition, may exceptionally been started on a GFD while awaiting the results of HLA and EMA testing[1]. If the findings do not allow a definite diagnosis and due to a poor clinical condition the SBB has to be postpone, additional workup such as looking for IgA anti-TG2 deposits in the mucosa may be helpful. Due to persistence of anti-TG2 deposits for months after a GFD has been initiated, the presence of deposits can be used as a high specific test for CD whenever the patient has started dietary restrictions before a definite diagnosis has been achieved[79,80]. Patients with associated autoimmune conditions may display false positive anti-TG2 or fluctuating results, usually at low levels[65,73]; however in type 1 diabetes, especially at the initial stages of the disease, higher levels of EMA and anti-TG2 can be detected, decreasing to below ULN on follow up.

3.1.6. Celiac Disease Diagnostic Approach in Clinical Practice

The new 2012 ESPGHAN guidelines include 2 practical algorithms for CD diagnosis, one to be applied to symptomatic cases (Figure 1) and another for asymptomatic individuals pertaining to high-risk groups (Figure 2). Neither of them are meant for mass screening or for fortuitously detected CD antibody

positivity[1]. It should be stressed that initial evaluation has to be performed while the child is on a gluten containing diet, thus before dietary restrictions are recommended.

In children and adolescents with otherwise unexplained signs and symptoms suggestive of CD it is recommended to start the diagnostic approach by IgA anti-TG2, together with total serum IgA to rule out IgA deficiency; in this situation IgG anti-TG2 testing is recommended (Figure 1).

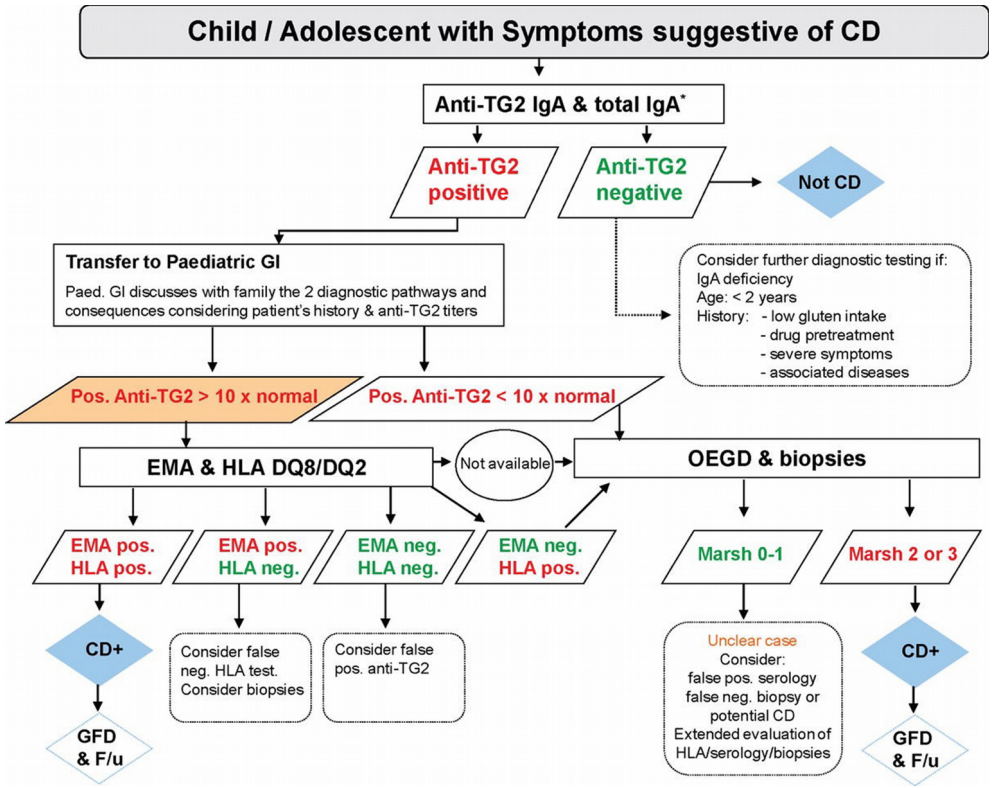

Figure 1. Diagnostic algorithm for children or adolescents with symptoms suggestive of CD. CD: celiac disease; EMA: endomysial antibodies; F/u: follow-up; GFD: gluten-free diet; GI: gastroenterologist; HLA: human leukocyte antigen; IgA: immunoglobulin A; IgG: immunoglobulin G; OEGD: oesophagogastroduodenoscopy; TG2: transglutaminase type 2. Adapted with permission from Lippincott Williams and Wilkins/Wolters Kluwer Health: Journal of Pediatric Gastroenterology & Nutrition, Husby S et al, ESPGHAN Guidelines for Diagnosis of Coeliac Disease[1], 2012.

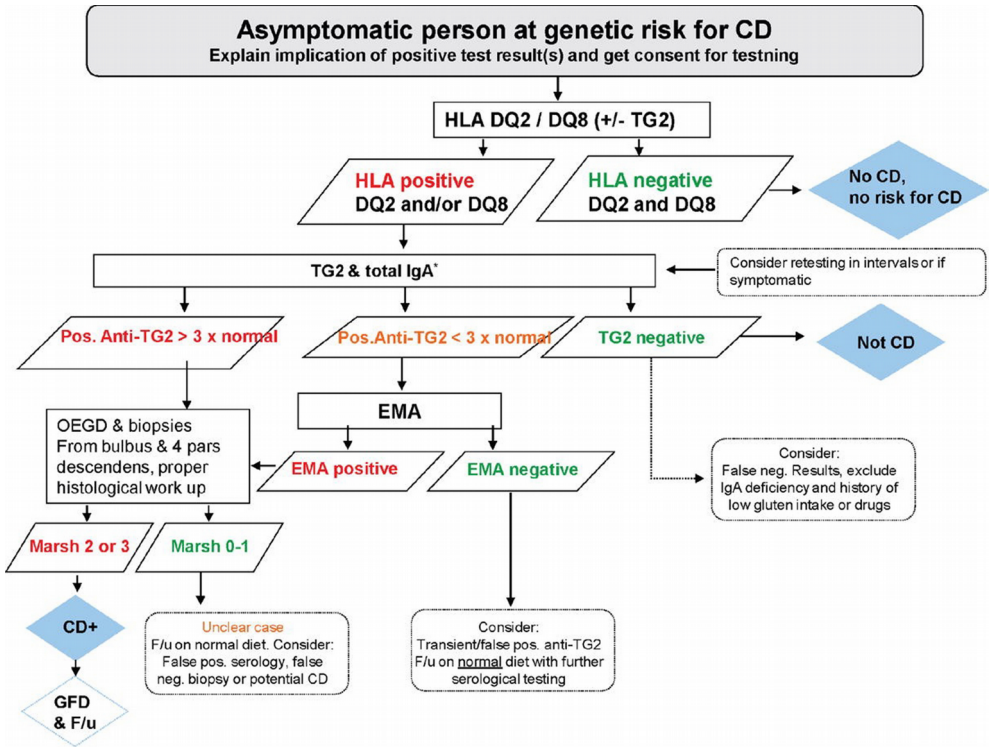

Figure 2. Diagnostic Algorithm for asymptomatic children and adolescents at genetic risk for CD (1st degree relatives or other at high-risk groups). See Fig. 1 for definitions. Adapted with permission from Lippincott Williams and Wilkins/Wolters Kluwer Health: Journal of Pediatric Gastroenterology & Nutrition, Husby S et al, ESPGHAN Guidelines for Diagnosis of Coeliac Disease[1], 2012.

In IgA sufficient patients, If **IgA anti-TG2 are negative** CD is unlikely. Several conditions such as low gluten intake, certain drugs (immunosuppressants), age (infants younger than 2 years) may impact on antibody results and should be taken into consideration. If symptoms and suspicion persists a SBB may be necessary independently of antibody results. Thus is seems reasonable that at this stage a pediatric gastroenterologist should be involved in decision taking.

For high **IgA anti-TG2 levels above 10 times ULN** the pediatric gastroenterologist should consider and discuss with the parents the option of

omitting the biopsies but performing additional investigations; this means that on a second (and thus different) blood sample HLA and EMA should be tested. If positive EMA antibodies and HLA-DQ2 or -DQ8, are found then the diagnosis of CD is confirmed and a GFD should be recommended; follow up is mandatory to ascertain improvement of symptoms and decline of antibodies, but no GC is further required. If any of them or both are negative, either a false positive anti-TG2 or a false negative EMA and/or HLA has to be considered; an extended workup including repeated testing and a SBB together with clinical follow up is mandatory to establish a definite diagnosis (Figure 1).

Skipping the biopsy is an option but not obligatory thus a SBB can be preferred for diagnosis confirmation despite very high anti-TG2. It remains mandatory if EMA or HLA-DQ2 and -DQ8 is not available.

If patients have positive **anti-TG2 antibodies but levels are lower than 10 times ULN**, a SBB and histological evaluation of the mucosa is mandatory to confirm CD diagnosis and this because low positive anti-TG2 can be related to non-CD conditions such as other autoimmune diseases, infections, tumors, or tissue damage and do not necessarily predict villous atrophy.

In **totally asymptomatic children or adolescents** who are being investigated because of pertaining to high-risk groups or associated conditions, the second algorithm (Figure 2) should be applied. In this group, HLA testing as the first step is probably cost-effective as HLA-DQ2 and -DQ8 negative individuals can be excluded from further follow-up studies, because of a minimal risk of developing CD. If HLA testing is not feasible the screening procedure may start with CD-specific antibody testing.

In HLA-DQ2 or -DQ8 positive individuals IgA anti-TG2 and serum total IgA determination should be performed or the corresponding IgG test in IgA deficient cases (Figure 2). If anti-TG2 are negative, as disease may still develop later in life, serological testing should be repeated at regular intervals. Truly there is no evidence on how frequently the testing should be performed. If high anti-TG2 are found, as persons belonging to this population more

often have false-positive anti-TG2 results, they should always be diagnosed after performing a SBB so as to have histological proof of CD diagnosis and thus support the need for a lifelong adherence to a strict gluten free diet[73]. If anti-TG2 levels are positive but low, that is <3 times ULN, a false-positive result has to be considered. In the absence of any signs or symptoms, follow up while still on a normal gluten-containing diet with repeated serological testing should be advised; in these cases, anti-EMA testing may be helpful to distinguish between false- and true-positive anti-TG2. If EMA is positive, the likelihood for CD increases and the patient should be referred for SBB. If EMA is negative, follow up and repeated testing is advisable.

A simple scoring system (Table 7) was also proposed by the working group which aimed at simplifying the diagnosis in obvious cases, thus enabling CD recognition at initial assessment, even in cases with no initial SBB and to avoid overdiagnosis in patients presenting only with non specific findings[1]. However prospectively validation in a large series of cases is required before it can be routinely recommended in clinical practice.

Another score system proposed by Catassi et al. (Table 8) is further discussed in section 3.2.9.; as compared to the previous one, histology evaluation is required in all cases[82].

3.2. Adults

Despite evidence of increasing rates of diagnosis, CD continues being an infradiagnosed disease in adults. It has been estimated that at least 75% of the cases remain undiagnosed[83]. Furthermore, there is often a delay in the diagnosis of the disease with a mean of 5 to 11 years from the onset of symptoms to diagnosis[18, 84]. These data may be explained by several facts:

1. The classic presentation of the disease is uncommon in adults. The major mode of presentation is diarrhea and nonspecific gastrointestinal symptoms which bear a large degree of overlap with functional dyspepsia, irritable bowel syndrome (IBS) or functional diarrhea[28].

Table 7. Diagnostic score[1]. The scoring takes into account 4 items: symptoms, antibodies, HLA, and biopsy findings, each contributing once. To make the diagnosis, a sum of 4 points is required.

	Points
Symptoms	
Malabsorption syndrome	2
Other CD- relevant symptom OR having T1DM OR being a 1 st-degree family member	1
Asymptomatic	0
Serum antibodies*	
EMA positivity and/ or high positivity (>10 ULN) for anti-TG2	2
Low positivity for anti-TG2 antibodies or isolated anti-DGP positivity	1
Serology was not performed	0
Serology performed but all* coeliac-specific antibodies negative	-1
HLA	
Full HLA- DQ2 (in cis or trans) or HLA-DQ8 heterodimers present	1
No HLA performed OR half DQ2 (only HLA-DQB1* 0202) present	0
HLA neither DQ2 nor DQ8	-1
Histology	
Marsh 3b or 3c (subtotal villous atrophy, flat lesion)	2
Marsh 2 or 3a (moderately decreased villous height/crypt depth ratio) OR Marsh 0-1 plus intestinal TG2 antibodies	1
Marsh 0-1 OR no biopsy performed	0

*Refers in IgA deficiency to IgG class EMA, anti-TG2 and DGP antibodies.

Comments and Explanations for Use.

*Biopsy items were graded by taking into account Villanacci scoring and the clinical utility of the results. We assumed that Marsh 0 or 1 results without any further information could be nonspecific. In contrast, demonstration of antibodies bound to tissue TG2 in the small bowel adds information to the diagnosis (when available). It is possible to diagnose CD as before even without this possibility. It is not necessary to have an EMA testing facility, but it is a clear advantage.

*Some findings that make CD improbable are resulting in negative scoring points.

*The sum of 4 points may be collected from findings registered at different time points during follow-up if they can be assumed to be gluten dependent. For example, an infant having villous atrophy before the introduction of gluten and normal biopsy at the age of 6 years while normally eating gluten will receive 0 for biopsy.

Table 8. Diagnostic criteria for Celiac Disease according to Catassi et al.[82].

	At least 4 out of 5, or 3 out of 4 if there are no HLA genotypes
1	Typical symptoms of celiac disease[1]
2	Positivity of serum celiac disease IgA class autoantibodies at high titer[2]
3	HLA DQ2 or DQ8 genotypes[3]
4	Celiac enteropathy at the small intestinal biosy[4]
5	Response to the gluten-free diet[5]

Notes: A family history of celiac disease adds evidence to the diagnosis; in symptom-free patients, particularly young children, it is advisable to confirm antibody positivity on 2 or more blood samples taken at least 3 months apart; in selected cases a gluten challenge after at least 2 years of gluten-free diet might be required for diagnosis confirmation.
[1] Examples of typical symptoms are chronic diarrhea, growth delay (children) or weight loss (adults) or iron deficiency anemia.
[2] Both IgA class anti-TG and EMA in IgA-sufficient or IgG class anti-TG and EMA in IgA-deficient subjects. The finding of IgG class anti-deamidated gliadin peptide adds evidence to the diagnosis.
[3] HLA-DQ2 positivity includes subjects with only half the heterodimer (positive HLA-DQB1*02).
[4] Including Marsh-Oberhuber 3 lesions, Marsh Oberhuber 1-2 lesions associated with positive celiac at low/high titer, or Marsh-Oberhuber 1-3 lesions associated with IgA subepithelial deposits.
[5] Histological in patients with sero-negative celiac disease or associated IgA deficiency.

2. Some antecedents, such as growth failure in childhood, iron-deficiency anemia, premature reduced bone mineral density, recurrent oral apthous, dermatological lesions or infertility may be often overlooked. Gastroenterologist's questions have focused exclusively on gastrointestinal symptoms, forgetting that CD is a disorder with a multisystemic expression.
3. Currently, active case-finding (serologic testing for CD in patients with symptoms or conditions closely associated with CD) is the favored strategy to increase detection of CD. However many adults with CD has mild forms of enteropathy (Marsh 1, 2 and 3a) in which a positive result of CD-specific serology may be lower than 30%[85,86].

Consequently, active case-finding may increase detection of CD among patients with symptoms although this strategy might be insufficient to detect all adults with CD[10].

With the objective of improve the recognition and diagnosis of CD several guides to clinical practice have been published[12-15]. In general, these guidelines recommend offering serologic testing for CD in patients with symptoms or conditions closely associated with CD (Table 2). The confirmation of a diagnosis of CD should be based on a combination of findings from the clinical scenario, CD-specific antibodies, upper endoscopy with duodenal biopsies, HLA-DQ2/DQ8 genotyping, and the response to a GFD. A summary of the specific recommendations from these guidelines to improve the diagnosis of CD is showed below.

3.2.1. When to Test for Celiac Disease?

There is no consensus regarding which symptoms, laboratory abnormalities, and/or associated diseases require evaluation for CD. The frequency of CD in common clinical scenarios varies from modestly elevated, such as irritable bowel syndrome, to substantially elevated, such as unexplained iron-deficiency anemia. Clinical guidelines[13,14] recommends to offer serological testing in patients with conditions in which CD occurs more frequently than in the general population and/or for whom a GFD may be beneficial (Table 2).

1. Patients with symptoms, signs, or laboratory evidence suggestive of malabsorption, such as chronic diarrhea with weight loss, steathorrhea, postprandial abdominal pain, and bloating.
2. Patients with unexplained gastrointestinal symptoms including dyspepsia, nausea and vomiting or recurrent abdominal pain.
3. Patients with extraintestinal symptoms such as unexplained iron-deficiency anemia, or other unspecified anemia, premature reduced bone mineral density, elevated serum aminotransferase levels when no other etiology is found or recurrent oral apthous ulcers.

4. Patients with any of the following conditions: Dermatitis herpetiformis, irritable bowel syndrome, autoimmune thyroid disease, type 1 diabetes, peripheral neuropathy, growth failure, discolored teeth or developmentally synchronous enamel loss, Down's and Turner's syndromes. Considerer offering serological test in the rest of associated conditions
5. Patients with a first-degree family member (parents, siblings or children) who has confirmed diagnosis of CD, specially if the show possible signs or symptoms or laboratory evidence of CD

3.2.2. How to Make the Diagnosis of Celiac Disease?

As in children, serologic testing of CD-specific antibodies is the preferred initial approach to find CD in adults, and TG2-based assays (EMA and anti-TG2) the most accurate tests. The sensitivity and specificity of the IgA anti-TG2 for untreated CD is about 95%, but its sensitivity is lower in case of mild histological lesions (no villous atrophy)[85,86]. The higher the titer of the test, the greater the likelihood of a true positive result. There are recognized differences in test performance between the various commercially available test kits, but overall there is consistency in the sensitivity and specificity of the test[87]. Antibodies directed against native gliadin are not recommended for the primary detection of CD[14].

IgA deficiency is more common in CD than in the general population. In patients in whom there is a high pre-test prevalence of CD, the measurement of IgA levels should be considered, especially if IgA-based celiac serology test is negative. One approach is to measure total IgA at the beginning of testing to determine whether IgA levels are sufficient and, if not, to incorporate IgG-based testing into the serology testing cascade (DGP-IgG and/or IgG anti-TG2)[14].

The antibodies directed against deaminated gliadin products as well as the self-antigen TG2 are dependent on the ingestion of gluten. The reduction or cessation of dietary gluten leads to a decrease in the levels of all these celiac-associated antibodies to normal concentrations. Therefore, all diagnostic

serologic testing should be done with patients on a gluten-containing diet. Combining several tests for CD in lieu of IgA anti-TG2 alone may marginally increase the sensitivity for CD but reduces specificity and therefore are not recommend in low-risk populations[14].

If the suspicion of CD is high, intestinal biopsy should be pursued even when serologies are negative (Figure 3).

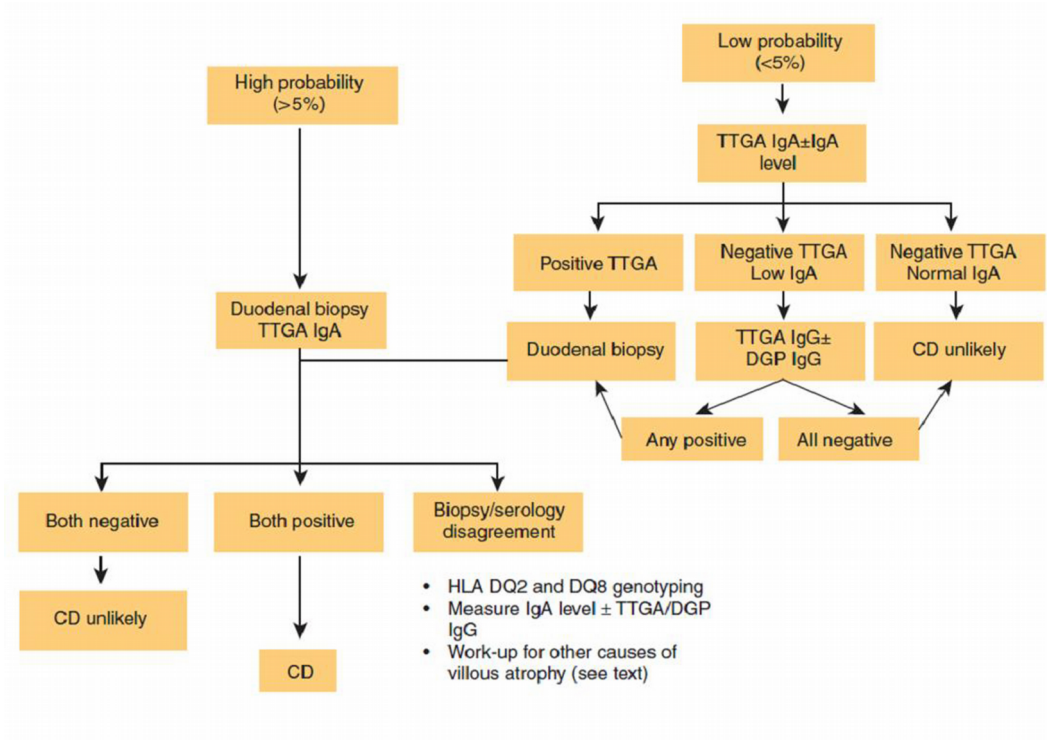

Figure 3. Celiac disease diagnostic testing algorithm according to American Journal Gastroenterology Clinical Guideline[14]. DGP: deamidated gliadin peptide; HLA: human leukocyte antigen; Ig: immunoglobulin; TTGA: tissue transglutaminase antibody. Reprinted by permission from Macmillan Publishers Ltd: American Journal Gastroenterology, Rubio-Tapia A et al, ACG Clinical Guidelines: Diagnosis and Management of Celiac Disease, 2013.

3.2.3. Confirmatory Testing in Celiac Disease

The confirmation of a diagnosis of CD should be based on a combination of findings from the medical history, physical examination, serology, and upper endoscopy with histological analysis of multiple biopsies of the duodenum[14].

Upper endoscopy with small bowel biopsy is a critical component of the diagnostic evaluation for persons with suspected CD and is recommend to confirm the diagnosis. Histological changes associated with the disease can be classified according to Marsh, Marsh modified (Oberhuber), or the more recent, simplified Corazza classification (Table 6)[88]. A positive CD-specific serology in patients with villous atrophy confirms the diagnosis of CD. However, a negative CD-specific serology in patients with enteropathy does not completely exclude the diagnosis of CD though it does make it much less likely. Histological response to GFD and HLA genotyping may help to rule out or confirm the diagnosis of CD in patients with sero-negative CD[14].

Histological abnormalities associated with CD can be patchy, therefore multiple biopsies of the duodenum (one or two biopsies of the bulb and at least four biopsies of the distal duodenum) are recommended to confirm the diagnosis of CD[89, 90]. Lymphocytic infiltration of the intestinal epithelium in the absence of villous atrophy is not specific for CD and other causes should be considered (Table 5)[91, 92].

The diagnosis may be confirmed when there is concordance between the serologic results and the histological findings and the symptoms resolve subsequently on a GFD. However, there are other situations in which it is possible to establish a diagnosis of CD although the result of CD-specific serology is negative[82].

3.2.4. Role of HLA-DQ2/DQ8 Genotyping in Celiac Disease

HLA-DQ2/DQ8 heterodimers are present in almost all patients with CD. Testing negative for both HLA-DQ genotypes makes CD diagnosis very unlikely. Among patients not carrying these heterodimers, the majority encoded half of the HLA-DQ2 heterodimer. Because HLA-DQ2 is present in

approximately 25-30% of the white population, testing for CD with either HLA-DQ type should not be used routinely in the initial diagnosis of CD because the predictive positive value is very low[14].

HLA-DQ2/DQ8 testing has been useful for exclusion of CD in selected clinical situations[14]. Examples of such clinical situations include but are not limited to: 1) Equivocal small-bowel histological finding (Marsh 1 or 2) in seronegative patients[93]; 2) Evaluation of patients on a GFD in whom no testing for CD was done before GFD[94]; 3) Patients with discrepant CD-specific serology and histology[95]; 4) Patients with suspicion of refractory CD where the original diagnosis of celiac remains in question; 5) Screening of CD in at-risk groups such as persons affected by Down' syndrome.[96]. The utility of HLA testing in other at-risk groups (such as type I diabetics or family members) is more limited because a high proportion of these subjects carry the CD susceptibility alleles.

3.2.5. Patients With Enteropathy But Negative CD-Specific Serology

This is a matter of crucial importance, especially in the adult population. In fact, the true prevalence of CD in this population has been underestimated, because both in population screening programs, as in symptomatic or high genetic risk people, intestinal biopsy is indicated only for positive serology. However, there is evidence that serology sensitivity is lower among adults with mild histological lesions (no villous atrophy; Marsh-Oberhuber 1 and 2)[85, 86].

The presence of mild histological lesions represents a difficult to interpret "gray area". Current data suggest that patients with lymphocytic duodenosis (>25 IELs per 100 epithelial cells), may suffer from gastrointestinal and extraintestinal symptoms, such as osteopenia or anemia, as frequently as patients with villous atrophy[97,98]. However, It should be noted that lymphocytic duodenosis, is common in the general population (prevalence of 5.4%)[99] and there are conditions other than CD in which lymphocytic duodenosis is possible. Examples of these include *H.pylori* infection,

medications (e.g., non-steroidal anti-inflammatory drugs), small-bowell bacterial overgrowth, food protein intolerance or autoimmune disorders[91,92]. Furthermore, celiac serology is positive only in 10-30% of patients with lymphocytic duodenosis secondary to CD. Consequently, diagnosis of CD in these patients is not easy and requires the following conditions[93]: First, it is necessary to exclude other possible etiologies such as *H. pylori* infection, medications or small-bowell bacterial overgrowth; Second, to prove the presence of either HLA-DQ2 or –DQ8 heterodimers; Finally, an unequivocal clinical and histological response to a gluten-free diet. The subset characterization of $\gamma\gamma\delta$+ IELs by immunohistochemical analysis or flow cytometry, as well as the presence of IgA anti-TG2 subepithelial deposits in the mucosa seem to be specific for CD[100,101]. However, these techniques require frozen or fresh nonfixed biopsies, and they are not straightforward for use in clinical practice.

3.2.6. Positive CD-Specific Serology But Absence of Enteropathy

False positive anti-TG2 results are rare but do occur and are usually low titer (typically less than twice the upper limit of normal). Repeating the test using an assay that uses human TG2 as the capture antigen may resolve the discrepancy. The duodenal biopsy should be reviewed by a pathologist familiar with CD to look for subtle abnormalities.

If these two steps do not reconcile the results, the patient can be placed on a high gluten diet and, after 6 to 12 weeks, it should be repeated the upper endoscopy with multiple additional biopsies of bulb and distal duodenum. Patients with positive serologic test and only mild histological lesions may respond to a GFD[102]. HLA-DQ2/DQ8 genotyping may also be useful for CD diagnosis in these patients with positive celiac-specific serology and normal duodenal histology[95].

3.2.7. Diagnosis Among Patients on a Gluten Free Diet

While standard diagnostic tests (specific serology and intestinal biopsy) have a high positive predictive value for CD, they should not be relied upon to exclude CD in patients already adhering to a GFD. The specific serologic and histologic features of CD do not normalize immediately upon the initiation of a GFD, but some patients might quickly revert to normal on a GFD. Hence, normal serologic and histologic findings on a GFD cannot be used to exclude CD definitively[14].

HLA-DQ2/DQ8 genotyping are not influenced by diet and can be used to evaluate the likelihood of CD in patients either on a normal or on a GFD. HLA-DQ2/DQ8 testing should be performed to try to exclude CD prior to embarking on a formal GC as a negative result will obviate the need for further workup[14].

Gluten challenge remains the gold standard for CD diagnosis in HLA-DQ2 or –DQ8-positive patients who have normal serologic and histologic findings when tested on a GFD. It must be noted that patients who develop severe symptoms following gluten ingestion are not suitable candidates for GC. Although gluten challenge with a diet containing at least 10 g of gluten per day for 6-8 weeks has long been the norm, there are few data to indicate the diagnostic efficacy of this approach or the optimum dose or duration of challenge[103].

Despite the disadvantages of neither confirming nor excluding a diagnosis of CD, some patients will opt to continue on a strictly GFD without undergoing formal gluten challenge; such patients should be managed in a similar fashion to those with known CD[14].

3.2.8. Differentiation of Celiac Disease from Non-celiac Gluten Sensitivity

Celiac disease should be differentiated from non-celiac gluten sensitivity in order to identify the risk for nutritional deficiency states, complications of CD, risk for CD and associated disorders in family members, and to influence the degree and duration of adherence to the GFD. Symptoms or symptoms

response to a GFD alone should not be used to diagnose CD, as there is often substantial overlap in symptoms between the two conditions. A diagnosis of non-celiac gluten sensitivity should be considered only after CD has been excluded with appropriate testing[14]. Objective tests including CD-specific serology and small-intestinal histology (both obtained while the patient is consuming a gluten-rich diet) and HLA-DQ2/DQ8 testing (to rule out CD if negative) are needed to differentiate between the two disorders[104].

3.2.9. Celiac Disease Diagnostic Approach in Clinical Practice

The diagnostic approach to an adult patient with suspected CD is complex, given the diversity of possible clinical settings. Figure 3 shows CD diagnostic testing algorithm proposed by ACG clinical guideline[14]. CD-specific serology (anti-TG2, EMA or anti-DGP) should be the initial diagnostic test to be performed in patients with signs, symptoms and/or conditions associated to CD.

When IgA anti-TG2 titers are higher than 10 times the upper limit of normal, the intestinal biopsy could be excluded, since the probability of detecting villous atrophy is quite high. Hills et al.[67] showed in adults that a IgA anti-TG2 level > 30U/ml (>10 UNL) using the Celikey test kit is absolutely predictive for CD (positive predictive value of 100%). Before taking this decision it is prudent to investigate and confirm the presence of EMA (performing the extraction at a different time of the first time) and checking for the HLA-DQ2/DQ8 heterodimers, since a positive result reinforces the diagnosis. In contrast, when IgA anti-TG2 level are lower than 10 UNL, multiple biopsies of duodenum should be performed, including one or two biopsies of the bulb (either 9- or 12-oclock position) and at least four biopsies of post-bulbar duodenum (2 bulb biopsies and 4 duodenal 2nd portion biopsies). If the histological results show enteropathy, a GFD should be started.

Further assessment is needed when specific serologic tests are negative but clinical suspicion of CD is high. In this situation, patients should undergo an upper endoscopy with duodenal biopsies to confirm the diagnosis of CD,

because sensitivity of CD-specific serology is lower among adults with non-atrophic lesions. HLA-DQ2/DQ8 genotyping might be useful to evaluate the likelihood of CD in these patients and performed intestinal biopsy only in HLA-DQ2 or –DQ8-positive patients. In patients with enteropathy but negative serologic test negative, HLA-DQ2/DQ8 genotyping might be useful to confirm or exclude a diagnosis of CD because testing negative for both HLA-DQ types makes diagnosis very unlikely[93].

It should be considered that, in any case, serology, genetic testing or duodenal biopsy results are pathognomonic. This means that in, certain cases, it is extremely difficult to confirm or rule out the disease. The wide variability of CD related findings suggests that it is difficult to conceptualize the diagnostic process into rigid algorithms that do not always cover the whole spectrum of clinical situations. Sometimes, it may be preferable the application of simple rules, which, in the hands of an experienced gastroenterologist, may be equally efficient. In this sense, Catassi and Fasano[82] proposed a 5-point scoring system that incorporates: 1) symptoms of CD; 2) positive CD serologies at high titer; 3) the presence of a HLA-DQ2 or -DQ8 haplotype; 4) characteristic histopathologic findings; and 5) a serologic or histologic response to the GFD. The presence of 4 out of the 5 criteria (or 3 out of 4, if HLA-DQ2/DQ8 testing is not performed) would meet diagnostic criteria for CD according to this proposed system, which has not yet been validated prospectively (Table 8).

References

1. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R et al. *European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease.* J Pediatr Gastroenterol Nutr. 2012; 54: 136-60.
   http://dx.doi.org/10.1097/MPG.0b013e31821a23d0
   PMid:22197856
2. Dicke WK. *Treatment of celiac disease.* Ned Tijdschr Geneeskd. 1951; 95: 124-30.
   PMid:14806692
3. Karell K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L 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.* Hum Immunol. 2003; 64: 469-77.
   http://dx.doi.org/10.1016/S0198-8859(03)00027-2
4. Petersen J, Montserrat V, Mujico JR, Loh KL, Beringer DX, van Lummel M et al. *T-cell receptor recognition of HLA-DQ2-gliadin complexes associated with celiac disease.* Nat Struct Mol Biol. 2014; 21: 480-8.
   http://dx.doi.org/10.1038/nsmb.2817
   PMid:24777060
5. de Meij TG, Budding AE, Grasman ME, Kneepkens CM, Savelkoul PH, Mearin ML. *Composition and diversity of the duodenal mucosa-associated microbiome in children with untreated coeliac disease.* Scand J Gastroenterol. 2013; 48: 530-6.
   http://dx.doi.org/10.3109/00365521.2013.775666
   PMid:23534388
6. Lebwohl B, Ludvigsson JF, Green PH. *The unfolding story of celiac disease risk factors.* Clin Gastroenterol Hepatol. 2014; 12: 632-5.
   http://dx.doi.org/10.1016/j.cgh.2013.10.031
   PMid:24211288 PMCid:PMC3960307
7. Steens RF, Csizmadia CG, George EK, Ninaber MK, Hira Sing RA, Mearin ML. *A national prospective study on childhood celiac disease in the Netherlands 1993-2000: an increasing recognition and a changing clinical picture.* J Pediatr. 2005; 147: 239-43.
   http://dx.doi.org/10.1016/j.jpeds.2005.04.013
   PMid:16126057
8. Csizmadia CG, Mearin ML, von Blomberg BM, Brand R, Verloove-Vanhorick SP. *An iceberg of childhood coeliac disease in the Netherlands.* Lancet. 1999; 353: 813-4.
   http://dx.doi.org/10.1016/S0140-6736(99)00243-3
9. Schweizer JJ, von Blomberg BM, Bueno-de Mesquita HB, Mearin ML. *Coeliac disease in The Netherlands.* Scand J Gastroenterol. 2004; 39: 359-64.
   http://dx.doi.org/10.1080/00365520310008503
   PMid:15125468

10. Catassi C, Kryszak D, Louis-Jacques O, Duerksen DR, Hill I, Crowe SE et al. *Detection of Celiac disease in primary care: a multicenter case-finding study in North America.* Am J Gastroenterol. 2007; 102: 1454-60.

http://dx.doi.org/10.1111/j.1572-0241.2007.01173.x
PMid:17355413

11. Ivarsson A, Myleus A, Norstrom F, van der Pals M, Rosen A, Hogberg L et al. *Prevalence of childhood celiac disease and changes in infant feeding.* Pediatrics. 2013; 131: e687-94.

http://dx.doi.org/10.1542/peds.2012-1015
PMid:23420914

12. Rostom A, Murray JA, Kagnoff MF. *American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease.* Gastroenterology. 2006; 131: 1981-2002.

http://dx.doi.org/10.1053/j.gastro.2006.10.004
PMid:17087937

13. National Institute for Health and Clinical Excellence. *Coeliac disease: recognition and assessment of coeliac disease.* London: National Institute for Health and Clinical Excellence. 2009. www.nice.org.uk/CG86

14. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA. *ACG Clinical Guidelines: Diagnosis and Management of Celiac Disease.* Am J Gastroenterol. 2013; 108: 656-76.

http://dx.doi.org/10.1038/ajg.2013.79
PMid:23609613 PMCid:PMC3706994

15. Ludvigsson JF, Bai JC, Biagi F, Card TR, Ciacci C, Ciclitira PJ et al. *Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology.* Gut. 2014; 63: 1210-28.

http://dx.doi.org/10.1136/gutjnl-2013-306578
PMid:24917550 PMCid:PMC4112432

16. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M et al. *Spectrum of gluten-related disorders: consensus on new nomenclature and classification.* BMC Med. 2012; 10: 13.

http://dx.doi.org/10.1186/1741-7015-10-13
PMid:22313950 PMCid:PMC3292448

17. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PH et al. *The Oslo definitions for coeliac disease and related terms.* Gut. 2013; 62: 43-52.

http://dx.doi.org/10.1136/gutjnl-2011-301346
PMid:22345659 PMCid:PMC3440559

18. Sanders DS, Hurlstone DP, Stokes RO, Rashid F, Milford-Ward A, Hadjivassiliou M et al. *Changing face of adult coeliac disease: experience of a single university hospital in South Yorkshire.* Postgrad Med J. 2002; 78: 31-3.

http://dx.doi.org/10.1136/pmj.78.915.31
PMid:11796869 PMCid:PMC1742229

19. Vivas S, Ruiz de Morales JM, Fernandez M, Hernando M, Herrero B, Casqueiro J et al. *Age-related clinical, serological, and histopathological features of celiac disease.* Am J Gastroenterol. 2008; 103: 2360-5.
http://dx.doi.org/10.1111/j.1572-0241.2008.01977.x
PMid:18702652

20. McGowan KE, Castiglione DA, Butzner JD. *The Changing Face of Childhood Celiac Disease in North America: Impact of Serological Testing.* Pediatrics. 2009; 124: 1572-1578.
http://dx.doi.org/10.1542/peds.2008-2373
PMid:19948628

21. Reilly NR, Fasano A, Green PH. *Presentation of celiac disease.* Gastrointest Endosc Clin N Am. 2012; 22: 613-21.
http://dx.doi.org/10.1016/j.giec.2012.07.008
PMid:23083982

22. Pelleboer RA, Janssen RL, Deckers-Kocken JM, Wouters E, Nissen AC, Bolz WE et al. *Celiac disease is overrepresented in patients with constipation.* J Pediatr (Rio J). 2012; 88: 173-6.
http://dx.doi.org/10.2223/JPED.2155

23. Reilly NR, Aguilar K, Hassid BG, Cheng J, Defelice AR, Kazlow P et al. *Celiac disease in normal-weight and overweight children: clinical features and growth outcomes following a gluten-free diet.* J Pediatr Gastroenterol Nutr. 2013; 53: 528-31.

24. Schaart MW, Mearin ML. *Early nutrition: prevention of celiac disease?* J Pediatr Gastroenterol Nutr. 2014; 59 Suppl 1: S18-20.
http://dx.doi.org/10.1097/01.mpg.0000450398.53650.f4
PMid:24979195

25. Ivarsson A, Persson LA, Nystrom L, Ascher H, Cavell B, Danielsson L et al. *Epidemic of coeliac disease in Swedish children.* Acta Paediatr. 2000; 89: 165-71.
http://dx.doi.org/10.1111/j.1651-2227.2000.tb01210.x
PMid:10709885

26. Vriezinga SL, Auricchio R, Bravi E, Castillejo G, Chmielewska A, Crespo Escobar P et al. *Randomized feeding intervention in infants at high risk for celiac disease.* N Engl J Med. 2014; 371: 1304-15.
http://dx.doi.org/10.1056/NEJMoa1404172
PMid:25271603

27. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Tonutti E, Amarri S et al. *Introduction of gluten, HLA status, and the risk of celiac disease in children.* N Engl J Med. 2014; 371: 1295-303.
http://dx.doi.org/10.1056/NEJMoa1400697
PMid:25271602

28. Green PH. *The many faces of celiac disease: clinical presentation of celiac disease in the adult population.* Gastroenterology. 2005; 128: S74-8.

http://dx.doi.org/10.1053/j.gastro.2005.02.016
PMid:15825130

29. M. Domínguez-Cajal S, Santolaria-Piedrafita I, Lera-Omiste B, Cuartero-Cuenca G, Pérez-Clavijo T. Guevara-López et al. *Patrones de presentación en una serie retrospectiva de pacientes con enfermedad celíaca según la nomenclatura aceptada en el consenso de Oslo, 2012. El comienzo del proyecto aretaea.* Gastroenterologia y Hepatologia. 2013; 36: 216.

30. Ciacci C, Cirillo M, Sollazzo R, Savino G, Sabbatini F, Mazzacca G. *Gender and clinical presentation in adult celiac disease.* Scand J Gastroenterol. 1995; 30: 1077-81.

http://dx.doi.org/10.3109/00365529509101610
PMid:8578167

31. Zipser RD, Patel S, Yahya KZ, Baisch DW, Monarch E. *Presentations of adult celiac disease in a nationwide patient support group.* Dig Dis Sci. 2003; 48: 761-4.

http://dx.doi.org/10.1023/A:1022897028030
PMid:12741468

32. Ford AC, Ching E, Moayyedi P. *Meta-analysis: yield of diagnostic tests for coeliac disease in dyspepsia.* Aliment Pharmacol Ther. 2009; 30: 28-36.

http://dx.doi.org/10.1111/j.1365-2036.2009.04008.x
PMid:19416130

33. Santolaria S, Alcedo J, Cuartero B, Diez I, Abascal M, Garcia-Prats MD et al. *Spectrum of gluten-sensitive enteropathy in patients with dysmotility-like dyspepsia.* Gastroenterol Hepatol. 2013; 36: 11-20.

http://dx.doi.org/10.1016/j.gastrohep.2012.07.011
PMid:23103052

34. O'Leary C, Wieneke P, Buckley S, O'Regan P, Cronin CC, Quigley EM et al. *Celiac disease and irritable bowel-type symptoms.* Am J Gastroenterol. 2002; 97: 1463-7.

http://dx.doi.org/10.1111/j.1572-0241.2002.05690.x
PMid:12094866

35. Ford AC, Chey WD, Talley NJ, Malhotra A, Spiegel BMR, Moayyedi P. *Yield of Diagnostic Tests for Celiac Disease in Individuals With Symptoms Suggestive of Irritable Bowel Syndrome Systematic Review and Meta-analysis.* Archives of Internal Medicine. 2009; 169: 651-658.

http://dx.doi.org/10.1001/archinternmed.2009.22
PMid:19364994

36. Fernandez-Bañares F, Esteve M, Salas A, Alsina M, Farre C, Gonzalez C et al. *Systematic evaluation of the causes of chronic watery diarrhea with functional characteristics.* Am J Gastroenterol. 2007; 102: 2520-8.

http://dx.doi.org/10.1111/j.1572-0241.2007.01438.x
PMid:17680846

37. Nachman F, Vazquez H, Gonzalez A, Andrenacci P, Compagni L, Reyes H et al. *Gastroesophageal reflux symptoms in patients with celiac disease and the effects of a gluten-free diet.* Clin Gastroenterol Hepatol. 2011; 9: 214-9.
http://dx.doi.org/10.1016/j.cgh.2010.06.017
PMid:20601132

38. Usai P, Manca R, Cuomo R, Lai MA, Russo L, Boi MF. *Effect of gluten-free diet on preventing recurrence of gastroesophageal reflux disease-related symptoms in adult celiac patients with nonerosive reflux disease.* J Gastroenterol Hepatol. 2008; 23: 1368-1372.
http://dx.doi.org/10.1111/j.1440-1746.2008.05507.x
PMid:18853995

39. Crowe SE. *In the clinic. Celiac disease.* Ann Intern Med. 2011; 154: ITC51-ITC5-15; quiz ITC5-16.

40. Meeuwisse GW. *Diagnostic criteria in coeliac disease.* Acta Paediatr Scand 1970; 59: 461-463.

41. Guandalini S, Ventura A, Ansaldi N, Giunta AM, Greco L, Lazzari R et al. *Diagnosis of coeliac disease: time for a change?* Arch Dis Child. 1989; 64: 1320-4; discussion 1324-5.
http://dx.doi.org/10.1136/adc.64.9.1320
PMid:2817956 PMCid:PMC1792735

42. Working Group of European Society of Paediatric Gastroenterology and Nutrition. *Revised criteria for diagnosis of coeliac disease.* Report. Arch Dis Child. 1990; 65: 909-11.
http://dx.doi.org/10.1136/adc.65.8.909
PMid:2205160 PMCid:PMC1792502

43. Koninckx CR, Giliams JP, Polanco I, Pena AS. *IgA antigliadin antibodies in celiac and inflammatory bowel disease.* J Pediatr Gastroenterol Nutr. 1984; 3: 676-82.
http://dx.doi.org/10.1097/00005176-198411000-00006
PMid:6502368

44. Troncone R, Ferguson A. *Anti-gliadin antibodies.* J Pediatr Gastroenterol Nutr. 1991; 12: 150-8.
http://dx.doi.org/10.1097/00005176-199102000-00002
PMid:2051264

45. Grodzinsky E, Jansson G, Skogh T, Stenhammar L, Falth-Magnusson K. *Anti-endomysium and anti-gliadin antibodies as serological markers for coeliac disease in childhood: a clinical study to develop a practical routine.* Acta Paediatr. 1995; 84: 294-8.
http://dx.doi.org/10.1111/j.1651-2227.1995.tb13631.x
PMid:7780251

46. Rossi TM, Albini CH, Kumar V. *Incidence of celiac disease identified by the presence of serum endomysial antibodies in children with chronic diarrhea, short stature, or insulin-dependent diabetes mellitus.* J Pediatr. 1993; 123: 262-4.
http://dx.doi.org/10.1016/S0022-3476(05)81699-3

47. de Lecea A, Ribes-Koninckx C, Polanco I, Calvete JF. *Serological screening (antigliadin and antiendomysium antibodies) for non-overt coeliac disease in children of short stature.* Acta Paediatr. Suppl 1996; 412: 54-5.

http://dx.doi.org/10.1111/j.1651-2227.1996.tb14252.x
PMid:8783760

48. Korponay-Szabo IR, Kovacs JB, Czinner A, Goracz G, Vamos A, Szabo T. *High prevalence of silent celiac disease in preschool children screened with IgA/IgG antiendomysium antibodies.* J Pediatr Gastroenterol Nutr. 1999; 28: 26-30.

http://dx.doi.org/10.1097/00005176-199901000-00008
PMid:9890464

49. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO et al. *Identification of tissue transglutaminase as the autoantigen of celiac disease.* Nat Med. 1997; 3: 797-801.

http://dx.doi.org/10.1038/nm0797-797
PMid:9212111

50. Troncone R, Maurano F, Rossi M, Micillo M, Greco L, Auricchio R et al. *IgA antibodies to tissue transglutaminase: An effective diagnostic test for celiac disease.* J Pediatr. 1999; 134: 166-71.

http://dx.doi.org/10.1016/S0022-3476(99)70410-5

51. Rostom A, Dube C, Cranney A, Saloojee N, Sy R, Garritty C et al. *The diagnostic accuracy of serologic tests for celiac disease: a systematic review.* Gastroenterology. 2005; 128: S38-46.

http://dx.doi.org/10.1053/j.gastro.2005.02.028
PMid:15825125

52. Prause C, Ritter M, Probst C, Daehnrich C, Schlumberger W, Komorowski L et al. *Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease.* J Pediatr Gastroenterol Nutr. 2009; 49: 52-8.

http://dx.doi.org/10.1097/MPG.0b013e318195dae3
PMid:19465869

53. Barker CC, Mitton C, Jevon G, Mock T. *Can tissue transglutaminase antibody titers replace small-bowel biopsy to diagnose celiac disease in select pediatric populations?* Pediatrics. 2005; 115: 1341-6.

http://dx.doi.org/10.1542/peds.2004-1392
PMid:15867045

54. Donaldson MR, Book LS, Leiferman KM, Zone JJ, Neuhausen SL. *Strongly positive tissue transglutaminase antibodies are associated with Marsh 3 histopathology in adult and pediatric celiac disease.* J Clin Gastroenterol. 2008; 42: 256-60.

http://dx.doi.org/10.1097/MCG.0b013e31802e70b1

55. Dahlbom I, Korponay-Szabo IR, Kovacs JB, Szalai Z, Maki M, Hansson T. *Prediction of clinical and mucosal severity of coeliac disease and dermatitis herpetiformis by quantification of IgA/IgG serum antibodies to tissue transglutaminase.* J Pediatr Gastroenterol Nutr. 2010; 50: 140-6.
http://dx.doi.org/10.1097/MPG.0b013e3181a81384
PMid:19841593

56. Mearin ML, Koninckx CR, Biemond I, Polanco I, Pena AS. *Influence of genetic factors on the serum levels of antigliadin antibodies in celiac disease.* J Pediatr Gastroenterol Nutr. 1984; 3: 373-7.
http://dx.doi.org/10.1097/00005176-198406000-00012
PMid:6588183

57. Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. *Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer.* J Exp Med. 1989; 169: 345-50.
http://dx.doi.org/10.1084/jem.169.1.345
PMid:2909659

58. Margaritte-Jeannin P, Babron MC, Bourgey M, Louka AS, Clot F, Percopo S et al. *HLA-DQ relative risks for coeliac disease in European populations: a study of the European Genetics Cluster on Coeliac Disease.* Tissue Antigens. 2004; 63: 562-7.
http://dx.doi.org/10.1111/j.0001-2815.2004.00237.x
PMid:15140032

59. Wolters VM, van de Nadort C, Gerritsen SA, Kneepkens CM, Ten Kate FJ, Gijsbers CF et al. *Is gluten challenge really necessary for the diagnosis of coeliac disease in children younger than age 2 years?* J Pediatr Gastroenterol Nutr. 2009; 48: 566-70.
http://dx.doi.org/10.1097/MPG.0b013e31818c526b
PMid:19367182

60. Ribes-Koninckx C, Mearin ML, Korponay-Szabo IR, Shamir R, Husby S, Ventura A et al. *Coeliac disease diagnosis: ESPGHAN 1990 criteria or need for a change? Results of a questionnaire.* J Pediatr Gastroenterol Nutr. 2011; 54: 15-9.
http://dx.doi.org/10.1097/MPG.0b013e31822a00bb
PMid:21716133

61. Giersiepen K, Lelgemann M, Stuhldreher N, Ronfani L, Husby S, Koletzko S et al. *Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report.* J Pediatr Gastroenterol Nutr. 2012; 54: 229-41.
http://dx.doi.org/10.1097/MPG.0b013e318216f2e5
PMid:22266486

62. Klapp G, Masip E, Bolonio M, Donat E, Polo B, Ramos D et al. *Celiac disease: the new proposed ESPGHAN diagnostic criteria do work well in a selected population.* J Pediatr Gastroenterol Nutr. 2013; 56: 251-6.
http://dx.doi.org/10.1097/MPG.0b013e318279887b
PMid:23111763

63. Lewis NR, Scott BB. *Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibody tests).* Aliment Pharmacol Ther. 2006; 24: 47-54.

http://dx.doi.org/10.1111/j.1365-2036.2006.02967.x
PMid:16803602

64. Kaukinen K, Maki M, Partanen J, Sievanen H, Collin P. *Celiac disease without villous atrophy: revision of criteria called for.* Dig Dis Sci. 2001; 46: 879-87.

http://dx.doi.org/10.1023/A:1010729207320
PMid:11330428

65. Kurppa K, Ashorn M, Iltanen S, Koskinen LLE, Saavalainen P, Koskinen O et al. *Celiac Disease without Villous Atrophy in Children: A Prospective Study.* Journal of Pediatrics. 2010; 157: 373-U48.

66. Vivas S, Ruiz de Morales JG, Riestra S, Arias L, Fuentes D, Alvarez N et al. *Duodenal biopsy may be avoided when high transglutaminase antibody titers are present.* World J Gastroenterol. 2009; 15: 4775-80.

http://dx.doi.org/10.3748/wjg.15.4775
PMid:19824110 PMCid:PMC2761554

67. Hill PG, Holmes GK. *Coeliac disease: a biopsy is not always necessary for diagnosis.* Aliment Pharmacol Ther. 2008; 27: 572-7.

http://dx.doi.org/10.1111/j.1365-2036.2008.03609.x
PMid:18194500

68. Baviera LC, Aliaga ED, Ortigosa L, Litwin N, Pena-Quintana L, Mendez V et al. *Celiac disease screening by immunochromatographic visual assays: results of a multicenter study.* J Pediatr Gastroenterol Nutr. 2007; 45: 546-50.

http://dx.doi.org/10.1097/MPG.0b013e31814794b9
PMid:18030231

69. Korponay-Szabo IR, Szabados K, Pusztai J, Uhrin K, Ludmany E, Nemes E et al. *Population screening for coeliac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study.* Bmj. 2007; 335: 1244-7.

http://dx.doi.org/10.1136/bmj.39405.472975.80
PMid:18063612 PMCid:PMC2137074

70. Ravelli A, Villanacci V, Monfredini C, Martinazzi S, Grassi V, Manenti S. *How patchy is patchy villous atrophy?: distribution pattern of histological lesions in the duodenum of children with celiac disease.* Am J Gastroenterol. 2010; 105: 2103-10.

http://dx.doi.org/10.1038/ajg.2010.153
PMid:20372112

71. Bonamico M, Thanasi E, Mariani P, Nenna R, Luparia RPL, Barbera C et al. *Duodenal Bulb Biopsies in Celiac Disease: A Multicenter Study.* J Pediatr Gastroenterol Nutr. 2008; 47: 618-622.

http://dx.doi.org/10.1097/MPG.0b013e3181677d6e
PMid:18979585

72. Corazza GR, Villanacci V, Zambelli C, Milione M, Luinetti O, Vindigni C et al. *Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease.* Clin Gastroenterol Hepatol. 2007; 5: 838-43.
http://dx.doi.org/10.1016/j.cgh.2007.03.019
PMid:17544877

73. Kaukinen K, Collin P, Maki M. *Latent coeliac disease or coeliac disease beyond villous atrophy?* Gut. 2007; 56: 1339-1340.

74. Weir DC, Glickman JN, Roiff T, Valim C, Leichtner AM. *Variability of histopathological changes in childhood celiac disease.* Am J Gastroenterol. 2010; 105: 207-12.
http://dx.doi.org/10.1038/ajg.2009.557
PMid:19809405

75. Marsh MN. *Grains of truth: evolutionary changes in small intestinal mucosa in response to environmental antigen challenge.* Gut. 1990; 31: 111-4.
http://dx.doi.org/10.1136/gut.31.1.111
PMid:2180789 PMCid:PMC1378351

76. Oberhuber G, Granditsch G, Vogelsang H. *The histopathology of coeliac disease: time for a standardized report scheme for pathologists.* Eur J Gastroenterol Hepatol. 1999; 11: 1185-94.
http://dx.doi.org/10.1097/00042737-199910000-00019
PMid:10524652

77. Lio D, Bonanno CT, D'Anna C, De Luca S, Gervasi F, Cavataio F et al. *Gluten stimulation induces an in vitro expansion of peripheral blood T gamma delta cells from HLA-DQ2-positive subjects of families of patients with celiac disease.* Exp Clin Immunogenet. 1998; 15: 46-55.
http://dx.doi.org/10.1159/000019052
PMid:9619399

78. Koskinen O, Collin P, Korponay-Szabo I, Salmi T, Iltanen S, Haimila K et al. *Gluten-dependent small bowel mucosal transglutaminase 2-specific IgA deposits in overt and mild enteropathy coeliac disease.* J Pediatr Gastroenterol Nutr. 2008; 47: 436-42.
http://dx.doi.org/10.1097/MPG.0b013e31817b6dec
PMid:18852635

79. Koskinen O, Collin P, Lindfors K, Laurila K, Maki M, Kaukinen K. *Usefulness of small-bowel mucosal transglutaminase-2 specific autoantibody deposits in the diagnosis and follow-up of celiac disease.* J Clin Gastroenterol. 2010; 44: 483-8.

80. Salmi TT, Collin P, Korponay-Szabo IR, Laurila K, Partanen J, Huhtala H et al. *Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits.* Gut. 2006; 55: 1746-53.
http://dx.doi.org/10.1136/gut.2005.071514
PMid:16571636 PMCid:PMC1856451

81. Jarvinen TT, Collin P, Rasmussen M, Kyronpalo S, Maki M, Partanen J et al. *Villous tip intraepithelial lymphocytes as markers of early-stage coeliac disease.* Scand J Gastroenterol. 2004; 39: 428-33.

http://dx.doi.org/10.1080/00365520310008773
PMid:15180179

82. Catassi C, Fasano A. *Celiac disease diagnosis: simple rules are better than complicated algorithms.* Am J Med. 2010; 123: 691-3.

http://dx.doi.org/10.1016/j.amjmed.2010.02.019
PMid:20670718

83. Katz KD, Rashtak S, Lahr BD, Melton LJ, 3rd, Krause PK, Maggi K et al. *Screening for celiac disease in a North American population: sequential serology and gastrointestinal symptoms.* Am J Gastroenterol. 2011; 106: 1333-9.

http://dx.doi.org/10.1038/ajg.2011.21
PMid:21364545 PMCid:PMC3130886

84. Green PHR, Stavropoulos SN, Panagi SG, Goldstein SL, McMahon DJ, Absan H et al. *Characteristics of adult celiac disease in the USA: results of a national survey.* Am J Gastroenterol. 2001; 96: 126-31.

http://dx.doi.org/10.1111/j.1572-0241.2001.03462.x
PMid:11197241

85. Rostami K, Kerckhaert J, Tiemessen R, von Blomberg BM, Meijer JW, Mulder CJ. *Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice.* Am J Gastroenterol. 1999; 94: 888-94.

http://dx.doi.org/10.1111/j.1572-0241.1999.983_f.x
PMid:10201452

86. Tursi A, Brandimarte G, Giorgetti GM. *Prevalence of antitissue transglutaminase antibodies in different degrees of intestinal damage in celiac disease.* J Clin Gastroenterol. 2003; 36: 219-21.

http://dx.doi.org/10.1097/00004836-200303000-00007
PMid:12590232

87. Naiyer AJ, Hernandez L, Ciaccio EJ, Papdakis K, Manavalan JS, Bhagat G et al. *Comparison of Commercially Available Serologic Kits for the Detection of Celiac Disease.* J Clin Gastroenterol. 2009; 43: 225-232.

http://dx.doi.org/10.1097/MCG.0b013e31816200e5
PMid:18724250

88. Walker MM, Murray JA. *An update in the diagnosis of coeliac disease.* Histopathology. 2010; 59: 166-79.

89. Hopper AD, Cross SS, Sanders DS. *Patchy villous atrophy in adult patients with suspected gluten-sensitive enteropathy: is a multiple duodenal biopsy strategy appropriate?* Endoscopy. 2008; 40: 219-24.

90. Evans KE, Aziz I, Cross SS, Sahota GR, Hopper AD, Hadjivassiliou M et al. *A prospective study of duodenal bulb biopsy in newly diagnosed and established adult celiac disease.* Am J Gastroenterol. 2011; 106: 1837-742.
http://dx.doi.org/10.1038/ajg.2011.171
PMid:21606978

91. Santolaria S, Dominguez M, Alcedo J, Abascal M, Garcia-Prats MD, Marigil M et al. *Lymphocytic duodenosis: etiological study and clinical presentations.* Gastroenterol Hepatol. 2013.

92. Rosinach M, Esteve M, Gonzalez C, Temino R, Marine M, Monzon H et al. *Lymphocytic duodenosis: Aetiology and long-term response to specific treatment.* Dig Liver Dis. 2012; 44: 643-8.
http://dx.doi.org/10.1016/j.dld.2012.03.006
PMid:22497904

93. Collin P, Wahab PJ, Murray JA. *Intraepithelial lymphocytes and coeliac disease.* Best Pract Res Clin Gastroenterol. 2005; 19: 341-50.
http://dx.doi.org/10.1016/j.bpg.2005.01.005
PMid:15925840

94. Green PH, Cellier C. *Celiac disease.* N Engl J Med. 2007; 357: 1731-43.
http://dx.doi.org/10.1056/NEJMra071600
PMid:17960014

95. Kaukinen K, Partanen J, Maki M, Collin P. *HLA-DQ typing in the diagnosis of celiac disease.* Am J Gastroenterol. 2002; 97: 695-99.
http://dx.doi.org/10.1111/j.1572-0241.2002.05471.x
PMid:11922565

96. Wouters J, Weijerman ME, van Furth AM, Schreurs MW, Crusius JB, von Blomberg BM et al. *Prospective human leukocyte antigen, endomysium immunoglobulin A antibodies, and transglutaminase antibodies testing for celiac disease in children with Down syndrome.* J Pediatr. 2009; 154: 239-42.
http://dx.doi.org/10.1016/j.jpeds.2008.08.007
PMid:18822429

97. Esteve M, Rosinach M, Fernandez-Bañares F, Farre C, Salas A, Alsina M et al. *Spectrum of gluten-sensitive enteropathy in first-degree relatives of patients with coeliac disease: clinical relevance of lymphocytic enteritis.* Gut. 2006; 55: 1739-45.
http://dx.doi.org/10.1136/gut.2006.095299
PMid:16709658 PMCid:PMC1856469

98. Zanini B, Caselani F, Magni A, Turini D, Ferraresi A, Lanzarotto F et al. *Celiac disease with mild enteropathy is not mild disease.* Clin Gastroenterol Hepatol. 2013; 11: 253-8.
http://dx.doi.org/10.1016/j.cgh.2012.09.027
PMid:23022697

99. Walker MM, Murray JA, Ronkainen J, Aro P, Storskrubb T, D'Amato M et al. *Detection of celiac disease and lymphocytic enteropathy by parallel serology and histopathology in a population-based study.* Gastroenterology. 2010; 139: 112-9.

http://dx.doi.org/10.1053/j.gastro.2010.04.007
PMid:20398668 PMCid:PMC2902605

100. Olivencia-Palomar P, Cano-Ruiz A, Martin-Scapa MA, Leon-Prieto F, Roy-Arino G, Redondo-Verge C. *Adult celiac disease and intraepithelial lymphocytes. New options for diagnosis?* Gastroenterol Hepatol. 2008; 31: 555-9.

101. Salmi TT, Collin P, Reunala T, Maki M, Kaukinen K. *Diagnostic methods beyond conventional histology in coeliac disease diagnosis.* Dig Liver Dis. 2010; 42: 28-32.

http://dx.doi.org/10.1016/j.dld.2009.04.004
PMid:19473894

102. Kurppa K, Collin P, Viljamaa M, Haimila K, Saavalainen P, Partanen J et al. *Diagnosing Mild Enteropathy Celiac Disease: A Randomized, Controlled Clinical Study.* Gastroenterology. 2009; 136: 816-823.

http://dx.doi.org/10.1053/j.gastro.2008.11.040
PMid:19111551

103. Leffler D, Schuppan D, Pallav K, Najarian R, Goldsmith JD, Hansen J et al. *Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease.* Gut. 2013; 62: 996-1004.

http://dx.doi.org/10.1136/gutjnl-2012-302196
PMid:22619366 PMCid:PMC3525791

104. Biesiekierski JR, Muir JG, Gibson PR. *Is Gluten a Cause of Gastrointestinal Symptoms in People Without Celiac Disease?* Curr Allergy Asthma Rep. 2013.