

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease

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ABSTRACT

Objective: Diagnostic criteria for coeliac disease (CD) from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) were published in 1990. Since then, the autoantigen in CD, tissue transglutaminase, has been identified; the perception of CD has changed from that of a rather uncommon enteropathy to a common multiorgan disease strongly dependent on the haplotypes human leukocyte antigen (HLA)-DQ2 and HLA-DQ8; and CD-specific antibody tests have improved.

Methods: A panel of 17 experts defined CD and developed new diagnostic criteria based on the Delphi process. Two groups of patients were defined with different diagnostic approaches to diagnose CD: children with symptoms suggestive of CD (group 1) and asymptomatic children at increased risk for CD (group 2). The 2004 National Institutes of Health/Agency for Healthcare Research and Quality report and a systematic literature search on antibody tests for CD in paediatric patients covering the years 2004 to 2009 was the basis for the evidence-based recommendations on CD-specific antibody testing.

Results: In group 1, the diagnosis of CD is based on symptoms, positive serology, and histology that is consistent with CD. If immunoglobulin A anti-tissue transglutaminase type 2 antibody titers are high (>10 times the upper limit of normal), then the option is to diagnose CD without duodenal biopsies by applying a strict protocol with further laboratory tests. In group 2, the diagnosis of CD is based on positive serology and histology. HLA-DQ2 and HLA-DQ8 testing is valuable because CD is unlikely if both haplotypes are negative.

Conclusions: The aim of the new guidelines was to achieve a high diagnostic accuracy and to reduce the burden for patients and their families. The performance of these guidelines in clinical practice should be evaluated prospectively.

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SYNOPSIS

Guidelines from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) for the diagnosis and treatment of coeliac disease (CD) have not been renewed for 20 years. During this time, the perception of CD has changed from a rather uncommon enteropathy to a common multiorgan disease with a strong genetic predisposition that is associated mainly with human leukocyte antigen (HLA)-DQ2 and HLA-DQ8. The diagnosis of CD also has changed as a result of the availability of CD-specific antibody tests, based mainly on tissue transglutaminase type 2 (TG2) antibodies.

Within ESPGHAN, a working group was established to formulate new guidelines for the diagnosis of CD based on scientific and technical developments using an evidence-based approach. The working group additionally developed a new definition of CD. A detailed evidence report on antibody testing in CD forms the basis of the guidelines and will be published separately. Guideline statements and recommendations based on a voting procedure have been provided. The goal of this synopsis is to summarise some of the evidence statements and recommendations of the guidelines for use in clinical practice.

Definitions

CD is an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals and characterised by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies,

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HLA-DQ2 or HLA-DQ8 haplotypes, and enteropathy. CD-specific antibodies comprise autoantibodies against TG2, including endomysial antibodies (EMA), and antibodies against deamidated forms of gliadin peptides (DGP).

Who Should Be Tested for CD?

CD may present with a large variety of nonspecific signs and symptoms. It is important to diagnose CD not only in children with obvious gastrointestinal symptoms but also in children with a less clear clinical picture because the disease may have negative health consequences. The availability of serological tests with high accuracy and other diagnostic tests allows a firm diagnosis to be made. The interpretation and consequences of the test results differ between symptomatic and asymptomatic patients in at-risk groups.

Testing for CD should be offered to the following groups:

Group 1: Children and adolescents with the otherwise unexplained symptoms and signs of chronic or intermittent diarrhoea, failure to thrive, weight loss, stunted growth, delayed puberty, amenorrhoea, iron-deficiency anaemia, nausea or vomiting, chronic abdominal pain, cramping or distension, chronic constipation, chronic fatigue, recurrent aphthous stomatitis (mouth ulcers), dermatitis herpetiformis-like rash, fracture with inadequate traumas/osteopenia/osteoporosis, and abnormal liver biochemistry.

Group 2: 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, and first-degree relatives with CD.

Diagnostic Tools

CD-specific Antibody Tests

CD-specific antibody tests measure anti-TG2 or EMA in blood. Tests measuring anti-DGP also could be reasonably specific. Laboratories providing CD-specific antibody test results for diagnostic use should continuously participate in quality control programmes at a national or an international level. Every antibody test used for the diagnosis of childhood CD should be validated against the reference standard of EMA or histology in a paediatric population ranging from infancy to adolescence.

A test is considered as reliable if it shows >95% agreement with the reference standard. The optimal threshold values for antibody positivity (cutoff value or upper limit of normal [ULN]) of a test should be established. Anti-TG2 and anti-DGP laboratory test results should be communicated as numeric values together with the specification of the immunoglobulin class measured, the manufacturer, the cutoff value defined for the specific test kit, and (if available) the level of "high" antibody values. It is not sufficient to state only positivity or negativity. Reports on EMA results should contain the specification of the investigated immunoglobulin class, cutoff dilution, interpretation (positive or negative), highest dilution still positive, and specification of the substrate tissue.

For the interpretation of antibody results, total IgA levels in serum, age of the patient, pattern of gluten consumption, and intake of immunosuppressive drugs should be taken into account. If gluten exposure was short or gluten had been withdrawn for a longer period of time (several weeks to years) the negative result is not reliable. For IgA-competent subjects, the conclusions should be drawn primarily from the results of IgA class antibody tests. For

subjects with low serum IgA levels (total serum IgA < 0.2 g/L), the conclusions should be drawn from the results of the IgG class CD-specific antibody tests.

HLA Testing for HLA-DQ2 and HLA-DQ8

Typing for HLA-DQ2 and HLA-DQ8 is a useful tool to exclude CD or to make the diagnosis unlikely in the case of a negative test result for both markers. HLA testing should be performed in patients with an uncertain diagnosis of CD, for example, in patients with negative CD-specific antibodies and mild infiltrative changes in proximal small intestinal biopsy specimens. If CD is considered in children in whom there is a strong clinical suspicion of CD, high specific CD antibodies are present, and small-bowel biopsies are not going to be performed, then the working group recommends performing HLA-DQ2 and HLA-DQ8 typing to add strength to the diagnosis. Prospective studies will make clear whether HLA typing is indeed an efficient and effective diagnostic tool in these patients. HLA testing may be offered to asymptomatic individuals with CD-associated conditions (group 2) to select them for further CD-specific antibody testing.

Histological Analysis of Duodenal Biopsies

The histological features of the small intestinal enteropathy in CD have a variable severity, may be patchy, and in a small proportion of patients with CD appear only in the duodenal bulb. The alterations are not specific for CD and may be found in enteropathies other than CD. Biopsies should be taken preferably during upper endoscopy from the bulb (at least 1 biopsy) and from the second or third portion of duodenum (at least 4 biopsies). The pathology report should include a description of the orientation, the presence or not of normal villi or degree of atrophy and crypt elongation, the villus-crypt ratio, the number of intraepithelial lymphocytes (IELs), and grading according to the Marsh-Oberhuber classification.

Diagnostic Approach for a Child or Adolescent With Symptoms or Signs Suggestive of CD

A test for CD-specific antibodies is the first tool that is used to identify individuals for further investigation to diagnose or to rule out CD. Patients who are consuming a gluten-containing diet should be tested for CD-specific antibodies. It is recommended that the initial test be IgA class anti-TG2 from a blood sample. If total serum IgA is not known, then this also should be measured. In subjects with either primary or secondary humoral IgA deficiency, at least 1 additional test measuring IgG class CD-specific antibodies should be done (IgG anti-TG2, IgG anti-DGP or IgG EMA, or blended kits for both IgA and IgG antibodies). In symptomatic patients in whom the initial testing was performed with a rapid CD antibody detection kit (point-of-care [POC] tests), the result should be confirmed by a laboratory-based quantitative test. Although published data indicate POC tests may achieve high accuracy for CD diagnosis, future studies must show whether they work equally well when applied in less selected populations and/or when handled by laypeople or untrained medical staff.

Tests measuring antibodies against DGP may be used as additional tests in patients who are negative for other CD-specific antibodies but in whom clinical symptoms raise a strong suspicion of CD, especially if they are younger than 2 years. Tests for the detection of IgG or IgA antibodies against native gliadin peptides (conventional gliadin antibody test) should not be used for CD

diagnosis. Tests for the detection of antibodies of any type (IgG, IgA, secretory IgA) in faecal samples should not be used.

If IgA class CD antibodies are negative in an IgA-competent symptomatic patient, then it is unlikely that CD is causing the symptom at the given time point. Further testing for CD is not recommended unless special medical circumstances (eg, younger than 2 years, restricted gluten consumption, severe symptoms, family predisposition or other predisposing disease, immunosuppressive medication) are present.

In seronegative cases for anti-TG2, EMA, and anti-DGP but with severe symptoms and a strong clinical suspicion of CD, small intestinal biopsies and HLA-DQ testing are recommended. If histology shows lesions are compatible with CD but HLA-DQ2/HLA-DQ8 heterodimers are negative, then CD is not likely and an enteropathy caused by a diagnosis other than CD should be considered. In these patients, the diagnosis of CD can be made only after a positive challenge procedure with repeated biopsies.

When duodenal biopsies, taken during routine diagnostic workup for gastrointestinal symptoms, disclose a histological pattern indicative of CD (Marsh 1–3 lesions), antibody determinations (anti-TG2 and, in children younger than 2 years, anti-DGP) and HLA typing should be performed. In the absence of CD-specific antibodies and/or HLA-DQ2 or HLA-DQ8 heterodimers, other causes of enteropathy (eg, food allergy, autoimmune enteropathy) should be considered.

What Should Be Done When CD-specific Antibody Tests Are Positive?

Children testing positive for CD-specific antibodies should be evaluated by a paediatric gastroenterologist or by a paediatrician with a similar knowledge of and experience with CD to confirm or exclude CD. A gluten-free diet (GFD) should be introduced only after the completion of the diagnostic process, when a conclusive diagnosis has been made. Health care professionals should be advised that starting patients on a GFD, when CD has not been excluded or confirmed, may be detrimental. A CD-specific antibody test also should be performed in children and adolescents before the start of a GFD because of suspected or proven allergy to wheat.

The clinical relevance of a positive anti-TG2 or anti-DGP result should be confirmed by histology, unless certain conditions are fulfilled that allow the option of omitting the confirmatory biopsies. If histology shows lesions that are consistent with CD (Marsh 2–3), then the diagnosis of CD is confirmed. If histology is normal (Marsh 0) or shows only increased IEL counts (>25 lymphocytes per 100 epithelial cells, Marsh 1), then further testing should be performed before establishing the diagnosis of CD.

In Which Patients Can the Diagnosis of CD Be Made Without Duodenal Biopsies?

In children and adolescents with signs or symptoms suggestive of CD and high anti-TG2 titers with levels >10 times ULN, the likelihood for villous atrophy (Marsh 3) is high. In this situation, the paediatric gastroenterologist may discuss with the parents and patient (as appropriate for age) the option of performing further laboratory testing (EMA, HLA) to make the diagnosis of CD without biopsies. Antibody positivity should be verified by EMA from a blood sample drawn at an occasion separate from the initial test to avoid false-positive serology results owing to mislabeling of blood samples or other technical mistakes. If EMA testing confirms specific CD antibody positivity in this second blood sample, then

the diagnosis of CD can be made and the child can be started on a GFD. It is advisable to check for HLA types in patients who are diagnosed without having a small intestinal biopsy to reinforce the diagnosis of CD.

Diagnostic Approach for an Asymptomatic Child or Adolescent With CD-associated Conditions

If it is available, HLA testing should be offered as the first-line test. The absence of DQ2 and DQ8 render CD highly unlikely and no further follow-up with serological tests is needed. If the patient is DQ8 and/or DQ2 positive, homozygous for only the β -chains of the HLA-DQ2 complex (DQB1*0202), or HLA testing is not done, then an anti-TG2 IgA test and total IgA determination should be performed, but preferably not before the child is 2 years old. If antibodies are negative, then repeated testing for CD-specific antibodies is recommended.

Individuals with an increased genetic risk for CD may have fluctuating (or transient) positive serum levels of CD-specific antibodies, particularly anti-TG2 and anti-DGP. Therefore, in this group of individuals (group 2) without clinical signs and symptoms, duodenal biopsies with the demonstration of an enteropathy should always be part of the CD diagnosis. If initial testing was performed with a rapid CD antibody-detection kit, then a positive test result always should be confirmed by a laboratory-based quantitative test. Negative rapid test results in asymptomatic individuals also should be confirmed by a quantitative test whenever the test has been carried out by laypeople or untrained medical staff and/or reliability of the test or circumstances of testing (eg, sufficient gluten intake, concomitant medication, IgA status) are unknown or questionable.

To avoid unnecessary biopsies in individuals with low CD-specific antibody levels (ie, <3 times ULN), it is recommended that the more specific test for EMA be performed. If the EMA test is positive, then the child should be referred for duodenal biopsies. If the EMA test is negative, then repeated serological testing on a normal gluten-containing diet in 3 to 6 monthly intervals is recommended.

Follow-up and Challenge Procedures

If the diagnosis of CD is made according to the diagnostic criteria mentioned above, the family should receive professional dietary counseling for a GFD. The patients should be followed up regularly for symptomatic improvement and normalisation of CD-specific antibody tests. The time until the antibody titers fall below the cutoff for normal depends on the initial level, but in general this should be achieved within 12 months after starting the GFD.

In patients fulfilling the diagnostic criteria for CD it is unnecessary to perform small-bowel biopsies on a GFD; however, if there is no clinical response to the GFD in symptomatic patients, after a careful dietary assessment to exclude lack of adherence to a GFD, further investigations are required. These investigations may include further biopsies.

Gluten challenge is not considered necessary except under unusual circumstances. These circumstances include situations in which there is doubt about the initial diagnosis. Gluten challenge should be preceded by HLA typing and assessment of mucosal histology and always should be performed under medical supervision, preferably by a paediatric gastroenterologist. Gluten challenge should be discouraged before the child is 5 years old and during the pubertal growth spurt, unless the child is HLA-DQ2 and HLA-DQ8 negative or has been placed on a GFD without proper testing. The daily gluten intake during gluten challenge should

contain at least the normal amount of gluten intake for children (approximately 15 g/day). IgA anti-TG2 antibody (IgG in low levels of serum IgA) should be measured during the challenge period. A patient should be considered to have relapsed (and hence the diagnosis of CD confirmed) if CD-specific antibodies become positive and a clinical and/or histological relapse is observed. In the absence of positive antibodies or symptoms the challenge should be considered completed after 2 years; however, additional biopsies on a normal diet are recommended because delayed relapse may occur later in life.

INTRODUCTION AND STRUCTURE

ESPGHAN guidelines for the diagnosis of CD were last published in 1990 (1) and at that time represented a significant improvement in both the diagnosis and management of CD. Since 1990, the understanding of the pathological processes of CD has increased enormously, leading to a change in the clinical paradigm of CD from a chronic, gluten-dependent enteropathy of childhood to a systemic disease with chronic immune features affecting different organ systems. Although CD may occur at any age (2), these guidelines focus on childhood and adolescence.

The disease etiology is multifactorial with a strong genetic influence, as documented in twin studies (3) and in studies showing a strong dependence on HLA-DQ2 and HLA-DQ8 haplotypes (4). A major step forward in the understanding of the pathogenesis of CD was the demonstration in patients with CD of gluten-reactive small-bowel T cells that specifically recognise gliadin peptides in the context of HLA-DQ2 and HLA-DQ8 (5). Furthermore, the discovery of TG2 as the major autoantigen in CD led to the recognition of the autoimmune nature of the disease (6). TG2 occurs abundantly in the gut and functions to deamidate proteins and peptides, including gliadin or gliadin fragments, leading to increased T-cell reactivity in patients with CD (7). This increased knowledge of CD pathogenesis has led to the further development of diagnostic serological tests based on antibody determination against gliadin and TG2-rich endomysium and later TG2.

Tests using DGP as substrate may be of significant value in CD diagnostic testing (8). Antibodies against TG2, EMA, and DGP are hence referred to as CD-specific antibodies, whereas antibodies against native (non-DGP) gliadin are largely nonspecific. Small-bowel biopsies have thus far been considered to be the reference standard for the diagnosis of CD; however, evidence has been accumulating on the diagnostic value of specific CD antibodies, and HLA typing has been used increasingly for diagnostic purposes. At the same time, the leading role of histology for the diagnosis of CD has been questioned for several reasons: histological findings are not specific for CD, lesions may be patchy and can occur in the duodenal bulb only, and interpretation depends on preparation of the tissue and is prone to a high interobserver variability (9). The diagnosis of CD may then depend not only on the results of small-bowel biopsies but also on information from clinical and family data and results from specific CD antibody testing and HLA typing.

In 2004, the US National Institutes of Health and the Agency for Healthcare Research and Quality (AHRQ) published a comprehensive evidence-based analysis of the diagnosis and management of CD (10), which was followed by specific clinical guidelines for children by the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (11). In 2008, the UK National Institute for Health and Clinical Evidence (NICE) published guidelines for the diagnosis and management of CD in general practice. These guidelines did not challenge the central and exclusive position of the result of small-bowel biopsies as the reference standard for the diagnosis of CD. A working group within ESPGHAN was established with the aim of formulating new

evidence-based guidelines for the diagnosis of CD in children and adolescents. During the work it became apparent that a new definition of CD was necessary, and such a definition is presented here. A major goal of the guidelines was to answer the question of whether duodenal biopsies with presumed characteristic histological changes compatible with CD could be omitted in some clinical circumstances in the diagnosis of CD. In addition, these guidelines present diagnostic algorithms for the clinical diagnosis of childhood CD.

METHODOLOGIES

Working Group

An ESPGHAN working group was established in 2007 with the aim of establishing evidence-based guidelines for the diagnosis of CD in children and adolescents. The members of the group were ESPGHAN members with a scientific and clinical interest in CD, including pathology and laboratory antibody determinations, and with a broad representation from European countries. A representative of the Association of European Coeliac Societies was a member of the working group. Two epidemiologists also participated in the working group.

Systematic Searches

The group decided to use an evidence-based approach to select diagnostic questions, followed by search and evaluation of the scientific literature to answer these questions. The guidelines were based on the available evidence analyses including the AHRQ report from 2004 (10). The search profile of the AHRQ report with regard to specific CD antibodies was used as a template for a new literature search. At first, a literature search was conducted on articles from January 2004 to August 2008, supplemented by a second search from September 2008 to September 2009. The articles found were assessed by epidemiologists and evidence-based medicine experts from the Centre for Health Technology Assessment at the University of Bremen, Germany (www.hta.uni-bremen.de).

Evidence Report

A key question was whether determination of specific CD antibodies was sufficiently accurate to permit avoidance of small-bowel biopsies to diagnose CD in all of the patients or in selected patients. The scientific evidence for this question was specifically sought and antibody analysis is the subject of a full evidence report (11a).

Grades of Evidence

Grading of evidence was sought with levels of evidence (LOE) based on the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system as a simplified version (12).

Strength of Recommendation

Evidence statements were formulated by the members of the working group and formed the basis of evidence statements and recommendations, including grading the evidence. The recommendations were based on the degree of evidence and when there was no evidence available on the consensus of experts from the working group. The strength of recommendation was chosen to be given with

arrows as strong (↑↑) or moderate (↑), as explained by Schünemann et al (13).

Voting

To achieve agreement in a range of clinical and diagnostic evidence statements and in recommendations within the areas “who to test,” “specific CD antibodies,” “HLA,” and “small-bowel biopsies,” a modified Delphi process that was based on the work of the GRADE working group. A voting discussion and repeated anonymous voting on the evidence statements and recommendations was conducted based on an online platform portal (Leitlinienentwicklung, Charité Hospital, Berlin, Germany, www.leitlinienentwicklung.de) to obtain consensus. Four working group members did not participate in the final voting, including the member from the patient organisation and the 2 epidemiologists.

Funding Sources

The production of the guidelines was funded by ESPGHAN with contributions from the coeliac patients’ associations of Germany, Great Britain, Italy, and Denmark within the Association of European Coeliac Societies and the national paediatric gastroenterology societies of Germany and Spain.

DEFINITION AND CLASSIFICATION OF CD

The working group decided to define CD as an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals, characterised by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 and HLA-DQ8 haplotypes, and enteropathy. Several classifications of CD have been used, most important with distinctions drawn among classical, atypical, asymptomatic, latent, and potential CD. 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 diarrhoea) and extraintestinal symptoms and signs (eg, anaemia, neuropathy, decreased bone density, increased risk of fractures). Table 1 provides an extensive list of symptoms and signs of CD in children and adolescents.

Silent CD is defined as the 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. *Latent CD* is defined by the 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. *Potential CD* is defined by the 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.

1. Who to Test

1.1. Evidence Background

CD may be difficult to recognise because of the variation in presentation and intensity of symptoms and signs, and many cases may actually occur without symptoms. It has been estimated that only 1 in 3 to 1 in 7 adult patients with CD are symptomatic (14). The object of this section is to list the symptoms and the concurrent conditions, which raise sufficient suspicion of CD to warrant further investigations, so-called CD case finding.

CD develops only after the introduction of gluten-containing foods into a child’s diet. The clinical symptoms of CD may appear in infancy, childhood, adolescence, or adulthood. A GFD in patients with CD improves or eliminates symptoms and normalises the specific CD antibodies and histological findings. Therefore, a normal gluten-containing diet with normal quantities of bread, pasta, and other gluten-containing foods should be consumed until the end of the diagnostic process. This should be particularly emphasised to families that consume a low gluten-containing diet because of family members diagnosed as having CD. When the diagnosis of CD is suspected in patients who are already receiving a GFD, it is essential that they be placed on a gluten-containing diet before initiating the diagnostic process. The length of time of gluten exposure depends on the duration of the GFD. There is no evidence in the scientific literature to suggest the precise amount of gluten that needs to be ingested to elicit a measurable serological and/or intestinal mucosal response (15). Patients without a conclusive diagnosis of CD, who are already receiving a GFD and do not want to reintroduce gluten into their diet, must be informed of the consequences of their decision.

Finally, a GFD is the only lasting treatment for CD. Adherence to a GFD in children results in remission of the intestinal lesions and promotes better growth and bone mineral density (16). It is the task of health care professionals to monitor and advise patients about adhering to a GFD because compliance with a GFD is variable and may be as low as 40% (17).

1.2. Evidence Review

Evaluation of the evidence for clinical symptoms of CD was performed in the AHRQ report from 2004 for 2 selected signs, anemia and low bone mineral density (10), and included in the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines (11). In the 2009 NICE guidelines, data were compiled for a series of symptoms and signs. This section is based on these analyses and supplemented with recent literature.

Symptoms and Signs

Gastrointestinal symptoms frequently appear in clinically diagnosed childhood CD, including diarrhoea in about 50% of patients (15,16,18) and chronic constipation (17). It is unclear whether chronic abdominal pain is indicative of CD because recurrent abdominal pain is so common in childhood. Abdominal pain has been reported as a presenting symptom in 90% of Canadian children with CD (18). A shift from gastrointestinal symptoms to extraintestinal symptoms seems to have occurred in children with CD (15,16,19). It is unclear whether this finding reflects a true clinical variation or an improved recognition of nongastrointestinal forms of CD because of increased awareness of the disease. Researchers have found good evidence that failure to thrive and stunted growth may be caused by CD. The risk of CD in patients with isolated stunted growth or short stature has been calculated as 10% to 40% (20). In some populations, CD is diagnosed in approximately 15% of children with iron-deficiency anaemia (21).

Associated Conditions

Good evidence exists for the increased prevalence of CD in first-degree relatives of patients with CD, patients with autoimmune diseases such as T1DM, and autoimmune thyroid disease (22) in some chromosomal aberration disorders and in selective IgA deficiency (Table 2). The prevalence of CD in T1DM has been investigated extensively and is 3% to 12%. The AHRQ report and

TABLE 1. Presenting features of children and adolescents with coeliac disease (CD)

Feature	Percentage of total no. children/adolescents with CD	Study population	Studies
Iron-deficiency anaemia	3–12	Adults and children	(19,27)
	16	Adults and children	
Other or unspecified anaemia	3–19	Adults and children	(28,105)
	23	Adults and children	
Anorexia	8	Adults and children	(15,19)
	26–35	Children	
Weight loss	44–60	Children and adults	(15,28)
	6	Children and adults	
Abdominal distension/bloating	28–36	Children	(15,16,27)
	10	Adults and children	
	20–39	Children	
Abdominal pain	12	Adults and children	(16,17,27,28)
	8	Adults and children	
	11–21	Children	
	90	Children	
Vomiting	26–33	Children	(15)
Flatulence	5	Adults and children	(27)
Diarrhoea	70–75	Children	(15,16,27,28)
	51	Adults and children	
	13	Adults and children	
	12–60	Children	
Short stature/growth failure	19	Adults and children	(19,28)
	20–31	Children	
Irritability	10–14	Children	(15)
Increased level of liver enzymes	5	Adults and children	(28)
Chronic fatigue	7	Adults and children	(28)
Failure to thrive	48–89	Children	(16)
Constipation	4–12	Children	(16)
Irregular bowel habits	4–12	Children	(16)

Adapted from the UK National Institute for Health and Clinical Evidence, with studies including children. Additional information was provided by a single paper. CD = coeliac disease.

the corresponding paper included 21 studies on T1DM with biopsy-proven CD, each with ≥ 50 participants (10). Two additional papers regarding children with T1DM have appeared: 1 reported 12% with CD (23) and 1 longitudinal study reported 7% (24). In addition, CD occurs more frequently than expected by chance in children with Turner syndrome (25) or Down syndrome. A 10-to 20-fold increase

in CD prevalence has been reported in subjects with selective IgA deficiency (26). A number of conditions (eg, epilepsy) have been suspected to be associated with CD, but the prevalence of 0.5% to 1% does not seem to differ significantly from the respective background populations. Such conditions have been omitted from Table 2.

TABLE 2. Conditions associated with CD apart from type 1 diabetes mellitus

Condition	CD, %	Study population	Studies
Juvenile chronic arthritis	1.5	Children	(106)
	2.5	Children	(107)
Down syndrome	0.3	Children and adults	(108)
	5.5	Children	
Turner syndrome	6.5	Children and adults	(25,108,109)
Williams syndrome	9.5	Children	(110)
IgA nephropathy	4	Adults	(111)
IgA deficiency	3	Children	(19,48)
Autoimmune thyroid disease	3		(22)
Autoimmune liver disease	13.5		(112)

Adapted from the UK National Institute for Health and Clinical Evidence, including only studies with children, except for immunoglobulin A nephropathy, in which data only on adults were available. CD = coeliac disease; IgA = immunoglobulin A.

1.3. Evidence Statements

1.3.1.

Patients with CD may present with a wide range of symptoms and signs or be asymptomatic. Symptoms in CD are adapted from the NICE guidelines. *Denotes added to the list from the NICE guidelines, + denotes a particularly common symptom.

- a. Gastrointestinal: Chronic diarrhea+, chronic constipation, abdominal pain+, nausea vomiting, distended abdomen+*.
- b. Extraintestinal: Failure-to-thrive+*, stunted growth+, delayed puberty, chronic anaemia+, decreased bone mineralisation (osteopenia/osteoporosis)+, dental enamel defects, irritability, chronic fatigue, neuropathy, arthritis/arthritis, amenorrhoea, increased levels of liver enzymes+.

LOE: 2.

References (15,16,19,26,27)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

1.3.2.

The following signs or diagnoses may be present when CD is diagnosed (*data from adults): short stature, amenorrhoea, recurrent aphthous stomatitis (mouth ulcers)*, dental enamel defects, dermatitis herpetiformis, osteopenia/osteoporosis, abnormal liver biochemistry.

LOE: 2.

References: (16,28)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

1.3.3.

CD has an increased prevalence in children and adolescents with first-degree relatives with CD (10%–20%), T1DM (3%–12%), Down syndrome (5%–12%), autoimmune thyroid disease (up to 7%), Turner syndrome (2%–5%), Williams syndrome (up to 9%), IgA deficiency (2%–8%), and autoimmune liver disease (12%–13%).

LOE: 1.

References: See Table 1.

Total number of votes: 13, Agree 13, Disagree: 0, Abstentions: 0

1.4. Recommendations

1.4.1.

(↑↑) Offer CD testing in children and adolescents with the following otherwise unexplained symptoms and signs: chronic abdominal pain, cramping or distension, chronic or intermittent diarrhoea, growth failure, iron-deficiency anaemia, nausea or vomiting, chronic constipation not responding to usual treatment, weight loss, chronic fatigue, short stature, delayed puberty, amenorrhoea, recurrent aphthous stomatitis (mouth ulcers), dermatitis herpetiformis–type rash, repetitive fractures/osteopenia/osteoporosis, and unexplained abnormal liver biochemistry.

Total number of votes: 13, Agree: 12, Disagree: 1, Abstentions: 0

1.4.2.

(↑↑) Offer CD testing in children and adolescents with the following conditions: T1DM, Down syndrome, autoimmune

thyroid disease, Turner syndrome, Williams syndrome, IgA deficiency, autoimmune liver disease, and first-degree relatives with CD.

Total number of votes: 13, Agree: 11, Disagree: 2, Abstentions: 0

1.4.3.

(↑↑) To avoid false-negative results, infants, children, and adolescents should be tested for CD only when they are consuming a gluten-containing diet. Paediatricians and gastroenterologists should always ask before testing whether the patients are consuming gluten.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

1.4.4.

(↑↑) In infants, CD antibodies should be measured only after the introduction of gluten-containing foods as complementary to the infant's diet.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

1.4.5.

A GFD should be introduced only after the completion of the diagnostic process, when a diagnosis of CD has been conclusively made. Health care professionals should be advised that putting patients on a GFD, when CD has not been excluded or confirmed, may be detrimental. GFD is a lifelong treatment, and consuming gluten later can result in significant illness.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

2. HLA Aspects

2.1. Evidence Background

The principal determinants of genetic susceptibility for CD are the major histocompatibility class II HLA class II DQA and DQB genes coded by the major histocompatibility region in the short arm of chromosome 6. More than 95% of patients with CD share the HLA-DQ2 heterodimer, either in the *cis* (encoded by HLA-DR3-DQA1*0501-DQB1*0201) or the *trans* configuration (encoded by HLA-DR11-DQA1*0505 DQB1 0301/DR7-DQA1*0201 DQB1 0202), and most of the remainder have the HLA-DQ8 heterodimer (encoded by DQA1*0301-DQB1*0302). CD is a multigenetic disorder, which means that the expression of these HLA-DQ2 or HLA-DQ8 molecules is necessary but not sufficient to cause disease because approximately 30% to 40% of the white population holds the HLA-DQ2 haplotype and only 1% develops CD. Outside the HLA region there are several genomic areas related to CD, controlling immune responses, among others the genes encoding for CTLA4, IL2, IL21, CCR3, IL12A, IL-18RAP, RGS1, SH2B3 and TAGAP (29–31). Their contribution to the genetics of CD is relatively small in comparison to that of HLA-DQ2 and HLA-DQ8. The strong relation between HLA genetic factors and CD is illustrated by the effect of the HLA-DQ2 gene dose on disease development; HLA-DQ2 homozygous individuals have an at least 5 times higher risk of disease development compared with HLA-DQ2 heterozygous individuals (32).

Table 3 presents the sensitivity of HLA-DQ2 and -DQ8 for CD as assessed by the Dutch evidence-based guidelines for CD and dermatitis herpetiformis (33a). Most of the studies included control

TABLE 3. Sensitivity of HLA-DQ2, HLA-DQ8, and HLA-DQ2 or HLA-DQ8 for CD

Author group, year	Type study	Origin	Tested CD group	N	Sensitivity, %		
					DQ2	DQ8	DQ2 or DQ8
Arnason, 1994 (113)	NIH; c-c	Iceland	Known CD	25	84		
Arranz, 1997 (114)	NIH; c-c	Spain	Known CD	50	92		
Balas, 1997 (39)	NIH; c-c	Spain	Known CD	212	95	4.3	99.1
Book, 2003 (115)	NIH; m-d	USA	1st-degree family member CD	34			97.1
Bouguerra, 1996 (116)	NIH; m-d	Tunis	Known CD	94	84		
Boy et al, 1994 (117)	NIH; c-c	Italy (Sardinia)	Known CD	50	96		
Catassi, 2001 (118)	NIH; m-d	Algeria	Saharawi Arabs	79	91		95.6
Colonna, 1990 (119)	NIH; c-c	Italy	Known CD	148	95		
Congia, 1994 (120)	NIH; c-c	Turkey	Known CD	65	91		
Congia, 1992 (121)	NIH; c-c	Italy	Known CD	25	96		
Csizmadia, 2000 (44)	NIH; m-d	The Netherlands	Down syndrome	10	100	20	100
Djilali-Saiah, 1994 (122)	NIH; c-c	France	Known CD	80	89		
Djilali-Saiah, 1998 (123)	NIH; c-c	France	Known CD	101	83		
Erkan, 1999 (124)	NIH; c-c	Turkey	Known CD	30	40		
Farre, 1999 (125)	NIH; m-d	Spain	1st-degree family member CD	60	93.3		
Fasano, 2003 (40)	NIH; m-d	USA	Population screening	98	83.7	22.5	100
Fernandez-Arquero, 1995 (126)	NIH; c-c	Spain	Known CD	100	92		
Ferrante, 1992 (127)	NIH; c-c	Italy	Known CD	50	88		
Fine, 2000 (128)	NIH; c-c	USA	Known CD	25	88		
Howell, 1995 (129)	NIH; c-c	England	Known CD	91	91		
Iltanen, 1999 (130,131)	NIH; c-c	Finland	Known CD	21	90		
Johnson, 2004 (132)	c-c	New York	Known CD	44	86	41	
Johnson, 2004 (132)	c-c	Paris, France	Known CD	66	93	21	
Karell, 2003 (33)	NIH; m-d	France	Known CD	92	87	6.5	93.5
	NIH; m-d	Italy	Known CD	302	93.7	5.6	89.4
	NIH; m-d	Finland	Known CD	100	91	5	96
	NIH; m-d	Norway	Known CD	326	91.4	5.2	96.6
	NIH; m-d	England	Known CD	188	87.8	8	95.7
Kaur, 2003 (133)	NIH; m-d	India	Known CD	35	97.1		
Lewis, 2000 (134)	NIH; m-d	USA	Family van CD	101	90		
Lio, 1998 (135)	NIH; c-c	Italy	Known CD	18	100		
Liu, 2002 (41)	NIH; m-d	Finland	Family member CD	260	96.9	2.7	99.6
Maki, 2003 (45)	NIH; m-d	Finland	Screening schoolchildren	56	85.7		
Margaritte-Jeannin, 2004 (35)	m-d	Italy	Known CD	128	86		
	m-d	France	Known CD	117	87		
	m-d	Scandinavia	Known CD	225	92		
Mazzilli, 1992 (136)	NIH; c-c	Italy	Known CD	50	92		
Michalski, 1995 (137)	NIH; c-c	Ireland	Known CD	90	97		
Mustalahti, 2002 (14)	NIH; m-d	Finland	Family member of CD of DH	29	100		
Neuhausen, 2002 (138)	NIH; m-d	Israel	Bedouin Arabs	23	82.6	56.5	100
Peña-Quintana, 2003 (139)	c-c	Spain, Gran Canaria	Known CD	118	92.4	0	92.4
Perez-Bravo 1999 (140)	NIH; m-d	Chile	Known CD	62	11.3	25.8	37.1
Ploski, 1993 (141)	NIH; c-c	Sweden	Known CD	94	95		
Ploski, 1996 (142)	NIH; m-d	Sweden	Known CD	135	92	4.4	96.3
Polvi, 1996 (34)	NIH; m-d	Finland	Known CD	45	100		100
Popat, 2002 (143)	NIH; m-d	Sweden	Known CD	62	93.6		
Ruiz del Prado, 2001 (144)	NIH; c-c	Spain	Known CD	38	95		
Sachetti, 1998 (145)	NIH; c-c	Italy	Known CD	122	87		
Sumnik, 2000 (146)	NIH; m-d	Czech	Diabetes	15	80	66.7	100

TABLE 3. (Continued)

CD population					Sensitivity, %		
Author group, year	Type study	Origin	Tested CD group	N	DQ2	DQ8	DQ2 or DQ8
Tighe, 1992 (147)	NIH; c-c	Italy	Known CD	43	91		
Tighe, 1993 (148)	NIH; c-c	Israel Ashkenazi Jews, known CD	34	71			
Tumer, 2000 (149)	NIH; c-c	Turkey	Known CD	33	52		
Tuysuz, 2001 (150)	NIH; m-d	Turkey	Known CD, children	55	84	16.4	90.9
Vidales, 2004 (42)	m-d	Spain	Known CD, children	136	94.1	2.1	95.6
Zubilaga, 2002 (151)	NIH; m-d	Spain	Known CD	135	92.6	3.7	96
Sensitivity							
No. studies					n = 55	n = 19	n = 20
Median					91	6.5	96.2
p10–p90					82.6–97.0	2.3–50.3	90.2–100
p25–p75					86.3–94.0	4.3–22.1	94.6–99.8

Data from the National Institutes of Health review (10) are referred to as NIH, m-d = mixed design study; cc = case control study. Data also from Richtlijn Coeliakie en Dermatitis Herpetiformis. Kwaliteitsinstituut voor de Gezondheidszorg CBO.

groups without results of small-bowel biopsies and were not designed primarily to assess the use of HLA typing in the diagnosis of CD. These studies reflect clearly the frequency of HLA-DQ2 and HLA-DQ8 in patients with CD. Table 4 presents the results of the studies included in the AHRQ report for the diagnosis of CD from 2004 (10) and a number of studies published after October 2003. All of the studies included more than 10 patients with CD. The results of the more recent studies did not change the conclusions regarding the sensitivity of HLA-DQ2 and HLA-DQ8 as stated by the AHRQ report. The sensitivity of HLA-DQ2 is high (median 91%; p25–p75 86.3%–94.0%), and if combined with HLA-DQ8 (at least 1 is positive), it is even higher (median 96.2%; p25–p75 94.6%–99.8%), making extremely small the chance of an individual who is negative for DQ2 and DQ8 to have CD; the small percentage of HLA-DQ2-negative and HLA-DQ8-negative patients is well documented (33–35).

The specificity of HLA-DQ2 and HLA-DQ8 for CD was assessed by the evidence-based Richtlijn Coeliakie en Dermatitis Herpetiformis (Dutch Guideline) (33a) in 31 studies, most of them including controls without small-bowel biopsy. The specificity of HLA-DQ2 is low (median 74%; p25–p75 65%–80%). The specificity of HLA-DQ8, evaluated in 9 studies, had a median of 80%

(p25–p75 75%–87.5%). The specificity of the combination HLA-DQ2/HLA-DQ8 varies widely in different study populations, from 12% to 68% with a median of 54%. A prospective study found that 43% of the non-CD controls were positive for DQ2 and/or DQ8 (specificity 57%) (36). In addition to the above-mentioned positivity for HLA-DQ2 and/or HLA-DQ8, the combination of the DQ complex can provide information on the risk for CD. Individuals, both HLA-DQ2 heterodimer positive and negative, who are homozygous for only the β -chains of the HLA-DQ2 complex (DQB1*02), have an increased risk for CD (35,37). For this reason HLA-DQ typing should be done by DNA testing for the 4 alleles in the HLA-DQ2 and HLA-DQ8 molecules. Traditionally, HLA typing has been relatively expensive, but new techniques (eg, using single tag nucleotide polymorphisms) will probably make HLA typing a relatively inexpensive test (30).

There is only 1 prospective study on the implementation of HLA-DQ typing in the diagnosis of CD (36). The diagnostic value of HLA typing, CD-specific antibodies, and small-bowel biopsies were prospectively assessed in 463 adult patients with clinically suspected CD. The study was included in the present report as major histocompatibility complex antigens, which are expressed for life. All 16 patients with CD (with villous atrophy and clinical

TABLE 4. Sensitivity and specificity of HLA-DQ2 and /or HLA-DQ8 for CD

Author group, year	Type of study	Origin	N CD	Sensitivity, %		Specificity, %	
				DQ2 and/or DQ8	N Control	DQ2 and/or DQ8	
Balas, 1997 (39)	NIH; c-c	Known CD vs controls, Spain	212	99	742	54	
Catassi, 2001 (118)	NIH; m-d	Saharawi Arabs, Algeria	79	96	136	58	
Fasano, 2003 (40)	NIH; m-d	EMApos vs, EMANeg USA	98	100	92	40	
Hadithi, 2007 (36)	m-d	Patients prospective, the Netherlands	16	100	447	57	
Liu, 2002 (41)	NIH; m-d	Family members of CD, Finland	260	100	237	32	
Neuhausen, 2002 (138)	NIH; m-d	Family of CD, Israel (Bedouins)	23	100	52	13	
Perez-Bravo, 1999 (140)	NIH; m-d	Known CD vs controls, Chile	62	37	124	85	
Sumnik, 2000 (146)	NIH; m-d	IDDM screen, Czech	15	100	186	12	
Tuysuz, 2001 (150)	NIH; m-d	Known CD vs controls, Turkey	55	91	50	68	

Data from the National Institutes of Health review (10) are referred to as NIH, m-d = mixed design study cc = case control study. Data also from Richtlijn Coeliakie en Dermatitis Herpetiformis. Kwaliteitsinstituut voor de Gezondheidszorg CBO.

response after GFD) were HLA-DQ2 or HLA-DQ8 positive, but there were no cases of CD among the 255 HLA-DQ2-negative and HLA-DQ8-negative patients. Because the chance of an individual negative for HLA-DQ2 or HLA-DQ8 having CD is extremely small, the main role of HLA-DQ typing in the diagnosis of CD is to exclude the disease or to make it unlikely.

Some evidence exists that HLA-DQ2/HLA-DQ8 typing plays a role in the case-finding strategy in individuals who belong to groups at risk for CD. These individuals include, among others, first-degree relatives of a confirmed case (3) and patients with immune-mediated as well as nonimmune conditions known to be associated with CD (Table 2). A negative result for HLA-DQ2/HLA-DQ8 renders CD highly unlikely in these children, and there is no need for subsequent CD antibodies testing of such individuals.

2.2. Evidence Statements

2.2.1.

There is a strong genetic predisposition to CD with the major risk attributed to the specific genetic markers known as HLA-DQ2 and HLA-DQ8.

LOE: 1.

References (10,38)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

2.2.2.

The vast majority of CD patients are HLA-DQ2 (full or incomplete heterodimer) and/or HLA-DQ8 positive.

LOE: 2.

References (38,39)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

2.2.3.

Individuals having neither DQ2 nor DQ8 are unlikely to have CD because the sensitivity of HLA-DQ2 is high (median 91%), and if combined with HLA-DQ8 (at least 1 of them positive), it is even higher (96%). The main role of HLA-DQ typing in the diagnosis of CD is to exclude the disease.

LOE: 2.

References (33,39–42)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

2.2.4.

HLA-DQ2 and/or HLA-DQ8 have poor specificity for CD (median 54%), indicating a low positive predictive value for CD.

LOE: 2.

References (36,39)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

2.2.5.

HLA-DQ typing should not be done by serology but by DNA testing for the 4 alleles in the HLA-DQ2 and HLA-DQ8 molecules. New techniques (eg, using tag single nucleotide polymorphisms) will make HLA typing available at a relatively low cost.

LOE: 2.

References (35,37,43)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

2.2.6.

HLA-DQ2/HLA-DQ8 typing has a role in the case-finding strategy in individuals who belong to groups at risk for CD. A negative result for HLA-DQ2/HLA-DQ8 renders CD highly unlikely in these children, and hence there is no need for subsequent CD antibodies testing in such individuals.

LOE: 2.

References (3,44,45)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

2.3. Recommendations

2.3.1.

(↑↑) Offer HLA-DQ2 and HLA-DQ8 typing in patients with uncertain diagnosis of CD, for example, in patients with negative CD-specific antibodies and mild infiltrative changes in small-bowel specimens. Negative results render CD highly unlikely in these children.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

2.3.2.

(↑↑) In patients with a clinical suspicion of CD, who are HLA-DQ2 negative and HLA-DQ8 negative, offer investigations for other causes of the symptoms (ie, different from CD).

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

2.3.3.

(↑↑) Start the screening for CD in groups at risk by HLA-DQ2 and HLA-DQ8 typing if the test is available. These groups include first-degree relatives of a patient with a confirmed case and patients with autoimmune and nonautoimmune conditions known to be associated with CD, such as T1DM, Down syndrome, and Turner syndrome.

Total number of votes: 13, Agree: 12, Disagree: 0, Abstentions: 1

2.3.4.

(↑) If CD can be diagnosed without performing small-bowel biopsies in children with strong clinical suspicion of CD and with high specific CD antibodies, consider performing HLA-DQ2/HLA-DQ8 typing in these children to add strength to the diagnosis.

Total number of votes: 13, Agree: 12, Disagree: 0, Abstentions: 1

3. Antibodies

3.1. Evidence Background

CD is characterised by highly specific autoantibodies directed against the common CD autoantigen TG2 (10) and by antibodies against DGP (46). EMA are directed against extracellular TG2 (47). Except for DGP antibodies these antibodies are typically of the IgA class. In IgA-deficient patients with CD, the same type of antibodies in IgG class can be detected (48).

Antibodies against TG2 bind in vivo to a patient's own TG2 expressed in the small bowel or in other tissues (eg, liver, muscles,

central nervous system) at sites accessible to the antibodies (47,49). Dermatitis herpetiformis is defined by the presence of granular IgA deposition in the dermal papillae of the skin containing antibodies against tissue transglutaminase type 3 (TG3). The appearance of CD-specific antibodies in the blood or in tissues may precede the development of structural abnormalities in the small bowel (50,51).

CD antibodies are not detectable in the blood of all patients with CD (10,52); however, TG2-specific antibodies may be present in small-intestine tissue or other tissues of seronegative patients (49,53). Negative antibody results in blood also can be obtained in subjects with dermatitis herpetiformis, after reduction of gluten consumption or during and after the use of immunosuppressive drugs (54–56).

3.2. Evidence Review

Antibody Detection

IgA and IgG class anti-TG2 antibodies can be detected in blood samples of patients by various immunoassays (enzyme-linked immunosorbent assay, radioimmunoassay, or others) using purified or recombinant TG2 antigens or tissue sections/fluids containing TG2. Most often serum is used, but plasma or whole blood also can be suitable sources (57). Immunofluorescent tests such as EMA require microscopic evaluation and may be subject to interobserver variability. Despite these limitations, the specificity of EMA test results is 98% to 100% in expert laboratories (10,52), and this test is considered the reference standard for CD-specific antibody detection. CD antibodies also can be detected by the use of synthetic peptides corresponding to deamidated gliadin sequences (46,58).

Antibody Values and Assay Performance

The values for serum anti-TG2 or anti-DGP levels obtained in a particular test depend on the source (human or animal) of the antigen, quality of the antigen, exposure of the antigen, calibrators, buffers, measuring methods, cutoff values and calculation mode of the results, so numerical values obtained with different kits may differ substantially. No universally accepted international standards are available that would allow the expression of antibody amount in absolute Ig concentrations; however, the majority of commercial kits use a calibration curve with antibody dilutions that provide numerical values that are proportional to antibody concentration in relative (arbitrary) units.

This is the preferred method for clinical evaluation. Antibody tests that calculate results from the percentage of absorbance values supply numerical values that correlate with the logarithmic values of antibody concentrations. Despite these differences, many commercial anti-TG2 antibody tests have equally high sensitivity and specificity on the same blood samples (59). Interlaboratory variability also exists (60). In addition, there may be considerable batch-to-batch variability within commercial anti-TG2 assays, which needs to be monitored by the use of independent quality control material.

The performance of a particular antibody test in a clinical setting depends on patient characteristics (age, genetic predisposition, IgA deficiency), pretest probability, stage of the disease, and ingested amounts of gluten. These factors should be taken into account when interpreting positive and negative antibody results and establishing the optimal cutoff limits (55,59,61). This can be done by receiver operating characteristics curve plotting sensitivity against 1–specificity. Anti-TG2 antibodies also can be detected in saliva. Sufficient sensitivity and specificity was not achieved with

conventional commercially available immunoassays (62,63), although the use of radiobinding assays appeared to be more favourable (64). There is no reliable method to detect specific CD antibodies from faecal samples (65).

Anti-TG2 antibody detection also can be done from the blood at the point of contact using rapid test kits (POC test) (57,66,67), but only as a semiquantitative test for circulating antibodies. Anti-TG2 antibodies detection by POC test may achieve a high accuracy for CD diagnosis, and the ESPGHAN evidence report on CD serology (11a) reported a pooled sensitivity of 96.4% and a pooled specificity of 97.7%; however, IgA-antiTG2 or EMA performed better. Published studies have thus far been based on populations with a high prevalence of CD because 60.3% of all of the patients had biopsy-confirmed CD. Assuming a prevalence of CD in 5% of all symptomatic children, the positive predictive value would be 68.6% and the negative predictive value would be 99.8% (11a). The expertise of the laboratory or of the observers has a great effect on the accuracy of the results in EMA and rapid tests (67).

Disease Prediction

The positivity for anti-TG2 and/or EMA is associated with a high probability for CD in children and adolescents (10,52); however, low levels of anti-TG2 have been described in a number of conditions unrelated to CD, such as other autoimmune diseases, infections, tumours, myocardial damage, liver disorders, and psoriasis (68–70). These antibodies are not associated with the EMA reaction, which explains why EMA has higher reliability for the diagnosis of CD. The ESPGHAN evidence report on CD serology (11a) estimates the pooled positive and negative likelihood ratios of EMA results in the studies performed between 2004 and 2009 as 31.8 (95% confidence limit 18.6–54.0) and 0.067 (95% confidence limit 0.038–0.12), respectively. Furthermore, EMA results were more homogeneous than results obtained with other CD antibody tests and had a high diagnostic odds ratio of 553.6. Taken together, these data mean that the presence of CD is likely if the EMA test result is positive (11a). Remarkably, EMA positivity also is associated with the later development of villous atrophy in the few reported cases of both adults and children with CD (50,71–73) who initially do not fulfill the histological criteria of CD because of normal small-intestinal architecture.

In the ESPGHAN report on CD antibodies, the specificity of anti-TG2 antibodies measured by enzyme-linked immunosorbent assay was lower than that of EMA testing and varied according to the test kit used (11a). It was not possible to obtain pooled performance estimates on sensitivity and specificity resulting from the heterogeneity in the evaluated studies, but for 11 of 15 study populations the sensitivity reached $\geq 90\%$ and for 13 of 15 study populations specificity reached $\geq 90\%$. Several studies confirmed that high concentrations of anti-TG2 antibodies in serum predict villous atrophy better than low or borderline values (55,74,75). These studies suggested that high anti-TG2 antibody levels can be defined as those exceeding 10 times ULN in concentration-dependent antibody tests based on calibration curves. Testing for anti-TG2 antibodies in serum is the preferred initial approach to find CD. The cutoff for such high values in a number of different commercial tests is examined in Appendix I.

Although tests for anti-DGP antibodies performed favourably and much better than antibodies against native gliadin, their performance was inferior compared with anti-TG2 or EMA assays (55,11a,76); however, their performance in patients not preselected by anti-TG2 or EMA testing must be resolved in prospective studies. In addition, their role in the diagnosis in children younger than 2 to 3 years required further assessment in large prospective

studies, especially in a head-to-head comparison with anti-TG2 or EMA detection (58,77,78). Conventional or native gliadin antibody tests have, in general, low specificity and sensitivity (10,11a). Some evidence exists, however, that their sensitivity may be higher in children younger than 2 years in comparison with EMA and anti-TG2 tests (79). Unfortunately, the specificity is low in this age group and makes anti-gliadin antibody tests unhelpful in clinical practice. It is thus advisable to obtain a small-intestine biopsy sample in young children with severe symptoms suggestive of CD, even when their serology is negative (73,80). If villous atrophy is found in children who are negative for CD-specific antibodies, then a later gluten challenge procedure always should be performed to confirm CD as a cause of the enteropathy.

IgA deficiency must be taken into consideration in a subgroup of children in the choice of diagnostic tests and the interpretation of the results. It is important to exclude IgA deficiency by measuring serum total IgA levels. IgA-deficient children can be evaluated on the basis of IgG class tests (26).

3.3. Evidence Statements

3.3.1.

CD is characterised by highly specific autoantibodies directed against the common CD autoantigen TG2 (“tissue” TG), including EMA and by antibodies against DGP.

LOE: 1.

References (10,11a)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.2.

In subjects with normal serum IgA values for age, a positive IgA class EMA result or a positive IgA class anti-TG2 antibody result is considered to be a CD-relevant antibody positivity. In the case of IgA deficiency, a positive IgG class EMA result, a positive IgG class anti-TG2 antibody, or a positive IgG class anti-DGP antibody is diagnostically relevant.

LOE: 1.

References (10,26,48,11a,78a)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.3.

It is not required that IgA-competent patients with CD be positive in both IgA and IgG class CD antibody tests. Isolated positivity for IgG class CD antibodies in a person with normal serum IgA levels does not have the same specificity and clinical relevance as the positivity of IgA class antibodies.

LOE: 2.

References (10,11a)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.4.

The numeric values obtained with different test kits in anti-TG2 or anti-DGP antibody measurements cannot be directly compared because they may differ in their measurement principles, calibrators, and calculation mode of results.

LOE: 2.

References (10,59,11a)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.5.

For blood anti-TG2 antibody tests that use calibration curves to express antibody concentration, values exceeding 10 times ULN may be denoted as high antibody positivity. For other tests, values considered to be high antibody positivity should be established by comparison with a panel of tests, which are listed in Appendix II.

LOE: 3.

References (55,74,75)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.6.

EMA testing in experienced hands has the highest specificity and positive likelihood ratio for CD among the available serology tools. It is more likely that CD is present if the EMA result is positive than if another CD antibody result is positive.

LOE: 1.

References (11a)

Total number of votes: 12, Agree: 12, Disagree: 0, Abstentions: 1

3.3.7.

The specificity and positive predictive value of serum anti-TG2 antibody measured by immunoassays other than EMA is lower than those of positive EMA results. Isolated positivity for anti-TG2, especially in the low positivity range, can occur in conditions that are unrelated to CD, such as other autoimmune conditions, infections, tumours, or tissue damage.

LOE: 1.

References (62,11a–70,81,82)

Total number of votes: 12, Agree: 12, Disagree: 0, Abstentions: 1

3.3.8.

High concentrations of anti-TG2 antibodies in blood (as defined in statement 2.3.5) predict villous atrophy better than low positive or borderline values.

LOE: 2.

References (55,74,75)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.9.

Rapid anti-TG2 antibody detection at the point of contact can perform with high accuracy similar to anti-TG2 antibody detection by laboratory measurements. The evaluation of rapid tests is less reliable if done by untrained or laypeople. Quantification as in serum immunoassays is not possible at present.

LOE: 1.

References (67,11a)

Total number of votes: 12, Agree: 12, Disagree: 0, Abstentions: 1

3.3.10.

Anti-TG2 antibody or EMA testing from a blood sample has a higher accuracy than antibody testing against DGP, unless special

patient characteristics are present (IgA deficiency, age younger than 2 years).

LOE: 1.

References: (11a,76)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.11.

Anti-TG2 antibodies are detectable in saliva samples from patients with CD, but the accuracy of available diagnostic tests is lower compared with serological tests.

LOE: 3.

References (64)

Total number of votes: 12, Agree: 12, Disagree: 0, Abstentions: 1

3.3.12.

Tests for the detection of IgG or IgA antibodies against native gliadin (conventional gliadin antibody test) are neither sufficiently sensitive nor sufficiently specific for the detection of CD.

LOE: 1.

References (10,11a)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.13.

Tests for the detection of CD antibodies of any isotype (IgG, IgA, secretory IgA) in fecal samples are unreliable.

LOE: 3.

References (65,11a)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.14.

The expertise of the laboratory and the selection of the test kit influence the accuracy of CD antibody tests.

LOE: 2.

References (59,60)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.3.15.

Demonstration of in vivo-bound anti-TG2 antibodies on the cell surface in the small bowel or in other tissues supports the diagnosis of CD.

LOE: 2.

References (49,50,53,67,73)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4. Recommendations

3.4.1.

(↑↑) Every antibody test used for the diagnosis of childhood CD must be validated in a paediatric population of at least 50 children with active CD and 100 control children of different ages

against the reference of EMA positivity detected under standard conditions in an expert laboratory.

(↑) Alternatively, a CD test can be validated in children against reference results of histology or against another anti-TG2 antibody test with performance similar to EMA. A test is considered as reliable if it shows >95% agreement with the reference test.

In both situations, seek statistical advice.

Total number of votes: 13, Agree: 12, Disagree: 1, Abstentions: 0

3.4.2.

(↑↑) The optimal threshold values for antibody positivity (ULN) of a test should be established. This is done by receiver operating characteristics curves plotting sensitivity against specificity at different cutoff levels.

(↑) In the case of new anti-TG2 antibody measuring tests, it is also advisable to establish the range of high positivity (in relation to ULN).

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.3.

(↑↑) Laboratories providing CD antibody test results for diagnostic use should participate continuously in a quality control programme at a national or a European level.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.4.

(↑↑) Anti-TG2 and anti-DGP laboratory test results should be reported as numeric values together with specification of the Ig class measured, the manufacturer, the cutoff value defined for the specific test kit, and (if available) the level of "high" antibody values. It is not sufficient to state only positivity or negativity. Information on the source of the antigen (natural, recombinant, human, nonhuman) should be provided for in-house methods.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.5.

(↑↑) Reports on EMA results should contain the specification of the investigated Ig class, the interpretation of the result (positive or negative), the cutoff dilution and the specification of the substrate tissue. It is also useful to have the information on the highest dilution that is still positive.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.6.

(↑) If a rapid or point-of-contact CD antibody test is used by a health care professional, the type of the device and class of the investigated antibodies and testing for IgA deficiency should be recorded.

Total number of votes: 12, Agree: 12, Disagree: 0, Abstentions: 1

3.4.7.

(↑↑) A diagnostic test for CD-specific antibody detection should be the first tool used to identify patients with symptoms and signs suggestive of CD for further diagnostic workup (eg, refined serological testing, HLA typing, small-intestine biopsies) or to rule out CD. Patients should be tested for CD-specific antibodies when on a gluten-containing diet.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.8.

(↑↑) For initial testing in symptomatic patients, a quantitative test detecting IgA class anti-TG2 or EMA from a blood sample is recommended. If total serum IgA is not known, measurement is recommended.

(↑↑) In subjects with either primary or secondary humoral IgA deficiency, at least 1 additional test measuring IgG class CD antibodies (IgG anti-TG2, IgG anti-DGP, or IgG EMA, or blended kits for both IgA and IgG antibodies) is recommended.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.9.

(↑) Rapid CD antibody detection kits meeting the requirements set forth above for CD antibody testing can be applied for initial testing.

(↑↑) Rapid testing is not meant to replace laboratory testing or to provide a final diagnosis.

Total number of votes: 12, Agree: 10, Disagree: 2, Abstentions: 1

3.4.10.

(↑↑) Tests for the detection of IgG or IgA antibodies against native gliadin (gliadin antibody or anti-gliadin antibody test) should not be used for detecting CD.

Total number of votes: 13, Agree: 12, Disagree: 13, Abstentions: 0

3.4.11.

(↑) Tests measuring IgG and/or IgA antibodies against deamidated gliadin peptides may be used as additional tests in children who are negative for other CD-specific antibodies but in whom clinical symptoms raise a strong suspicion of CD, especially if they are younger than 2 years old.

Total number of votes: 13, Agree: 12, Disagree: 1, Abstentions: 0

3.4.12.

(↑) The use of tests for the detection of antibodies of any type (IgG, IgA, secretory IgA) in faecal samples are not recommended for clinical evaluation.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.13.

(↑) Measurements of anti-TG2 or anti-DGP antibodies with the purpose of demonstrating a decrease in antibody levels after

dietary gluten restriction should be made with the same testing method as before treatment.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.14.

(↑↑) For the interpretation of antibody results, serum total IgA levels, the age of the patient, and the pattern of gluten consumption should be taken into account.

(↑↑) If gluten exposure was short or gluten had been withdrawn for a longer period of time (several weeks to years), the negative result is not reliable.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.15.

(↑↑) For IgA-competent subjects, the conclusions should be drawn primarily from the results of the IgA class antibody tests.

(↑↑) For IgA-deficient subjects, the conclusions should be drawn from the results of the IgG class CD antibody tests.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.16.

(↑↑) If IgA class CD antibodies are negative in an IgA-competent symptomatic subject, it is unlikely that CD is causing the symptom. Further testing for CD is not recommended unless special medical circumstances (child younger than 2 years, restricted gluten consumption, severe symptoms, family predisposition or other predisposing disease, immunosuppressive medications) are present.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.17.

(↑) Children found to test positive for CD-specific antibodies should be evaluated by a paediatric gastroenterologist to prove or to exclude the presence of CD.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.18.

(↑↑) Skin immunofluorescent study-proven dermatitis herpetiformis also can be regarded as confirmation of gluten sensitivity.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

3.4.19.

(↑) If an IgA-competent subject is negative for all of the IgA class CD antibodies but has IgG class anti-TG2 or EMA or anti-DGP positivity, a decision on additional testing should be made after considering all of the laboratory and clinical parameters, including the clarification of a previous reduction of gluten intake.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4. Biopsy

4.1. Evidence Review

Histology

A distinct pattern of histological abnormalities has been observed in CD (83). The features include partial to total villous atrophy, elongated crypts, decreased villus/crypt ratio, increased mitotic index in the crypts, increased IEL density, increased IEL mitotic index, infiltration of plasma cells, lymphocytes, mast cells, and eosinophils and basophils into the lamina propria. In addition, the absence of an identifiable brush border may be seen as well as abnormalities in the epithelial cells, which become flattened, cuboidal, and pseudostratified. It has become clear that a whole spectrum of histological signs may be present, ranging from a normal villous architecture to severe villous atrophy (83). According to the Marsh classification, lesions include infiltrative, hyperplastic, and atrophic patterns. This classification was modified (84,85). The pathology report should always include a description of the orientation, evaluation of villi (normal or degree of atrophy), crypts, villus/crypt ratio, and number of IELs. IELs in numbers >25/100 epithelial cells suggest an infiltrative lesion (86); however, these changes are not pathognomonic of CD and most of them may be seen in other entities, such as cow's milk or soy protein hypersensitivity, intractable diarrhea of infancy, heavy infestation with *Giardia lamblia*, immunodeficiencies, tropical sprue, and bacterial overgrowth. Hence, changes, even the most severe, should always be interpreted in the context of the clinical and serological setting and with consideration of the gluten content of the diet. Finally, there are subjects, often belonging to at-risk groups, with infiltrative lesions or even completely normal mucosa and yet positive CD-specific antibodies (72,87,88). Little information is available on their natural history and on the need for a GFD in these subjects.

Low-grade Enteropathy

In the case of mild histological lesions (no villous atrophy, Marsh 1), histology shows low specificity for the diagnosis of CD. In fact, only 10% of subjects presenting infiltrative changes have CD (83,89,90). Positive antibody levels increase the likelihood of CD; however, under these circumstances the sensitivity of serology is much less (55,91). Immunohistochemical analysis of biopsies may improve specificity: a high count of $\gamma\delta$ cells (or $\gamma\delta$ /CD3 ratio) in intestinal mucosae showing Marsh 1 to Marsh 2 changes increases the chances of CD, but requires frozen, nonfixed biopsies. In paraffin-embedded biopsies, counting villous tip IELs also increases the specificity for CD (92,93). The presence of IgA anti-TG2 deposits in the mucosa seems to be specific for CD and to predict the evolution to more severe histological patterns (53).

How to Perform a Biopsy

Biopsies can be retrieved by upper endoscopy or by suction capsule (94–98). Although duodenal biopsies obtained by suction capsule are usually of better quality, upper endoscopy has several advantages (eg, shorter procedure time, absence of radiation, multiple biopsies obtained to overcome the possibility of focal lesions). Furthermore, endoscopy allows other differential diagnoses to be considered as well as endoscopic patterns suggestive of CD (eg, absence of folds, scalloped folds, mosaic pattern of the mucosa between the folds), although the reliability of these observations is limited to patients with total or subtotal villous atrophy (85,99).

Analysis of multiple biopsies is important. Patchiness of the lesion has been reported (99–102), and in fact, recent work suggests that different degrees of severity may be present, even in the same fragment (103). The site where a biopsy is taken remains a matter for discussion. In a few patients, lesions may be limited to the duodenal bulb (100,101), although this has not been confirmed by others (103). In conclusion, biopsies should be taken from the second/third portion of the duodenum (at least 4 samples), and at least 1 biopsy should be taken from the duodenal bulb.

When Should a Biopsy Be Taken After Diagnosis?

Patients diagnosed as having CD do not need a histological reevaluation on a GFD. The disappearance of symptoms when present and/or normalisation of CD-associated antibodies are sufficient to support the diagnosis. If there is no response to GFD, then a careful dietary assessment should be taken to exclude lack of compliance and inadvertent exposure to a gluten-containing diet. Further investigations are then required, which could include new biopsies.

When and How to Perform a Gluten Challenge

Gluten challenge is not necessary in most cases to diagnose CD, but it may be performed under special circumstances, including situations in which doubt exists about the initial diagnosis. Age at diagnosis of younger than 2 years does not represent a reason for challenge, unless the diagnosis was made in the absence of positive CD-specific antibodies (anti-TG2 antibody and EMA) (104). Gluten challenge should be discouraged before a child is 5 years old and during the pubertal growth spurt. Once decided upon, gluten challenge always should be performed under strict medical supervision, preferably by a paediatric gastroenterologist. It should be preceded by HLA testing if not performed previously and by an assessment of duodenal histology. Furthermore, the challenge should be performed ensuring that a normal amount of gluten in the diet is ingested. IgA anti-TG2 antibody (IgG anti-TG2 in IgA deficiency) should be measured during the challenge period. A patient is considered to have relapsed (and hence the diagnosis of CD confirmed) if CD antibodies become positive and a clinical and/or histological relapse is observed. In the absence of positive serology/symptoms, the challenge for practical purposes is considered complete after 2 years, although follow-up should be continued because relapse may occur at a later time.

4.2. Evidence Statements

4.2.1.

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 to villous atrophy.

LOE: 1.

References (83,84)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.2.2.

Patchiness of the lesions may be present.

LOE: 1.

References (99,101,102)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.2.3.

Lesions may be present only at the level of the duodenal bulb.
LOE: 2.

References (101,102)

Total number of votes: 12, Agree: 12, Disagree: 0, Abstentions: 1

4.2.4.

High IgA anti-TG2 antibody levels are correlated with more severe histological lesions.

LOE: 1.

References (11,55,75)

Total number of votes: 13, Agree: 12, Disagree: 0, Abstentions: 1

4.2.5.

Milder lesions (Marsh 1) are nonspecific because only 10% of subjects presenting this pattern have proven CD.

LOE: 1.

References (89,90)

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.2.6.

In the presence of mild histological lesions a high $\gamma\delta$ cell count increases the likelihood for the diagnosis of CD.

LOE: 2.

References (89,90)

Total number of votes: 13, Agree: 12, Disagree: 0, Abstentions: 1

4.2.7.

In the presence of mild histological lesions, the presence of IgA anti-TG2 deposits in the mucosa increases the likelihood for the diagnosis of CD.

LOE: 2.

References (73,80)

Total number of votes: 13, Agree: 12, Disagree: 0, Abstentions: 1

4.3. Recommendations

4.3.1.

(\uparrow) Histological assessment may be omitted in symptomatic patients (see list in Who to Test) who have high IgA anti-TG2 levels (10 times above ULN), verified by EMA positivity, and are HLA-DQ2 and/or HLA-DQ8 heterodimer positive.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.2.

(\uparrow) If all of the criteria in 4.3.1 were fulfilled and the histological assessment was omitted before the initiation of a

GFD, then follow-up should include significant symptomatic improvement and normalisation of CD-specific antibody tests.

Total number of votes: 13, Agree: 12, Disagree: 1, Abstentions: 0

4.3.3.

(\uparrow) If anti-TG2 antibodies are positive only in low concentrations and EMA testing is negative, then the diagnosis of CD is less likely. A small intestinal biopsy should be performed to clarify whether CD is present.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.4.

($\uparrow\uparrow$) In seronegative patients with strong clinical suspicion of CD, small-intestine biopsies are recommended.

(\uparrow) If histology shows lesions compatible with CD, then HLA-DQ testing should also be performed; however, an enteropathy other than CD should be considered. In these patients, CD must be confirmed by a challenge procedure with repeated biopsies.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.5.

(\uparrow) In the absence of anti-TG2/EMA, the diagnosis of CD is unlikely. In the case of mild lesions (eg, Marsh 1), additional supportive evidence (extended serology, HLA, IgA anti-TG2 intestinal deposits, high IEL $\gamma\delta$ count) should be looked for before establishing the diagnosis of CD.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.6.

(\uparrow) When duodenal biopsies, taken during diagnostic workup or by chance, disclose a histological pattern with Marsh 1 to Marsh 3 lesions, antibody determinations (anti-TG2 and in children younger than 2 years, anti-DGP) and HLA typing should be performed. In the absence of positive CD antibodies or compatible HLA typing other causes of enteropathy (eg, food allergy, autoimmune enteropathy) should be considered.

Total number of votes: 13, Agree: 12, Disagree: 1, Abstentions: 0

4.3.7.

($\uparrow\uparrow$) It is preferable to take biopsies during upper endoscopy.

Total number of votes: 12, Agree: 12, Disagree: 0, Abstentions: 1

4.3.8.

(\uparrow) Biopsies should be taken from the bulb (at least 1) and from the second or third portion of the duodenum (at least 4).

Total number of votes: 12, Agree: 12, Disagree: 0, Abstentions: 1

4.3.9.

(\uparrow) The pathology report should include description of the orientation, evaluation of villi (normal or degree of atrophy), crypts,

villus/crypt ratio, and number of IELs. Grading according to the Marsh-Oberhuber classification is recommended.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.10.

(†) Patients on a GFD fulfilling the diagnostic criteria of CD do not need biopsies.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.11.

(†) If there is no clinical response to a GFD in symptomatic patients, then after a careful dietary assessment to exclude lack of compliance, further investigations are recommended. These investigations may include additional biopsies.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.12.

(†) Gluten challenge is not considered mandatory, except under unusual circumstances. These circumstances include situations in which there is doubt about the initial diagnosis, including patients with no CD-specific antibodies before starting a GFD.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.13.

(†) If gluten challenge is indicated, then it should not be performed before the patient is 5 to 6 years old or during the pubertal growth spurt.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.14.

(††) Gluten challenge should be performed under medical supervision, preferably by a paediatric gastroenterologist.

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.15.

(†) HLA typing and assessment of duodenal histology should be considered before gluten challenge is instituted.

Total number of votes: 12, Agree: 12, Disagree: 0, Abstentions: 1

4.3.16.

(††) The daily dietary intake during gluten challenge should contain a normal amount of gluten (approximately 15 g/day).

Total number of votes: 13, Agree: 13, Disagree: 0, Abstentions: 0

4.3.17.

(††) During the challenge period, IgA anti-TG2 antibody (IgG in the case of IgA deficiency) should be measured. A patient

should be considered to have relapsed (and hence the diagnosis of CD confirmed) if CD serology becomes positive and a clinical and/or histological relapse is observed. In the absence of positive antibodies/symptoms, the challenge should be considered to be completed after 2 years and biopsies performed. Follow-up should be continued because relapse may occur after >2 years.

Total number of votes: 13, Agree: 11, Disagree: 2, Abstentions: 0

ALGORITHMS

Two algorithms have been developed based on the evidence-based evidence statements and recommendations. The first algorithm (Fig. 1) can be applied to children and adolescents with otherwise unexplained signs and symptoms suggestive of CD. In this patient group, the algorithm provides the option to omit duodenal biopsies and histology, but only if certain conditions are fulfilled. The second algorithm (Fig. 2) should be applied to children and adolescents with no signs or symptoms suggestive of CD, who are investigated because of their increased risk for the disease (first-degree relatives of CD patients or other chronic, immune-mediated or chromosomal diseases listed in Table 2). In such individuals, the clinical workup should look for previously undetected disease signs such as iron-deficiency anaemia or elevated liver enzymes, and when these are present, the symptomatic algorithm applies. It must be emphasised that algorithms may not fit 100% of cases and may always allow exceptions; however, the 2 algorithms should fit at least 95% of children and adolescents under consideration. These guidelines did not aim to prepare algorithms for mass screening or for other nonclinical situations resulting from accidentally detected CD antibody positivity.

Algorithm 1: Child or Adolescent With Otherwise Unexplained Symptoms and Signs Suggestive of CD

The initial approach to symptomatic patients is to test for anti-TG2 IgA antibodies and in addition for total IgA in serum to exclude IgA deficiency. As an alternative for total IgA in serum, direct testing for IgG anti-DGP antibodies can be performed. The decision to initiate IgA anti-TG2 in this population is based on the high sensitivity and specificity of the test, the widespread availability, and low costs compared with EMA IgA antibodies. It is not cost-effective to add further CD-specific tests to the initial diagnostic workup in symptomatic patients.

If IgA anti-TG2 antibodies are negative and serum total IgA is normal for age (or IgG anti-DGP antibodies are negative), then CD is unlikely to be the cause of the symptoms; however, certain conditions that are known to give false-negative anti-TG2 results must be considered. These include a diet low in gluten, protein-losing enteropathy, intake of immunosuppressive drugs, and patients younger than 2 years old. In young children, extended tests for both IgA and IgG CD-specific antibodies should be performed after consideration of cow's-milk protein allergy with a trial of cow's-milk-free diet. If symptoms are severe, then duodenal biopsies may be warranted.

If anti-TG2 antibody testing is positive, then patients should be referred to a paediatric gastroenterologist for further diagnostic workup, which is dependent on serum antibody levels. Patients with positive anti-TG2 antibody levels lower than 10 times ULN given by the manufacturer of this particular test should undergo upper endoscopy with multiple biopsies. The paediatric gastroenterologist should discuss with the parents and the patient who is positive for anti-TG2 antibody levels ≥ 10 times ULN (as appropriate for age)

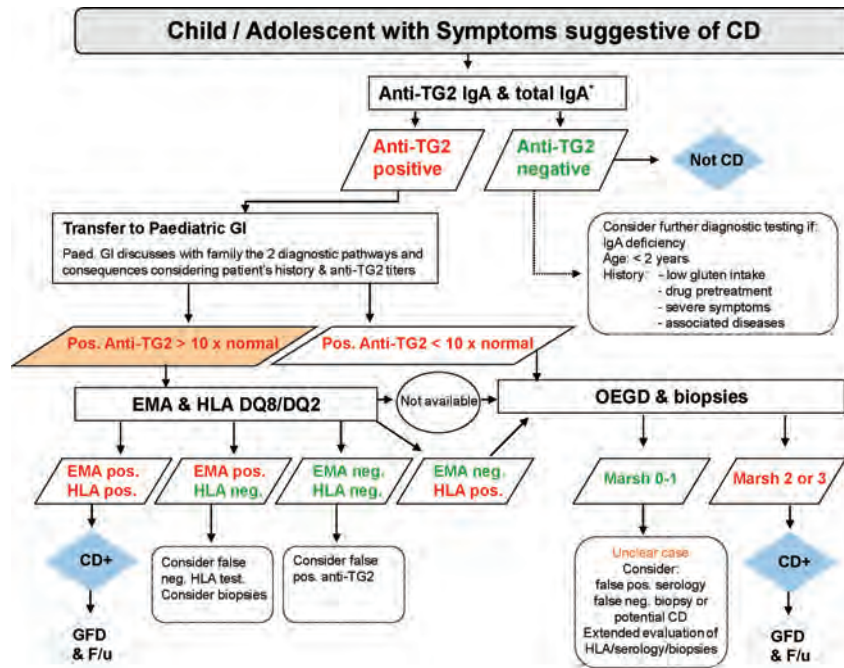


FIGURE 1. Symptomatic patient. CD = coeliac disease; EMA = endomysial antibodies; F/u = follow-up; GFD = gluten-free diet; GI = gastroenterologist; HLA = human leukocyte antigen; IgA = immunoglobulin A; IgG = immunoglobulin G; OEGD = oesophagogastroduodenoscopy; TG2 = transglutaminase type 2.

the option of omitting the biopsies and the implications of doing so. If the parents (patient) accept this option, then blood should be drawn for HLA and EMA testing. It is important that EMA testing be performed from a different blood sample than anti-TG2 testing to exclude false-positive results because of mislabelling of the previous sample or other errors in processing and reporting. Because EMA testing depends on the quality and experience of

the laboratory, the clinician must collaborate with a laboratory with documented experience and high standards in immunohistochemistry. If the patient tests positive for EMA antibodies and positive for HLA-DQ2 or HLA-DQ8, then the diagnosis of CD is confirmed. A GFD is started and the patient is studied for improvement of symptoms and decline of antibodies. A later gluten challenge in these children is not required.

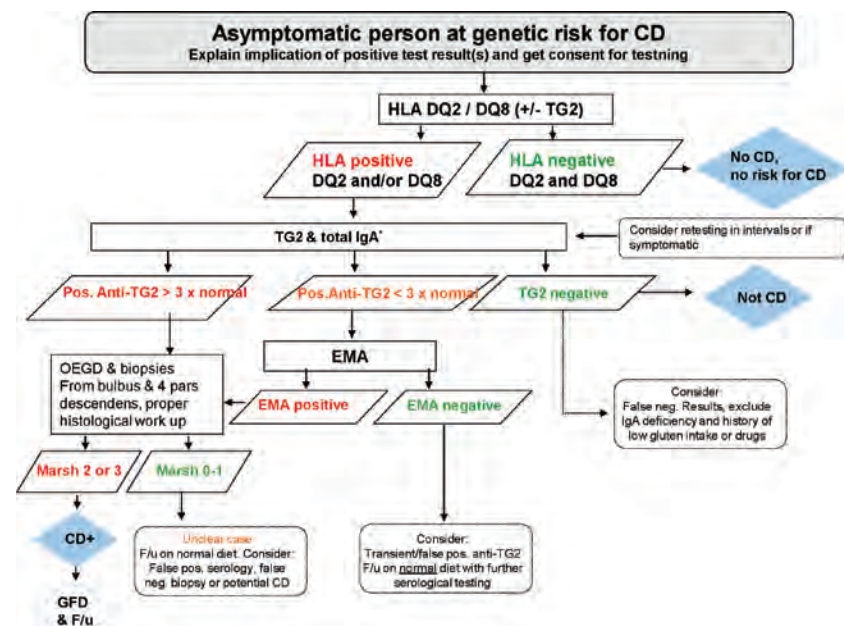


FIGURE 2. Asymptomatic patient. See Fig. 1 for definitions.

In the rare case of negative results for HLA and/or EMA in a child with TG2 antibody titres ≥ 10 times ULN, the different possibilities for false-positive and false-negative test results must be considered. Under these circumstances, the diagnostic workup should be extended, including repeated testing and duodenal biopsies. A small number of cases remain unclear even after extended evaluation of antibodies and histology specimens. They may require longer follow-up and demonstration of gluten dependency of the symptoms/other findings on a case-by-case basis.

Some deviation from this stepwise procedure may occur in anti-TG2-positive children with classical symptoms (failure to thrive, diarrhoea, distended abdomen, and anaemia), who are in such poor clinical condition that postponing a GFD and awaiting the results of HLA and EMA testing may put the child at further risk. Under these circumstances, the paediatric gastroenterologist at his or her discretion may start the child on a GFD while awaiting the test results of anti-EMA and HLA testing. This exception is justified considering the risk-to-benefit ratio: General anaesthesia bears a higher risk in these children and the likelihood for CD is high in a child with anti-TG2 titers ≥ 10 times ULN. In the unexpected case of negative results for HLA or EMA, however, the diagnostic workup should be extended to include duodenal biopsies and a later gluten challenge.

Algorithm 2: Child or Adolescent Without Symptoms Suggestive of CD Who Belongs to a High-risk Group

In totally asymptomatic individuals belonging to groups with a high risk for CD (defined by their own or family history, Table 2) CD always should be diagnosed using duodenal biopsies. A different algorithm than above is recommended because people belonging to this population more often have false-positive anti-TG2 results (61). Considering that CD is a lifelong disorder with the need for adherence to a restrictive and demanding diet, the opinion of the working group was that in asymptomatic individuals, histological proof is needed to accept the diagnosis.

In this group HLA-DQ2 and HLA-DQ8 testing as the initial action is probably cost-effective because a significant proportion of the patients can be excluded from further studies because they do not harbour DQ2 or DQ8 (44). If HLA testing is not feasible, however, then the screening procedure may begin with CD-specific antibody testing.

In individuals with DQ2 or DQ8 positivity or without HLA testing, IgA anti-TG2 and serum total IgA determination should be performed. If IgA anti-TG2 is negative and IgA deficiency is excluded, then CD is unlikely; however, the disease may still develop later in life. Therefore, serological testing should be repeated at regular intervals. No data support any firm recommendations, but it was the opinion of the working group members that a child should be investigated by serology every 2 to 3 years to avoid the detrimental effects of unrecognised CD on growth and bone health.

If anti-TG2 antibodies are positive, then signs related to CD should be searched for (eg, anaemia, elevated liver enzymes) and it should be decided whether the patient qualifies for the symptomatic algorithm 1. If such signs are absent and anti-TG2 concentration is >3 times ULN, the patient should be referred to a paediatric gastroenterologist for endoscopy with multiple duodenal biopsies (at least 4 from the descending part of the duodenum and at least 1 from the duodenal bulb).

If anti-TG2 levels are positive but low, that is <3 times ULN, then a false-positive result is possible. In the absence of any signs or symptoms the person may be followed up on a normal gluten-

containing diet and serological testing should be repeated. In these patients, anti-EMA testing may help to distinguish between false- and true-positive low anti-TG2 titres. If EMA is positive, then the likelihood for CD increases because of the high specificity of EMA. In this situation, the patient should be referred for endoscopy in spite of low anti-TG2 titres. If EMA are negative, then the patient should be followed up on a normal diet and anti-TG2 testing should be repeated every 3 to 6 months until the antibody levels either turn negative or the levels increase to levels at which endoscopy is indicated.

If a seropositive asymptomatic at-risk person does not demonstrate conclusive evidence of CD after the extended evaluation of biopsies, then such a person should be followed up on a normal gluten-containing diet and be reevaluated at regular intervals.

CONCLUSIONS AND FUTURE DIRECTIONS

The main conclusions of these guidelines are that the diagnosis of CD depends on gluten-dependent symptoms, CD-specific antibody levels, the presence of HLA-DQ2 and/or HLA-DQ8, and characteristic histological changes (villous atrophy and crypt hyperplasia) in the duodenal biopsy. High TG2-antibody levels (≥ 10 times ULN for a standard curve-based calculation) as measured by a qualified laboratory show high diagnostic accuracies. In the presence of high antibody levels the diagnosis of CD may be based on a combination of symptoms, antibodies, and HLA, thus omitting the duodenal biopsy. The diagnosis is confirmed by an antibody decline and preferably a clinical response to a GFD. Gluten challenge and repetitive biopsies will be necessary only in selected patients in whom diagnostic uncertainty remains.

The present guidelines replace the existing ESPGHAN guidelines, but require a period of implementation and testing. It will be important to be precise in the clinical evaluation of patients and to perform prospective research studies. Likewise, it will be important for laboratories performing analyses of CD antibodies and HLA determinations to develop methodologies and continuously participate in quality control programmes. In the future, new diagnostic tools, for example a new serological test, may be evaluated in symptomatic patients with CD and controls that are defined and classified by all 4 diagnostic criteria (symptoms/signs, antibodies, HLA, and histology). In particular, POC tests have not been sufficiently validated to include in a diagnostic algorithm.

Based on the current evidence symptoms, CD-specific antibodies, HLA, and biopsy findings contribute to the CD diagnosis. A wide spectrum of findings is present within each item, from suggestive to contradictory. For example, a malabsorption syndrome is more convincing than alopecia or being a person belonging to a risk group. Likewise, histological lesions with severe villous atrophy (Marsh 3b and 3c) are more convincing than Marsh 1 lesions. In the hierarchy of antibodies, EMA has the highest impact, whereas negativity for all antibodies on an unrestricted diet or absence of both HLA-DQ2 and HLA-DQ8 strongly contradict the presence of CD. Nonetheless, an unconventional HLA-DQ type cannot totally exclude CD, and typical gluten-responsive lesions can be present even in the absence of CD antibodies in serum. A scoring system (Appendix II) may be applied so that a stronger finding in 1 item may compensate for a missing abnormality in another and the sum could be taken into account. The main functions of a scoring approach are to assist in the interpretation of the spectrum of diagnostic findings and to protect against overdiagnosis in insufficiently documented or borderline cases. In addition, the scoring system may provide further diagnostic reassurance in typical cases in which genetic testing or immunohistochemistry are not available. Such scoring systems must be evaluated formally in prospective

clinical studies before they can be recommended in regular clinical use. They do not alter the present recommendations.

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APPENDIX I COMPARISON OF HIGH SERUM ANTI-TG2 ANTIBODY LEVELS OBTAINED BY DIFFERENT COMMERCIAL TESTS

Several research studies have shown that presence of small-intestine villous atrophy can be predicted if the levels of circulating anti-TG2 antibodies are high (55,74,75,150). TG2-specific antibodies can be measured only in relative units, so numerical values for such “high” values are kit specific and show considerable variations. In addition, the calculation of results (number and value of calibrators) also differs.

There are 2 main ways to calculate serum antibody results: the majority of available commercial anti-TG2 tests calculate test results by comparison to a dilution curve prepared from the serial dilutions of a positive sample which correspond to fixed concentrations (standard curve). Such values are proportional to the serum concentration of antibodies. A few tests use the more simple calculation of dividing the specific test signal (absorbance after subtracting the background) by the signal obtained with an internal, kit-specific positive sample. These values are logarithmic; as a consequence, numeric values are higher than those derived by standard curve calculations for samples with values below the positive control but lower for samples exceeding the positive calibrator. In other words, the dynamic range of a logarithmic test is narrower than that of a standard curve-based immunoassay.

It is, therefore, essential to make comparisons between different coeliac antibody test results using the same positive samples. This issue has not been sufficiently investigated in academic studies. The United Kingdom National External Quality Assessment Service provides an external quality control service across Europe and distributes 6 serum samples per year, which are then measured by a large number of clinical laboratories, each using its own kit or method. In this way, large pools of results are continuously generated by the most often used commercial anti-TG2 kits, which represent current clinical testing practices and can be updated steadily. With the help of the United Kingdom National External Quality Assessment Service, we analysed the returns for 3 positive samples with different antibody positivity levels distributed in 2009. Assays were included if they had been applied by at least 5 different laboratories (on average 22, range 5–108). Table A shows values for 3 representative samples (I–III) that yielded 13.6, 18, and 30.1 U/L median values with Phadia’s Varelisa (Celikey, Freiburg, Germany) assay, which had been used in the research Hill et al (74) and Dahlbom et al (55). These authors found that serum antibody results exceeding 10 times ULN of this test (30 U/L) were invariably associated with villous atrophy. The bold column indicates the kit-specific values obtained with the 30.1 U/L (high positive) sample. A total of 99.1% of the laboratories measured this sample as positive. The last column shows these values divided by the respective ULN of those tests. It is concluded that antibody test results above 10 times ULN values represent “high” values in the respective tests. Figure A shows that most tests can distinguish slightly (4 times ULN), moderately (6 times ULN), and highly (10 times ULN) positive anti-TG2 levels resulting in almost parallel albeit numerically different curves. These results are regarded as an example for the characteristics of different tests and final conclusions could be drawn only from more systematic studies or from a longer survey.

TABLE A. Median values obtained in 2009 for the same UKNEQAS positive test samples in 306 European clinical laboratories by the 14 most frequently applied serum anti-TG2 IgA antibody assays

Test kits	Sample I (13.6 U)	Sample II (18 U)	Sample III (30.1 U), high positive	Cutoff	Times ULN for the high sample
Aesku	48	63	135	15	9.0
Binding Site	18	24.1	33.3	4	8.3
BMD Luminex	32.5	27	43	15	
Diasorin	28.6	37.5	57	8	7.1
Euroimmun	171.9	186	200	20	10.0
Eurospital*	70	80.1	95	7	13.6
Generic assays	39.9	44.3	89	20	4.5
Genesis	36.9	48.8	69	7	9.9
Immco	25.9	29.8	48.3	20	2.4

TABLE A. (Continued)

Test kits	Sample I (13.6 U)	Sample II (18 U)	Sample III (30.1 U), high positive	Cutoff	Times ULN for the high sample
Inova*	56	69	95.5	20	4.8
Orgentec	25.8	33.2	65.5	10	6.6
Phadia ELIA	35	45	69	7	9.9
Phadia Immuno CAP	34.9	43.5	71	7	10.1
Phadia Varelisa	13.6	18	30.1	3 [†]	10.0

The Aesku test measures the combination of anti-TG2 and anti-gliadin antibodies and thus may have different characteristics. IgA = immunoglobulin A; UKNEQAS = United Kingdom National External Quality Assessment Service.

* These tests calculate results in a logarithmic manner.

[†] Optimal cutoff in research studies.

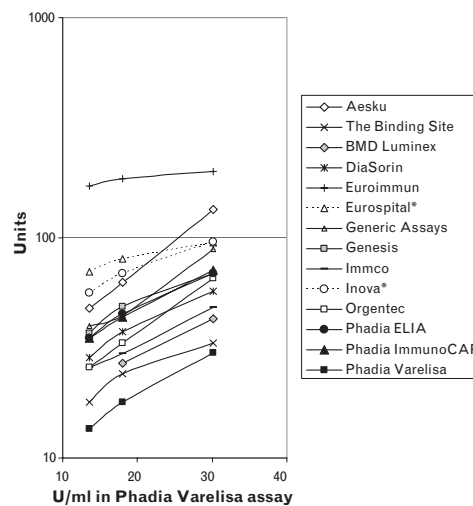


FIGURE A. A plot of kit-specific values against the values obtained by Phadia’s Varelisa assay. Dotted line denotes logarithmic assays not using calibrator curves (in 2009).

APPENDIX II A SIMPLE SCORING SYSTEM FOR THE DIAGNOSIS OF CD

The aims of the scoring system are as follows:

- To positively diagnose coeliac disease at the initial assessment and be able to accept a diagnosis made in the past with biopsy
- To simplify the diagnosis of CD in patients with obvious findings
- To protect against overdiagnosis when only nonspecific findings are present

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 1st-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

	Points
HLA	
Full HLA-DQ2 (in <i>cis</i> or <i>trans</i>) 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 villus 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, TG2 and DGP antibodies.

Comments and Explanations for Use

Biopsy items were graded by taking into account Villanacci scoring (85) 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.

REFERENCES

- Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. *Arch Dis Child* 1990; 65:909-11.
- Rewers M. Epidemiology of coeliac disease: what are the prevalence, incidence, and progression of coeliac disease? *Gastroenterology* 2005; 128(4 Suppl 1):S47-51.
- Greco L, Romino R, Coto I, et al. The first large population based twin study of coeliac disease. *Gut* 2002;50:624-8.
- Lundin KE, Sollid LM, Qvigstad E, et al. T lymphocyte recognition of a coeliac disease-associated cis-or trans-encoded HLA-DQ alpha/beta-heterodimer. *J Immunol* 1990;145:136-9.
- van de WY, Kooy YM, van Veelen PA, et al. Small intestinal T cells of coeliac disease patients recognize a natural pepsin fragment of gliadin. *Proc Natl Acad Sci U S A* 1998;95:10050-4.
- Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of coeliac disease. *Nat Med* 1997; 3:797-801.
- Sjostrom H, Lundin KE, Molberg O, et al. Identification of a gliadin T-cell epitope in coeliac disease: general importance of gliadin deamidation for intestinal T-cell recognition. *Scand J Immunol* 1998;48:111-5.
- Mothes T. Deamidated gliadin peptides as targets for coeliac disease-specific antibodies. *Adv Clin Chem* 2007;44:35-63.
- Corazza GR, Villanacci V, Zambelli C, et al. Comparison of the interobserver reproducibility with different histologic criteria used in coeliac disease. *Clin Gastroenterol Hepatol* 2007;5:838-43.
- Rostom A, Dube C, Cranney A, et al. Coeliac disease. *Evid Rep Technol Assess (Summ)* 2004;104:1-6.
- Hill ID, Dirks MH, Liptak GS, et al. Guideline for the diagnosis and treatment of coeliac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2005;40:1-19.
- Giersiepen K, Lelgemann M, Stuhldreher N, et al. Accuracy of diagnostic antibody tests for coeliac disease in children: summary from an evidence report. European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Working Group on Coeliac Disease Diagnosis. *J Pediatr Gastroenterol Nutr* In press.
- Ebell MH, Siwek J, Weiss BD, et al. Strength of recommendation taxonomy (SORT): a patient-centered approach to grading evidence in the medical literature. *J Am Board Fam Pract* 2004;17:59-67.
- Schunemann HJ, Best D, Vist G, et al. Letters, numbers, symbols and words: how to communicate grades of evidence and recommendations. *CMAJ* 2003;169:677-80.
- Mustalahti K, Sulkanen S, Holopainen P, et al. Coeliac disease among healthy members of multiple case coeliac disease families. *Scand J Gastroenterol* 2002;37:161-5.
- Bottaro G, Failla P, Rotolo N, et al. Changes in coeliac disease behaviour over the years. *Acta Paediatr* 1993;82:566-8.
- Garampazzi A, Rapa A, Mura S, et al. Clinical pattern of coeliac disease is still changing. *J Pediatr Gastroenterol Nutr* 2007;45:611-4.
- Rashid M, Cranney A, Zarkadas M, et al. Coeliac disease: evaluation of the diagnosis and dietary compliance in Canadian children. *Pediatrics* 2005;116:e754-9.
- Simmons JH, Klingensmith GJ, McFann K, et al. Impact of coeliac autoimmunity on children with type 1 diabetes. *J Pediatr* 2007;150:461-6.
- Bottaro G, Cataldo F, Rotolo N, et al. The clinical pattern of sub-clinical/silent coeliac disease: an analysis on 1026 consecutive cases. *Am J Gastroenterol* 1999;94:691-6.
- van Rijn JC, Grote FK, Oostdijk W, et al. Short stature and the probability of coeliac disease, in the absence of gastrointestinal symptoms. *Arch Dis Child* 2004;89:882-3.
- Ferrara M, Coppola L, Coppola A, et al. Iron deficiency in childhood and adolescence: retrospective review. *Hematology* 2006;11: 183-6.
- Valentino R, Savastano S, Tommaselli AP, et al. Prevalence of coeliac disease in patients with thyroid autoimmunity. *Horm Res* 1999; 51:124-7.
- Hansen D, Brock-Jacobsen B, Lund E, et al. Clinical benefit of a gluten-free diet in type 1 diabetic children with screening-detected coeliac disease: a population-based screening study with 2 years' follow-up. *Diabetes Care* 2006;29:2452-6.
- Salardi S, Volta U, Zucchini S, et al. Prevalence of coeliac disease in children with type 1 diabetes mellitus increased in the mid-1990s: an 18-year longitudinal study based on anti-endomysial antibodies. *J Pediatr Gastroenterol Nutr* 2008;46:612-4.
- Bonamico M, Pasquino AM, Mariani P, et al. Prevalence and clinical picture of coeliac disease in Turner syndrome. *J Clin Endocrinol Metab* 2002;87:5495-8.
- Korponay-Szabo IR, Dahlbom I, Laurila K, et al. Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for coeliac disease in selective IgA deficiency. *Gut* 2003;52:1567-71.
- Emami MH, Taheri H, Kohestani S, et al. How frequent is coeliac disease among epileptic patients? *J Gastrointest Liver Dis* 2008;17: 379-82.
- Dickey W, McMillan SA, McCrum EE, et al. Association between serum levels of total IgA and IgA class endomysial and antigliadin antibodies: implications for coeliac disease screening. *Eur J Gastroenterol Hepatol* 1997;9:559-62.

29. Monsuur AJ, Wijnga C. Understanding the molecular basis of celiac disease: what genetic studies reveal. *Ann Med* 2006;38:578–91.
30. Monsuur AJ, de Bakker PI, Zernakova A, et al. Effective detection of human leukocyte antigen risk alleles in celiac disease using tag single nucleotide polymorphisms. *PLoS One* 2008;3:e2270.
31. van Heel DA, Franke L, Hunt KA, et al. A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. *Nat Genet* 2007;39:827–9.
32. Mearin ML, Biemond I, Pena AS, et al. HLA-DR phenotypes in Spanish coeliac children: their contribution to the understanding of the genetics of the disease. *Gut* 1983;24:532–7.
33. Karell K, Louka AS, Moodie SJ, 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.
- 33a. Richtlijn Coeliakie en Dermatitis Herpetiformis. Kwaliteitsinstituut voor de Gezondheidszorg CBO. Haarlem, The Netherlands: Nederlandse Vereniging van Maag-Darm. Leverartsen; 2008.
34. Polvi A, Eland C, Koskimies S, et al. HLA DQ and DP in Finnish families with celiac disease. *Eur J Immunogenet* 1996;23:221–34.
35. Margaritte-Jeannin P, Babron MC, Bourgey M, 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.
36. Hadithi M, von Blomberg BM, Crusius JB, et al. Accuracy of serologic tests and HLA-DQ typing for diagnosing celiac disease. *Ann Intern Med* 2007;147:294–302.
37. van Belzen MJ, Koelleman BP, Crusius JB, et al. Defining the contribution of the HLA region to cis DQ2-positive coeliac disease patients. *Genes Immun* 2004;5:215–20.
38. Sollid LM, Markussen G, Ek J, et al. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. *J Exp Med* 1989;169:345–50.
39. Balas A, Vicario JL, Zambrano A, et al. Absolute linkage of celiac disease and dermatitis herpetiformis to HLA-DQ. *Tissue Antigens* 1997;50:52–6.
40. Fasano A, Berti I, Gerarduzzi T, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med* 2003;163:286–92.
41. Liu J, Juo SH, Holopainen P, et al. Genomewide linkage analysis of celiac disease in Finnish families. *Am J Hum Genet* 2002;70:51–9.
42. Vidales MC, Zubillaga P, Zubillaga I, et al. Allele and haplotype frequencies for HLA class II (DQA1 and DQB1) loci in patients with celiac disease from Spain. *Hum Immunol* 2004;65:352–8.
43. Al-toma A, Goerres MS, Meijer JW, et al. Human leukocyte antigen-DQ2 homozygosity and the development of refractory celiac disease and enteropathy-associated T-cell lymphoma. *Clin Gastroenterol Hepatol* 2006;4:315–9.
44. Csizmadia CG, Mearin ML, Oren A, et al. Accuracy and cost-effectiveness of a new strategy to screen for celiac disease in children with Down syndrome. *J Pediatr* 2000;137:756–61.
45. Maki M, Mustalahti K, Kokkonen J, et al. Prevalence of celiac disease among children in Finland. *N Engl J Med* 2003;348:2517–24.
46. Schwartz E, Kahlenberg F, Sack U, et al. Serologic assay based on gliadin-related nonapeptides as a highly sensitive and specific diagnostic aid in celiac disease. *Clin Chem* 2004;50:2370–5.
47. Korponay-Szabo IR, Halttunen T, Szalai Z, et al. In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. *Gut* 2004;53:641–8.
48. Cataldo F, Lio D, Marino V, et al. IgG(1) antiendomysium and IgG antitissue transglutaminase (anti-tTG) antibodies in coeliac patients with selective IgA deficiency. Working Groups on Celiac Disease of SIGEP and Club del Tenue. *Gut* 2000;47:366–9.
49. Hadjivassiliou M, Maki M, Sanders DS, et al. Autoantibody targeting of brain and intestinal transglutaminase in gluten ataxia. *Neurology* 2006;66:373–7.
50. Kurppa K, Ashorn M, Iltanen S, et al. Celiac disease without villous atrophy in children: a prospective study. *J Pediatr* 2010;157:373–80.
51. Maki M, Holm K, Koskimies S, et al. Normal small bowel biopsy followed by coeliac disease. *Arch Dis Child* 1990;65:1137–41.
52. 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.
53. Salmi TT, Collin P, Korponay-Szabo IR, et al. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. *Gut* 2006;55:1746–53.
54. Bargetzi MJ, Schonberger A, Tichelli A, et al. Celiac disease transmitted by allogeneic non-T cell-depleted bone marrow transplantation. *Bone Marrow Transplant* 1997;20:607–9.
55. Dahlbom I, Korponay-Szabo IR, Kovacs JB, et al. 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.
56. Karpai S, Torok E, Kosnai I. IgA class antibody against human jejunum in sera of children with dermatitis herpetiformis. *J Invest Dermatol* 1986;87:703–6.
57. Raivio T, Kaukinen K, Nemes E, et al. Self transglutaminase-based rapid coeliac disease antibody detection by a lateral flow method. *Aliment Pharmacol Ther* 2006;24:147–54.
58. Prause C, Ritter M, Probst C, et al. Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease. *J Pediatr Gastroenterol Nutr* 2009;49:52–8.
59. Naiyer AJ, Hernandez L, Ciaccio EJ, et al. Comparison of commercially available serologic kits for the detection of celiac disease. *J Clin Gastroenterol* 2009;43:225–32.
60. Li M, Yu L, Tiberti C, et al. A report on the International Transglutaminase Autoantibody Workshop for Celiac Disease. *Am J Gastroenterol* 2009;104:154–63.
61. Vecsei A, Arenz T, Heilig G, et al. Influence of age and genetic risk on anti-tissue transglutaminase IgA titers. *J Pediatr Gastroenterol Nutr* 2009;48:544–9.
62. Di Tola M, Barilla F, Trappolini M, et al. Antitissue transglutaminase antibodies in acute coronary syndrome: an alert signal of myocardial tissue lesion? *J Intern Med* 2008;263:43–51.
63. Pastore L, Campisi G, Compilato D, et al. Orally based diagnosis of celiac disease: current perspectives. *J Dent Res* 2008;87:1100–7.
64. Bonamico M, Ferri M, Nenna R. Tissue transglutaminase autoantibody detection in human saliva: a powerful method for celiac disease screening. *J Pediatr* 2004;144:632–6.
65. Kappler M, Krauss-Etschmann S, Diehl V, et al. Detection of secretory IgA antibodies against gliadin and human tissue transglutaminase in stool to screen for coeliac disease in children: validation study. *BMJ* 2006;332:213–4.
66. Baviera LC, Aliaga ED, Ortigosa L, et al. Celiac disease screening by immunochromatographic visual assays: results of a multicenter study. *J Pediatr Gastroenterol Nutr* 2007;45:546–50.
67. 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.
68. Bizzaro N, Tampona M, Villalta D, Platzgummer S, Liguori M, Tozzoli R, et al. Low specificity of anti-tissue transglutaminase antibodies in patients with primary biliary cirrhosis. *J Clin Lab Anal* 2006;20:184–9.
69. Ferrara F, Quaglia S, Caputo I, et al. Anti-transglutaminase antibodies in non-coeliac children suffering from infectious diseases. *Clin Exp Immunol* 2010;159:217–23.
70. Villalta D, Bizzaro N, Tonutti E, et al. IgG anti-transglutaminase autoantibodies in systemic lupus erythematosus and Sjogren syndrome. *Clin Chem* 2002;48:1133.
71. Collin P, Helin H, Maki M, et al. Follow-up of patients positive in reticulon and gliadin antibody tests with normal small-bowel biopsy findings. *Scand J Gastroenterol* 1993;28:595–8.
72. Kurppa K, Collin P, Viljamaa M, et al. Diagnosing mild enteropathy celiac disease: a randomized, controlled clinical study. *Gastroenterology* 2009;136:816–23.
73. Koskinen O, Collin P, Korponay-Szabo I, 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.

74. Hill PG, Holmes GK. Coeliac disease: a biopsy is not always necessary for diagnosis. *Aliment Pharmacol Ther* 2008;27:572–7.
75. Vivas S, Ruiz de Morales JG, Riestra S, et al. Duodenal biopsy may be avoided when high transglutaminase antibody titers are present. *World J Gastroenterol* 2009;15:4775–80.
76. Lewis NR, Scott BB. Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease. *Aliment Pharmacol Ther* 2010;31:73–81.
77. Agardh D. Antibodies against synthetic deamidated gliadin peptides and tissue transglutaminase for the identification of childhood celiac disease. *Clin Gastroenterol Hepatol* 2007;5:1276–81.
78. Liu E, Li M, Emery L, et al. Natural history of antibodies to deamidated gliadin peptides and transglutaminase in early childhood celiac disease. *J Pediatr Gastroenterol Nutr* 2007;45:293–300.
- 78a. National Institute for Health and Clinical Excellence. CG87 type 2 diabetes newer agents: NICE guidelines. <http://guidance.nice.org.uk/CG87/NICEGuidance/pdf/English>. Accessed August 31, 2011.
79. Lagerqvist C, Dahlbom I, Hansson T, Jidell E, Juto P, Olcen P, et al. Antigliadin immunoglobulin A best in finding celiac disease in children younger than 18 months of age. *J Pediatr Gastroenterol Nutr* 2008;47:428–35.
80. Koskinen O, Collin P, Lindfors K, et al. 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.
81. Sardy M, Csikos M, Geisen C, et al. Tissue transglutaminase ELISA positivity in autoimmune disease independent of gluten-sensitive disease. *Clin Chim Acta* 2007;376:126–35.
82. Damasiewicz-Bodzek A, Wielkoszynski T. Serologic markers of celiac disease in psoriatic patients. *J Eur Acad Dermatol Venereol* 2008;22:1055–61.
83. Marsh MN. Grains of truth: evolutionary changes in small intestinal mucosa in response to environmental antigen challenge. *Gut* 1990;31:111–4.
84. 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.
85. Corazza GR, Villanacci V. Coeliac disease. *J Clin Pathol* 2005;58:573–4.
86. Dickson BC, Streutker CJ, Chetty R. Coeliac disease: an update for pathologists. *J Clin Pathol* 2006;59:1008–16.
87. Paparo F, Petrone E, Tosco A, et al. Clinical, HLA, and small bowel immunohistochemical features of children with positive serum anti-endomysium antibodies and architecturally normal small intestinal mucosa. *Am J Gastroenterol* 2005;100:2294–8.
88. Simell S, Hoppu S, Hekkala A, et al. Fate of five celiac disease-associated antibodies during normal diet in genetically at-risk children observed from birth in a natural history study. *Am J Gastroenterol* 2007;102:2026–35.
89. Biagi F, Bianchi PI, Campanella J, et al. The prevalence and the causes of minimal intestinal lesions in patients complaining of symptoms suggestive of enteropathy: a follow-up study. *J Clin Pathol* 2008;61:1116–8.
90. Kakar S, Nehra V, Murray JA, et al. Significance of intraepithelial lymphocytosis in small bowel biopsy samples with normal mucosal architecture. *Am J Gastroenterol* 2003;98:2027–33.
91. Rostami K, Kerckhaert J, Tiemessen R, et al. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. *Am J Gastroenterol* 1999;94:888–94.
92. Jarvinen TT, Kaukinen K, Laurila K, et al. Intraepithelial lymphocytes in celiac disease. *Am J Gastroenterol* 2003;98:1332–7.
93. Jarvinen TT, Collin P, Rasmussen M, et al. Villous tip intraepithelial lymphocytes as markers of early-stage coeliac disease. *Scand J Gastroenterol* 2004;39:428–33.
94. Achkar E, Carey WD, Petras R, et al. Comparison of suction capsule and endoscopic biopsy of small bowel mucosa. *Gastrointest Endosc* 1986;32:278–81.
95. Barakat MH, Ali SM, Badawi AR, et al. Peroral endoscopic duodenal biopsy in infants and children. *Acta Paediatr Scand* 1983;72:563–9.
96. Branski D, Faber J, Freier S, et al. Histologic evaluation of endoscopic versus suction biopsies of small intestinal mucosae in children with and without celiac disease. *J Pediatr Gastroenterol Nutr* 1998;27:6–11.
97. Granot E, Goodman-Weill M, Pizov G, et al. Histological comparison of suction capsule and endoscopic small intestinal mucosal biopsies in children. *J Pediatr Gastroenterol Nutr* 1993;16:397–401.
98. Mee AS, Burke M, Vallon AG, et al. Small bowel biopsy for malabsorption: comparison of the diagnostic adequacy of endoscopic forceps and capsule biopsy specimens. *Br Med J (Clin Res Ed)* 1985;291:769–72.
99. Ravelli A, Bolognini S, Gambarotti M, et al. Variability of histologic lesions in relation to biopsy site in gluten-sensitive enteropathy. *Am J Gastroenterol* 2005;100:177–85.
100. Bonamico M, Thanasi E, Mariani P, et al. Duodenal bulb biopsies in celiac disease: a multicenter study. *J Pediatr Gastroenterol Nutr* 2008;47:618–22.
101. Rashid M, MacDonald A. Importance of duodenal bulb biopsies in children for diagnosis of celiac disease in clinical practice. *BMC Gastroenterol* 2009;9:78.
102. Weir DC, Glickman JN, Roiff T, et al. Variability of histopathological changes in childhood celiac disease. *Am J Gastroenterol* 2010;105:207–12.
103. Ravelli A, Villanacci V, Monfredini C, et al. 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.
104. Korponay-Szabo IR, Kovacs JB, Lorincz M, et al. Prospective significance of antiendomysium antibody positivity in subsequently verified celiac disease. *J Pediatr Gastroenterol Nutr* 1997;25:56–63.
105. Vilppula A, Collin P, Maki M, et al. Undetected coeliac disease in the elderly: a biopsy-proven population-based study. *Dig Liver Dis* 2008;40:809–13.
106. George DK, Evans RM, Gunn IR. Familial chronic fatigue. *Postgrad Med J* 1997;73:311–3.
107. Lepore L, Martellosi S, Pennesi M, et al. Prevalence of celiac disease in patients with juvenile chronic arthritis. *J Pediatr* 1996;129:311–3.
108. Goldacre MJ, Wotton CJ, Seagroatt V, et al. Cancers and immune related diseases associated with Down's syndrome: a record linkage study. *Arch Dis Child* 2004;89:1014–7.
109. Mortensen KH, Cleemann L, Hjerrild BE, et al. Increased prevalence of autoimmunity in Turner syndrome—influence of age. *Clin Exp Immunol* 2009;156:205–10.
110. Giannotti A, Tiberio G, Castro M, et al. Coeliac disease in Williams syndrome. *J Med Genet* 2001;38:767–8.
111. Collin P, Syrjanen J, Partanen J, et al. Celiac disease and HLA DQ in patients with IgA nephropathy. *Am J Gastroenterol* 2002;97:2572–6.
112. Caprai S, Vajro P, Ventura A, et al. Autoimmune liver disease associated with celiac disease in childhood: a multicenter study. *Clin Gastroenterol Hepatol* 2008;6:803–6.
113. Arnason A, Skaftadottir I, Sigmundsson J, et al. The association between coeliac disease, dermatitis herpetiformis and certain HLA-antigens in Icelanders. *Eur J Immunogenet* 1994;21:457–60.
114. Arranz E, Telleria JJ, Sanz A, et al. HLA-DQA1*0501 and DQB1*02 homozygosity and disease susceptibility in Spanish coeliac patients. *Exp Clin Immunogenet* 1997;14:286–90.
115. Book L, Zone JJ, Neuhausen SL. Prevalence of celiac disease among relatives of sib pairs with celiac disease in US families. *Am J Gastroenterol* 2003;98:377–81.
116. Bouguerra F, Babron MC, Eliaou JF, et al. Synergistic effect of two HLA heterodimers in the susceptibility to celiac disease in Tunisia. *Genet Epidemiol* 1997;14:413–22.
117. Boy MF, La NG, Balestrieri A, et al. Distribution of HLA-DPB1, -DQB1 -DQA1 alleles among Sardinian celiac patients. *Dis Markers* 1995;12:199–204.
118. Catassi C, Doloretta MM, Ratsch IM, et al. The distribution of DQ genes in the Saharawi population provides only a partial explanation for the high celiac disease prevalence. *Tissue Antigens* 2001;58:402–6.

119. Colonna M, Mantovani W, Corazza GR, et al. Reassessment of HLA association with celiac disease in special reference to the DP association. *Hum Immunol* 1990;29:263–74.
120. Congia M, Cucca F, Frau F, et al. A gene dosage effect of the DQA1*0501/DQB1*0201 allelic combination influences the clinical heterogeneity of celiac disease. *Hum Immunol* 1994;40:138–42.
121. Congia M, Frau F, Lampis R, et al. A high frequency of the A30, B18, DR3, DRw52, DQw2 extended haplotype in Sardinian celiac disease patients: further evidence that disease susceptibility is conferred by DQ A1*0501, B1*0201. *Tissue Antigens* 1992;39:78–83.
122. Djilali-Saiah I, Caillat-Zucman S, Schmitz J, et al. Polymorphism of antigen processing (TAP, LMP) and HLA class II genes in celiac disease. *Hum Immunol* 1994;40:8–16.
123. Djilali-Saiah I, Schmitz J, Harfouch-Hammoud E, et al. CTLA-4 gene polymorphism is associated with predisposition to coeliac disease. *Gut* 1998;43:187–9.
124. Erkan T, Kutlu T, Yilmaz E, et al. Human leukocyte antigens in Turkish pediatric celiac patients. *Turk J Pediatr* 1999;41:181–8.
125. Farre C, Humbert P, Vilar P, et al. Serological markers and HLA-DQ2 haplotype among first-degree relatives of celiac patients. Catalanian Coeliac Disease Study Group. *Dig Dis Sci* 1999;44:2344–9.
126. Fernandez-Arquero M, Figueredo MA, Maluenda C, et al. HLA-linked genes acting as additive susceptibility factors in celiac disease. *Hum Immunol* 1995;42:295–300.
127. Ferrante P, Petronzelli F, Mariani P, et al. Oligotyping of Italian celiac patients with the 11th International Histocompatibility Workshop reagents. *Tissue Antigens* 1992;39:38–9.
128. Fine KD, Do K, Schulte K, et al. High prevalence of celiac sprue-like HLA-DQ genes and enteropathy in patients with the microscopic colitis syndrome. *Am J Gastroenterol* 2000;95:1974–82.
129. Howell WM, Leung ST, Jones DB, et al. HLA-DRB, -DQA, and -DQB polymorphism in celiac disease and enteropathy-associated T-cell lymphoma. Common features and additional risk factors for malignancy. *Hum Immunol* 1995;43:29–37.
130. Iltanen S, Holm K, Partanen J, et al. Increased density of jejunal gammadelta+ T cells in patients having normal mucosa—marker of operative autoimmune mechanisms? *Autoimmunity* 1999;29:179–87.
131. Iltanen S, Rantala I, Laippala P, et al. Expression of HSP-65 in jejunal epithelial cells in patients clinically suspected of coeliac disease. *Autoimmunity* 1999;31:125–32.
132. Johnson TC, Diamond B, Memeo L, et al. Relationship of HLA-DQ8 and severity of celiac disease: comparison of New York and Parisian cohorts. *Clin Gastroenterol Hepatol* 2004;2:888–94.
133. Kaur G, Sarkar N, Bhatnagar S, et al. Pediatric celiac disease in India is associated with multiple DR3-DQ2 haplotypes. *Hum Immunol* 2002; 63:677–82.
134. Lewis C, Book L, Black J, et al. Celiac disease and human leukocyte antigen genotype: accuracy of diagnosis in self-diagnosed individuals, dosage effect, and sibling risk. *J Pediatr Gastroenterol Nutr* 2000; 31:22–7.
135. Lio D, Bonanno CT, D'Anna C, 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.
136. Mazzilli MC, Ferrante P, Mariani P, et al. A study of Italian pediatric celiac disease patients confirms that the primary HLA association is to the DQ(alpha 1*0501, beta 1*0201) heterodimer. *Hum Immunol* 1992;33:133–9.
137. Michalski JP, McCombs CC, Arai T, et al. HLA-DR, DQ genotypes of celiac disease patients and healthy subjects from the West of Ireland. *Tissue Antigens* 1996;47:127–33.
138. Neuhausen SL, Weizman Z, Camp NJ, et al. HLA DQA1-DQB1 genotypes in Bedouin families with celiac disease. *Hum Immunol* 2002;63:502–7.
139. Peña-Quintana L, Torres-Galvan MJ, Deniz-Naranjo MC, et al. Assessment of the DQ heterodimer test in the diagnosis of celiac disease in the Canary Islands (Spain). *J Pediatr Gastroenterol Nutr* 2003;37:604–8.
140. Perez-Bravo F, Araya M, Mondragon A, et al. Genetic differences in HLA-DQA1* and DQB1* allelic distributions between celiac and control children in Santiago, Chile. *Hum Immunol* 1999;60:262–7.
141. Ploski R, Ek J, Thorsby E, et al. On the HLA-DQ(alpha 1*0501, beta 1*0201)-associated susceptibility in celiac disease: a possible gene dosage effect of DQB1*0201. *Tissue Antigens* 1993;41:173–7.
142. Ploski R, Ascher H, Sollid LM. HLA genotypes and the increased incidence of coeliac disease in Sweden. *Scand J Gastroenterol* 1996;31:1092–7.
143. Popat S, Hearle N, Wixey J, et al. Analysis of the CTLA4 gene in Swedish coeliac disease patients. *Scand J Gastroenterol* 2002;37:28–31.
144. Ruiz del Prado MY, Olivares Lopez JL, Lazaro AA, et al. HLA system. Phenotypic and gene frequencies in celiac and healthy subjects from the same geographical area. *Rev Esp Enferm Dig* 2001;93:106–13.
145. Sacchetti L, Calcagno G, Ferrajolo A, et al. Discrimination between celiac and other gastrointestinal disorders in childhood by rapid human lymphocyte antigen typing. *Clin Chem* 1998;44 (8 Pt 1):1755–7.
146. Sumnik Z, Kolouskova S, Cinek O, et al. HLA-DQA1*05-DQB1*0201 positivity predisposes to coeliac disease in Czech diabetic children. *Acta Paediatr* 2000;89:1426–30.
147. Tighe MR, Hall MA, Barbado M, et al. HLA class II alleles associated with celiac disease susceptibility in a southern European population. *Tissue Antigens* 1992;40:90–7.
148. Tighe MR, Hall MA, Ashkenazi A, et al. Celiac disease among Ashkenazi Jews from Israel. A study of the HLA class II alleles and their associations with disease susceptibility. *Hum Immunol* 1993;38:270–6.
149. Tumer L, Altuntas B, Hasanoglu A, et al. Pattern of human leukocyte antigens in Turkish children with celiac disease. *Pediatr Int* 2000;42:678–81.
150. Tuysuz B, Dursun A, Kutlu T, et al. HLA-DQ alleles in patients with celiac disease in Turkey. *Tissue Antigens* 2001;57:540–2.