JPGN Journal of Pediatric Gastroenterology and Nutrition Publish Ahead of Print

DOI: 10.1097/MPG.0b013e31821a23d0

ESPGHAN guidelines for the diagnosis of coeliac disease in children and adolescents. An evidence-based approach

By

Husby S*, Koletzko S*, Korponay-Szabó IR*, Mearin ML*, Phillips A*, Shamir R*, Troncone R*, Giersiepen K*, Branski D, Catassi C, Lelgeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer KP. (The ESPGHAN Working Group on Coeliac Disease Diagnosis)

*(all contributed equally to the paper and are listed as first authors).

Corresponding Author and Reprints:

Steffen Husby, MD

dk-5000 Odense C

Denmark

Fax: 45 6- 591 1862

E-mail: stephen.freedman@sickkids.ca

Abstract: Objective: Diagnostic criteria for coeliac disease (CD) from ESPGHAN were published in 1990.

Since then the main autoantigen in CD, tissue transglutaminase, has been identified, the perception of

CD has changed from a rather uncommon enteropathy to a common multiorgan disease strongly depending on the haplotypes HLA-DQ2 and 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: 1)

children with symptoms suggestive of CD and 2) asymptomatic children at increased risk for CD. A systematic literature search on antibody tests for CD in paediatric patients covering the years 2004 - 2009 was the basis for the evidence based recommendations on CD specific antibody testing.

Results: In group 1 the diagnosis is based on symptoms, positive serology and histology consistent with CD. If anti-TG2-IgA antibody titers are very high (>10 times the upper limit of normal) the option

is given 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 DQ8

testing is valuable as CD is very unlikely if both haplotypes are negative.

Conclusions: The aim of the new guidelines is to achieve a high diagnostic accuracy and to reduce the

burden for the patients and their families. The performance of the new guidelines in clinical practice should be prospectively evaluated.

Keywords: coeliac disease, guidelines, evidence-based

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 twenty 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 mainly associated with HLA-DQ2 and DQ8. The diagnosis of CD has also changed due to the availability of specific CD antibody tests, mainly based on tissue transglutaminase (TG2) antibodies.

Within ESPGHAN a working group was established in order to formulate new guidelines for the diagnosis of CD based on scientific and technical developments in 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 summarize some of the 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, HLA-DQ2 or DQ8 haplotypes and enteropathy.

CD-specific antibodies comprise autoantibodies against transglutaminase type-2 (TG2, 'tissue' transglutaminase) 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 non-specific 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 since the disease may have negative health consequences. The availability of serological tests with a high accuracy as well as other diagnostic tests allows a firm diagnosis to be made. The interpretation and consequences of the test results differ between the symptomatic patient and the asymptomatic subject in at risk groups.

Testing for CD should be offered to the following two 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, abnormal liver biochemistry

Group 2: Asymptomatic children and adolescents with increased risk for CD:

type 1 diabetes mellitus, Down's syndrome, autoimmune thyroid disease, Turner syndrome, Williams' syndrome, selective IgA deficiency, autoimmune liver disease, and 1st degree relatives with CD.

Diagnostic tools

CD specific antibody tests

CD specific antibody tests measure anti-TG2 or EMA in blood. Also tests measuring anti-DGP 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 (cut-off 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 specification of the immunoglobulin class measured, the manufacturer, the cut-off 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, the cut-off dilution, interpretation (positive or negative), the highest dilution still positive and the 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 lower than 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 it very unlikely in case of a negative test result for both markers. HLA testing should be performed in cases with an uncertain diagnosis of CD, for example in cases with negative CD-specific antibodies and mild infiltrative changes in proximal small intestinal biopsy specimens. If CD is considered in children where there is a strong clinical suspicion of CD, high specific CD antibodies are present, and small bowel biopsies are not going to be performed, the working group recommends performing HLA-DQ2/DQ8 typing to add strength to the diagnosis. Prospective studies will make clear if HLA-typing is indeed an efficient and effective diagnostic tool in these cases. HLA testing may be offered to asymptomatic persons with CD associated conditions (group 2, see above) 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, they may be patchy and in a small proportion of CD patients only appear in the duodenal bulb. The alterations are not specific for CD and they may be found in enteropathies other than CD. Biopsies

should be taken preferably during upper endoscopy from the bulb (at least one biopsy) and from the second or third portion of duodenum (at least four biopsies). The pathology report should include description of the orientation, the presence or not of normal villi or degree of atrophy and crypt elongation, villous-crypt ratio, number of intraepithelial lymphocytes and a grading according to Marsh-Oberhuber.

Diagnostic approach to a child or adolescent with symptoms or signs suggestive of CD

A test for CD specific antibodies is the first tool used to identify individuals for further investigation to diagnose or to rule out CD. Patients should be tested for CD specific antibodies when on a gluten containing diet. It is recommended that the initial test should be to test IgA class anti-TG2 from a blood sample. If total serum IgA is not known, this should also be measured. In subjects with either primary or secondary humoral IgA deficiency, at least one 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 where the initial testing was performed with a rapid CD antibody detection kit (point-of–care tests, POC) the result should be confirmed by a laboratory based quantitative test. Although published data indicate POC tests may achieve a high accuracy for CD diagnosis future studies will have to show if they work equally well when applied in less selected populations and/or when handled by lay people 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 two years of age. 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 subject, 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 (age below 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 compatible with CD and DQ2/DQ8 heterodimers are negative, CD is not likely and an enteropathy caused by a diagnosis other than CD should be considered. In these cases the diagnosis of CD can only be made after a positive challenge procedure with repeated biopsies.

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

.

What to do in case of positive results of CD specific antibody tests

Children found positive for CD specific antibodies should be evaluated by a paediatric gastroenterologist or by apaediatrician with a similar knowledge and experience of CDo confirm or

to 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 should also be performed in children and adolescents before the start of a GFD because of suspected or proven wheat allergy.

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

In which patients may the diagnosis of CD be made without duodenal biopsies?

In children and adolescents with signs or symptoms suggestive of CD and very high anti-TG2 (or anti-DGP) titers with levels exceeding 10 times upper limit of normal (>10xULN) 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) in order to make the diagnosis of CD without biopsies. Antibody positivity should be verified by EMA from a blood sample drawn at a separate occasion to the initial test in order to avoid false positive serology results due to mislabeling of blood samples or other technical mistakes. If EMA testing confirms specific CD antibody positivity in this second blood sample the diagnosis of CD can be made and the child started on a GFD. It is advisable to check for HLA types in patients diagnosed without small intestinal biopsy to reinforce the diagnosis of CD.

Diagnostic approach to an asymptomatic child or adolescent with CD associated conditions.

If available offer HLA testing as the first line test. 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*022), or HLA testing is not done, an anti-TG2 IgA test and total IgA determination should be performed, but preferably not before the age of 2 years. If antibodies are negative repeated testing for CD specific antibodies is recommended.

Persons with 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 (group2) 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, a positive test result should always be confirmed by a laboratory based quantitative test. Negative rapid test results in asymptomatic individuals should also be confirmed by a quantitative test whenever the test has been carried out by lay people or untrained medical staff and /or reliability of the test or circumstances of testing (i.e. sufficient gluten intake, concomitant medication, IgA-status) is unknown or questionable.

To avoid unnecessary biopsies in individuals with low CD specific antibody levels i.e. less than 3 times upper limit of normal (<3xULN) it is recommended to perform the more specific test for EMA. If the EMA test is positive the child should be referred for duodenal biopsies. If EMA test is negative repeated serological testing on a normal gluten-containing diet in 3 – 6 monthly intervals are recommended.

Follow up and challenge procedures

If the diagnosis of CD was made according to the diagnostic criteria mentioned above, the family should receive professional dietary counseling for a GFD. The patients should be followed up on a regular basis for symptomatic improvement as well as normalization of CD specific antibody tests. The time until the antibody titers fall below the cut-off 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, in these unclear symptomatic cases in whom there is no clinical response to the GFD further investigations are required after a careful dietary assessment to exclude lack of adherence to a GFD. They may include further biopsies.

Gluten challenge is not considered necessary except under unusual circumstances. These include situations when there is doubt about the initial diagnosis. Gluten challenge should be preceded by HLA typing and assessment of mucosal histology and should always be performed under medical supervision preferably by a paediatric gastroenterologist. Gluten challenge should be discouraged before the age of 5-years and during the pubertal growth spurt, unless the child is HLA-DQ2 and 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 (around 15g/day). IgA anti-TG2 antibody (IgG in the case of low levels of serum IgA) should be measured during the challenge period. A patient should be considered 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

over after two years. However, further biopsies on a normal diet. are recommended as delayed relapse may occur later in life.

2. 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, as well as the management of CD. Since then 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, CD may occur at any age (2) but in this context we shall focus on childhood and adolescence.

The disease etiology is multifactorial with very strong genetic influence as documented in twin studies (3) and in studies showing a strong dependence on HLA-DQ2 and –DQ8 haplotypes (4). A major step forward in the understanding of the pathogenesis of CD was the demonstration in CD patients of gluten-reactive small bowel T cells that specifically recognize gliadin peptides in the context of HLA-DQ2 and –DQ8(5). Furthermore, the discovery of tissue transglutaminase (TG2) as the major autoantigen in CD led to recognition of the autoimmune nature of the disease (6). TG2 occurs abundantly in the gut and functions as to deamidate proteins and peptides including gliadin or gliadin fragments leading to increased T cell reactivity in CD patients (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 deamidated gliadin peptides (DGP) as substrate may be of significant value in CD diagnostic testing (8). Antibodies against TG2, endomysium (EMA) and DGP are hence referred to as CD specific antibodies whereas antibodies against native (non-deamidated) gliadin are largely non-specific. Small bowel biopsies have so far been considered as the reference standard for the diagnosis of CD. However, during the last decades evidence has accumulated on the diagnostic value of specific CD antibodies, and HLA typing has increasingly been used 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, 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 on results from specific CD antibody testing and HLA typing.

The USA National Institute of Health (NIH) and the Agency for Healthcare Research and Quality (AHRQ) published in 2004 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 Association for Paediatric Gastroenterology Hepatology and Nutrition (NASPGHAN) (11) Recently the National Institute for Health and Clinical Evidence UK (NICE) published guidelines for the diagnosis and management of CD in general practice (2008). However, 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 to formulate 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 in this report. A major goal in the guidelines has been

to answer the question whether duodenal biopsies with presumed characteristic histological changes compatible with CD could be omitted in some clinical circumstances to diagnose CD. In addition these guidelines present diagnostic algorithms for the clinical diagnosis of childhood CD.

3. Methods

Working group

An ESPGHAN working group was established in 2007 with the purpose to establish 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 patients Societies (AOECS) was a member of the working group. Two epidemiologists also participated in the working group.

Systematic searches

It was 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 made from January 2004 up to August 2008 supplemented by a second search from September 2008 up to September 2009. The papers found were assessed by epidemiologists and evidence 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 had sufficient accuracy to permit avoidance of small bowel biopsies to diagnose CD in all or in selected cases. The scientific evidence for this question was specifically sought and antibody analysis was the subject of a full evidence report (see Giersiepen et al. 2010).

Grades of evidence

Grading of evidence was sought with levels of evidence (LOE) based on the GRADE system (GRADE 2004) as a simplified version (12).

Strength of recommendation

Evidence reviews/draft statements were formulated by the members of the working group and formed the basis for statements and recommendations with a grading of the evidence. The recommendations were based on the degree of evidence and when there was not 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 in Schünemann et al (13).

Voting

To achieve agreement in a range of clinical and diagnostic statements and in recommendations within the areas "who to test", "specific CD antibodies", "HLA" and "small bowel biopsies", a modified Delphi process was used (Lomaz 1991; GRADE 2004). A voting discussion and repeated anonymous voting on the statements and recommendations was done based on an online platform portal (Leitlinienentwicklung, Charité Hospital, Berlin, Germany) at www. leitlinienentwicklung.de in order to obtain consensus. Four working group members did not participate in the final voting including the member from the patient organisation and the two epidemiologists.

Funding sources

The production of the guidelines was funded by ESPGHAN with contributions from the Coeliac Patients Associations within the AOECS from Germany, Great Britain, Italy, Denmark and the national paediatric gastroenterology societies in Germany and Spain.

4. Definition and classification of CD

The working group decided to define CD as follows: CD is 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 DQ8 haplotypes and enteropathy.

Several classifications of CD have been used in the past, most importantly with the distinction between classical, atypical, asymptomatic, latent and potential CD. As atypical symptoms may be considerably more common than classic symptoms the ESPGHAN working group decided to use the following nomenclature:

Gastrointestinal symptoms and signs e.g. chronic diarrhea,

Extra-intestinal symptoms and signs e.g. anaemia, neuropathy, decreased bone density and increased risk of fractures.

An extensive list of symptoms and signs of CD in children and adolescents is shown (Table 1).

Silent CD is defined as the presence of positive CD specific antibodies, HLA and small bowel biopsy findings 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 other time of her/his 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 specific CD 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 at a later time.

5 Who to test

5.1 Evidence background

CD may be difficult to recognize 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 1:3 to 1:7 adult CD patients 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, the so called CD case-finding.

CD develops only after the introduction of gluten-containing foods into the child's diet. The clinical symptoms of CD may appear in infancy, childhood, adolescence or adulthood. A gluten free diet (GFD) in CD patients improves or eliminates symptoms, and normalizes 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 consuming a low gluten containing diet because of family members diagnosed with CD. When the diagnosis of CD is suspected in subjects who are already on a GFD, it is essential that they are put 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 literature to suggest the precise amount of gluten that needs to be ingested in order to elicit a measurable serological and/or intestinal mucosal response (15). Patients without a conclusive diagnosis of CD, who are already on a GFD and do not want to re-introduce gluten into the diet, have to be informed of the consequences of their decision.

Finally, a GFD is currently 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 since compliance with a GFD is variable and may be as low as 40% (17).

5.2 Evidence review

Evaluation of the evidence for clinical symptoms of CD has been performed in the AHRQ report from 2004 for two selected signs, anemia and low bone mineral density (10) and included in the NASPGHAN guidelines (11). In the NICE guidelines of 2009 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 are frequent in clinically diagnosed childhood CD, including diarrhoea in about 50 % of patients , (15, 16, 18) as well as chronic constipation (17). It is unclear if chronic abdominal pain may be indicative of CD, as recurrent abdominal pain is so common in childhood. However, abdominal pain has been reported as a presenting symptom in 90 % of Canadian children with CD (17). A shift from gastrointestinal symptoms to extra-intestinal symptoms seems to have occurred in children with CD (15, 16, 19) It is unclear whether this finding reflects a true clinical

variation or improved recognition of non-gastrointestinal forms of CD because of increased awareness of the disease. There is good evidence that failure-to-thrive and stunted growth may be caused by CD. The risk of CD in isolated stunted growth or short stature has been calculated as 10-40 % (20). In some populations CD is diagnosed in approximately 15 % of children with iron deficiency anaemia (21).

Associated conditions

There is good evidence of an increased prevalence of CD in first-degree relatives of patients with CD, patients with autoimmune diseases such as type 1 diabetes mellitus (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 extensively investigated and is 3-12 %. The AHRQ report and the corresponding paper included 21 studies on T1DM with biopsy-proven CD, each with 50 or more participants (10). Two additional papers have appeared on children with T1DM: one reported 12% with CD (23) and one longitudinal study that reported 7% (24). Also CD occurs more frequently than expected by chance in children with Turner (25) or Down's syndrome. A 10-20 fold increase of CD prevalence has been reported in subjects with selective IgA deficiency (26). A number of conditions, e.g. epilepsy, have been suspected to be associated with CD, but the prevalences of 0.5-1 % do not seem to differ significantly from the respective background populations. Such conditions have been omitted from Table 2, which is modified from the NICE guideline.

5.3 Evidence statements

5.3.1.

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) Extra-intestinal: Failure-to-thrive+*, stunted growth+, delayed puberty, chronic anaemia+, decreased bone mineralisation (osteopenia/osteoporosis)+,dental enamel defects, irritability, chronic fatigue, neuropathy, arthritis/arthralgia,amenorrhea, increased levels of liver enzymes+

LOE: 2.

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

Total number of votes: 13; Agree: 13, Disagree: 0, Abstensions: 0

5.3.2

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, Abstensions: 0

5.3.3.

CD has an increased prevalence in children and adolescents with: first degree relatives with CD (10-20%), T1DM (2–12%), Down's 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

5.4 Recommendations

5.4.1. (↑↑) Offer testing for CD to 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 and chronic fatigue. Short stature,delayed puberty, ameorrhoea, recurrent aphthous stomatitis (mouth ulcers),dermatitis herpetiformis-type rash, repetitive fractures/osteopenia/osteoporosis, unexplained abnormal liver biochemistry.

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

5.4.2. (↑↑) Offer testing for CD in children and adolescents with the following conditions:

T1DM, Down's syndrome, autoimmune thyroid disease,Turner syndrome, Williams' syndrome, IgA deficiency, autoimmune liver disease and 1st degree relatives with CD.

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

5.4.3. (↑↑) 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 if the patients are consuming gluten or not..

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

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

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

5.4.5. 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 life-long treatment, and consuming gluten later can result in significant illness.

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

6 HLA aspects

6.2 Evidence Background

The principal determinants of genetic susceptibility for CD are the major histocompatibility class II human leukocyte antigen (HLA) class II DQA and DQB genes coded by the major histocompatibility region in the short arm of chromosome 6. More than 95% of CD patients share the HLA-DQ2 heterodimer, either in the *cis* (encoded by HLA-DR3-DQA1*0501-DQB1*0201) or in 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 (encoded by DQA1*0301-DQB1*0302). However, 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, since approximately 30% to 40% of the Caucasian 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, IL18RAP, RGS1, SH2B3 and TAGAP (29-31) . However, their contribution to the genetics of CD is relatively small in comparison to HLA-DQ2 and –DQ8. The strong relationship between HLA genetic factors and CD is illustrated by the impact of the HLA-DQ2 gene dose on the chance of disease development: HLA-DQ2 homozygous individuals have an at least five times higher risk of disease development compared to HLA-DQ2 heterozygous individuals (32).

Table 3 presents the sensitivity of HLA-DQ2 and –DQ8 for CD as assessed by the Dutch EB guidelines for CD and dermatitis herpetiformis. Most of the studies included control groups without results of small bowel biopsies and were not primary designed to assess the use of HLA typing in the diagnosis of CD. However, these studies reflect clearly the frequency of HLA-DQ2 and -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 the studies included more than 10 CD patients. The results of the more recent studies did not change the conclusions over the sensitivity of HLA-DQ2 and DQ8 as stated by the AHRQ report. The sensitivity of HLA-DQ2 is quite high (median 91%; p. 25-p75 86.3-94.0%), and if combined with HLA-DQ8 (at least one of them positive) it is even higher (96.2%; p25-p75 94.6 -99.8%), making the chance of an individual negative for DQ2 and DQ8 to have CD extremely small. However, the small percentage of HLA-DQ2/8-negative patients is well documented (33-35).

The specificity of HLA-DQ2 and-DQ8 for CD was assessed by the EB Dutch Guideline in 31 studies, most of them including controls without small bowel biopsy. The specificity of HLA-DQ2 is quite 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-DQ2/8 varies widely in different study populations, from 12% to 68% with a median of 54%. In a recent

prospective study 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 -DQ8 molecules. Traditionally HLA typing has been relative expensive, but new techniques e.g. using single tag nucleotide polymorphisms (SNP) will very probably make HLA typing a relatively cheap test (30).

There is only one 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 clinical suspected CD. The study was included in the present report as MHC antigens are expressed for life. All 16 CD patients (with villous atrophy and clinical response after GFD) were HLADQ2 or DQ8 positive, but there were no cases of CD among the 255 HLA-DQ2- and -DQ8-negative patients. Because the chance of an individual negative for HLA- DQ2 or -DQ8 to have 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 very unlikely.

There is some evidence that HLADQ2/DQ8 typing has a role in the case-finding strategy in individuals who belong to groups at risk for CD. These include a.o. first degree relatives of a confirmed case (3), and patients with immune-mediated aswell as non-immune conditions known to be associated with CD (Table 2). A negative result for HLA DQ2/DQ8 renders CD highly unlikely in these children, and there is no need for subsequent CD antibodies testing of such individuals.

6.3 Evidence statements

6.2.1

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

6.2.2

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

(LOE 2)

References:

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

6.2.3

Individuals having neither DQ2 nor DQ8 are very unlikely to have CD, since the sensitivity of HLA-DQ2 is quite high (median 91%), and if combined with HLA-DQ8 (at least one 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

6.2.4

HLA-DQ2 and/or DQ8 have a 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, Abstension: 0

6.2.5

HLA-DQ typing should not be done by serology, but by DNA testing for the 4 alleles in the HLA-DQ2 and -DQ8 molecules. New techniques, e.g. using tag single nucleotide polymorphisms (SNP), 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, Abstension: 0

6.2.6

HLADQ2/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/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

6.4 Recommendations

6.3.1

(↑↑) It is recommended to offer

HLA-DQ2 and –DQ8 typing in cases with uncertain diagnosis of CD, for example in cases 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

6.3..2

(↑↑) In patients with a clinical suspicion of CD, who are HLA-DQ2 and -DQ8 negative,

it is recommended to offer investigations for other causes of the symptoms, i.e. different from CD. .

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

6.3.3

(↑↑) It is recommended to start the screening for CD in groups at risk by HLA-DQ2 and –DQ8 typing if the test is available. These groups include first degree relatives of a confirmed case and patients with autoimmune and non-autoimmune conditions known to be associated with CD, such as type 1 diabetes mellitus, Downs' syndrome and Turner's syndrome.

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

6.3.4

(↑) If CD may be diagnosed without performing small bowel biopsies in children with strong clinical suspicion of CD and with high specific CD antibodies, it is

recommended to consider performing HLA-DQ2/DQ8 typing in these children to add strength to the diagnosis.

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

7 Antibodies

7.1 Evidence background

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

Antibodies against TG2 bind *in vivo* to the patient's own TG2 expressed in the small bowel or in other tissues (e.g. liver, muscles, central nervous system) at sites accessible for 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 CD patients (10, 52), however, TG2-specific antibodies may be present in small intestinal or other tissues of seronegative patients (49, 53). Negative antibody results in blood can also be obtained in subjects with dermatitis herpetifomis, after reduction of gluten consumption or during and after the use of immunosuppressive drugs (54-56).

7.2 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 (ELISA), Radio Immuno Assay (RIA) or others) using purified or recombinant TG2 antigens or tissue sections/fluids containing TG2. Most often serum is used, but plasma or whole blood can also 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 98-100% in expert laboratories (10, 10, 52) and this test is currently considered as the reference standard of CD-specific antibody detection. CD antibodies can also 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), quality and exposure of the antigen, calibrators, buffers, measuring methods, cut-off values and calculation mode of the results, so numerical values obtained with different kits may substantially differ. Currently, no universally accepted international standards are available that would allow the expression of antibody amount in absolute immunoglobulin concentrations. However, the majority of commercial kits use a calibration curve with antibody dilutions that provide numerical values proportional to antibody concentration in relative (arbitrary) units. This is the preferred method for clinical evaluation. The antibody tests calculating the results from the % of absorbance values supply numerical values that correlate with the logarithmic values of antibody concentrations. Despite of these differences, many commercial anti-TG2 antibody tests have equally high sensitivity and specificity on the same blood samples (59) . Inter-laboratory 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), pre-test 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 cut-off limits (55, 59, 61). This can be done by receiver operated characteristics (ROC) curve plotting sensitivity against 1-specificity. Anti-TG2 antibodies can also be detected in saliva. However, sufficient sensitivity and specificity was not achieved with conventional commercially available immunoassays (62, 63), although the use of radiobinding assays appeared more favourable in one report (64). There is no reliable method to detect specific CD antibodies from faecal samples (65).

Anti-TG2 antibody detection can also be done from the blood at the point of contact using rapid test kits (POC-test) (57, 66, 67), however, only as a semi-quantitative 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 (Giersiepen 2010) reported a pooled sensitivity of 96.4% and a pooled specificity of 97.7% . However, IgA-antiTG2 or EMA performed better. Published studies have so far been based on populations with a very high prevalence of CD, as 60.3% of all 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 99.8% (Giersiepen 2010) . The expertise of the laboratory or of the observers has high impact 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 (Giersiepen 2010) estimates the pooled positive and negative likelihood ratios of EMA results in the studies performed between 2004 and 2009 as 31.8 (95% confidence limits 18.6-54.0) and 0.067 (95% confidence limits 0.038-0.12), respectively. Further, EMA results were more homogeneous than results obtained with other CD antibody tests and had a very high diagnostic odds ratio of 553.6. Taken together, this data means that the presence of CD is very likely if the EMA test result is positive (Giersiepen 2010). Remarkably, EMA positivity is also associated with the later development of villous atrophy in the few reported cases of both adults with CD (71, 72) and children (50, 73) who initially do not fulfill the histological criteria of CD because of normal small intestinal architecture.

In the ESPGHAN report on CD antibodies (Giersiepen 2010) the specificity of anti-TG2 antibodies measured by ELISA was lower than those of EMA testing and varied according to the test kit used (Giersiepen 2010). It was not possible to obtain pooled performance estimates on sensitivity and specificity due to the heterogeneity in the evaluated studies, but for 11 out of 15 study populations the sensitivity reached ≥90% and for 13 out of 15 study populations specificity reached ≥ 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 the upper limit of normal 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 cut-off for such high values in a number of different commercial tests is discussed in Appendix II.

Although tests for anti-DGP antibodies performed favourably and much better than antibodies against native gliadin their performance was inferior compared to anti-TG2 or EMA assays (55, 76) (Giersiepen 2010). However, their performance in patients not preselected by anti-TG2 or EMA testing needs to be resolved in prospective studies. In addition their role in the diagnosis in young children below 2-3 years needs to be further assessed 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)(Giersiepen 2010). However, there is some evidence that their sensitivity may be higher in children below 2 years of age in comparison to EMA and anti-TG2 tests (79). Unfortunately the specificity is very low in this age group and makes anti-gliadin antibody tests not helpful for clinical practice. It is thus advisable to obtain a small intestinal 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 negative for CD specific antibodies a later gluten challenge procedure should always be performed to confirm CD as a cause of the enteropathy.

IgA deficiency needs to be taken into consideration in a subgroup of children in terms of 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).

7.3 Statements

7. 3.1

CD is characterised by highly specific autoantibodies directed against the common CD autoantigen transglutaminase type-2 (TG2, 'tissue' transglutaminase) including EMA and antibodies against deamidated forms of gliadin peptides (DGP).

LOE: 1,

References: (10), Giersiepen 2010

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

7.3.2

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), NICE 2009, Giersiepen 2010, (26, 48, 70)

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

7.3.3

It is not required that IgA competent CD patients are 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), Giersiepen 2010

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

7.3.4

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

LOE: 2,

References: (10, 59), Giersiepen 2010

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

7.3.5

For blood anti-TG2 antibody tests that use calibration curves to express antibody concentration, values exceeding 10 times the upper limit of the cut-off for normal (ULN) may be denoted as high antibody positivity. For other tests, values to be considered as high antibody positivity should be established by comparison with a panel of tests listed in Appendix II.

LOE: 3,

References: (55, 74, 75)

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

7.3.6

EMA testing in experienced hands has the highest specificity and positive likelihood ratio for CD among currently 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: Giersiepen 2010

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

7.3.7

The specificity and positive predictive value of serum anti-TG2 antibody measured by other immunoassays 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 unrelated to CD, such as other autoimmune conditions, infections, tumours, or tissue damage.

LOE: 1,

References: Giersiepen 2010 (62, 68-70, 81, 82)

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

7.3.8

High concentrations of anti-TG2 antibodies in blood (as defined in statement 7.3.5

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

7.3.9:

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

LOE: 1,

References: Giersiepen 2010, (67)

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

7.3.10

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 below 2 years).

LOE: 1

References: Giersiepen 2010, (76)

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

7.3.11:

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

LOE: 3

References:(64)

Total number of votes: 12, Distribution of answers: agree: 12, disagree: 0 of 12, Abstentions: 1

7.3.12

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), Giersiepen 2010

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

7.3.13

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

LOE: 3

References: Giersiepen 2010, (65)

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

7.3.14

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

7.3.15

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, 50, 67, 73)

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

7.4 Recommendations

7.4.1

(↑↑) Every antibody test used for the diagnosis of childhood CD needs to 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 similar performance as EMA A test is considered as reliable if it shows >95% agreement with the reference test.

In both situations it is recommended to seek statistical advice.

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

7.4..2

(↑↑) The optimal threshold values for antibody positivity (cut-off value or upper limit of normal, ULN) of a test should be established. This is done by receiver operated characteristics (ROC) curves plotting sensitivity against specificity at different cut-off 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

7.4.3:

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

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

7.4.4:

(↑↑) Anti-TG2 and anti-DGP laboratory test results should be reported as numeric values together with specification of the immunoglobulin class measured, the manufacturer, the cut-off 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, non-human) should be provided for in-house methods.

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

7.4.5:

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

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

7.4.6:

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

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

7.4.7.

(↑↑) It is recommended that a diagnostic test for CD specific antibody detection is the first tool to identify cases with symptoms and signs suggestive of CD for further diagnostic work up (refined serological testing, HLA typing, small intestinal biopsies and others), 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

7.4.8.

(↑↑) For initial testing in symptomatic cases, 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 one 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

7.4.9

(↑) 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

7.4.10

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

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

7.4.11

(↑) 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 two years of age.

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

7.4.12

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

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

7.4.13

(↑) It is recommended that measurements of anti-TG2 or anti-DGP antibodies with the purpose of demonstrating a decrease of antibody levels after dietary restriction are done with the same testing method as before treatment.

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

7.4.14

(↑↑) 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

7.4.15.

(↑↑) For IgA competent subjects, the conclusions should be drawn primarily from the results of 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

7.4.16.

(↑↑) 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 (age below 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

7.4.17.

(↑) Children found 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

7.4.18.

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

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

7.4.19.

(↑) If an IgA-competent subject is negative for all IgA class CD antibodies but has IgG class anti-TG2 or EMA or anti-DGP positivity, decision on further testing should be made after considering all 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

8 Biopsy

8.1 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 intraepithelial lymphocyte (IEL) density, increased IEL mitotic index, infiltration of plasma cells, lymphocytes, mast cells, eosinophils and basophils into the lamina propria. In addition, 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 has been modified by Oberhuber (84), and later by Corazza (85). The pathology report should always include a description of the orientation, evaluation of villi (normal or degree of atrophy), crypts, villous/crypt ratio, number of IELs. IELs in numbers more than 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, the changes, even the most severe, should always be interpreted in the context of the clinical and serological setting, and considering 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 of a GFD in these subjects.

Low-grade enteropathy

In the case of mild histological lesions (no villous atrophy, Marsh 1) the histology has a 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, sensitivity of serology is much less under these circumstances (55, 91). Immunohistochemical analysis of biopsies may improve specificity: high count of γδ cells (or γδ/CD3 ratio) in intestinal mucosae showing Marsh 1-2 changes increases the chance of CD, but requires frozen, non-fixed 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 a better quality, upper endoscopy has several advantages (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 (absence of folds, scalloped folds, mosaic pattern of the mucosa between the folds), although the reliability of those observations is limited to cases 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 to take a biopsy is still a matter of 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 four), and at least one from the duodenal bulb.

When should a biopsy be taken after diagnosis

Patients diagnosed with CD do not need a histological re-evaluation on a GFD. 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 a careful dietary assessment should be taken to exclude lack of compliance and inadvertent exposure to a gluten containing diet. Then further investigations are required which could include new biopsies.

When and how to perform a gluten challenge

Gluten challenge is not necessary for most cases to diagnose CD but it may be performed in special circumstances, including situations where there is doubt about the initial diagnosis. Age at diagnosis of less than two 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 the age of 5 years and during the pubertal growth spurt. Once decided, gluten challenge should always be performed under strict medical supervision, preferably by a paediatric gastroenterologist. It should be preceded by HLA testing if this was not done before, and an assessment of duodenal histology. Furthermore, the challenge should be performed assuring 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 will be 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 will be considered complete after two years, although follow up should be continued since relapse may occur at a later timepoint.

8.2 Evidence statements

8.2.1

The histological features of the small intestinal 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

8.2.2

Patchiness of the lesions may be present.

LOE: 1

References: (99, 101, 102)

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

Statement 8.2.3

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

8.2.4

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

8.2.5

Milder lesions (Marsh 1) are non-specific as 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

8.2.6

In the presence of mild histological lesions a high γδ 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

8.2.7

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: (74,81)

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

8.2 Recommendations

8.3.1

(↑) Histological assessment may be omitted in symptomatic cases (see list in the section "Who to test"), who have high IgA anti-TG2 levels (10 x above upper normal limit), verified by EMA positivity, and are HLA DQ2 and/or DQ8 heterodimer positive.

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

8.3.2

(↑) If all criteria in 8.3.1 were fulfilled and histological assessment was omitted prior to the start of GFD, follow up should include significant symptomatic improvement as well as normalization of CD specific antibody tests.

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

8.3.3.

(↑) If anti-TG2 antibodies are positive only in low concentrations and EMA testing is negative, the diagnosis of CD is less likely. A small intestinal biopsy should be performed so as to clarify whether CD is present or not.

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

8.3.4.

(↑↑) In seronegative cases with strong clinical suspicion of CD small intestinal biopsies are recommended.

(↑) If histology shows lesions compatible with CD, HLA-DQ testing should also be performed. However, an enteropathy other than CD should be considered. In these cases CD needs to be confirmed by a challenge procedure with repeated biopsies.

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

8.3.5

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

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

8.3.6

(↑) When duodenal biopsies, taken during diagnostic work-up or by chance, disclose a histological pattern with Marsh 1- Marsh 3 lesions, antibody determinations (anti-TG2 and in children below 2 y old anti-DGP) and HLA-typing should be performed. In the absence of positive CD antibodies or compatible HLA-typing other causes of enteropathy (e.g. food allergy, autoimmune enteropathy) should be considered.

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

8.3.7

(↑↑) It is recommended that it is preferable to take biopsies during upper endoscopy.

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

8.3.8

(↑) It is recommended that biopsies should be taken from the bulb (at least one) and from the second or third portion of the duodenum (at least four).

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

8.3.9

(↑) It is recommended that the pathology report includes description of the orientation, evaluation of villi (normal or degree of atrophy), crypts, villous/crypt ratio and number of IELs.
Grading according to Marsh-Oberhuber is recommended.

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

8.3.10

(↑) Patients fulfilling the diagnostic criteria of CD do not need biopsies on a gluten-free

diet (GFD).

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

8.3.11

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

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

8.3.12

(↑) Gluten challenge is not considered mandatory, except under unusual circumstances. These include situations where 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

8.3.13

(↑) If gluten challenge is indicated it should not be recommended before the age of 5-6 years and during the pubertal growth spurt.

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

8.3.14

(↑↑) It is recommended that gluten challenge is performed under medical supervision preferably by a pediatric gastroenterologist.

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

8.3.15

(↑) It is recommended to consider HLA typing and assessment of duodenal histology before gluten challenge is instituted.

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

8.3.16

(↑↑) The daily dietary intake during gluten challenge is recommended to contain a normal amount of gluten (around 15g/day).

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

8.3.17

(↑↑) It is recommended that during the challenge period IgA anti-TG2 antibody (IgG in the case of IgA deficiency) is measured. A patient should be considered relapsed (and hence the diagnosis of CD confirmed) if CD serology becomes positive and a clinical and/or histological relapse observed. In the absence of positive antibodies/symptoms the challenge should be considered over after two years and biopsies performed. Follow up should be continued since relapse may occur after more than two years.

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

Algorithms

Two algorithms have been developed based on the evidence based statements and recommendations. The first algorithm can be applied to children and adolescents with otherwise

unexplained signs and symptoms suggestive of CD. In this patient group the algorithm gives the option to omit duodenal biopsies and histology, but only if certain conditions are fulfilled.

The second algorithm should be applied to children and adolescents with no signs or symptoms suggestive of CD, who are investigated due to their increased risk for the disease (1st degree relatives of CD patients or other chronic, immune-mediated or chromosomal diseases listed in Table 2). In such persons the clinical workup should look for previously undetected disease signs, like iron-deficiency anaemia or elevated liver enzymes, and when these are present, the symptomatic algorithm applies.

It needs to be emphasized that algorithms may not fit 100% of cases and may always allow exceptions. However, the two algorithms should fit at least 95 % of children and adolescents under consideration.

These guidelines were not aiming to prepare algorithms for mass screening or for other non-clinical situations resulting from accidentally detected CD antibody positivity.

Algorithm 1: Child or adolescent with otherwise unexplained symptoms and signs suggestive of CD (Fig. 1)

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 start with IgA anti-TG2 in this population is based on the high sensitivity and specificity of the test, the widespread availability and low costs compared to anti-EMA IgA antibodies. It is not cost effective to add additional CD specific tests to the initial diagnostic work up 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) CD is very unlikely to be the cause of the symptoms. However, certain conditions which are known to give false negative anti-TG2 results need to be considered. These

include a diet low in gluten, protein-losing enteropathy, intake of immunosuppressive drugs and young patients below 2 years of age. In the 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 duodenal biopsies may be warranted.

If anti-TG2 antibody testing is positive the patients should be referred to a paediatric gastroenterologist for further diagnostic work up, which depends on the serum antibody levels. Patients with positive anti-TG2 antibody levels lower than 10 times the ULN given by the manufacturer of this particular test should undergo upper endoscopy with multiple biopsies.

In patients with positive anti-TG2 antibody levels at or higher than 10 times the ULN the paediatric gastroenterologist should discuss with the parents and the patient (as appropriate for age) the option of omitting the biopsies and the implications. If the parents (patient) accept this option blood should be drawn for HLA and EMA testing. It is important that EMA testing is performed from a different blood sample than anti-TG2 testing to exclude false positive results due to mislabelling of the previous sample or other mistakes in processing and reporting. Since EMA testing depends on the quality and experience of the laboratory the clinician needs to collaborate with a laboratory with documented experience and high standards in immunohistochemistry. If the patient is positive for EMA antibodies and positive for DQ2 or DQ8 HLA testing the diagnosis of CD is confirmed. A gluten free diet is started and the patient is followed for improvement of symptoms and decline of antibodies. A later gluten challenge in these children is not required.

In the rare case of negative results for HLA and/or anti-EMA in a child with TG2 antibody titres ≥10x ULN the different possibilities for false positive and false negative test results need to be considered. In these circumstances the diagnostic work up 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 need a longer follow up and demonstration of gluten-dependency of the symptoms/ other findings on a case by case basis.

There may be some deviation from this stepwise procedure in anti-TG2 positive children with classical symptoms (failure to thrive, diarrhoea, distended abdomen, and anaemia), who are in such a 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/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 very high in a child with anti TG2 titers ≥10 time ULN. However, in the unexpected case of negative results for HLA or anti-EMA the diagnostic work up 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 (Fig. 2)

In totally asymptomatic persons belonging to groups with a high risk for CD (defined by their own or the family history, see Table 2) CD should always be diagnosed using duodenal biopsies. A different algorithm than above is recommended as persons 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 working group had the opinion that in asymptomatic persons histological proof is needed to accept the diagnosis.

In this group HLA testing as the first is probably cost effective because a significant proportion of the patients can be excluded from further follow up studies because they not harbour DQ2 or DQ8

(44). However, if HLA testing is not feasible the screening procedure may start with CD specific antibody testing.

In persons with DQ2 or DQ8 positivity or without HLA testing IgA anti-TG2and serum total IgA determination should be performed. If IgA anti-TG2 is negative and IgA deficiency is excluded CD is very unlikely. However the disease may still develop later in life. Therefore serological testing should be repeated at regular intervals. There are not data to support any firm recommendations, but it was the opinion of the working group members that a child should be investigated by serology every 2 – 3 years in order to avoid detrimental effects of unrecognized CD on growth and bone health.

If anti-TG2 antibodies are positive, signs related to CD should be searched for (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 on from the duodenal bulb).

If anti-TG2 levels are positive but low, that is less than 3 times ULN a false positive result is possible. In the absence of any signs or symptoms the person may be followed on a normal gluten containing diet and serological testing should be repeated. In these cases anti-EMA testing may help to distinguish between false and correct positive low anti-TG2 titers. If EMA is positive the likelihood for CD increases due to 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 the child should be followed on a normal diet and anti-TG2 testing should be repeated every 3 – 6 months until the antibody levels either turn negative or the levels increase to levels where endoscopy is indicated.

If a seropositive asymptomatic at risk person does not have a conclusive evidence for CD after the extended evaluation of biopsies it is advised to follow such a person on a normal gluten containing diet and re-evaluate them 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 DQ8 and characteristic histological changes (villous atrophy and crypt hyperplasia) in the duodenal biopsy. High TG2-antibody levels (≥ULN x 10 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 only be necessary in selected cases where diagnostic uncertainty remains.

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

Based on the current evidence symptoms, CD-specific antibodies, HLA and biopsy findings all contribute to the CD diagnosis. However, a wide spectrum of findings is present within each item

from very 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 Marsh1 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 DQ8 strongly contradict the presence of CD. Nonetheless, a non-conventional 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 I) may be applied so a stronger finding in one item may compensate for a missing abnormality in the other and the sum could be taken into account. The main functions of a scoring approach are to help interpret the spectrum of diagnostic findings and to protect against over-diagnosis in insufficiently documented or borderline cases. In addition, the scoring system may provide further diagnostic reassurance in typical cases where genetic testing or immunohistochemistry are not available. Such scoring systems need to be formally evaluated in prospective clinical studies before they can be recommended in regular clinical use. They do not alter the current recommendations.

Statements of conflicts of interest

The following authors stated no conflicts of interest: D. Branski, K. Giersiepen, S. Husby, M. Lelgemann, M.L. Mearin, A. Phillips, R. Shamir, R.T. Troncone, A. Ventura,

The following authors declared potential conflicts of interest: S. Koletzko (research support from Euroimmun, Phadia, Inova for one research project), I. Korponay-Szabo (patent application on POC-test, licensed by the Univ.of Tampere to AniBiotech), C. Ribes-Koninckx (research support from Phadia), M. Maki (consultancies for Finnish Food Safety Authority Evira, The Finnish Funding Agency for Technology and Innovation Tekes; The Finnish Innovation Funds Sitra; International Life Science Institute; Coeliac Research Fund, Australia; Domm International; Finn Medi; Sinebrychoff; Moilas; Raisio; Phadia; Anibiotech; Kustannus Duodecim, Finland; Vactech;

Eurospital; Inova;Association des Amidonniers at Féculiers, France; Nexpep; Alvine Pharmaceuticals; Shire; GlaxoSmithKline; Alba Therapeutics; ChemoCentryx; Zedira), C. Catassi (consultant for Menarini Diagnostics, Italy).

Acknowledgements

We thank the AOECS for help and a positive attitude towards the project. We thank the ESPGHAN council for interest and professional understanding of the concept of these new guidelines. We thank Mrs. Tunde Koltai, representative for AOECS in the working group. We thank librarian Mrs. Kirsten Keller for valuable assistance. We thank Joan Frandsen for secretarial assistance in the preparation of the manuscript.

List of abbreviations

AHRQ	Agency for Healthcare Research and Quality, USA
AOECS	Association of European Coeliac patients Societies
CD	Coeliac disease
DGP	Deamidated gliadin peptides
EMA	Endomysial antibodies
ESPGHAN	European Society for Pediatric Gastroenterology Hepatology and Nutrition
GFD	Gluten free diet
GRADE	
NASPGHAN	North American Association for Pediatric Gastroenterology Hepatology and Nutrition
NICE	National Institute for Health and Clinical Evidence UK
NIH	National Institute of Health USA

TG2	Tissue transglutaminase 2
T1DM	Type 1 diabetes mellitus
ULN	Upper limit of normal

Reference List

Reference List

(1) Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. Revised criteria for diagnosis of coeliac disease. 65 ed. 1990. p. 909-11.

(2) Rewers M. Epidemiology of celiac disease: what are the prevalence, incidence, and progression of celiac disease? Gastroenterology 2005 Apr;128(4 Suppl 1):S47-S51.

(3) Greco L, Romino R, Coto I, Di CN, Percopo S, Maglio M, et al. The first large population based twin study of coeliac disease. Gut 2002 May;50(5):624-8.

(4) Lundin KE, Sollid LM, Qvigstad E, Markussen G, Gjertsen HA, Ek J, et al. T lymphocyte recognition of a celiac disease-associated cis- or trans-encoded HLA-DQ alpha/beta-heterodimer. J Immunol 1990 Jul 1;145(1):136-9.

(5) van de WY, Kooy YM, van Veelen PA, Pena SA, Mearin LM, Molberg O, et al. Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. Proc Natl Acad Sci U S A 1998 Aug 18;95(17):10050-4.

(6) Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997 Jul;3(7):797-801.

(7) Sjostrom H, Lundin KE, Molberg O, Korner R, McAdam SN, Anthonsen D, 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 Aug;48(2):111-5.

(8) Mothes T. Deamidated gliadin peptides as targets for celiac disease-specific antibodies. Adv Clin Chem 2007;44:35-63.

(9) Corazza GR, Villanacci V, Zambelli C, Milione M, Luinetti O, Vindigni C, et al. Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease. Clin Gastroenterol Hepatol 2007 Jul;5(7):838-43.

(10) Rostom A, Dube C, Cranney A, Saloojee N, Sy R, Garritty C, et al. Celiac disease. Evid Rep Technol Assess (Summ) 2004 Jun;(104):1-6.

(11) Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, et al. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2005 Jan;40(1):1-19.

(12) Ebell MH, Siwek J, Weiss BD, Woolf SH, Susman J, Ewigman B, et al. Strength of recommendation taxonomy (SORT): a patient-centered approach to grading evidence in the medical literature. J Am Board Fam Pract 2004 Jan;17(1):59-67.

(13) Schunemann HJ, Best D, Vist G, Oxman AD. Letters, numbers, symbols and words: how to communicate grades of evidence and recommendations. CMAJ 2003 Sep 30;169(7):677-80.

(14) Mustalahti K, Sulkanen S, Holopainen P, Laurila K, Collin P, Partanen J, et al. Coeliac disease among healthy members of multiple case coeliac disease families. Scand J Gastroenterol 2002 Feb;37(2):161-5.

(15) Bottaro G, Failla P, Rotolo N, Sanfilippo G, Azzaro F, Spina M, et al. Changes in coeliac disease behaviour over the years. Acta Paediatr 1993 Jun;82(6-7):566-8.

(16) Garampazzi A, Rapa A, Mura S, Capelli A, Valori A, Boldorini R, et al. Clinical pattern of celiac disease is still changing. J Pediatr Gastroenterol Nutr 2007 Nov;45(5):611-4.

(17) Rashid M, Cranney A, Zarkadas M, Graham ID, Switzer C, Case S, et al. Celiac disease: evaluation of the diagnosis and dietary compliance in Canadian children. Pediatrics 2005 Dec;116(6):e754-e759.

(18) Simmons JH, Klingensmith GJ, McFann K, Rewers M, Taylor J, Emery LM, et al. Impact of celiac autoimmunity on children with type 1 diabetes. J Pediatr 2007 May;150(5):461-6.

(19) Bottaro G, Cataldo F, Rotolo N, Spina M, Corazza GR. The clinical pattern of subclinical/silent celiac disease: an analysis on 1026 consecutive cases. Am J Gastroenterol 1999 Mar;94(3):691-6.

(20) van Rijn JC, Grote FK, Oostdijk W, Wit JM. Short stature and the probability of coeliac disease, in the absence of gastrointestinal symptoms. Arch Dis Child 2004 Sep;89(9):882-3.

(21) Ferrara M, Coppola L, Coppola A, Capozzi L. Iron deficiency in childhood and adolescence: retrospective review. Hematology 2006 Jun;11(3):183-6.

(22) Valentino R, Savastano S, Tommaselli AP, Dorato M, Scarpitta MT, Gigante M, et al. Prevalence of coeliac disease in patients with thyroid autoimmunity. Horm Res 1999;51(3):124-7.

(23) Hansen D, Brock-Jacobsen B, Lund E, Bjorn C, Hansen LP, Nielsen C, et al. Clinical benefit of a gluten-free diet in type 1 diabetic children with screening-detected celiac disease: a population-based screening study with 2 years' follow-up. Diabetes Care 2006 Nov;29(11):2452-6.

(24) Salardi S, Volta U, Zucchini S, Fiorini E, Maltoni G, Vaira B, et al. Prevalence of celiac disease in children with type 1 diabetes mellitus increased in the mid-1990 s: an 18-year

longitudinal study based on anti-endomysial antibodies. J Pediatr Gastroenterol Nutr 2008 May;46(5):612-4.

(25) Bonamico M, Pasquino AM, Mariani P, Danesi HM, Culasso F, Mazzanti L, et al. Prevalence and clinical picture of celiac disease in Turner syndrome. J Clin Endocrinol Metab 2002 Dec;87(12):5495-8.

(26) Korponay-Szabo IR, Dahlbom I, Laurila K, Koskinen S, Woolley N, Partanen J, et al. Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for coeliac disease in selective IgA deficiency. Gut 2003 Nov;52(11):1567-71.

(27) Emami MH, Taheri H, Kohestani S, Chitsaz A, Etemadifar M, Karimi S, et al. How frequent is celiac disease among epileptic patients? J Gastrointestin Liver Dis 2008 Dec;17(4):379-82.

(28) Dickey W, McMillan SA, McCrum EE, Evans AE. Association between serum levels of total IgA and IgA class endomysial and antigliadin antibodies: implications for coeliac disease screening. Eur J Gastroenterol Hepatol 1997 Jun;9(6):559-62.

(29) Monsuur AJ, Wijmenga C. Understanding the molecular basis of celiac disease: what genetic studies reveal. Ann Med 2006;38(8):578-91.

(30) Monsuur AJ, de Bakker PI, Zhernakova A, Pinto D, Verduijn W, Romanos J, et al. Effective detection of human leukocyte antigen risk alleles in celiac disease using tag single nucleotide polymorphisms. PLoS One 2008;3(5):e2270.

(31) van Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M, et al. A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. Nat Genet 2007 Jul;39(7):827-9.

(32) Mearin ML, Biemond I, Pena AS, Polanco I, Vazquez C, Schreuder GT, et al. HLA-DR phenotypes in Spanish coeliac children: their contribution to the understanding of the genetics of the disease. Gut 1983 Jun;24(6):532-7.

(33) Karell K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L, et al. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. Hum Immunol 2003 Apr;64(4):469-77.

(34) Polvi A, Eland C, Koskimies S, Maki M, Partanen J. HLA DQ and DP in Finnish families with celiac disease. Eur J Immunogenet 1996 Jun;23(3):221-34.

(35) Margaritte-Jeannin P, Babron MC, Bourgey M, Louka AS, Clot F, Percopo S, et al. HLA-DQ relative risks for coeliac disease in European populations: a study of the European Genetics Cluster on Coeliac Disease. Tissue Antigens 2004 Jun;63(6):562-7.

(36) Hadithi M, von Blomberg BM, Crusius JB, Bloemena E, Kostense PJ, Meijer JW, et al. Accuracy of serologic tests and HLA-DQ typing for diagnosing celiac disease. Ann Intern Med 2007 Sep 4;147(5):294-302.

(37) van Belzen MJ, Koeleman BP, Crusius JB, Meijer JW, Bardoel AF, Pearson PL, et al. Defining the contribution of the HLA region to cis DQ2-positive coeliac disease patients. Genes Immun 2004 May;5(3):215-20.

(38) Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. J Exp Med 1989 Jan 1;169(1):345-50.

(39) Balas A, Vicario JL, Zambrano A, Acuna D, Garcia-Novo D. Absolute linkage of celiac disease and dermatitis herpetiformis to HLA-DQ. Tissue Antigens 1997 Jul;50(1):52-6.

(40) Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, 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 Feb 10;163(3):286-92.

(41) Liu J, Juo SH, Holopainen P, Terwilliger J, Tong X, Grunn A, et al. Genomewide linkage analysis of celiac disease in Finnish families. Am J Hum Genet 2002 Jan;70(1):51-9.

(42) Vidales MC, Zubillaga P, Zubillaga I, fonso-Sanchez MA. Allele and haplotype frequencies for HLA class II (DQA1 and DQB1) loci in patients with celiac disease from Spain. Hum Immunol 2004 Apr;65(4):352-8.

(43) Al-toma A, Goerres MS, Meijer JW, Pena AS, Crusius JB, Mulder CJ. Human leukocyte antigen-DQ2 homozygosity and the development of refractory celiac disease and enteropathy-associated T-cell lymphoma. Clin Gastroenterol Hepatol 2006 Mar;4(3):315-9.

(44) Csizmadia CG, Mearin ML, Oren A, Kromhout A, Crusius JB, von Blomberg BM, et al. Accuracy and cost-effectiveness of a new strategy to screen for celiac disease in children with Down syndrome. J Pediatr 2000 Dec;137(6):756-61.

(45) Maki M, Mustalahti K, Kokkonen J, Kulmala P, Haapalahti M, Karttunen T, et al. Prevalence of Celiac disease among children in Finland. N Engl J Med 2003 Jun 19;348(25):2517-24.

(46) Schwertz E, Kahlenberg F, Sack U, Richter T, Stern M, Conrad K, et al. Serologic assay based on gliadin-related nonapeptides as a highly sensitive and specific diagnostic aid in celiac disease. Clin Chem 2004 Dec;50(12):2370-5.

(47) Korponay-Szabo IR, Halttunen T, Szalai Z, Laurila K, Kiraly R, Kovacs JB, et al. In vivo targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. Gut 2004 May;53(5):641-8.

(48) Cataldo F, Lio D, Marino V, Picarelli A, Ventura A, Corazza GR. 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 Sep;47(3):366-9.

(49) Hadjivassiliou M, Maki M, Sanders DS, Williamson CA, Grunewald RA, Woodroofe NM, et al. Autoantibody targeting of brain and intestinal transglutaminase in gluten ataxia. Neurology 2006 Feb 14;66(3):373-7.

(50) Kurppa K, Ashorn M, Iltanen S, Koskinen LL, Saavalainen P, Koskinen O, et al. Celiac Disease without Villous Atrophy in Children: A Prospective Study. J Pediatr 2010 Apr 16.

(51) Maki M, Holm K, Koskimies S, Hallstrom O, Visakorpi JK. Normal small bowel biopsy followed by coeliac disease. Arch Dis Child 1990 Oct;65(10):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 Jul 1;24(1):47-54.

(53) Salmi TT, Collin P, Korponay-Szabo IR, Laurila K, Partanen J, Huhtala H, et al. Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits. Gut 2006 Dec;55(12):1746-53.

(54) Bargetzi MJ, Schonenberger A, Tichelli A, Fried R, Cathomas G, Signer E, et al. Celiac disease transmitted by allogeneic non-T cell-depleted bone marrow transplantation. Bone Marrow Transplant 1997 Oct;20(7):607-9.

(55) Dahlbom I, Korponay-Szabo IR, Kovacs JB, Szalai Z, Maki M, Hansson T. Prediction of clinical and mucosal severity of coeliac disease and dermatitis herpetiformis by quantification of IgA/IgG serum antibodies to tissue transglutaminase. J Pediatr Gastroenterol Nutr 2010 Feb;50(2):140-6.

(56) Karpati S, Torok E, Kosnai I. IgA class antibody against human jejunum in sera of children with dermatitis herpetiformis. J Invest Dermatol 1986 Dec;87(6):703-6.

(57) Raivio T, Kaukinen K, Nemes E, Laurila K, Collin P, Kovacs JB, et al. Self transglutaminase-based rapid coeliac disease antibody detection by a lateral flow method. Aliment Pharmacol Ther 2006 Jul 1;24(1):147-54.

(58) Prause C, Ritter M, Probst C, Daehnrich C, Schlumberger W, Komorowski L, et al. Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease. J Pediatr Gastroenterol Nutr 2009 Jul;49(1):52-8.

(59) Naiyer AJ, Hernandez L, Ciaccio EJ, Papadakis K, Manavalan JS, Bhagat G, et al. Comparison of commercially available serologic kits for the detection of celiac disease. J Clin Gastroenterol 2009 Mar;43(3):225-32.

(60) Li M, Yu L, Tiberti C, Bonamico M, Taki I, Miao D, et al. A report on the International Transglutaminase Autoantibody Workshop for Celiac Disease. Am J Gastroenterol 2009 Jan;104(1):154-63.

(61) Vecsei A, Arenz T, Heilig G, Arenz S, Bufler P, Koletzko S. Influence of age and genetic risk on anti-tissue transglutaminase IgA titers. J Pediatr Gastroenterol Nutr 2009 May;48(5):544-9.

(62) Di TM, Barilla F, Trappolini M, Palumbo HF, Gaudio C, Picarelli A. Antitissue transglutaminase antibodies in acute coronary syndrome: an alert signal of myocardial tissue lesion? J Intern Med 2008 Jan;263(1):43-51.

(63) Pastore L, Campisi G, Compilato D, Lo ML. Orally based diagnosis of celiac disease: current perspectives. J Dent Res 2008 Dec;87(12):1100-7.

(64) Bonamico M, Ferri M, Nenna R, Verrienti A, Di MU, Tiberti C. Tissue transglutaminase autoantibody detection in human saliva: a powerful method for celiac disease screening. J Pediatr 2004 May;144(5):632-6.

(65) Kappler M, Krauss-Etschmann S, Diehl V, Zeilhofer H, Koletzko S. Detection of secretory IgA antibodies against gliadin and human tissue transglutaminase in stool to screen for coeliac disease in children: validation study. BMJ 2006 Jan 28;332(7535):213-4.

(66) Baviera LC, Aliaga ED, Ortigosa L, Litwin N, Pena-Quintana L, Mendez V, et al. Celiac disease screening by immunochromatographic visual assays: results of a multicenter study. J Pediatr Gastroenterol Nutr 2007 Nov;45(5):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 Dec 15;335(7632):1244-7.

(68) Bizzaro N, Tampoia 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(5):184-9.

(69) Ferrara F, Quaglia S, Caputo I, Esposito C, Lepretti M, Pastore S, et al. Anti-transglutaminase antibodies in non-coeliac children suffering from infectious diseases. Clin Exp Immunol 2010 Feb;159(2):217-23.

(70) Villalta D, Bizzaro N, Tonutti E, Tozzoli R. IgG anti-transglutaminase autoantibodies in systemic lupus erythematosus and Sjogren syndrome. Clin Chem 2002 Jul;48(7):1133.

(71) Collin P, Helin H, Maki M, Hallstrom O, Karvonen AL. Follow-up of patients positive in reticulin and gliadin antibody tests with normal small-bowel biopsy findings. Scand J Gastroenterol 1993 Jul;28(7):595-8.

(72) Kurppa K, Collin P, Viljamaa M, Haimila K, Saavalainen P, Partanen J, et al. Diagnosing mild enteropathy celiac disease: a randomized, controlled clinical study. Gastroenterology 2009 Mar;136(3):816-23.

(73) Koskinen O, Collin P, Korponay-Szabo I, Salmi T, Iltanen S, Haimila K, et al. Gluten-dependent small bowel mucosal transglutaminase 2-specific IgA deposits in overt and mild enteropathy coeliac disease. J Pediatr Gastroenterol Nutr 2008 Oct;47(4):436-42.

(74) Hill PG, Holmes GK. Coeliac disease: a biopsy is not always necessary for diagnosis. Aliment Pharmacol Ther 2008 Apr 1;27(7):572-7.

(75) Vivas S, Ruiz de Morales JG, Riestra S, Arias L, Fuentes D, Alvarez N, et al. Duodenal biopsy may be avoided when high transglutaminase antibody titers are present. World J Gastroenterol 2009 Oct 14;15(38):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 Jan;31(1):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 Nov;5(11):1276-81.

(78) Liu E, Li M, Emery L, Taki I, Barriga K, Tiberti C, et al. Natural history of antibodies to deamidated gliadin peptides and transglutaminase in early childhood celiac disease. J Pediatr Gastroenterol Nutr 2007 Sep;45(3):293-300.

(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 Oct;47(4):428-35.

(80) Koskinen O, Collin P, Lindfors K, Laurila K, Maki M, Kaukinen K. Usefulness of small-bowel mucosal transglutaminase-2 specific autoantibody deposits in the diagnosis and follow-up of celiac disease. J Clin Gastroenterol 2010 Aug;44(7):483-8.

(81) Sardy M, Csikos M, Geisen C, Preisz K, Kornsee Z, Tomsits E, et al. Tissue transglutaminase ELISA positivity in autoimmune disease independent of gluten-sensitive disease. Clin Chim Acta 2007 Feb;376(1-2):126-35.

(82) Damasiewicz-Bodzek A, Wielkoszynski T. Serologic markers of celiac disease in psoriatic patients. J Eur Acad Dermatol Venereol 2008 Sep;22(9):1055-61.

(83) Marsh MN. Grains of truth: evolutionary changes in small intestinal mucosa in response to environmental antigen challenge. Gut 1990 Jan;31(1):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 Oct;11(10):1185-94.

(85) Corazza GR, Villanacci V. Coeliac disease. J Clin Pathol 2005 Jun;58(6):573-4.

(86) Dickson BC, Streutker CJ, Chetty R. Coeliac disease: an update for pathologists. J Clin Pathol 2006 Oct;59(10):1008-16.

(87) Paparo F, Petrone E, Tosco A, Maglio M, Borrelli M, Salvati VM, et al. Clinical, HLA, and small bowel immunohistochemical features of children with positive serum antiendomysium antibodies and architecturally normal small intestinal mucosa. Am J Gastroenterol 2005 Oct;100(10):2294-8.

(88) Simell S, Hoppu S, Hekkala A, Simell T, Stahlberg MR, Viander M, 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 Sep;102(9):2026-35.

(89) Biagi F, Bianchi PI, Campanella J, Badulli C, Martinetti M, Klersy C, 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 Oct;61(10):1116-8.

(90) Kakar S, Nehra V, Murray JA, Dayharsh GA, Burgart LJ. Significance of intraepithelial lymphocytosis in small bowel biopsy samples with normal mucosal architecture. Am J Gastroenterol 2003 Sep;98(9):2027-33.

(91) Rostami K, Kerckhaert J, Tiemessen R, von Blomberg BM, Meijer JW, Mulder CJ. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. Am J Gastroenterol 1999 Apr;94(4):888-94.

(92) Jarvinen TT, Kaukinen K, Laurila K, Kyronpalo S, Rasmussen M, Maki M, et al. Intraepithelial lymphocytes in celiac disease. Am J Gastroenterol 2003 Jun;98(6):1332-7.

(93) Jarvinen TT, Collin P, Rasmussen M, Kyronpalo S, Maki M, Partanen J, et al. Villous tip intraepithelial lymphocytes as markers of early-stage coeliac disease. Scand J Gastroenterol 2004 May;39(5):428-33.

(94) Achkar E, Carey WD, Petras R, Sivak MV, Revta R. Comparison of suction capsule and endoscopic biopsy of small bowel mucosa. Gastrointest Endosc 1986 Aug;32(4):278-81.

(95) Barakat MH, Ali SM, Badawi AR, Khuffash FA, Fernando N, Majeed HA, et al. Peroral endoscopic duodenal biopsy in infants and children. Acta Paediatr Scand 1983 Jul;72(4):563-9.

(96) Branski D, Faber J, Freier S, Gottschalk-Sabag S, Shiner M. Histologic evaluation of endoscopic versus suction biopsies of small intestinal mucosae in children with and without celiac disease. J Pediatr Gastroenterol Nutr 1998 Jul;27(1):6-11.

(97) Granot E, Goodman-Weill M, Pizov G, Sherman Y. Histological comparison of suction capsule and endoscopic small intestinal mucosal biopsies in children. J Pediatr Gastroenterol Nutr 1993 May;16(4):397-401.

(98) Mee AS, Burke M, Vallon AG, Newman J, Cotton PB. Small bowel biopsy for malabsorption: comparison of the diagnostic adequacy of endoscopic forceps and capsule biopsy specimens. Br Med J (Clin Res Ed) 1985 Sep 21;291(6498):769-72.

(99) Ravelli A, Bolognini S, Gambarotti M, Villanacci V. Variability of histologic lesions in relation to biopsy site in gluten-sensitive enteropathy. Am J Gastroenterol 2005 Jan;100(1):177-85.

(100) Bonamico M, Thanasi E, Mariani P, Nenna R, Luparia RP, Barbera C, et al. Duodenal bulb biopsies in celiac disease: a multicenter study. J Pediatr Gastroenterol Nutr 2008 Nov;47(5):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, Valim C, Leichtner AM. Variability of histopathological changes in childhood celiac disease. Am J Gastroenterol 2010 Jan;105(1):207-12.

(103) Ravelli A, Villanacci V, Monfredini C, Martinazzi S, Grassi V, Manenti S. How Patchy Is Patchy Villous Atrophy?: Distribution Pattern of Histological Lesions in the Duodenum of Children With Celiac Disease. Am J Gastroenterol 2010 Apr 6.

(104) Korponay-Szabo IR, Kovacs JB, Lorincz M, Goracz G, Szabados K, Balogh M. Prospective significance of antiendomysium antibody positivity in subsequently verified celiac disease. J Pediatr Gastroenterol Nutr 1997 Jul;25(1):56-63.

(105) Barker CC, Mitton C, Jevon G, Mock T. Can tissue transglutaminase antibody titers replace small-bowel biopsy to diagnose celiac disease in select pediatric populations? Pediatrics 2005 May;115(5):1341-6.

Figure legends

Fig. 1

Symptomatic patient

Fig. 2 Asymptomatic patient

Appendix II. Figure

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 I

A simple scoring system for the diagnosis of coeliac disease (CD)

Aims:

1. to positively diagnose coeliac disease at the initial assessment and be able to accept a diagnosis made in the past with biopsy

2. to simplify the diagnosis of CD in cases with very obvious findings
3. 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.

A/ Symptoms

	points
• Malabsorption syndrome	2
• Other CD-relevant symptom ***OR*** having type-1 diabetes mellitus ***OR*** being a first degree family member	1
• Asymptomatic	0

B/ 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

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

C/ HLA

• Full HLA-DQ2 (in *cis or trans*) or DQ8 heterodimers present	1
• No HLA performed ***OR*** half DQ2 (only HLA-DQB1*0202) present	0
• HLA neither DQ2 nor DQ8	-1

D/ Histology

• Marsh IIIb or IIIc (subtotal villous atrophy, flat lesion)	2
• Marsh II or IIIa (moderately decreased villus height/crypt depth ratio)	

OR Marsh 0-I plus intestinal TG2 antibodies 1

- Marsh 0-I ***OR*** no biopsy performed 0

Comments and explanations for use:

Biopsy items were graded by taking into account Villanacci's scoring (85) and the clinical utility of the results. We assumed that Marsh 0 or Marsh I results without any further information could be non-specific. 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 previously even WITHOUT this possibility.*It is not necessary to have EMA testing facility, but it is a clear advantage. Some findings which make CD very inprobable 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 as gluten-dependent. E.g. an infant with villous atrophy before the introduction of gluten and normal biopsy at the age of 6 years while on a normal diet eating gluten will obtain 0 for biopsy.

Appendix II

Comparison of high serum anti-TG2 levels obtained by different commercial tests.

Data kindly provided by the United Kingdom National External Quality Assessment Service for Immunology and Immunochemistry (UKNEQAS, Sheffield, UK)

Several research studies have shown that presence of small intestinal villous atrophy can be predicted if the levels of circulating anti-TG2 antibodies are high (55, 74, 75, 105). Currently, TG2-specific antibodies only can be measured 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 two main ways to calculate serum antibody results: the majority of currently available commercial anti-TG2 tests calculates 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 a more simple calculation, that is 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; in consequence, numeric values are higher than those 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 more narrow 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 yet been sufficiently investigated in academic studies. UKNEQAS provides an external quality control service across Europe and distributes 6 serum samples each year which are then measured by a large number of clinical laboratories, each using his own kit or method. In this way, large pools of results are continuously generated by t anti-TG2 kits which represent current clinical testing practices and can steadily be updated. With the help of UKNEQAS, we analysed the returns for three 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). The table shows values for three representative samples (I-III) that yielded 13.6, 18 and 30.1 U/l median values with Phadia's Varelisa [Celikey™] assay which had been used in thepapers of Hill (75) and Dahlbom (55). These authors found that serum antibody results exceeding 10 times the upper limit of normal (ULN) of this test (30 U/l) were invariably associated with villous atrophy. The gray column indicates the kit-specific values obtained with the 30.1 U/l (high positive) sample, 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 x times the ULN values represent 'high' values in the respective tests. The figure (Appendix II. Fig) shows that most tests can distinguish slightly (4xULN), moderately (6xULN) and highly (10xULN) positive anti-TG2 levels resulting in almost parallel albeit numerically different curves. These results may be regarded as an example of the performance of different tests in a high number of clinical laboratories. Final conclusions should be drawn from more systematic studies or from a longer survey.

Table. 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	Cut-off	(x) 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	2.9
Diasorin	28.6	37.5	57	8	7.1
Euroimmun	171.9	186	200	20	10.0
Eurospital[a]	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
Inova[a]	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[b]	10.0

a: these test calculate results in logarithmically, b: optimal cut-off in research studies

The Aesku test is measuring the combination of anti-TG2 and anti-gliadin antibodies and thus may have different characteritics.

Appendix II. Fig.

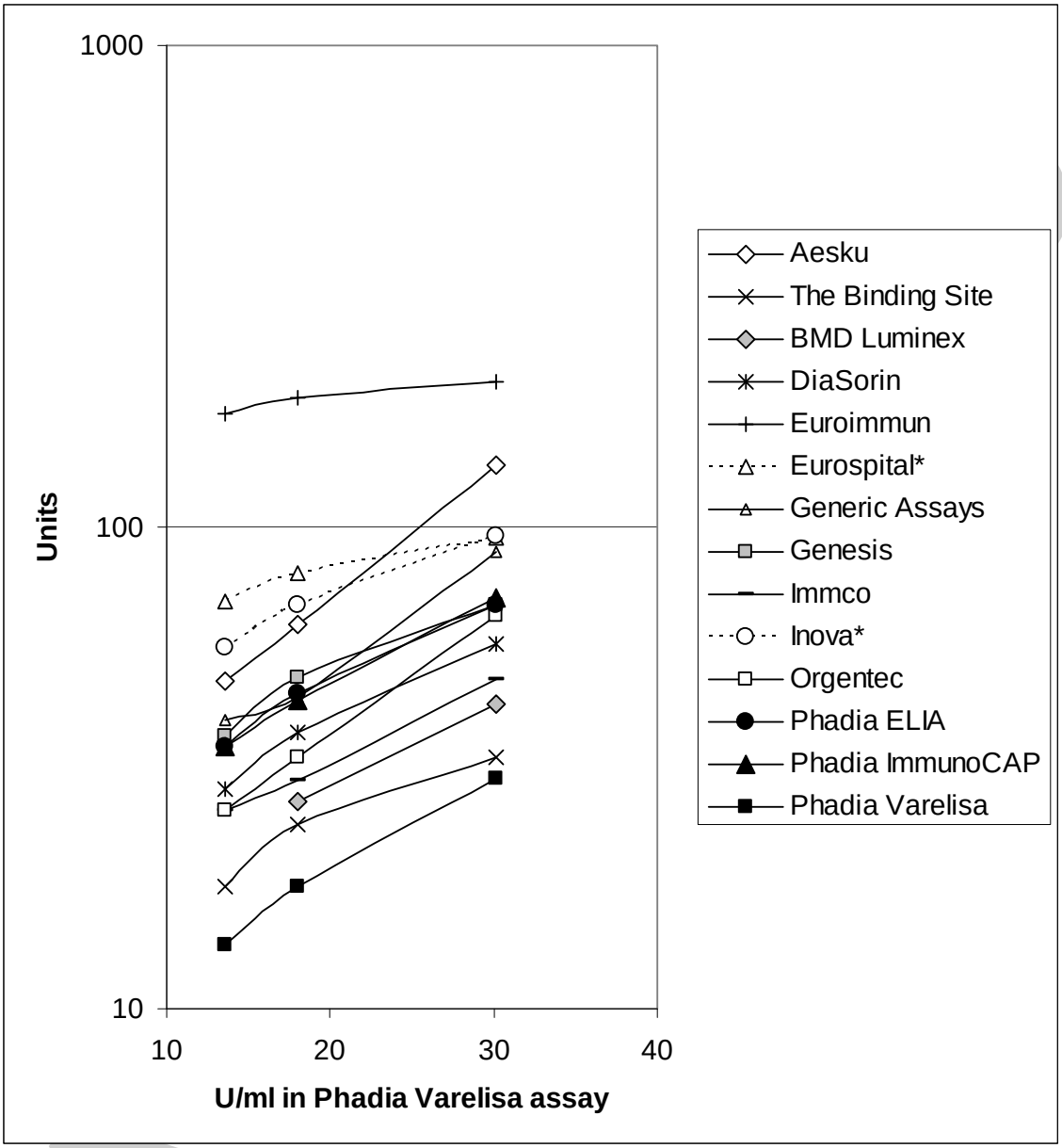

Table 1 Presenting features of children and adolescents with coeliac disease (CD)

(adapted from NICE*, only with studies including children)

Feature	% of total number of CD	Study population	Studies
Iron-deficiency anaemia	39 – 12	adults and children adults and children	(19, 27)
Other or unspecified anaemia	16 3 -19 23	adults and children adults and children	(28, 105)
Anorexia	8 26- 35	adults and children children	(15, 19)
Weight loss	44-60 6	children and adults children and adults	(15, 28)
Abdominal distension/bloating	28-36 10 20-39	children adults and children children	(15, 16, 27)
Abdominal pain	12 8. 11 - 21 90	adults and children adults and children children children	(16, 16, 17, 27, 28)
Vomiting	26-33	children	(15)
Flatulence	5	adults and children	(27)
Diarrhoea	70- 75 51	Children adults and children	(15, 16, 16, 27, 28, 28, 28, 28)

Feature	% of total number of CD	Study population	Studies
	13 12 -60	adults and children children	
Short stature/growth failure	19 20-31	adults and children children	(19, 28)
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)

*Additional information was provided by a single paper

Table 2 Conditions associated with CD apart from Type-1 diabetes (adapted from NICE, only studies including children, except for IgA nephropathy where only data on adults were available)

Condition	**%Coeliac disease**	**Study population**	**Study author and year**
Juvenile Chronic Arthritis	1.5	children	(106)
	2.5	children	(107)
Down's syndrome	0.3 5.5	children and adults children	(108)
Turner's syndrome	6 5	Children and adults	(25, 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)

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

Author, Year	Type study	CD population		Sensitivity (%)			
		Origin	**Tested CD group**	**N**	**DQ2**	**DQ8**	**DQ2or8**
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, 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
Dijilali-Saiah, 1994(122)	NIH; c-c	France	known CD	80	89		

Dijilali-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	USA, New York	known CD	44	86	41	
Johnson, 2004(132)	c-c	France, Paris	known CD	66	93	21	
Karell, 2003(33)	NIH; m-d	France	known CD	92	87	6,5	93,5
Karell, 2003(33)	NIH; m-d	Italy	known CD	302	93,7	5,6	89,4
Karell, 2003(33)	NIH; m-	Finland	known CD	100	91	5	96

	d						
Karell, 2003(33)	NIH; m-d	Norway	known CD	326	91,4	5,2	96,6
Karell, 2003(33)	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 school children	56	85,7		
Margaritte-Jeannin, 2004(35)	m-d	Italy	known CD	128	86		
Margaritte-Jeannin, 2004(35)	m-d	France	known CD	117	87		
Margaritte-Jeannin, 2004(35)	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
Pettersson, 1993 [52]	NIH; c-c	Sweden	known CD	65	92		
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
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							
Number of researches					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 taken from the NIH review are marked with NIH, m-d: mixed design study; cc: case control study

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

Author, Year	**Type of study**	**Origin**	**N CD**	**Sensitivity (%) DQ2 and/or DQ8**	**N control**	**Specificity (%) 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 taken from the NIH review are marked with NIH, m-d: mixed design study; cc: case control study

Fig. 1 Symptomatic patient:

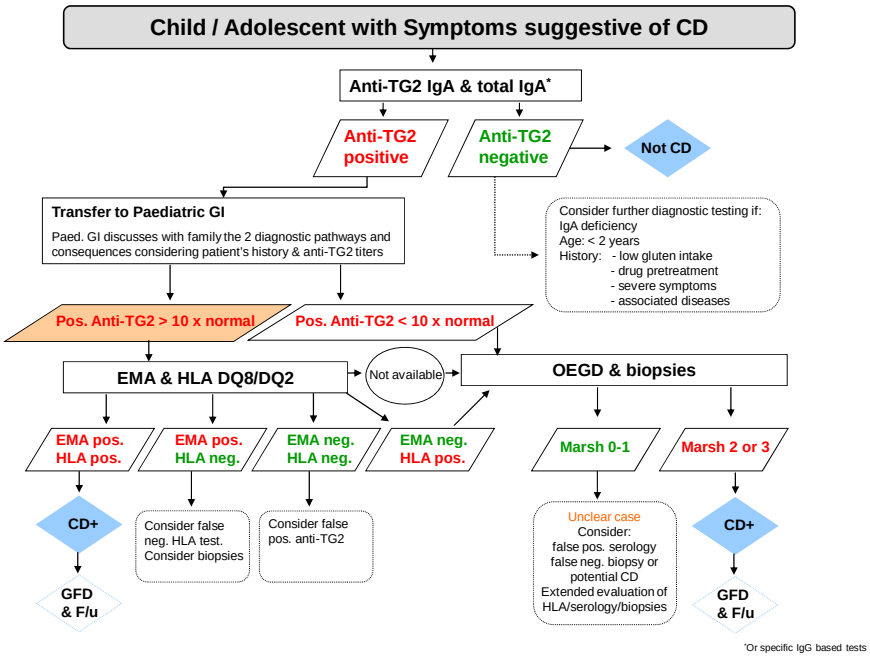

Fig. 2 Asymptomatic patient: