

European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020

*Steffen Husby, †Sibylle Koletzko, ‡Ilma Korponay-Szabó, §Kalle Kurppa, ||Maria Luisa Mearin, ¶Carmen Ribes-Koninckx, #Raanan Shamir, **Riccardo Troncone, **Renata Auricchio, ††Gemma Castillejo, ††Robin Christensen, §§Jernej Dolinsek, ||||Peter Gillett, ¶¶Asbjørn Hróbjartsson, ##Tunde Koltai, §Markku Maki, ††Sabrina Mai Nielsen, ***Alina Popp, †††Ketil Størdal, †Katharina Werkstetter, and †††Margreet Wessels

ABSTRACT

Objectives: The ESPGHAN 2012 coeliac disease (CD) diagnostic guidelines aimed to guide physicians in accurately diagnosing CD and permit omission of duodenal biopsies in selected cases. Here, an updated and expanded evidence-based guideline is presented.

Methods: Literature databases and other sources of information were searched for studies that could inform on 10 formulated questions on symptoms, serology, human leukocyte antigen genetics, and histopathology. Eligible articles were assessed using QUADAS2. GRADE provided a basis for statements and recommendations.

Results: Various symptoms are suggested for case finding, with limited contribution to diagnostic accuracy. If CD is suspected, measurement of total serum IgA and IgA-antibodies against transglutaminase 2 (TGA-IgA) is superior to other combinations. We recommend against deamidated gliadin peptide antibodies (DGP-IgG/IgA) for initial testing. Only if total IgA is low/undetectable, an IgG-based test is indicated. Patients with positive results should be referred to a paediatric gastroenterologist/specialist. If TGA-IgA is ≥ 10 times the upper limit of normal ($10 \times$ ULN) and the family agrees, the no-biopsy diagnosis may be applied, provided endomysial antibodies (EMA-IgA) will test positive in a second blood sample. Human leukocyte antigen DQ2-/DQ8 determination and symptoms are not obligatory criteria. In children with positive TGA-IgA $< 10 \times$ ULN at least 4 biopsies from the distal duodenum and at least 1 from the bulb should be taken. Discordant results between TGA-IgA and histopathology may require re-evaluation of biopsies. Patients with no/mild histological changes (Marsh 0/I) but confirmed autoimmunity (TGA-IgA/EMA-IgA+) should be followed closely.

Conclusions: CD diagnosis can be accurately established with or without duodenal biopsies if given recommendations are followed.

Key Words: children and adolescents, coeliac disease, diagnostic tests, meta-analysis

(*JPGN* 2020;70: 141–157)

Received March 25, 2019; accepted August 27, 2019.

From the *Hans Christian Andersen Children's Hospital, Odense University Hospital, DK-5000 Odense C, Denmark, the †Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, LMU Munich, Germany and Department of Pediatrics, Gastroenterology and Nutrition, School of Medicine Collegium Medicum University of Warmia and Mazury, Olsztyn, Poland, the ‡Heim Pál National Paediatric Institute, Coeliac Disease Centre, Budapest, and Department of Paediatrics, University of Debrecen Medical Faculty, Debrecen, Hungary, the §Tampere Centre for Child Health Research, Tampere University, and Department of Pediatrics, Tampere University Hospital, Tampere, Finland, the ||Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands, the ¶Paediatric Gastroenterology Unit, La Fe University Hospital, Valencia, Spain, the #Institute for Gastroenterology, Nutrition and Liver Diseases, Schneider Children's Medical Center, Petach Tikva, Sackler Faculty of Medicine, Tel Aviv University, Israel, the **Department of Medical Translational Sciences and European Laboratory for the Investigation of Food-Induced

What Is Known

- Coeliac disease is underdiagnosed because of the heterogeneous presentation of clinical signs and symptoms.
- To diagnose coeliac disease, different approaches are applied (history, clinical examination, serology, human leukocyte antigen testing, histopathology), but neither 1 of them has been considered sufficient alone to make a reliable diagnosis.
- For the first time, the European Society of Paediatric Gastroenterology, Hepatology and Nutrition 2012 guidelines allowed serology-based diagnosis, omitting the necessity of histopathology in selected cases, but the evidence came mainly from retrospective studies.

What Is New

- For initial testing, the combination of total IgA and IgA class antibodies against transglutaminase 2 is more accurate than other test combinations.
- The no-biopsy approach for coeliac disease diagnosis is safe in children with high serum IgA class antibody concentration against transglutaminase 2 values (≥ 10 times the upper limit of normal) with appropriate tests and positive endomysial antibodies (EMA-IgA) in a second serum sample.
- Children with positive IgA class antibodies against transglutaminase 2 but lower titers (< 10 times upper limit of normal) should undergo biopsies to decrease the risk of false positive diagnosis.
- Human leukocyte antigen testing and presence of symptoms are not obligatory criteria for a serology-based diagnosis without biopsies.

Diseases, University Federico II, Naples, Italy, the ††Paediatric Gastroenterology Unit, Department of Pediatrics, Hospital Universitari Sant Joan de Reus, Reus, Spain, the †††Musculoskeletal Statistics Unit: The Parker Institute, Bispebjerg and Frederiksberg Hospital & Department of Rheumatology, Odense University Hospital, Denmark, the §§Unit of Pediatric Gastroenterology and Nutrition, University Medical Centre Maribor, Maribor, Slovenia, the ||||Paediatric Gastroenterology, Hepatology and Nutrition Department, Royal Hospital for Sick Children, Edinburgh, Scotland, UK, the ¶¶Centre for Evidence Based Medicine Odense (CEBMO), Odense University Hospital, Denmark, the ##Association of European Coeliac Society/Belgium, Hungarian Coeliac Society/Hungary, the ***University of Medicine and Pharmacy "Carol Davila", National Institute for Mother and Child Health, Bucharest, Romania, the †††Norwegian Institute of Public Health, Oslo and Ostfold Hospital Trust, Norway, and the ††††Department of Pediatrics, Rijnstate Hospital, Arnhem, the Netherlands.

The recognition of the broad clinical spectrum of coeliac disease (CD) has evolved during the last decades. It became evident that CD is a common disease occurring at all ages and with a variety of signs and symptoms. In 2012, the CD working group of European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) revised their diagnostic guidelines for CD (1). One main message of these guidelines was that the diagnosis of CD can be made without biopsies in a subgroup of paediatric patients because coeliac enteropathy (with Marsh 2 or 3 changes) was nearly invariably present in patients with very high coeliac auto-antibody levels in serum. For this so-called no-biopsy approach, all of the following criteria had to be fulfilled:

1. Symptoms suggestive of CD (particularly malabsorption)
2. Serum levels of ≥ 10 times the upper limit of normal (ULN) of IgA antibodies against type-2 (tissue) transglutaminase (TGA-IgA)
3. Positive endomysial antibodies (EMA-IgA) in a second serum sample
4. Positive coeliac human leukocyte antigen (HLA) risk alleles DQ2 and/or DQ8
5. Omitting duodenal biopsies should only be considered in patients/parents who understand the diagnosis and are committed to a gluten-free diet. The diagnosis and follow-up of CD should be made by a paediatric gastroenterologist or paediatrician with extensive knowledge of CD.

Although later published guidelines intended for adults (2,3) did not give the option for the no-biopsy approach, the 2012 ESPGHAN guidelines attracted considerable interest. Several recent prospective studies have favourably evaluated their performance (4,5) and on this basis, it is timely to update and expand the 2012 guidelines.

METHODS

Guideline Development Process

In 2016, ESPGHAN established a working group to develop an updated evidence-based clinical guideline for the diagnosis of CD. Ten focused clinical questions were formulated according to PICO format: Population, Indicator, Comparator, and Outcome. For each question, a bibliographic search was conducted; informative studies, systematically assessed for the risk of bias and clinical applicability, were included in the evidence base; meta-analysed study results were summarized and graded for certainty of evidence; and the implications for clinical practice were discussed and recommendations formulated and graded for strength.

The working group consisted of paediatric gastroenterologists, a GRADE methodologist (AH), biostatisticians and a member of the Association of European Coeliac Societies (AOECS). Smaller working groups focused on each clinical question and all questions were discussed jointly at 4 face-to-face meetings and 12 telephone conferences.

Address correspondence and reprint requests to Steffen Husby, MD, Odense University Hospital, dk-5000 Odense C, Denmark (e-mail: Steffen.Husby@rsyd.dk).

Steffen Husby, Sibylle Koletzko, Ilma Korponay-Szabó, Kalle Kurppa, M. Luisa Mearin, Carmen Ribes-Koninckx, Raanan Shamir, and Riccardo Troncone, contributed equally.

This article has been developed as a Journal CME and MOC Part II Activity by NASPGHAN. Visit <https://naspghan.org/professional-resources/continuing-education-resources/cme/> to view instructions, documentation, and the complete necessary steps to receive CME and MOC credits for reading this article.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are

Population, Indicator, Comparator, and Outcomes

The 10 questions addressed in this guideline reflect the 2016 NICE guidelines on CD (6), and the resulting recommendations are listed in Table 1. Histological analysis of duodenal biopsies was considered as the reference standard in diagnostic accuracy and the predefined outcomes of most interest were sensitivity, specificity, and positive/negative-predictive values (PPV/NPV).

Search for and Inclusion of Studies

Eligibility criteria: For each question, study characteristics (limited to children and adolescents whenever appropriate, setting, index test, reference standards, target conditions and study design) were specified.

In collaboration with an information specialist, the following databases were searched for eligible studies published from 2000 to 2016: Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, Database of Abstracts of Reviews of Effects, Health Technology Assessment Database, EMBASE (Ovid), MEDLINE (Ovid), and MEDLINE In-Process (Ovid).

Studies were screened and, for potentially eligible studies, full texts were assessed. The final choice of studies was agreed by subgroup discussion and consensus. Covidence (www.covidence.org) was used to organize the flow of references and studies.

Assessment of Risk of Bias and Clinical Applicability

All included studies were risk-assessed, using the QUADAS-2 tool (7) and data extraction and assessment was conducted by 2 independent reviewers.

Evidence Synthesis Process

Methods and results of studies were summarized according to the question posed. For questions 3 to 6, a meta-analysis for sensitivity and specificity and/or PPV was performed. For questions 1 and 2 and 7 to 10, results were summarized qualitatively (Table 1). A clear distinction was made between prospective and retrospective studies, and for diagnostic accuracy, between cross-sectional (cohort) studies and case-control studies. All figures and tables with "S" are supplementary materials, which are available online.

Diagnostic Accuracy Measures and Synthesis of Results

For a test to be useful at ruling out a disease, it must have high sensitivity and to be useful at confirming a disease it must have high

provided in the HTML text of this article on the journal's Web site (www.jpgn.org).

All guideline members' conflicts of interest have been noted and registered on the ESPGHAN website. The guideline was funded by ESPGHAN and was developed in collaboration with AOECS.

Disclaimer: ESPGHAN is not responsible for the practices of physicians and provides guidelines and position papers as indicators of best practice only. Diagnosis and treatment is at the discretion of physicians.

Copyright © 2019 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

DOI: 10.1097/MPG.0000000000002497

TABLE 1. Questions for the 2020 European Society Paediatric Gastroenterology, Hepatology and Nutrition criteria for the diagnosis of coeliac disease

Question	Text	Recommendation	Grading (strength)
1	Is there a difference in the prevalence of CD in children with constipation, abdominal pain, signs of irritable bowel syndrome (IBS), dyspepsia, malabsorption, iron deficiency anaemia, oral aphthae as compared with the general population?	We recommend considering testing for CD in children and adolescents with symptoms, signs and conditions shown in Table 2.	↑
2	What will HLA-DQ2 and DQ8 determination add to the diagnostic certainty of CD-diagnosis?	We recommend that HLA-DQ2 and DQ8 typing is not required in patients with positive TGA-IgA, if they qualify for CD diagnosis with biopsies or have high-serum TGA-IgA ($\geq 10 \times$ ULN) and EMA-IgA positivity. If a patient tests negative for HLA DQ2 and DQ8, the risk of CD is very low, whereas a positive result does not confirm the diagnosis	↑↑
3	How does the algorithm proposed to avoid biopsies in symptomatic patients work in asymptomatic subjects?	We give a conditional recommendation that, taking available evidence into account, CD can be diagnosed without duodenal biopsies in asymptomatic children, using the same criteria as in patients with symptoms. We recommend that the decision whether or not to perform diagnostic duodenal biopsies should be made during a shared decision-making process together with the parent(s) and, if appropriate, with the child	↑
4	Which serological test is the most appropriate to diagnose CD?	We recommend that in subjects with normal serum IgA values for age, TGA-IgA should be used as the initial serological test regardless of age	↑↑
5	Should more than 1 serological test be used and, if so, what should be the sequence of testing?	We recommend testing for total IgA and TGA-IgA as initial screening in children with suspected CD. In patients with low total IgA concentrations, an IgG-based test (DGP, EMA or TGA) should be performed as a second step. Testing for EMA, DGP or AGA antibodies (IgG and IgA) as initial screening in clinical practice is not recommended	↑↑
6	A diagnosis of CD may be safely done (positive-predictive value >95%) with omission of biopsy, at which cutoff for TGA-IgA (ULN $\times 10$, $\times 7$, $\times 5$)?	We recommend that for CD diagnosis without biopsies, TGA-IgA serum concentration of at least $10 \times$ ULN should be obligatory. Only antibody tests with proper calibrator curve-based calculation, and having the $10 \times$ ULN value within their measurement range, should be used. Omitting biopsies in IgA-deficient cases with positive IgG-based serological tests is not recommended	↑↑
7	Is endomysial antibody test (EMA) testing necessary in every case to diagnose CD with omission of biopsy?	We recommend that in children with TGA $\geq 10 \times$ ULN, and parents/patient agreement to the no-biopsy approach, the CD diagnosis should be confirmed by a positive EMA-IgA test in a second blood sample	↑↑
8	What is the inter- and intra-observer variability regarding CD diagnosis of histopathology results of duodenal and bulb biopsies? What degree of lesion is considered to be untreated CD? Do duodenal bulb biopsies increase the detection rate of CD? Is a reference pathologist needed in clinical practice?	At least 4 biopsies from the distal duodenum and at least 1 from the duodenal bulb should be taken for histology assessment during a gluten-containing diet. Reading of biopsies should be performed on optimally orientated biopsies. A villous to crypt ratio of < 2 indicates mucosal lesions. In cases of discordant results between TGA-results and histopathology, re-cutting of biopsies and/or second opinion from an experienced pathologist should be requested	↑↑
9	Does Marsh 0 or 1 (increased IEL counts only) compared with Marsh 0 have a different long-term outcome regarding diagnosis of CD in children with coeliac autoimmunity (positive TGA or EMA)?	We recommend before diagnosing potential CD to check the gluten content of the diet and the correct orientation of biopsies. Once confirmed, potential CD requires clinical and laboratory surveillance (serology, further biopsies) to monitor possible evolution to villous atrophy. For follow-up, it is important to refer the patient to tertiary care centres with expertise in CD	↑
10	How often are other clinically relevant diagnoses missed if upper (oesophageal-gastroduodenal) endoscopy is not performed in patients diagnosed by the no-biopsy approach?	We recommend that the decision to omit upper endoscopy with biopsies can be taken without the consideration of missing other pathologies or diagnoses	↑↑

Two arrows (↑↑) indicate a strong recommendation in favour, 1 arrow (↑) indicates a weak conditional recommendation; and similarly for strong and weak conditional recommendations against (↓↓ or ↓) as suggested by the GRADE Working Group (11).

Statements and recommendations from the 2012 Guidelines not investigated in the frame of these 10 questions remain in force (see supplementary file S 23, <http://links.lww.com/MPG/B719>). CD = coeliac disease; HLA = human leukocyte antigen.

specificity. As most seronegative patients do not get a biopsy, and thus true negatives are often missing, no study could provide valid data on sensitivity for case finding. Additionally, a high positive predictive value (PPV) was more appropriate for some questions, such as determining the TGA-IgA level for the no-biopsy approach.

For summarizing sensitivity and specificity for different groups, bivariate binomial meta-regressions were used together with investigation of statistically significant differences between the different groups [by following the method suggested by the Cochrane Diagnostic Test Accuracy Working Group (8)]. For each group, Youden index was calculated as $J = \text{sensitivity} + \text{specificity} - 1$ (9). A Forest Plot showing sensitivity and specificity was constructed for each group and summary receiver operating characteristics curves (ROC) were plotted for each group based on the models.

For selecting the optimal cut-off point for sensitivity and specificity, a multiple cut-offs model was used based on a restricted maximum likelihood (REML)-based multi-level random effects model (10). Computations were carried out in R version 3.3.3 (www.r-project.org/) using the packages “lme4,” “meta,” and “diagmeta” (<https://CRAN.R-project.org/package=diagmeta>), as well as Review Manager version 5.3.

Quality of the Evidence

GRADE was used to rate the overall quality of evidence for risk of bias, publication bias, imprecision, inconsistency, indirectness, and magnitude of effect (11). The GRADE ratings of very low-, low-, moderate-, or high-quality evidence reflect the extent of confidence that the diagnostic measures obtained are correct. Although the formal GRADE approach focused on a quantitative estimate (typically from a meta-analysis) whenever possible, similar principles were applied to assess the certainty of a qualitative summary, recognising the increased uncertainty in this procedure. Also, though standard outcomes of accuracy studies, such as sensitivity and specificity, can be regarded as surrogate outcomes, the correct diagnosis of CD is clearly linked to a well-established and effective intervention (gluten-free diet). Thus, studies were not downrated/downgraded for indirectness or lack of directly patient-relevant important outcomes.

Strength of Recommendations

The recommendation included a grading of strength, according to the GRADE approach and as suggested by the GRADE Working Group.

The implications of a strong recommendation for patients would be: “most parents and children in your situation would want the recommended diagnostic test and only a small proportion would not.” For clinicians, the implications would be that most patients should receive the recommended diagnostic test. For a conditional recommendation, clinicians should realise that different diagnostic tests will be appropriate for different children with suspected CD; that is, the clinician must help each suspected patient (and parents) to arrive at a decision consistent with their values and preferences.

Ethics and Regulations

All guideline members' conflicts of interest have been noted and registered on the ESPGHAN website. The guideline was funded by ESPGHAN and was developed in collaboration with AOECs.

RESULTS

Symptoms and Signs

Question 1: Is there a difference in the prevalence of CD in children with constipation, abdominal pain, signs of irritable bowel syndrome (IBS), dyspepsia, malabsorption, iron deficiency anaemia, or oral aphthae compared with the general population?

On the basis of results from the literature search (Fig. S1, Supplemental Digital Content, <http://links.lww.com/MPG/B719>), 13 relevant studies were selected and evaluated (Table S1, Table S11, Supplemental Digital Content, <http://links.lww.com/MPG/B719>).

Prospective Studies

Two studies addressed the issue of functional gastrointestinal disorders (FGID) but without healthy controls [$n = 78$ (12) and $n = 1047$ (13)]. They found a prevalence of CD in children with IBS of 4.4% and 2.2%, respectively; whilst functional abdominal pain and dyspepsia prevalence in CD ranged from 0.3% to 1%. In 101 children with functional constipation lasting >2 months (14), 4 cases had positive TGA-IgA, 3 were biopsied, and 1 had CD, resulting in a prevalence of 1%. In a large prospective birth cohort ($n = 6706$) with 3 monthly testing for TGA-IgA in serum symptoms were assessed by parental questionnaire without their knowledge of TGA-IgA results (15). At 3 and 4 years of age constipation and abdominal discomfort were more frequently reported in those with confirmed TGA-IgA positivity compared with age- and sex-matched participants remaining TGA-IgA-negative. In the ProCeDE study (4), stool consistency was prospectively assessed by the Bristol stool scale in 653 children and adolescents with newly diagnosed CD: 13% documented hard stool (type 1 or 2) compared with 17% reporting soft/liquid stool (type 6 or 7). Although there was no control group, the data indicate that constipation is almost as frequent as diarrhoea in children with CD. Chronic diarrhoea was investigated in 825 cases and 825 controls (16) with a 9% CD prevalence in the diarrhoea cases as compared with 0.6% in controls. In another study, 24 cases of CD (23%) were diagnosed in 103 children with chronic diarrhoea compared with 18 (19%) of 97 disease controls (17).

Oral aphthae were assessed in 50 CD cases and 50 controls and the prevalence was 62% and 13% respectively (18). Similarly, delayed dental eruption was observed in 38% and 11% and specific enamel defects in 48% and 0%, respectively. The prevalence of CD in Iranian teenagers and adults ($n = 247$, age range 13–40 years) reporting recurrent oral aphthae, was 2.8%, the youngest CD patient being 13 years old. This prevalence was significantly higher than the 0.9% found in the general population (19).

In 302 patients positive for antithyroid antibodies (age range 3.1–24.9 years), the prevalence of biopsy-confirmed CD disease was 2.3%. However, when patients with type 1 diabetes or Down syndrome were excluded, the prevalence decreased to 1.3% (20).

Iron deficiency anaemia (IDA) is a typical complication of malabsorption in CD. In the large ProCeDE cohort of children diagnosed based on symptoms, iron deficiency anaemia was reported in 17% (4). When CD children were identified by TGA-IgA screening in a large paediatric population-based cohort in Germany, no significant differences were found between 97 TGA-IgA seropositive children compared with 12,509 seronegatives; however, serum ferritin was significantly lower in seropositives indicating lower iron stores (21). When 135 iron deficient anaemia patients without gastrointestinal symptoms were screened,

6 cases (4.4%) had CD, in contrast to 0 cases 223 healthy asymptomatic children without anaemia from the same region (22).

Retrospective Studies

A chart review (23), including 165 paediatric CD patients, concluded that abdominal pain (in 52.7%) and constipation (in 38.9%) were the most frequent presenting features for CD. One additional case-control study, found a positive serology in 1.1% of abdominal pain cases and in 1.2% of controls (24). No duodenal biopsies were performed.

Statement

A broad spectrum of symptoms and signs have been reported in patients at the time of CD diagnosis. Classical symptoms of malabsorption seem to be more specific and include failure to thrive, weight loss, and chronic diarrhoea. For less specific symptoms, there is evidence that patients with diarrhoea-predominant IBS-like symptoms, iron deficiency anaemia, chronic constipation, and enamel defects have increased risk for CD. For other nonspecific gastrointestinal symptoms like abdominal pain, dyspepsia, and bloating, there is insufficient evidence.

Recommendation

We recommend considering testing for CD in children and adolescents with symptoms, signs and conditions shown in Table 2 [1].

TABLE 2. Symptoms and signs suggesting coeliac disease

Gastrointestinal	Chronic or intermittent diarrhea* Chronic constipation not responding to usual treatment Chronic abdominal pain Distended abdomen*
Extraintestinal symptoms	Recurrent nausea, recurrent vomiting Weight loss, failure-to-thrive*, stunted growth/ short stature* Delayed puberty, amenorrhoea Irritability, chronic fatigue Neuropathy Arthritis/arthralgia Chronic iron-deficiency anaemia Decreased bone mineralization (osteopenia/osteoporosis), repetitive fractures Recurrent aphthous stomatitis, Dermatitis herpetiformis-type rash Dental enamel defects Abnormal liver biochemistry
Specific conditions	First-degree relatives with CD Autoimmune conditions: T1DM, thyroid disease, liver disease Down syndrome, Turner syndrome Williams-Beuren syndrome IgA deficiency

CD = coeliac disease; T1DM = type-1 diabetes mellitus.

*Common symptoms.

Voting

Statement and Recommendation: Agree: 18 Disagree: 0 Abstain: 0

HLA Aspects

Question 2: What will HLA-DQ2 and DQ8 determination add to the diagnostic certainty of CD-diagnosis?

ESPGHAN 2012 recommendations for a no-biopsy approach included testing for HLA-DQ2 and DQ8 in individuals with very high TGA-IgA titres and EMA-IgA positivity, often described as a “triple test” (TGA/EMA and DQ) in several publications. This term, however, may imply that all 3 tests can be performed from 1 blood sample, which does not conform to the guidelines. The recommendation presumed that DQ typing added further accuracy to the diagnosis, given that this is unlikely among DQ2/DQ8 negative individuals. HLA testing, however, is not universally available and quite costly in some countries, and if it does not improve the no-biopsy diagnosis, it should be omitted. In an Australasian coeliac population (n = 356), 99.6% were DQ2/DQ8-positive (25) and the production of TGA-IgA and/or EMA was shown to be HLA-DQ-dependent. It may be concluded that the higher percentage of HLA DQ2/DQ8-negative CD patients (up to 5%) in earlier publications had several causes, with 1 of these being the HLA testing method. SNP-based tests are cheap to perform and recognize the common variants (DQ2.5, DQ8, DQ2.2, DQ7.5), whereas in depth allele typing is required to identify rare variant alleles. Therefore, the accuracy to exclude CD by HLA testing still depends on the method used. Other causes for so called “HLA DQ2/DQ8 negative CD patients” are the inclusion of a more heterogeneous group of patients with CD-compatible symptoms, and in some cases, false-positive histopathology considering an interobserver-variability of 5–7% regarding CD diagnosis (see Question 8 below).

A QUADAS-2 analysis was performed based on 8 papers (Table S2, Supplemental Digital Content, <http://links.lww.com/MPG/B719>) and the evidence for the value of HLA-testing as a criterion of the no-biopsy approach was graded (Table S12, Supplemental Digital Content, <http://links.lww.com/MPG/B719>).

Prospective Studies

Two prospective studies were of high quality in relation to this question (4,5). Werkstetter et al (4) analysed 645 paediatric patients with positive TGA-IgA and biopsy-proven CD where high TGA-IgA titres (>10× ULN), positive EMA and Marsh 2 or 3 lesions were found in 399 patients, compatible with the no-biopsy strategy. All 399 were positive for DQ2 and/or DQ8, and it was concluded that HLA typing did not add to the certainty of CD diagnosis in these patients. Wolf et al (5) reported 409 CD patients with TGA-IgA titres higher than 10× ULN, positive EMA and biopsy-proven CD. HLA testing was available in 227 and all typed positive for HLA-DQ2 or DQ8.

In another study (26), 82 CD patients had villous atrophy, 81 were positive for HLA-DQ2/DQ8 (98.8%) and for TGA and/or EMA. The single case negative for both genetics and serology tests was later found to have noncoeliac enteropathy.

In a Finnish study (27) including relatives of CD patients, all 114 with biopsy-proven CD and TGA-IgA and EMA-IgA positivity had a coeliac-type HLA. In a screening study in 12-year olds in Sweden (28), 153 children had biopsy-proven CD, and all were HLA-DQ2 and/or DQ8 positive.

Retrospective Studies

Of the three retrospective studies (29–31), 1 (29) reported 401 DQ2 and/or DQ8-positive (99%) patients among 405 with a TGA-IgA titre $\geq 10 \times$ ULN. One centre had previously reported all cases undergoing duodenal biopsy because of suspicion of CD (31). Of 150 with complete data, 116 were positive for TGA-IgA, EMA and HLA and were all diagnosed with CD. Four patients (2.7%) were initially considered to have neither DQ2 nor DQ8 heterodimers, but were heterozygous for *0202 HLA-DQB1 allele, so actually all carried a permissibility gene. In a further multicentre study, 368 of 749 CD cases were genotyped, with 98.1% positive for DQ2/DQ8 and 1.9% negative for those haplotypes (30).

Statement

HLA- typing does not add to the certainty of the diagnosis if the other criteria for CD diagnosis are fulfilled. Testing for HLA DQ2 and DQ8 may be useful in other circumstances. If no risk alleles are found, CD is unlikely.

Recommendation

We recommend that HLA typing is not required in patients with positive TGA-IgA, if they qualify for CD diagnosis with biopsies or if they have high serum TGA-IgA ($\geq 10 \times$ ULN) and EMA-IgA positivity. If a patient tests negative for HLA DQ2 and DQ8, the risk of CD is very low, while a positive result does not confirm the diagnosis [↑↑].

Voting

Statement: agree: 17	Disagree: 0	Abstain: 1
Recommendation: agree: 15	Disagree: 2	Abstain: 1

Antibodies

Question 3: Does the algorithm proposed to avoid biopsies in symptomatic patients work in asymptomatic subjects?

Eleven articles were considered suitable and underwent QUADAS-2 analysis (Table S3 and S13, Supplemental Digital Content,

<http://links.lww.com/MPG/B719>). Even in these selected articles, however, limitations were present, as different populations with diverse study designs, reference standards and varying sample size and assays applied. In addition to the data presented in the publications, the original data of the asymptomatic children were included from 4 of 6 prospective studies (4,5,32,33). The majority of asymptomatic individuals were screened as they belonged to at-risk groups. Three of the 6 prospective studies concerned patients suspected for CD (4,5,34), whereas the other 3 were birth cohorts with genetic susceptibility for CD (HLA-DQ2/DQ8 positive) (32,33,35). The 2 cross-sectional studies were mass-screening studies in the general population, not seeking medical attention for any complaint or risk (36,37). The 3 retrospective studies included patients who were at risk of CD (29,38,39). As studies with a large number of CD cases with coexisting type 1 diabetes have not been included in our literature search, this specific group of patients has not been addressed in this question.

In an analysis of data from 555 asymptomatic children with TGA-IgA titres $\geq 10 \times$ ULN (Table S21, <http://links.lww.com/MPG/B719>), 552 had diagnostic small bowel biopsies, with 520 (94.2%) having Marsh class 2 or 3 duodenal lesion. The Forest plot (Fig. 1) shows a considerable variation with PPV from as low as 0.69, therefore, pooling of results is statistically not appropriate. The 3 studies with the smallest sample size had the lowest PPV, whereas the rest had values above 0.90, but with 95% confidence intervals including values down to 0.79. The outcome is further described in the supplementary material (S21, Supplemental Digital Content, <http://links.lww.com/MPG/B719>).

Statement

Recent studies suggest that the no-biopsy approach to diagnose CD can be applied in asymptomatic children. In asymptomatic children, however, the PPV of high TGA-IgA $\geq 10 \times$ ULN may be lower than in symptomatic children, which needs to be considered during the decision-making process.

Recommendation

We give a conditional recommendation that, taking available evidence into account, CD can be diagnosed without duodenal biopsies in asymptomatic children, using the same criteria as in patients with symptoms. We recommend that the decision whether or not to perform diagnostic duodenal biopsies should be made during a shared decision-making process together with the parent(s) and, if appropriate, with the child [↑].

Study	TP	TP + FP	PPV (95% CIs)
Nevoral 2013	103	114	0.90 [0.83; 0.95]
Lionetti 2014	21	24	0.88 [0.68; 0.97]
Vriezinga 2014	27	27	1.00 [0.87; 1.00]
Trovato 2015	37	40	0.92 [0.80; 0.98]
Webb 2015	63	64	0.98 [0.92; 1.00]
Cilleruelo 2016	9	13	0.69 [0.39; 0.91]
Donat 2016	65	69	0.94 [0.86; 0.98]
Jansen 2017	16	19	0.84 [0.60; 0.97]
Paul 2017	84	84	1.00 [0.96; 1.00]
Werkstetter 2017	50	51	0.98 [0.90; 1.00]
Wolf 2017	45	47	0.96 [0.85; 0.99]

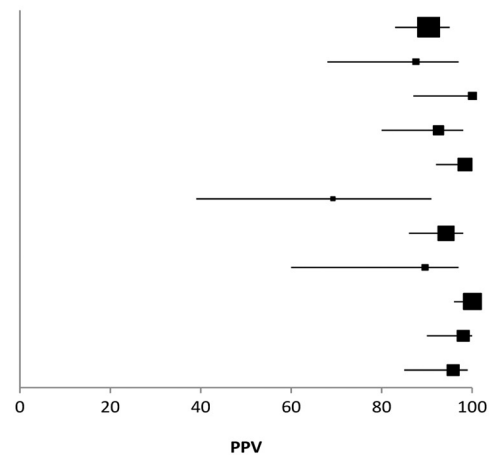


FIGURE 1. Forest plot for positive-predictive values for Question 3.

Downloaded from http://journals.lww.com/jpgn by BMDMSepHKav1zEoun1tQFN4a+kLhEZgbsHh4XMI0hCwCX1AW on 09/04/2022

Voting

Statement:	Agree: 16	Disagree: 2	Abstain: 0
Recommendation:	Agree: 14	Disagree: 3	Abstain: 1

Question 4: Which serological test is the most appropriate to diagnose CD?

Eighteen articles were selected for a detailed QUADAS-2 analysis (Table S4, Supplemental Digital Content, <http://links.lww.com/MPG/B719>), along with 5 prospective and 13 retrospective studies. Three tests were thoroughly evaluated: TGA-IgA, DGP-IgG, and EMA-IgA.

Prospective Studies

The prospective studies (5,40–43) were in general large ones with a low risk of bias and of high quality (Table S14, Supplemental Digital Content, <http://links.lww.com/MPG/B719>). The largest study (5) found EMA-IgA to have excellent accuracy. Three out of 5 recent articles showed surprisingly low specificity for EMA-IgA (41,42,44), with 2 of them coming from the same centre. As the 3 articles provided insufficient information about the technical aspects of serology and histology assessment with an allowed time gap up to 6 months between serology and biopsies (allowing time for the effect of gluten-restriction before biopsies), it was not possible to decipher the reasons for the discrepant results.

Retrospective Studies

The retrospective studies had higher degrees of bias, in particular as to patient selection, and were judged to be of lower quality. Overall, the Forest plot for TGA-IgA, DGP-IgG, and EMA-IgA (Fig. 2) showed considerable heterogeneity.

A bivariate binomial meta-regression meta-analysis disclosed similar accuracies for the three antibody species (Fig. S2, Supplemental Digital Content, <http://links.lww.com/MPG/B719>) that showed overall significant differences between the tests for both sensitivity ($P=0.005$) and specificity ($P=0.016$), with summary ROC curves showing summary points and 95% confidence intervals for TGA-IgA, DGP-IgG, and EMA (Fig. S2, Supplemental Digital Content, <http://links.lww.com/MPG/B719>). The highest value obtained was for EMA that had the highest sensitivity but TG2-IgA had the highest specificity and Youden J statistic.

Statement and Recommendation

The three specific coeliac antibodies (TGA-IgA, EMA-IgA, DGP-IgG) show different performance. TGA-IgA scored highest by a comparison of assay accuracy and is therefore regarded as the most appropriate primary test for CD in the diagnostic work up of children with suspected CD. We recommend that in subjects with normal serum IgA values for age, TGA-IgA should be used as the initial test regardless of age [↑↑].

Voting

Statement:	Agree: 17	Disagree: 0	Abstain: 1
Recommendation:	Agree: 17	Disagree: 0	Abstain: 1

Question 5: Should more than one serological test be used and, if so, what should be the sequence of testing?

We searched the literature to find whether any combination of tests (either 2 separate tests or a blended test kit for both IgA and IgG detection) is better for initial testing than TGA-IgA plus total IgA. Evidence from studies restricted to young children below 2 or 3 years of age was downgraded, if the diagnosis of CD in cases with negative autoantibodies was not confirmed by a gluten challenge, as recommended in the current guidelines. Of 107 studies identified, 10 were of sufficient quality to be considered for the final analysis (Table S5 and Table S15, Supplemental Digital Content, <http://links.lww.com/MPG/B719>), and further described in more detail (supplementary material S22, Supplemental Digital Content, <http://links.lww.com/MPG/B719>).

Prospective Studies

TGA-IgA plus DGP-IgG With or Without DGP-IgA

The only unbiased prospective study was performed in adults (45), in which 2297 unselected adults were screened with TGA-IgA, DGP-IgG, and DGP-IgA. A total of 56 were positive on at least 1 antibody test and duodenal biopsies were performed in 40. Of 8 biopsy-confirmed CD cases, 7 were positive for TGA-IgA, 5 for DGP-IgG and 5 for DGP-IgA, with 4 positive in all 3 tests. False-positive results were found in 2 for TGA-IgA, in 5 for DGP-IgG, and in 28 for DGP-IgA. In order to find the only CD-case with negative TGA-IgA, almost 2300 tests for DGP-IgG had to be performed plus 4 unneeded endoscopies. Wolf et al (5) prospectively included children below 18 years of age, referred because of either a positive serology for CD and/or symptoms. A total of 898 children were centrally tested for total IgA, TGA-IgA, DGP-IgG, and EMA-IgA. When TGA-IgA plus total IgA was compared with TGA-IgA and DGP-IgG (TGA-DGP procedure) for initial testing, 592 were diagnosed with CD, 245 as no CD, and 24 had no final diagnosis. The TGA-DGP procedure detected 6 additional CD patients, 5 of which were also negative for EMA IgA, whereas the remaining child was positive for EMA-IgG and TGA-IgG. The TGA-DGP compared with the TG2-IgA procedure resulted in 16 unnecessary endoscopies (negative TGA-IgA but false positive DGP-IgG).

DGP-IgA With Total IgA and AGA-IgA

Vriezinga et al (32) reported a European multi-centre placebo-controlled intervention trial in infants at genetic risk for CD (all HLA DQ2 or DQ8 positive). Participants were regularly tested from age 4 months for TGA-IgA and AGA-IgA, and with an IgG-based test in case of low total IgA. Biopsies were offered to those with persistent positive TGA-IgA levels, high or increasing AGA-IgA, and symptoms strongly suggesting CD regardless of serology results. All IgA-competent children with biopsy-proven CD were positive for TGA-IgA, whilst all 17 TGA-IgA negative children biopsied, based on symptoms or AGA-IgA positive results, had either a normal mucosa or a transient enteropathy. Transient positivity of AGA-IgA occurred in a third of infants randomized to early gluten exposure and was not predictive for later CD.

Retrospective Studies

TGA-IgA and DGP-IgG With or Without DGP-IgA

Of 5 retrospective studies, 4 were performed in young children only (42,46–48), whilst 1 included children and adults (49). A further description is included in the supplementary material (S22, Supplemental Digital Content, <http://links.lww.com/MPG/B719>). These studies, however, have major limitations:

the biopsy rate of patients with a positive test result in either TGA-IgA or DPG was low, there was no reference pathologist and no gluten challenge in children below 2 years of age with villous atrophy but negative autoantibodies. In summary, these studies do not support to add DGP to TGA-IgA testing for initial screening.

Marsh 1 in 9 (4.5%), villous atrophy because of other diagnosis than CD in 3 (1.5%) and the remaining 6 patients (3%) having histopathology as CD. The authors concluded that TGA-IgG did not add to the accuracy.

TGA-IgA and TGA-IgG

Of 2911 persons (age range 1–80 years) with a positive coeliac serology during a 17-year period, 233 individuals with an isolated positivity for TGA-IgG were identified (50). Biopsies were performed in 178/233 (78%), with a normal histology in 160 (90%),

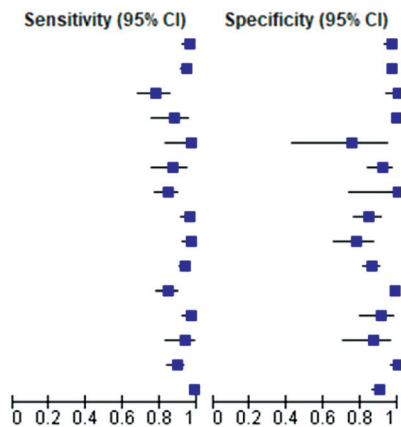
TGA-IgA With Total IgA and AGA-IgA

One study (46) evaluated whether AGA-IgA testing in addition to TGA-IgA testing improves case finding in children below 2 years of age. Of 4122 children tested, 312 (8%) where TGA-IgA or EMA positive, whilst 85 were only AGA-IgA positive. Clinical data was available in 62 and duodenal biopsy results in 33 of them, leading to CD in 5 children. The remaining 57 children

A Analyses

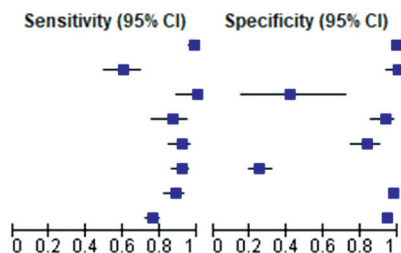
TGA-IgA

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Aita 2013 a	150	5	6	166	0.96 [0.92, 0.99]	0.97 [0.93, 0.99]
Basso 2011 a	311	11	18	363	0.95 [0.91, 0.97]	0.97 [0.95, 0.99]
Brusca 2011 a	74	0	21	55	0.78 [0.68, 0.86]	1.00 [0.94, 1.00]
Dahlbom 2013 a	44	1	6	191	0.88 [0.76, 0.95]	0.99 [0.97, 1.00]
Hojesak 2012 a	30	3	1	9	0.97 [0.83, 1.00]	0.75 [0.43, 0.95]
Jaskowski 2010 a	48	6	7	70	0.87 [0.76, 0.95]	0.92 [0.84, 0.97]
Klapp 2013	116	0	22	12	0.84 [0.77, 0.90]	1.00 [0.74, 1.00]
Mubarak 2011 a	105	16	4	87	0.96 [0.91, 0.99]	0.84 [0.76, 0.91]
Mubarak 2012 a	116	14	4	49	0.97 [0.92, 0.99]	0.78 [0.66, 0.87]
Olen 2012 a	259	36	17	224	0.94 [0.90, 0.96]	0.86 [0.81, 0.90]
Oyert 2015 c	132	13	24	962	0.85 [0.78, 0.90]	0.99 [0.98, 0.99]
Panetta 2011 a	104	4	3	42	0.97 [0.92, 0.99]	0.91 [0.79, 0.98]
Parizade 2009 a	45	4	3	27	0.94 [0.83, 0.99]	0.87 [0.70, 0.96]
Teesalu 2009 b	154	0	19	97	0.89 [0.83, 0.93]	1.00 [0.96, 1.00]
Wolf 2017 a	549	36	9	324	0.98 [0.97, 0.99]	0.90 [0.86, 0.93]



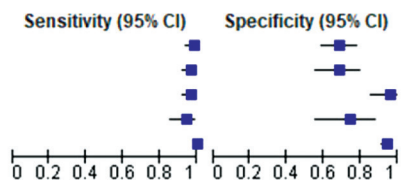
DGP-IgG

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Aita 2013 b	154	1	2	170	0.99 [0.95, 1.00]	0.99 [0.97, 1.00]
Brusca 2011 b	57	0	38	55	0.60 [0.49, 0.70]	1.00 [0.94, 1.00]
Hojesak 2012 b	31	7	0	5	1.00 [0.89, 1.00]	0.42 [0.15, 0.72]
Jaskowski 2010 b	48	5	7	72	0.87 [0.76, 0.95]	0.94 [0.85, 0.98]
Mubarak 2011 b	100	17	9	86	0.92 [0.85, 0.96]	0.83 [0.75, 0.90]
Olen 2012 b	172	164	16	56	0.91 [0.87, 0.95]	0.25 [0.20, 0.32]
Oyert 2015 d	138	19	18	956	0.88 [0.82, 0.93]	0.98 [0.97, 0.99]
Wolf 2017 b	417	20	133	353	0.76 [0.72, 0.79]	0.95 [0.92, 0.97]



EMA

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Mubarak 2011 c	107	32	2	71	0.98 [0.94, 1.00]	0.69 [0.59, 0.78]
Mubarak 2012 b	116	20	4	43	0.97 [0.92, 0.99]	0.68 [0.55, 0.79]
Panetta 2011 b	105	2	3	45	0.97 [0.92, 0.99]	0.96 [0.85, 0.99]
Parizade 2009 c	54	8	3	23	0.95 [0.85, 0.99]	0.74 [0.55, 0.88]
Wolf 2017 c	502	20	1	325	1.00 [0.99, 1.00]	0.94 [0.91, 0.96]



B

	Sensitivity	Specificity	Youden's J statistic
TGA	0.936 (0.904 0.958)	0.957 (0.912 0.979)	0.893
DGP	0.907 (0.802 0.959)	0.929 (0.708 0.986)	0.836
EMA	0.983 (0.959 0.993)	0.827 (0.681 0.915)	0.810

FIGURE 2. (A) Forest plots for sensitivity and specificity for Question 4. Determination of TG2-IgA, DGP-IgG and EMA-IgA as primary analysis. (B) Meta-analysis for Question 4. Meta-regressions showed that there is statistical evidence (chi-square = 10.4, $P = 0.005$) that the expected sensitivity differs between the assays, as well as statistical evidence (chi-square = 8.3, $P = 0.016$) that the expected specificity differs between the assays.

with isolated AGA positivity, including 4 with villous lesions, received a diagnosis other than CD.

Statement

Current evidence indicates that adding DGP-IgG, DGP-IgA, or AGA-IgA testing to TGA-IgA testing seldom improves sensitivity after excluding patients with low total IgA. Specificity markedly decreases, especially in children below 4 years of age, in which isolated DGP or AGA positivity is a common transient phenomenon.

Recommendation

We recommend testing for total IgA and TGA-IgA as initial screening in children with suspected CD. In patients with low total IgA concentrations, an IgG-based test (DGP, EMA, or TGA) should be performed as a second step. Testing for EMA, DGP or AGA antibodies (IgG and IgA) as initial screening in clinical practice is not recommended [↑↑].

Voting

Statement and recommendation: Agree: 18 Disagree: 0 Abstain: 0

Question 6: At which cut-off for TGA-IgA (ULN 10×, 7×, 5×) may a diagnosis of CD safely be done (positive predictive value > 95%) with omission of biopsies?

Higher serum levels of TGA-IgA are strongly associated with higher degree of villous atrophy if TGA-IgA is measured by a calibration curve-based immunoassay. ESPGHAN 2012 guidelines (1) suggested that the no-biopsy approach can be considered when TGA-IgA values $\geq 10\times$ ULN but this guideline evaluates the evidence for $\geq 10\times$ ULN and possibly lower cut-offs to predict Marsh 2 to 3 lesions and CD.

A search yielded 44 studies where PPV of high TGA-IgA levels were compared with the histological outcome. Of these, only 36 utilised antibody tests suitable for calculating multiples of ULN. After narrowing the scope to those paediatric studies, where exact numbers of true positives and false positives could be extracted, 19 retrospective (29,31,38–40,42,43,46,51–60,94) and 3 prospective studies (4,5,41) remained for QUADAS-2 and further analysis (Table S6 and Table S16, Supplemental Digital Content, <http://links.lww.com/MPG/B719>). These 49 datasets constituted 9 conventional ELISA assays (Biosystems, DiaSorin, Euroimmun, Eurospital, Immco, Inova, Orgentec, Phadia, R-Biopharm), 2 fluorescent immunoassays (Phadia), and 2 chemiluminescence tests (Immulate, Inova).

From the 30 datasets evaluating the TGA-IgA cut-off at $\geq 10\times$ ULN, 28 reported >95% PPV and 21 reported >99% PPV (Fig. 3). The PPVs were higher in studies where both Marsh 2 and 3 were accepted as CD (all >97% PPV) compared with studies, which required strictly Marsh 3 for CD diagnosis. At cut-off levels 5 to 7.5× ULN PPV values varied between 92.3% and 100%, still 4/7 datasets showing PPV >99%. At cut-off levels 2 to 4× ULN PPV values varied between 86 and 100%, again with 4/7 datasets still showing PPV >99% (Fig. 3). The study of Werkstetter et al (4), included 8 TGA-IgA assays in the central head-to-head analysis, which showed a PPV of 99% even at lower cut-offs than 10× ULN (presented only as graphs), but the same study also demonstrated high inter-test and inter-laboratory variability at these lower ranges. Notably, in local laboratories, a PPV >99% was only reached at 10× ULN. At the cut-off levels between 3 and 10× ULN, the test

kit and the diagnostic approach (Marsh 3 only or Marsh 2–3 as CD) influenced the clinical outcome. In most retrospective studies, no reference pathologists were involved and the histology evaluation was not blinded. Interestingly, all three prospective studies providing a blinded reference pathologist yielded excellent PPV values (100%, 99.1%, and 98.9%) suggesting that high TGA IgA levels strongly support the CD diagnosis and discrepant results occur more likely because of technical difficulties with the histology.

TGA-IgG cut-off levels reliably predicting CD in IgA-deficient persons could not be derived from the literature. Therefore, the 10× ULN cut-off is not validated for TGA-IgG. Differences in calibrators and slow IgG antibody kinetics warrant special caution with IgA-deficient subjects where levels of EMA and TGA-IgG may remain high for several years after starting a gluten-free diet (61).

Statement

High serum TGA-IgA levels $\geq 10\times$ ULN predict enteropathy (Marsh 2/3) and should be used as a criterion for CD diagnosis without biopsies. Due to inter-laboratory and inter-test variability, the reliability of positive TGA-IgA levels $< 10\times$ ULN and that of TGA-IgG are prone to technical error and not sufficient for the no-biopsy approach.

Recommendation

We recommend that for CD diagnosis without biopsies, TGA-IgA serum concentration of at least 10× ULN should be obligatory. Only antibody tests with proper calibrator curve-based calculation, and having the 10× ULN value within their measurement range, should be used. We recommend against omitting biopsies in IgA-deficient cases with positive IgG-based serological tests [↑↑].

Voting

Statement: Agree: 17 Disagree: 1 Abstain: 0
Recommendation: Agree: 17 Disagree: 1 Abstain: 0

Question 7: Is endomysial antibody (EMA-IgA) testing necessary in every case to diagnose CD without biopsy?

The recommendations for a no-biopsy approach in patients with high TGA-IgA levels rests on a second serum sample taken for EMA-IgA on a separate occasion on a gluten-containing diet (62). This consideration aims at avoiding mislabelling of samples or technical errors and confirming coeliac auto-immunity using another test assay with high specificity.

EMAs are directed against the transglutaminase 2 (TG2) antigen present in the anatomical endomysium in a tissue section and the EMA test is based on indirect immunofluorescence performed on primate oesophageal or human umbilical cord substrate. The test is considered positive if a serum dilution of 1:5 or higher gives a visible binding pattern. The EMA-IgA test performance, however, depends on a subjective interpretation of the results, which may be critical at low titres. The inter-lab variability of EMA titers is highly depending on lab condition. In addition, the test is more time-consuming and expensive than measurement of TGA-IgA. Ten studies were identified for QUADAS2 analysis (Table S7, Supplemental Digital Content, <http://links.lww.com/MPG/B719>) and further evaluation (Table S17, Supplemental Digital Content, <http://links.lww.com/MPG/B719>).

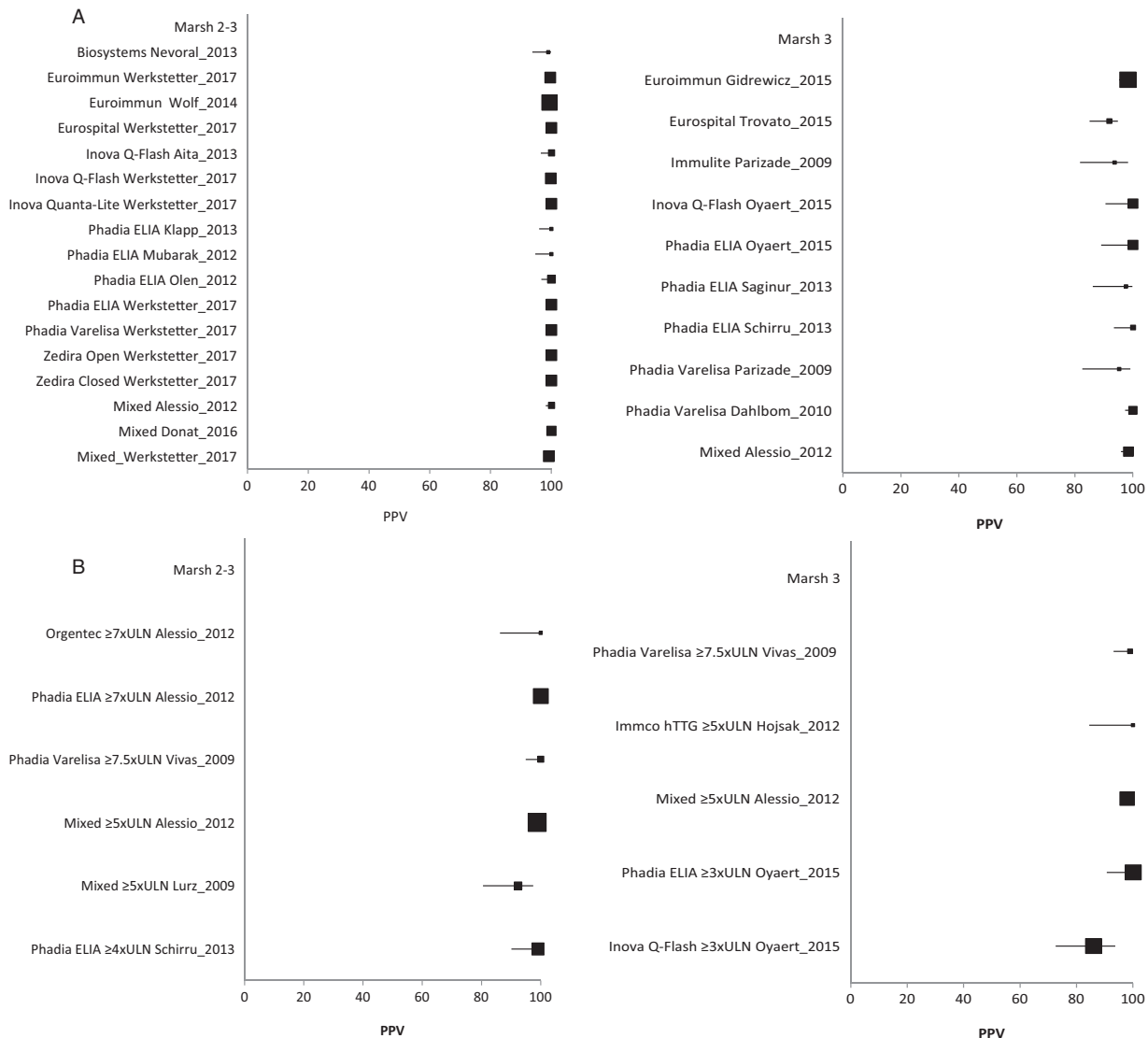


FIGURE 3. (A) Question 6: positive predictive value of $\geq 10 \times$ ULN TGA-IgA serum concentrations for coeliac disease. (B) Question 6: positive predictive values of at least 3 to $7.5 \times$ ULN TGA-IgA serum concentrations for coeliac disease.

Prospective Studies

In three prospective studies, a total of 1788 symptomatic children were included of whom 1357 had a final diagnosis of CD (4,5,41) (Table S17, Supplemental Digital Content, <http://links.lww.com/MPG/B719>). In total, 895 out of 1357 had TGA-IgA levels of $\geq 10 \times$ ULN qualifying for the no-biopsy approach, 4 of which had a negative EMA; 1 of these 4 had a final diagnosis of no CD and 3 remained unclear. Thus, 1 to 4 of 895 patients qualifying for the no-biopsy approach (symptoms with TGA-IgA $\geq 10 \times$ ULN) need to be tested with EMA-IgA to find 1 case with a final diagnosis of no CD. This yields a “number needed to test” of 224 to 895 to identify a non-CD case among those with TGA-IgA $\geq 10 \times$ ULN.

In the study by Wolf, 5 patients considered not to have CD (n = 2) or unclear diagnosis (n = 3) out of 405 patients with TGA-IgA $\geq 10 \times$ ULN also had a positive EMA-IgA (6). Four out of 5 had $< 10 \times$ ULN for TGA-IgA at the first sample assayed locally, suggesting transient high levels and a need for 2 separate samples

to apply the no-biopsy criteria, or alternatively uncertain histology. In the study by Werkstetter (4), 4 cases regarded as possible false positives also had TGA-IgA $< 10 \times$ ULN or were negative in the second sample.

The studies have not formally assessed whether a second TGA-IgA test could serve as an alternative approach to cater for the possibility of transient increases or technical errors.

Retrospective Studies

Seven retrospective studies did not report symptoms ((31,38,43,44,56,63,64). Only 1 of these presented stratified tables in categories of TGA-IgA levels and with EMA-IgA for the group with TGA-IgA $\geq 10 \times$ ULN. Two of the papers presented data suitable to answer the question. In total, 4 out of 565 individuals with TGA-IgA $\geq 10 \times$ ULN were considered as false positives for a diagnosis of CD based on biopsy. These appeared in the same study and all had a negative EMA-IgA. The authors reported that in 3 of those children, the TGA-IgA result was normal after 2–5 months

Downloaded from http://journals.lww.com/jpgn by BNDMf5epHkav1zEoun1tQfN4a+kULhEZgbsHh04XMI0hCwCXT1AW nYQp/1Q/H/D/3/3D00OdRvY7/1V/S/F/4/C/3V/C4/OAV/pDDa8KKGKvY07my+78= on 09/04/2022

whilst still on a gluten-containing diet. Thus, these cases could be due to a transient antibody increase, sample mixing or technical errors. The numbers needed to test with EMA-IgA to avoid a false positive diagnosis was 141.

Statement

Although high TGA-IgA ($\geq 10 \times \text{ULN}$) results are rare in children with normal histopathology, a positive EMA-IgA result will further decrease the rate of false positive results.

Recommendation

We recommend that in children with TGA $\geq 10 \times \text{ULN}$, and parents/patient agreement to the no-biopsy approach, the CD diagnosis should be confirmed by a positive EMA-IgA test in a second blood sample [↑↑].

Voting

Statement and Recommendation: Agree: 18 Disagree: 0 Abstain: 0

Biopsy

Question 8: What is the inter- and intra-observer variability regarding CD diagnosis of histopathology results of duodenal and bulb biopsies? What degree of lesion is considered to be untreated CD? Do duodenal bulb biopsies increase the detection rate of CD? Is a reference pathologist needed in clinical practice?

Currently, the histological lesions in CD are graded using grouped classifications, mostly based on Marsh-Oberhuber (65,66) and literature shows unsatisfactory inter-observer agreement between evaluators (67–69). The use of validated standard operating procedures (SOPs) with correct orientation and cutting of the duodenal specimen is considered critical for an accurate interpretation of the mucosal architectural changes (69–71). Villus height-crypt depth ratio of less than 2 in some parts of at least 1 duodenal biopsy is considered to be in agreement with CD. Marsh-Oberhuber grading can only be given with proper tissue orientation, as is the case for villus height crypt depth morphometry. In a recent inter-observer agreement study in paediatric patients, only approximately half of the biopsies were considered optimally oriented and satisfactory results were obtained with respect to CD with a Kappa value of 0.84. When specific Marsh-Oberhuber gradings were, however, compared by different evaluators, poor agreement in grading the injury was observed. In the study by Werkstetter et al (4), there was disagreement between the local and the central pathologist regarding the diagnoses of no-CD (Marsh 0 or 1) or CD (Marsh 2 or 3a–c) in 7%, whereas discordant judgement considering all classes (Marsh 0, 1, 2, 3a, 3b, or 3c) was reported in 58%. Some pathologists tended to give a suggestive or clear diagnosis, even in cases with very poor quality of biopsies, instead of requesting adequate samples (4).

The traditional histological evaluation of CD has undergone marked changes in recent years, as bulb biopsies have been recommended (1). These new recommendations came as a consequence of reported cases showing histologic lesions only in the duodenal bulb. The literature search identified 3 relevant paediatric studies for the inter-observer agreement of the histopathology results and 13 studies relevant for the duodenal bulb histopathology

evaluation in children, 3 of them of high quality (67,72) (Table S8, Table S18, Supplemental Digital Content, <http://links.lww.com/MPG/B719>). A recent finding from a large multi-centre study confirms that sometimes the mucosal injury is found only in the bulb (4). Some studies have, however, questioned the added value of intestinal bulb biopsies in improving CD diagnosis, especially in children (73–75).

Statement

The inter-observer variability of the grading of small-bowel histopathology lesions is high, indicating that histopathology cannot serve as the sole reference standard. A higher detection rate for CD may be achieved with more duodenal samples, including at least 1 from the bulb. Histopathology reading can be improved by validated standard operating procedures (SOPs). Biopsies of low quality or lacking correct orientation are not suitable for CD diagnosis.

Recommendation

At least 4 biopsies from the distal duodenum and at least 1 from the duodenal bulb should be taken for histology assessment during a gluten-containing diet. Reading of biopsies should be performed on optimally orientated biopsies. A villous to crypt ratio of < 2 indicates mucosal lesions. In cases of discordant results between TGA-IgA results and histopathology, re-cutting of biopsies and/or second opinion from an experienced pathologist should be requested [↑↑].

Voting

Statement and Recommendation: Agree: 18 Disagree: 0 Abstain: 0

Question 9: Does Marsh 1 (increased IEL counts only) compared to Marsh 0 have a different long-term outcome regarding diagnosis of CD in children with coeliac autoimmunity (positive TGA or EMA)?

The Marsh classification is based on stages identifiable during mucosal remodelling (76). Marsh 1 lesions are in most cases not associated with TGA-IgA or EMA autoimmunity and in these cases not related to CD. If Marsh 1 lesions are found in seropositive persons (“potential CD”), particularly in those with moderately high titres of TGA-IgA, the question arises whether this is sufficient to diagnose CD. Six articles were identified as being suitable for QUADAS-2 analysis (Table S9, Supplemental Digital Content, <http://links.lww.com/MPG/B719>) and for further GRADE evaluation (Table S19, Supplemental Digital Content, <http://links.lww.com/MPG/B719>).

In 18 out of 20 subjects with potential CD, a higher than normal number of γ/δ IELs were found versus 11 of 13 active CD patients and 20 out of 42 controls (77). In the end 38%, potential CD patients were classified as CD (55% of those Marsh 1 and 14% of those Marsh 0) on the basis of a discriminating equation taking into account CD3 IELs, γ/δ IELs, and lamina propria CD25+ cells (78). Presence of TGA-IgA in the mucosa is found by immunofluorescence in the majority of patients with potential CD [see Tables S9 and S19, Supplemental Digital Content, <http://links.lww.com/MPG/B719>, and (79,80)].

In children, evolution of potential CD to CD has been reported to occur in 33% (81) to 100% of cases. Other possible outcomes are persistent seropositivity in the presence of normal

mucosa, fluctuation or permanent seroconversion to negative auto-antibodies (81). There are no specific studies addressing the outcome of Marsh 0 versus Marsh 1 histology in biopsies but the increase of γ/δ IELs contribute to a discriminating equation predicting the evolution to villous atrophy.

Children with potential CD may already present with symptoms (82) and or signs, like iron deficient anaemia (83). Symptomatic patients range from 27% (81) to 100% (84). The rate of responders to gluten free diet (GFD) is variable from 54% (85) to 100% (84), although a placebo effect for subjective symptoms cannot be excluded. Depending on the severity of symptoms and after exclusion of other causing diseases, a GFD may be recommended for a symptomatic child, based upon a decision shared with the parents. Care must be given to follow-up with clinical evaluation for improvement and serological testing.

Statement

The term potential CD identifies subjects with positive TGA-IgA and EMA and no or minor small bowel histological changes. Reasons for this situation, however, may also be low gluten intake before biopsies, sampling error or incorrect orientation of the biopsies for reading, leading to misdiagnosis of potential instead of true CD. Marsh 1 is not considered sufficient to diagnose CD but some observations suggest that potential CD cases with Marsh 1 small bowel lesions have a higher chance to evolve to villous atrophy in comparison to Marsh 0.

Recommendation

We recommend before diagnosing potential CD to check the gluten content of the diet and the correct orientation of biopsies. Once confirmed, potential CD requires clinical and laboratory surveillance (serology, further biopsies) to monitor possible evolution to villous atrophy. For follow-up, it is important to refer the patient to tertiary care centres with expertise in CD [↑].

Voting

Statement and recommendation: Agree: 18 Disagree: 0 Abstain: 0

Question 10: How often are other clinically relevant diagnoses missed if upper (oesophageal-gastro-duodenal) endoscopy is not performed in patients diagnosed by the non-biopsy approach?

When CD is diagnosed by endoscopy, other conditions may be detected, which may remain undetected in children diagnosed without biopsies. Concern has been expressed that these conditions will be missed in children diagnosed with CD based on the nonbiopsy approach (86). These may be coincidental findings, occurring with similar prevalence in individuals with and without suspected CD. Alternatively, other conditions detected could be truly associated and occur more frequently in individuals with CD but may resolve with a GFD (87). Ideally, in order to assess whether the risk of overlooking other conditions justifies routine endoscopy, the prevalence of these conditions should be known in individuals without suspicion of CD. It may in general be said that CD patients should be monitored while on a GFD to ensure that no additional GI issues might have been missed.

Of the 6 relevant studies (Table S10, Supplemental Digital Content, <http://links.lww.com/MPG/B719>), 5 were retrospective. In the retrospective studies, biopsies from the oesophagus and gastric mucosa were not taken routinely, making the findings difficult to

interpret and prone to selection bias. No serology results were reported in the retrospective studies and a no-biopsy approach could not be determined (Table S20, Supplemental Digital Content, <http://links.lww.com/MPG/B719>).

Macroscopic Peptic Mucosal Lesions and *Helicobacter pylori* Infections

In a mixed paediatric and adult cohort of 240 patients with biopsy-proven CD, peptic lesions in the stomach or duodenum were found in 12%. No control group was reported. In another retrospective study, abnormal findings were reported in 11 of 115 paediatric patients (86). One prospective study systematically assessed macroscopic findings and *H pylori* status during upper endoscopy at the time of CD diagnosis in children (n = 653) (4). *H pylori* infection was searched for in 442 patients with only 21 (4.5%) found positive. This figure is very low considering that children were recruited also from high *H pylori*-prevalent countries like Iran, Russia, Israel, and from Eastern Europe and suggest a negative association between CD and *H pylori* infection. In the total cohort (n = 653), erosions were found in the oesophagus in 24 (4%), in the stomach in 21 (3%), and in the duodenum in 43 (6%) children including 2 with shallow ulcers. Only 3 (4.7%) of 64 children with gastroduodenal lesions were *H pylori*-positive, an infection rate equal to the total cohort. Duodenitis including shallow ulcerations in the absence of *H pylori* in CD children has been reported (88) and may indicate a higher vulnerability to gastric secretion of the inflamed mucosa in CD. Whether the rate of reflux oesophagitis of 4% in CD is higher than a paediatric background population is unclear. Dysmotility with delayed gastric emptying in untreated CD may promote reflux disease. No long-term data are available in affected children during a GFD.

Eosinophilic Oesophagitis

Four studies described eosinophilic oesophagitis (EoE) in CD and non-CD cases (4,89–91). The first case-control study, with controls undergoing endoscopy for other reasons except CD, found a similar frequency in children with CD and in a highly selected control group without CD (89). In a cross-sectional study from a large pathology database, including patients with available oesophageal and duodenal biopsies, a weak association between EoE and CD was found, which was not significant for children (90). The third study found signs of oesophageal eosinophilia in 4% of children with CD but had no comparator group (91). Lastly, in the only population-based study, not a single case of EoE was identified from 653 children with CD (92). A systematic review did not find an association between EoE and CD (93).

Statement

There is no evidence to support that relevant diagnoses are missed if upper endoscopy with biopsies are omitted to diagnose CD.

Recommendation

We recommend that the decision to omit upper endoscopy with biopsies can be taken without the consideration of missing other pathologies or diagnoses [↑↑].

Voting

Statement and Recommendation: Agree: 18 Disagree: 0 Abstain: 0

Algorithm

On the basis of the evidence, the algorithms from the 2012 ESPGHAN guidelines have been modified into a common algorithm (Fig. 4) in subjects with normal IgA (Fig. 4A), with low or absent IgA (Fig. 4B) and with instructions for duodenal biopsies (Fig. 4C).

CONCLUSIONS AND FUTURE DIRECTIONS

These guidelines take into consideration new evidence (Table 3), mostly arising from studies inspired by the publication of the previous guidelines (1). Not all of the statements in the 2012 guidelines were supported by a similar degree of evidence. The most informative studies conducted in recent years have confirmed

the substantial correctness of the 2012 guidelines (see supplementary material S23, Supplemental Digital Content, <http://links.lww.com/MPG/B719>), but at the same time indicate that we should consider the process far from being concluded.

Serology

The specificity of TGA-IgA at low titres, particularly in the absence of EMA-IgA, and the consequent clinical decision needs further investigation. The importance of EMA-IgA and TGA-IgA in the recommended repeat blood sample in the serological diagnosis has not been fully clarified by the existing literature. As EMA-IgA allows to selectively detect antibodies against certain TG2 epitopes, new submolecular TGA assays with coeliac epitope-specific target

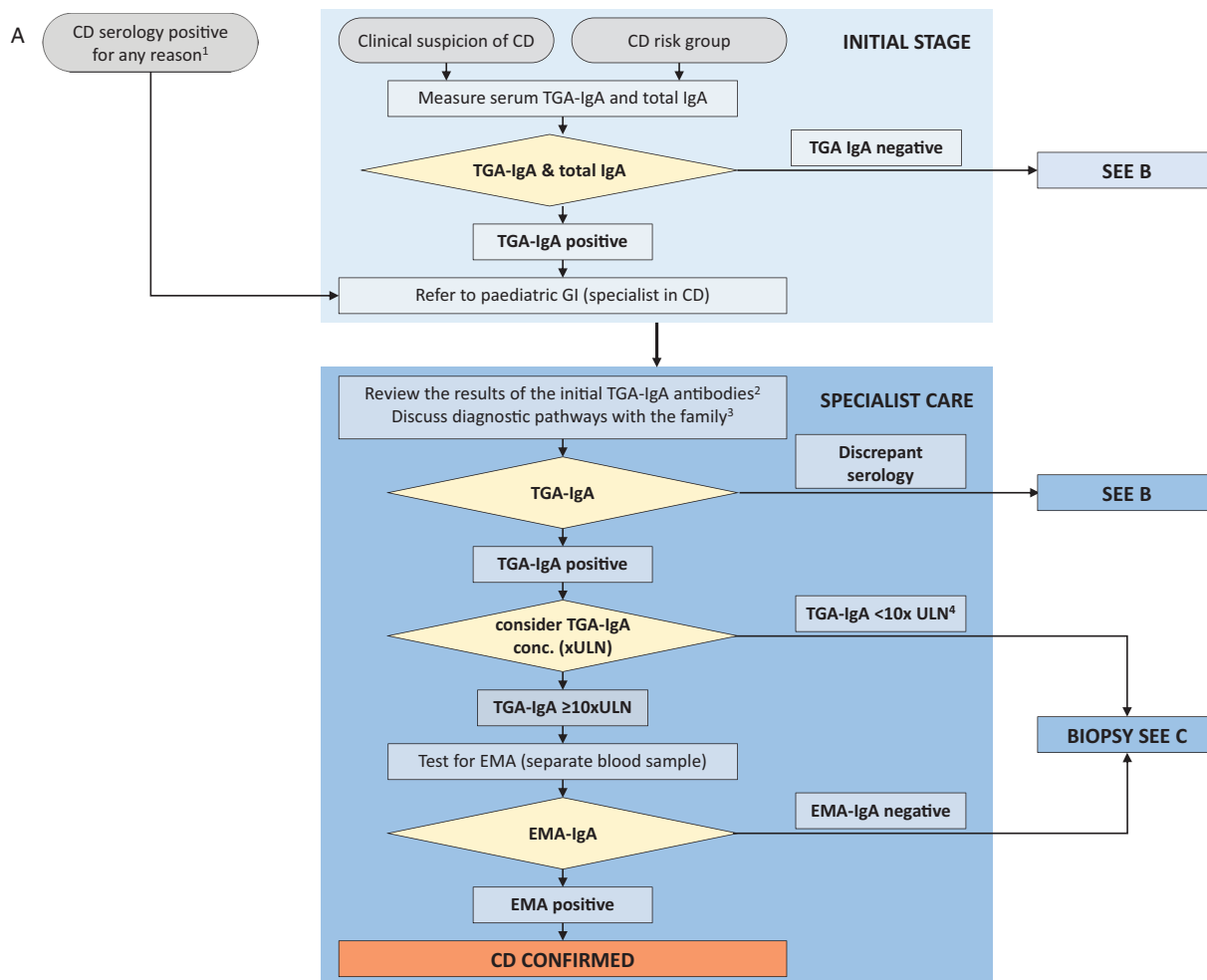


FIGURE 4. Algorithm for diagnosis of coeliac disease: A) in IgA-competent subjects, B) in IgA deficient subjects, C) for biopsy. ¹Other than TGA-IgA, including point-of-care tests and DGP. ² Check the value also in relation to the cut-off and repeat the test if questionable or borderline. No need to retest if done with validated assay with calibration curve. Test with conventional TGA-IgA test if positive POCT and TGA has not been measured quantitatively. ³Convey the message that the diagnosis of coeliac disease with or without biopsy confirms the need for a lifelong gluten-free diet and that re-evaluation after introduction of the diet would need prolonged re-exposure to gluten with a series of further investigations. ⁴If TGA-IgA is only borderline positive confirm sufficient gluten intake and consider re-testing of TGA-IgA and EMA. ⁵Low for age or <0.2 g/L above the age of 3 years. ⁶For example, dermatitis herpetiformis, in which serology is frequently negative. ⁷The cut-off for normal numbers of IEL is >25 cells/100 enterocytes. The action done at the initial stage in primary care (light blue) is separated from specialist care (dark blue).

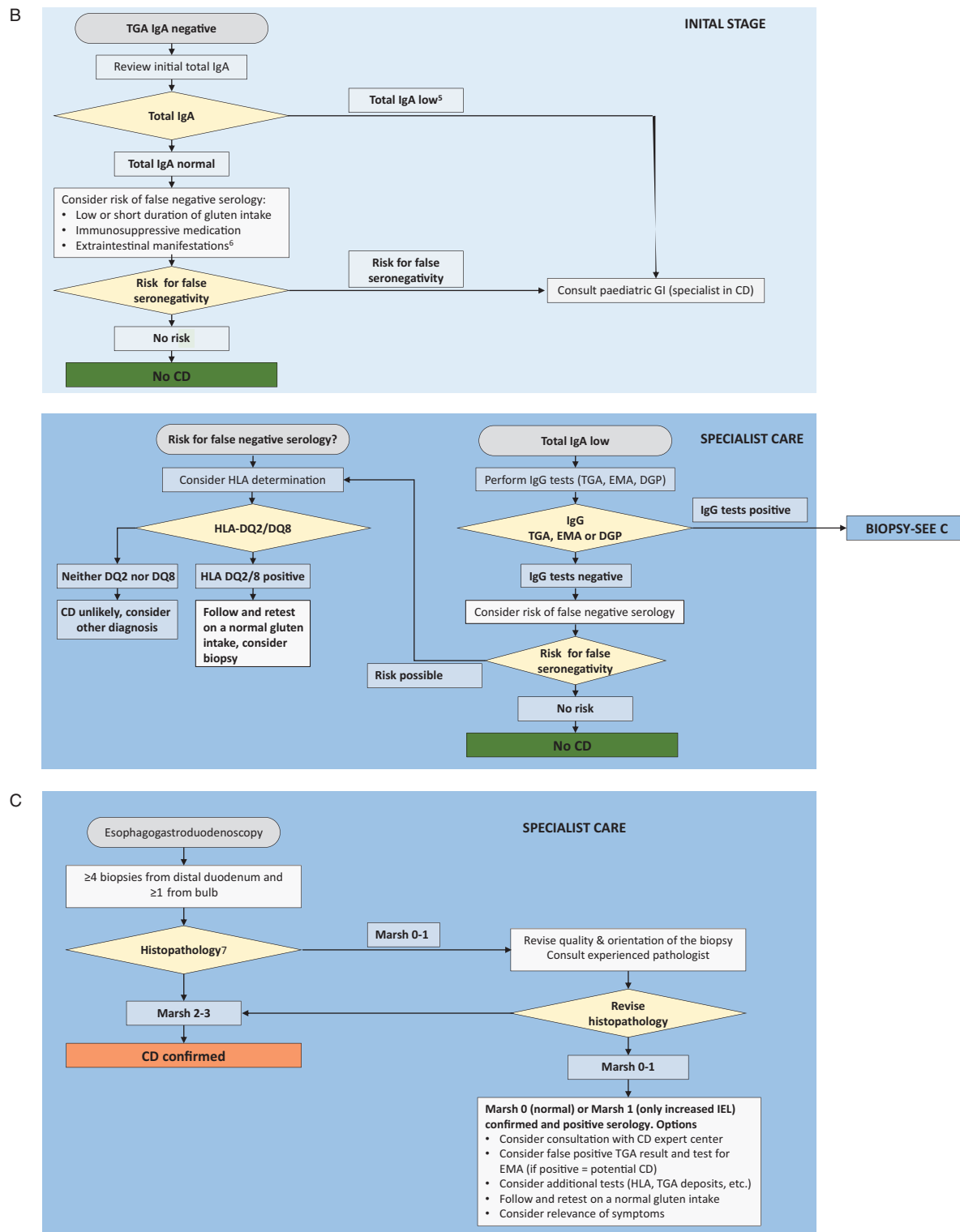


FIGURE 4. (Continued)

Downloaded from http://journals.lww.com/jpgn by BMDM5epHKav1zEoun1tQfN4a+kLhEZgbsHh04XMI0hCwCX1AW nYQp/1QrHD33D00OdRy17TVSFI4C3V4/OAV/pDDa8KKGKvY07my+78= on 09/04/2022

TABLE 3. Major changes from 2012 to 2020 European Society Paediatric Gastroenterology, Hepatology and Nutrition guidelines

	2012 guidelines	2020 guidelines
1	If CD can be diagnosed without performing small-bowel biopsies in children with strong clinical suspicion of CD and with high specific CD antibodies, consider performing HLA-DQ2/HLADQ8 typing in these children to add strength to the diagnosis	We recommend that HLA-DQ2 and DQ8 typing is not required in patients with positive TGA-IgA, if they qualify for CD diagnosis with biopsies or have high-serum TGA-IgA ($\geq 10 \times$ ULN) and EMA-IgA positivity. If a patient tests negative for HLA DQ2 and DQ8, the risk of CD is very low, whereas a positive result does not confirm the diagnosis
2	Start the screening for CD in groups at risk by HLA-DQ2 and HLA-DQ8 typing if the test is available. These groups include first-degree relatives of a patient with a confirmed case and patients with autoimmune and non-autoimmune conditions known to be associated with CD, such as T1DM, Down and Turner syndrome	We give a conditional recommendation that, taking available evidence into account, CD can be diagnosed without duodenal biopsies in asymptomatic children, using the same criteria as in patients with symptoms. We recommend that the decision whether or not to perform diagnostic duodenal biopsies should be made during a shared decision-making process together with the parent(s) and, if appropriate, with the child.
3	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 antibodies but in whom clinical suggestions raise a strong suspicion of CD, especially if they are younger than 2 years old.	We recommend testing for total IgA and TGA-IgA as initial screening in children with suspected CD. In patients with low total IgA concentrations, an IgG-based test (DGP, EMA or TGA) should be performed as a second step. Testing for EMA, DGP or AGA antibodies (IgG and IgA) as initial screening in clinical practice is not recommended.

For other statements and recommendations still in force from the 2012 guidelines, see the supplementary file S23, <http://links.lww.com/MPG/B719>. CD = coeliac disease; DGP = deamidated gliadin peptide; EMA = endomysial antibody test; HLA = human leukocyte antigen; TGA-IgA = IgA-antibodies against transglutaminase 2; ULN = upper limit of normal.

antigen(s) may bring advances, including the required specificity to distinguish these epitope-specific targets from background reaction and noncoeliac TG2 antibodies in other diseases. Assay differences for the TGA assays should be further evaluated and work is necessary to establish a common standard, which could allow direct comparison between the tests and provide a reliable antibody level cut-off, for the no-biopsy approach. Currently, internal calibrators of TGA-IgA tests are patient serum samples and contain different amounts of polyclonal antibodies with individual epitope pattern and may be replaced by monospecific recombinant antibody reagents with a defined epitope. Refinement of the diagnostic tests is still possible, either by technical advances with more precise tests or with discovery of new putative diagnostic targets (87).

HLA

The 2012 guidelines recommended that HLA typing should be used in “at-risk” groups to help rule CD out or to risk-stratify those patients who are positive. For some at-risk groups, such as T1DM it may not be cost-effective because of the high percentage of HLA positives but may be in other groups (for example, in 1st degree relatives or in Down syndrome). Future research should concentrate on the utility of HLA testing in at-risk groups and its cost-effectiveness using health economic models, as well as the acceptability and family understanding of HLA testing.

Histology

Validated SOPs for handling and reading of biopsies should be implemented in routine diagnostics and teaching and multicentre ring testing are warranted. Whole biopsy scanning and digital image analysis will allow for online training, and virtual histopathology results can be re-evaluated whenever intra-observer variability estimates are warranted. Also, duodenal bulb specimens should be properly oriented and evaluated when used in diagnostics, and more studies are needed in this area.

Potential Coeliac Disease

This issue is of particular relevance as it questions the very definition of CD. The term potential refers to the possibility for the

patient to evolve to villous atrophy. We, however, know there are “potential” CD patients with symptoms responding to GFD and it is difficult not to consider them as CD patients, despite the absence of villous atrophy. Data are being collected on the clinical features and natural history of this condition to determine: rate of gluten responsiveness in symptomatic patients; rate of evolution to villous atrophy; differences in outcome between Marsh 0 and 1; markers predicting the evolution to villous atrophy; and long-term risks if asymptomatic patients are maintained on gluten-containing diet.

Finally, it will be important to monitor the actual implementation of these new guidelines with surveillance of their strict application without any “shortcuts,” for example, use of the serological diagnosis without proper restriction as to antibody concentrations and EMA second testing, thus omitting biopsies when it should not. More data from high-quality studies on the no-biopsy approach in children without symptoms, particularly in those with type 1 diabetes are warranted.

The long-term impact of the implementation of this new no-biopsy approach also remains to be assessed in terms of compliance with a gluten free diet.

Acknowledgments: The preparation of these guidelines has been supported by ESPGHAN. We thank Dr Klaus Giersiepen for important help in the early phases of this work and Drs Tove Frandsen and Julie Bolvig Hansen for their great help with the literature search.

REFERENCES

- Husby S, Koletzko S, Korponay-Szabo IR, et al., ESPGHAN Working Group on Coeliac Disease Diagnosis; ESPGHAN Gastroenterology Committee; European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012;54:136–60.
- Ludvigsson JF, Bai JC, Biagi F, et al., BSG Coeliac Disease Guidelines Development Group; British Society of Gastroenterology. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. *Gut* 2014;63:1210–28.
- Rubio-Tapia A, Hill ID, Kelly CP, et al., American College of Gastroenterology. ACG clinical guidelines: diagnosis and management of celiac disease. *Am J Gastroenterol* 2013;108:656–76.

4. Werkstetter KJ, Korponay-Szabo IR, Popp A, et al., ProCeDE study group. Accuracy in diagnosis of celiac disease without biopsies in clinical practice. *Gastroenterology* 2017;153:924–35.
5. Wolf J, Petroff D, Richter T, et al. Validation of antibody-based strategies for diagnosis of pediatric celiac disease without biopsy. *Gastroenterology* 2017;153:410.e17–9.e17.
6. Downey L, Houten R, Murch S, et al., Guideline Development Group. Recognition, assessment, and management of coeliac disease: summary of updated NICE guidance. *BMJ* 2015;351:h4513.
7. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;155:529–36.
8. Loeffel MM, Deeks JJ, Gatsonis C, et al. Systematic reviews of diagnostic test accuracy. *Ann Intern Med* 2008;149:889–97.
9. Bohning D, Bohning W, Holling H. Revisiting Youden's index as a useful measure of the misclassification error in meta-analysis of diagnostic studies. *Stat Methods Med Res* 2008;17:543–54.
10. Steinhauser S, Schumacher M, Rucker G. Modelling multiple thresholds in meta-analysis of diagnostic test accuracy studies. *BMC Med Res Methodol* 2016;16:97.
11. Schunemann HJ, Oxman AD, Brozek J, et al., GRADE Working Group. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. *BMJ* 2008;336:1106–10.
12. Cristofori F, Fontana C, Magista A, et al. Increased prevalence of celiac disease among pediatric patients with irritable bowel syndrome: a 6-year prospective cohort study. *JAMA Pediatr* 2014;168:555–60.
13. Kansu A, Kuloglu Z, Demir A, et al. Yield of coeliac screening in abdominal pain-associated functional gastrointestinal system disorders. *J Paediatr Child Health* 2015;51:1066–70.
14. Dehghani SM, Ehsaei Z, Honar N, et al. Frequency of celiac disease in children with chronic functional constipation in Shiraz-Iran. *Middle East J Digest Dis* 2015;7:166–9.
15. Agardh D, Lee HS, Kurppa KS, et al. Clinical features of celiac disease: a prospective birth cohort. *Pediatrics* 2015;135:627–34.
16. Imanzadeh F, Sayyari AA, Yaghoobi M, et al. Celiac disease in children with diarrhea is more frequent than previously suspected. *J Pediatr Gastroenterol Nutr* 2005;40:309–11.
17. Sharma A, Poddar U, Yachha SK. Time to recognize atypical celiac disease in Indian children. *Indian J Gastroenterol* 2007;26:269–73.
18. Bramanti E, Cicciu M, Matacena G, et al. Clinical evaluation of specific oral manifestations in pediatric patients with ascertained versus potential coeliac disease: a cross-sectional study. *Gastroenterology Res Pract* 2014;2014:934159.
19. Shakeri R, Zamani F, Sotoudehmanesh R, et al. Gluten sensitivity enteropathy in patients with recurrent aphthous stomatitis. *BMC Gastroenterol* 2009;9:44.
20. Sattar N, Lazare F, Kacer M, et al. Celiac disease in children, adolescents, and young adults with autoimmune thyroid disease. *J Pediatr* 2011;158:272.e1–5.e1.
21. Laass MW, Schmitz R, Uhlig HH, et al. The prevalence of celiac disease in children and adolescents in Germany. *Deutsches Arzteblatt international* 2015;112:553–60.
22. Kalayci AG, Kanber Y, Birinci A, et al. The prevalence of coeliac disease as detected by screening in children with iron deficiency anaemia. *Acta Paediatr* 2005;94:678–81.
23. Khatib M, Baker RD, Ly EK, et al. Presenting pattern of pediatric celiac disease. *J Pediatr Gastroenterol Nutr* 2016;62:60–3.
24. Fitzpatrick KP, Sherman PM, Ipp M, et al. Screening for celiac disease in children with recurrent abdominal pain. *J Pediatr Gastroenterol Nutr* 2001;33:250–2.
25. Anderson RP, Henry MJ, Taylor R, et al. A novel serogenetic approach determines the community prevalence of celiac disease and informs improved diagnostic pathways. *BMC Med* 2013;11:188.
26. Clouzeau-Girard H, Rebouissoux L, Taupin JL, et al. HLA-DQ genotyping combined with serological markers for the diagnosis of celiac disease: is intestinal biopsy still mandatory? *J Pediatr Gastroenterol Nutr* 2011;52:729–33.
27. Kurppa K, Salminen J, Ukkola A, et al. Utility of the new ESPGHAN criteria for the diagnosis of celiac disease in at-risk groups. *J Pediatr Gastroenterol Nutr* 2012;54:387–91.
28. Sandstrom O, Rosen A, Lagerqvist C, et al. Transglutaminase IgA antibodies in a celiac disease mass screening and the role of HLA-DQ genotyping and endomysial antibodies in sequential testing. *J Pediatr Gastroenterol Nutr* 2013;57:472–6.
29. Donat E, Ramos JM, Sanchez-Valverde F, et al., SEGHPN Working Group on Coeliac Disease. ESPGHAN 2012 Guidelines for coeliac disease diagnosis: validation through a retrospective Spanish Multi-centric Study. *J Pediatr Gastroenterol Nutr* 2016;62:284–91.
30. Tucci F, Astarita L, Abkari A, et al. Celiac disease in the Mediterranean area. *BMC Gastroenterol* 2014;14:24.
31. Klapp G, Masip E, Bolonio M, et al. Celiac disease: the new proposed ESPGHAN diagnostic criteria do work well in a selected population. *J Pediatr Gastroenterol Nutr* 2013;56:251–6.
32. Vriezinga SL, Auricchio R, Bravi E, et al. Randomized feeding intervention in infants at high risk for celiac disease. *N Engl J Med* 2014;371:1304–15.
33. Lionetti E, Castellana S, Francavilla R, et al., SIGENP (Italian Society of Pediatric Gastroenterology, Hepatology, and Nutrition) Working Group on Weaning and CD Risk. Introduction of gluten, HLA status, and the risk of celiac disease in children. *N Engl J Med* 2014;371:1295–303.
34. Paul SP, Sandhu BK, Spray CH, et al. Evidence supporting serology-based pathway for diagnosing celiac disease in asymptomatic children from high-risk groups. *J Pediatr Gastroenterol Nutr* 2018;66:641–4.
35. Cilleruelo ML, Fernandez-Fernandez S, Jimenez-Jimenez J, et al. Prevalence and natural history of celiac disease in a cohort of at-risk children. *J Pediatr Gastroenterol Nutr* 2016;62:739–45.
36. Jansen M, van Zelm M, Groeneweg M, et al. The identification of celiac disease in asymptomatic children: the Generation R Study. *J Gastroenterol* 2018;53:377–86.
37. Webb C, Norstrom F, Myleus A, et al. Celiac disease can be predicted by high levels of anti-tissue transglutaminase antibodies in population-based screening. *J Pediatr Gastroenterol Nutr* 2015;60:787–91.
38. Nevoril J, Kotalova R, Hradsky O, et al. Symptom positivity is essential for omitting biopsy in children with suspected celiac disease according to the new ESPGHAN guidelines. *Eur J Pediatr* 2013.
39. Trovato CM, Montuori M, Anania C, et al. Are ESPGHAN “biopsy-sparing” guidelines for celiac disease also suitable for asymptomatic patients? *Am J Gastroenterol* 2015;110:1485–9.
40. Oyaert M, Vermeersch P, De Hertogh G, et al. Combining antibody tests and taking into account antibody levels improves serologic diagnosis of celiac disease. *Clin Chem Lab Med* 2015;53:1537–46.
41. Mubarak A, Wolters VM, Gmelig-Meyling FH, et al. Tissue transglutaminase levels above 100 U/mL and celiac disease: a prospective study. *World J Gastroenterol* 2012;18:4399–403.
42. Parizade M, Bujanover Y, Weiss B, et al. Performance of serology assays for diagnosing celiac disease in a clinical setting. *Clin Vaccine Immunol* 2009;16:1576–82.
43. Panetta F, Torre G, Colistro F, et al. Clinical accuracy of anti-tissue transglutaminase as screening test for celiac disease under 2 years. *Acta Paediatr* 2011;100:728–31.
44. Mubarak A, Wolters VM, Gerritsen SA, et al. A biopsy is not always necessary to diagnose celiac disease. *J Pediatr Gastroenterol Nutr* 2011;52:554–7.
45. Horwitz A, Skaaby T, Karhus LL, et al. Screening for celiac disease in Danish adults. *Scand J Gastroenterol* 2015;50:824–31.
46. Hojsak I, Mozer-Glassberg Y, Segal Gilboa NW, et al. Celiac disease screening assays for children younger than 3 years of age: the performance of three serological tests. *Dig Dis Sci* 2012;57:127–32.
47. Frulio G, Polimeno A, Palmieri D, et al. Evaluating diagnostic accuracy of anti-tissue Transglutaminase IgA antibodies as first screening for Celiac Disease in very young children. *Clin Chim Acta* 2015;446:237–40.
48. Aberg AK, Olcen P. Serologic screening for celiac disease in children: a comparison between established assays and tests with deamidated gliadin-derived peptides plus conjugates for both IgA and IgG antibodies. *APMIS* 2009;117:808–13.
49. Vermeersch P, Geboes K, Marien G, et al. Defining thresholds of antibody levels improves diagnosis of celiac disease. *Clin Gastroenterol Hepatol* 2013;11:398–403quiz e32.
50. Absah I, Rishi AR, Gebrail R, et al. Lack of utility of anti-tTG IgG to diagnose celiac disease when anti-tTG IgA is negative. *J Pediatr Gastroenterol Nutr* 2017;64:726–9.

51. Aita A, Rossi E, Basso D, et al. Chemiluminescence and ELISA-based serum assays for diagnosing and monitoring celiac disease in children: a comparative study. *Clin Chim Acta* 2013;421:202–7.
52. Alessio MG, Tonutti E, Brusca I, et al., Study Group on Autoimmune Diseases of Italian Society of Laboratory Medicine. Correlation between IgA tissue transglutaminase antibody ratio and histological finding in celiac disease. *J Pediatr Gastroenterol Nutr* 2012;55:44–9.
53. Dahlbom I, Korponay-Szabo IR, Kovacs JB, et al Prediction of clinical and mucosal severity of coeliac disease and dermatitis herpetiformis by quantification of IgA/IgG serum antibodies to tissue transglutaminase. *J Pediatr Gastroenterol Nutr* 2010;50:140–6.
54. Wolf J, Hasenclever D, Petroff D, et al. Antibodies in the diagnosis of coeliac disease: a biopsy-controlled, international, multicentre study of 376 children with coeliac disease and 695 controls. *PLoS One* 2014;9:e97853.
55. Lurz E, Scheidegger U, Spalinger J, et al. Clinical presentation of celiac disease and the diagnostic accuracy of serologic markers in children. *Eur J Pediatr* 2009;168:839–45.
56. Gidrewicz D, Potter K, Trevenen CL, et al. Evaluation of the ESPGHAN Coeliac Guidelines in a North American Pediatric Population. *Am J Gastroenterol* 2015;110:760–7.
57. Olen O, Gudjonsdottir AH, Browaldh L, et al. Antibodies against deamidated gliadin peptides and tissue transglutaminase for diagnosis of pediatric celiac disease. *J Pediatr Gastroenterol Nutr* 2012;55:695–700.
58. Prause C, Ritter M, Probst C, et al. Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease. *J Pediatr Gastroenterol Nutr* 2009;49:52–8.
59. Vivas S, Ruiz de Morales JG, Riestra S, et al. Duodenal biopsy may be avoided when high transglutaminase antibody titers are present. *World J Gastroenterol* 2009;15:4775–80.
60. Schirru E, Danjou F, Cicotto L, et al. Anti-actin IgA antibodies identify celiac disease patients with a Marsh 3 intestinal damage among subjects with moderate anti-TG2 levels. *BioMed Res Int* 2013;2013:630463.
61. Korponay-Szabo IR, Dahlbom I, Laurila K, et al. Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for coeliac disease in selective IgA deficiency. *Gut* 2003;52:1567–71.
62. Giersiepen K, Lelgemann M, Stuhldreher N, et al., ESPGHAN Working Group on Coeliac Disease Diagnosis. Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. *J Pediatr Gastroenterol Nutr* 2012;54:229–41.
63. Donaldson MR, Book LS, Leiferman KM, et al. Strongly positive tissue transglutaminase antibodies are associated with Marsh 3 histopathology in adult and pediatric celiac disease. *J Clin Gastroenterol* 2008;42:256–60.
64. Burgin-Wolff A, Mauro B, Faruk H. Intestinal biopsy is not always required to diagnose celiac disease: a retrospective analysis of combined antibody tests. *BMC Gastroenterol* 2013;13:19.
65. Marsh MN. Grains of truth: evolutionary changes in small intestinal mucosa in response to environmental antigen challenge. *Gut* 1990;31:111–4.
66. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999;11:1185–94.
67. Montén C, Bjelkenkrantz K, Gudjonsdottir AH, et al. Validity of histology for the diagnosis of paediatric coeliac disease: a Swedish multicentre study. *Scand J Gastroenterol* 2016;51:427–33.
68. Webb C, Halvarsson B, Norstrom F, et al. Accuracy in celiac disease diagnostics by controlling the small-bowel biopsy process. *J Pediatr Gastroenterol Nutr* 2011;52:549–53.
69. Villanacci V, Lorenzi L, Donato F, et al. Histopathological evaluation of duodenal biopsy in the PreventCD project. An observational interobserver agreement study. *APMIS* 2018;126:208–14.
70. Adelman DC, Murray J, Wu TT, et al. Measuring change in small intestinal histology in patients with celiac disease. *Am J Gastroenterol* 2018;113:339–47.
71. Kuitunen P, Kosnai I, Savilahti E. Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. *J Pediatr Gastroenterol Nutr* 1982;1:525–31.
72. Mangiavillano B, Masci E, Parma B, et al. Bulb biopsies for the diagnosis of celiac disease in pediatric patients. *Gastrointest Endosc* 2010;72:564–8.
73. Ravelli A, Villanacci V, Monfredini C, et al. How patchy is patchy villous atrophy?: distribution pattern of histological lesions in the duodenum of children with celiac disease. *Am J Gastroenterol* 2010;105:2103–10.
74. Ravelli A, Bolognini S, Gambarotti M, et al. Variability of histologic lesions in relation to biopsy site in gluten-sensitive enteropathy. *Am J Gastroenterol* 2005;100:177–85.
75. Taavela J, Popp A, Korponay-Szabo IR, et al. A prospective study on the usefulness of duodenal bulb biopsies in celiac disease diagnosis in children: urging caution. *Am J Gastroenterol* 2016;111:124–33.
76. Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). *Gastroenterology* 1992;102:330–54.
77. Koskinen O, Collin P, Korponay-Szabo I, et al. Gluten-dependent small bowel mucosal transglutaminase 2-specific IgA deposits in overt and mild enteropathy coeliac disease. *J Pediatr Gastroenterol Nutr* 2008;47:436–42.
78. Tosco A, Maglio M, Paparo F, et al. Discriminant score for celiac disease based on immunohistochemical analysis of duodenal biopsies. *J Pediatr Gastroenterol Nutr* 2015;60:621–5.
79. Maglio M, Tosco A, Auricchio R, et al. Intestinal deposits of anti-tissue transglutaminase IgA in childhood celiac disease. *Dig Liver Dis* 2011;43:604–8.
80. Tosco A, Aitoro R, Auricchio R, et al. Intestinal anti-tissue transglutaminase antibodies in potential coeliac disease. *Clin Exp Immunol* 2013;171:69–75.
81. Auricchio R, Tosco A, Piccolo E, et al. Potential celiac children: 9-year follow-up on a gluten-containing diet. *Am J Gastroenterol* 2014;109:913–21.
82. Kurppa K, Ashorn M, Iltanen S, et al. Celiac disease without villous atrophy in children: a prospective study. *J Pediatr* 2010;157:373–808.e1.
83. Repo M, Lindfors K, Maki M, et al. Anemia and iron deficiency in children with potential celiac disease. *J Pediatr Gastroenterol Nutr* 2017;64:56–62.
84. Kurppa K, Ashorn M, Iltanen SK, et al. Celiac disease without villous atrophy in children: a prospective study. *J Pediatr* 2010;157:373.e1–80.e1.
85. Mandile R, Discepolo V, Scapaticci S, et al. The effect of gluten-free diet on clinical symptoms and the intestinal mucosa of patients with potential celiac disease. *J Pediatr Gastroenterol Nutr* 2018;66:654–6.
86. Guandalini S, Newland C. Can we really skip the biopsy in diagnosing symptomatic children with celiac disease. *J Pediatr Gastroenterol Nutr* 2013;57:e24.
87. Husby S, Koletzko S, Korponay-Szabo I. Authors' response. *J Pediatr Gastroenterol Nutr* 2013;57:e24–5.
88. Alper A, Hardee S, Rojas-Velasquez D, et al. Prevalence and clinical, endoscopic, and pathological features of duodenitis in children. *J Pediatr Gastroenterol Nutr* 2016;62:314–6.
89. Ahmed OI, Qasem SA, Abdulsattar JA, et al. Esophageal eosinophilia in pediatric patients with celiac disease: is it a causal or an incidental association? *J Pediatr Gastroenterol Nutr* 2015;60:493–7.
90. Jensen ET, Eluri S, Leibold B, et al. Increased risk of esophageal eosinophilia and eosinophilic esophagitis in patients with active celiac disease on biopsy. *Clin Gastroenterol Hepatol* 2015;13:1426–31.
91. Leslie C, Mews C, Charles A, et al. Celiac disease and eosinophilic esophagitis: a true association. *J Pediatr Gastroenterol Nutr* 2010;50:397–9.
92. Hommeida S, Alsawas M, Murad MH, et al. The association between celiac disease and eosinophilic esophagitis: mayo experience and meta-analysis of the literature. *J Pediatr Gastroenterol Nutr* 2017;65:58–63.
93. Lucendo AJ, Arias A, Tenias JM. Systematic review: the association between eosinophilic oesophagitis and coeliac disease. *Aliment Pharmacol Ther* 2014;40:422–34.
94. Saginur M, AlRefaee FAM, Spady DW, et al. Antitissue transglutaminase antibody determination versus upper endoscopic biopsy diagnosis of paediatric celiac disease. *Paediatr Child Health* 2013;18:246–50.