Journal of Pediatric Gastroenterology and Nutrition, Publish Ahead of Print

DOI : 10.1097/MPG.0000000000002497

European Society Paediatric Gastroenterology, Hepatology and Nutrition guidelines for diagnosing coeliac disease 2019

Short Title:

ESPGHAN guidelines for diagnosing coeliac disease 2019

Authors:

Steffen **Husby**[1], Odense, Denmark*

Sibylle **Koletzko**[2], Munich, Germany*

Ilma **Korponay-Szabó**[3], Budapest and Debrecen, Hungary*

Kalle **Kurppa**[4], Tampere, Finland*

M. Luisa **Mearin**[5], Leiden, The Netherlands*

Carmen **Ribes-Koninckx**[6], Valencia, Spain*

Raanan **Shamir**[7], Tel Aviv, Israel*

Riccardo **Troncone**[8], Naples, Italy*

Renata **Auricchio**[8], Naples, Italy

Gemma **Castillejo**[9], Reus, Spain

Robin **Christensen**[10], Copenhagen, Denmark

Jernej **Dolinsek**[11], Maribor, Slovenia

Peter **Gillett**[12], Edinburgh, Scotland

Asbjørn **Hróbjartsson**[13], Odense, Denmark

Tunde **Koltai**[14] Budapest, Hungary

Markku **Maki**[4], Tampere, Finland

Sabrina Mai **Nielsen**[10], Copenhagen, Denmark

Alina **Popp**, Bucharest[15], Romania

Ketil **Størdal**[16], Oslo, Norway

Katharina **Werkstetter**[2], Munich, Germany

Margreet **Wessels**[17], Arnhem, The Netherlands.

*contributed equally.

Affiliations:

[1]Hans Christian Andersen Children's Hospital, Odense University Hospital, DK-5000 Odense C, Denmark.

[2]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.

[3]Heim Pál National Paediatric Institute, Coeliac Disease Centre, Budapest, and Department of Paediatrics, University of Debrecen Medical Faculty, Debrecen, Hungary

[4]Tampere Centre for Child Health Research, Tampere University, and Department of Pediatrics, Tampere University Hospital, Tampere, Finland

[5]Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands

[6]Pediatric Gastroenterology Unit. La Fe University Hospital. Valencia. Spain

[7]Institute for Gastroenterology, Nutrition and Liver Diseases, Schneider Children's Medical Center, Petach Tikva, Sackler Faculty of Medicine, Tel Aviv University, Israel.

[8]Department of Medical Translational Sciences and European Laboratory for the Investigation of Food-Induced Diseases, University Federico II, Naples, Italy.

[9]Pediatric Gastroenterology Unit, Department of Pediatrics, Hospital Universitari Sant Joan de Reus, Reus, Spain.

[10] Musculoskeletal Statistics Unit: The Parker Institute, Bispebjerg and Frederiksberg Hospital & Department of Rheumatology, Odense University Hospital, Denmark.

[11]Unit of Pediatric Gastroenterology and Nutrition, University Medical Centre Maribor, Maribor, Slovenia

[12]Paediatric Gastroenterology, Hepatology and Nutrition Department, Royal Hospital for Sick Children, Edinburgh EH9 1LF Scotland, UK

[13]Centre for Evidence Based Medicine Odense (CEBMO), Odense University Hospital, Denmark

[14]Association of European Coeliac Society/Belgium, Hungarian Coeliac Society/Hungary

[15]University of Medicine and Pharmacy "Carol Davila", National Institute for Mother and Child Health, Bucharest, Romania

[16] Norwegian Institute of Public Health, Oslo and Ostfold Hospital Trust, Norway.

[17]Department of Pediatrics, Rijnstate Hospital, Arnhem, the Netherlands

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.

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 ten formulated questions on symptoms, serology, HLA 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 (10xULN) and the family agrees, the no-biopsy diagnosis may be applied, provided endomysial antibodies (EMA-IgA) will test positive in a second blood sample. HLA DQ2-/DQ8 determination and symptoms are not obligatory criteria. In children with positive TGA-IgA <10xULN at least 4 biopsies from the distal duodenum and at least one 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.

Keywords: Coeliac disease; Children and adolescents; diagnostic tests; Meta-Analysis

What is known

- Coeliac disease (CD) is underdiagnosed due to the heterogeneous presentation of clinical signs and symptoms.
- To diagnose CD, different approaches are applied (history, clinical examination, serology, HLA testing, histopathology), but neither one of them has been considered sufficient alone to make a reliable diagnosis.
- For the first time, the ESPGHAN 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 (TGA-IgA) is more accurate than other tests combinations.
- The no-biopsy approach for CD diagnosis is safe in children with high TGA-IgA 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 TGA-IgA but lower titers (<10 times upper limit of normal) should undergo biopsies to decrease the risk of false positive diagnosis.
- HLA testing and presence of symptoms are not obligatory criteria for a serology based diagnosis without biopsies.

Introduction

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 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. 10 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.

PICOs

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 when 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 2000-2016: 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 two independent reviewers.

Evidence synthesis process

Methods and results of studies were summarized according to the question posed. For questions 3-6, a meta-analysis for sensitivity and specificity and/or PPV was performed. For questions 1-2 and 7-10, 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 specificity. Since 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's index was calculated as J = sensitivity + 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 focusses on a quantitative estimate (typically from a meta-analysis) when 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; i.e. 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 to the general population?

Based on 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), four cases had positive TGA-IgA, three were biopsied and one had CD, resulting in a prevalence of 1%. In a large prospective birth cohort (n= 6.706) 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 to 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 to 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 diarrhea in children with CD. Chronic diarrhoea was investigated in 825 cases and 825 controls (16) with a 9.0% CD prevalence in the diarrhoea cases as compared to 0.6% in controls. In another study, 24 cases of CD (23%) were diagnosed in 103 children with chronic diarrhoea compared to 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 case being 13 years old. This prevalence was significantly higher than the 0.9% found in the general population (19).

In 302 patients positive for anti-thyroid antibodies (age range 3.1-24.9y), the prevalence of biopsy-confirmed CD disease was 2.3%. However, when patients with type 1 diabetes or Down´s 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 pediatric population based cohort in Germany, no significant differences were found between 97 TGA-IgA seropositive children compared to 12,509 seronegatives, however serum ferritin was significant 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 [↑].

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. However, this term may imply that all three tests can be performed from one 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. However, HLA testing 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 one of the being the HLA testing method. SNP-based tests are cheap to perform and recognize the common variants (DQ2.5, DQ8, DQ2.2, DQ7.5), while 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 (>10xULN), 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 10XULN, 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 non-coeliac 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 celiac-type HLA. In a screening study in 7208 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), one(29) reported 401 DQ2 and/or DQ8-positive (99%) patients among 405 with a TGA-IgA titre ≥10xULN. One center had previously

reported all cases undergoing duodenal biopsy due to 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 (≥10xULN) 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?

11 Papers were considered suitable and underwent QUADAS-2 analysis (Table S3 and S13, Supplemental Digital Content, http://links.lww.com/MPG/B719). However, even in these selected papers, limitations were present, since 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 four of six prospective studies (4, 5, 32, 33) . The majority of asymptomatic individuals were screened because they belonged to at risk groups. Three of the six prospective studies concerned patients suspected for CD (4, 5, 34), whereas the other three were birth cohorts with genetic susceptibility for CD (HLA-DQ2/DQ8 positive) (32, 33, 35). The two cross-sectional studies were mass-screening studies in the general population, not seeking medical attention for any complaint or risk (36, 37). The three retrospective studies included patients who were at risk of CD (29, 38, 39). Since 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 ≥10x ULN (Table S21), 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 three 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. However, in asymptomatic children the PPV of high TGA-IgA≥10xULN 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 [↑] .

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?

18 Papers 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 five recent papers showed surprisingly low specificity for EMA-IgA (41, 42, 44), with two of them coming from the same center. As the three papers 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 prior to 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's J statistic.

Statement: 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.

Recommendation: 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 two 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 one 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 DGPIgA, 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, while the remaining child was positive for EMA-IgG and TGA-IgG. The TGA-DGP compared to the TG2-IgA procedure resulted in 16 unnecessary endoscopies (negative TGA-IgA but false positive DGP).

TGA-IgA plus 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 a) persistent positive TGA-IgA levels, b) high or increasing AGA-IgA and c) 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 plus DGP-IgG with or without DGP-IgA: Of 5 retrospective studies, 4 were performed in young children only (42, 46-48), whilst one included children and adults ((49). A further description is included in the supplementary material (S22, Supplemental Digital Content, http://links.lww.com/MPG/B719). However, these studies 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 auto-antibodies. In summary, these studies do not support to add DGP to TGA-IgA testing for initial screening.

TGA-IgA plus 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%), Marsh 1 in 9 (4.5 %), villous atrophy due to 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 plus 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 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 (ULNx10, x7, x5) 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 ≥10xULN but this guideline evaluates the evidence for ≥10xULN and possibly lower cut-offs to predict Marsh 2-3 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) +ref. 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 comprised 9 conventional ELISA assays

(Biosystems, DiaSorin, Euroimmun, Eurospital, Immco, Inova, Orgentec, Phadia, R-Biopharm), two fluorescent immunoassays (Phadia) and two chemiluminescence tests (Immulite, Inova).

From the 30 datasets evaluating the TGA IgA cut-off at ≥10xULN, 28 reported >95% PPV and 21 reported >99% PPV (Fi. 3). The PPVs were higher in studies where both Marsh 2 and 3 were accepted as CD (all >97% PPV) compared to studies which required strictly Marsh 3 for CD diagnosis. At cut-off levels 5-7.5xULN PPV values varied between 92.3% and 100%, still 4/7 datasets showing PPV >99%. At cut-off levels 2-4xULN 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 10xULN (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 10xULN. At the cut-off levels between 3-10xULN, 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 are more likely due to 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 10xULN 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 ≥10xULN 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 < 10xULN 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 10xULN should be obligatory. Only antibody tests with proper calibrator curve-based calculation, and having the 10xULN 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. However, the EMA-IgA test performance depends on a subjective interpretation of the results which may be critical at low titers. 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).

Prospective studies:

In three prospective studies, a total of 1,788 symptomatic children were included of whom1357 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 ≥10xULN qualifying for the no-biopsy approach, four of which had a negative EMA; one of these four had a final diagnosis of no CD and three remained unclear. Thus, 1-4/895 patients qualifying for the no-biopsy approach (symptoms + TGA-IgA ≥10xULN) need to be tested with EMA-IgA to find one case with a final diagnosis of no CD. This yields a "number needed to test" of 224-895 to identify a non-CD case among those with TGA-IgA ≥10xULN.

In the study by Wolf (6), five patients considered not to have CD (n=2) or unclear diagnosis (n=3) out of 405 patients with TGA-IgA≥10x ULN also had a positive EMA-IgA. Four out of five had <10x ULN for TGA-IgA at the first sample assayed locally, suggesting transient high levels and a need for two separate samples to apply the no-biopsy criteria, or alternatively uncertain histology. In the study by Werkstetter(4), four cases regarded as possible false positives also had TGA-IgA<10x 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 one of these presented stratified tables in categories of TGA-IgA levels and with EMA-IgA for the

group with TGA-IgA ≥10x ULN. Two of the papers presented data suitable to answer the question. In total, 4 out of 565 individuals with TGA-IgA ≥10x 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 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 (≥10xULN) 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 ≥10X 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 two in some parts of at least one 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. However, when specific Marsh-Oberhuber gradings were compared by different evaluators, poor agreement in grading the injury was observed. In the study by Werkstetter(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%, while 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 three relevant paediatric studies for the inter-observer agreement of the histopathology results and 13 studies relevant for the duodenal bulb histopathology evaluation in children, three 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). However, some studies have 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 one 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 four biopsies from the distal duodenum and at least one 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. 6 papers 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 vs 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 table S9, S19, Supplemental Digital Content, http://links.lww.com/MPG/B719, and (79, 80)).

In children, evolvement 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 autoantibodies (81). There are no specific studies addressing the outcome of Marsh 0 vs. 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 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. However, reasons for this situation may also be low gluten intake prior to 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 non-biopsy 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 these patients should be monitored while on a GFD to ensure that no additional GI issues might have been missed.

Of the six relevant studies (Table S10, Supplemental Digital Content, http://links.lww.com/MPG/B719), five 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 *Helicobacter pylori (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* prevalence 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 esophagitis of 4% in CD is higher than a pediatric 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 (EoE):

Four studies described eosinophilic oesophagitis 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

Based on 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 sub molecular TGA assays with coeliac epitope specific target antigen(s) may bring advances, including the required specificity to distinguish these epitope specific targets from background reaction and non-coeliac 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 due to 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 multi-centre 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 CD. 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. However, we 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: 1) rate of gluten responsiveness in symptomatic patients; 2) rate of evolution to villous atrophy; 3) differences in outcome between Marsh 0 and 1; 4) markers predicting the evolution to villous atrophy; and 5) 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", e.g. 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.

Acknowledgements

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.

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.

References:

1. Husby S, Koletzko S, Korponay-Szabo IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012;54(1):136-60.
2. Ludvigsson JF, Bai JC, Biagi F, et al. Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology. Gut. 2014;63(8):1210-28.
3. Rubio-Tapia A, Hill ID, Kelly CP, et al. ACG Clinical Guidelines: Diagnosis and Management of Celiac Disease. Am J Gastroenterol. 2013;108(5):656-76.
4. Werkstetter KJ, Korponay-Szabo IR, Popp A, et al. Accuracy in Diagnosis of Celiac Disease Without Biopsies in Clinical Practice. Gastroenterology. 2017;153(4):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(2):410-9.e17.
6. Downey L, Houten R, Murch S, et al. 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(8):529-36.
8. Leeflang MM, Deeks JJ, Gatsonis C, et al. Systematic reviews of diagnostic test accuracy. Ann Intern Med. 2008;149(12):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(6):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(1):97.
11. Schunemann HJ, Oxman AD, Brozek J, et al. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. BMJ. 2008;336(7653):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 pediatrics. 2014;168(6):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(11):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 journal of digestive diseases. 2015;7:166-9.
15. Agardh D, Lee HS, Kurppa K, S, et al. Clinical features of celiac disease: a prospective birth cohort. Pediatrics. 2015;135(4):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(3):309-11.
17. Sharma A, Poddar U, Yachha SK. Time to recognize atypical celiac disease in Indian children. Indian J Gastroenterol. 2007;26(6):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 research and practice. 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(2):272-5.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(33-34):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(6):678-81.

23. Khatib M, Baker RD, Ly EK, et al. Presenting Pattern of Pediatric Celiac Disease. J Pediatr Gastroenterol Nutr. 2016;62(1):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(3):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(6):729-33.

27. Kurppa K, Salminiemi 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(3):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(4):472-6.

29. Donat E, Ramos JM, Sanchez-Valverde F, et al. ESPGHAN 2012 Guidelines for Coeliac Disease Diagnosis: Validation Through a Retrospective Spanish Multicentric Study. J Pediatr Gastroenterol Nutr. 2016;62(2):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(3):251-6.

32. Vriezinga SL, Auricchio R, Bravi E, C, et al. Randomized feeding intervention in infants at high risk for celiac disease. N Engl J Med. 2014;371(14):1304-15.

33. Lionetti E, Castellaneta S, Francavilla R, et al. Introduction of gluten, HLA status, and the risk of celiac disease in children. N Engl J Med. 2014;371(14):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(4):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(5):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(3):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(6):787-91.

38. Nevoral 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(10):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(10):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(32):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(11):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(5):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(5):554-7.

45. Horwitz A, Skaaby T, Karhus LL, et al. Screening for celiac disease in Danish adults. Scand J Gastroenterol. 2015;50(7):824-31.

46. Hojsak I, Mozer-Glassberg Y, Segal Gilboa N, Wet al. Celiac disease screening assays for children younger than 3 years of age: the performance of three serological tests. Dig Dis Sci. 2012;57(1):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(11):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(4):398-403; quiz 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(5):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. Correlation between IgA tissue transglutaminase antibody ratio and histological finding in celiac disease. J Pediatr Gastroenterol Nutr. 2012;55(1):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(2):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(5):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(7):839-45.

56. Gidrewicz D, Potter K, Trevenen CL, et al. Evaluation of the ESPGHAN Celiac Guidelines in a North American Pediatric Population. Am J Gastroenterol. 2015;110(5):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(6):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(1):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(38):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 research international. 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(11):1567-71.

62. Giersiepen K, Lelgemann M, Stuhldreher N, , et al. Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report. J Pediatr Gastroenterol Nutr. 2012;54(2):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(3):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(1):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(10):1185-94.

67. Monten 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(4):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(5):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(3):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(3):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(4):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(3):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(9):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(1):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(1):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(1):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(4):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(5):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(8):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(1):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(6):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(3):373-80, 80.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(1):56-62.

84. Kurppa K, Ashorn M, Iltanen S, Ket al. Celiac disease without villous atrophy in children: a prospective study. J Pediatr. 2010;157(3):373-80, 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(4):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(4):e24.

87. Husby S, Koletzko S, Korbonay-Szabo I. Authors' response. J Pediatr Gastroenterol Nutr. 2013;57(4):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(2):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(4):493-7.

90. Jensen ET, Eluri S, Lebwohl B,et al. Increased Risk of Esophageal Eosinophilia and Eosinophilic Esophagitis in Patients With Active Celiac Disease on Biopsy. Clin Gastroenterol Hepatol. 2015;13(8):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(4):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(1):58-63.

93. Lucendo AJ, Arias A, Tenias JM. Systematic review: the association between eosinophilic oesophagitis and coeliac disease. Aliment Pharmacol Ther. 2014;40(5):422-34.

94. Saginur M, AlRefaee FAM, Spady DW, et. al. Antitissue trasnglutaminase antibody determination versus upper endoscopic biopsy diagnosis of paediatric celiac disease. Paediatr Child Health. 2013;18(5):246-250.

Fig. 1 Forest plot for PPVs for Question 3

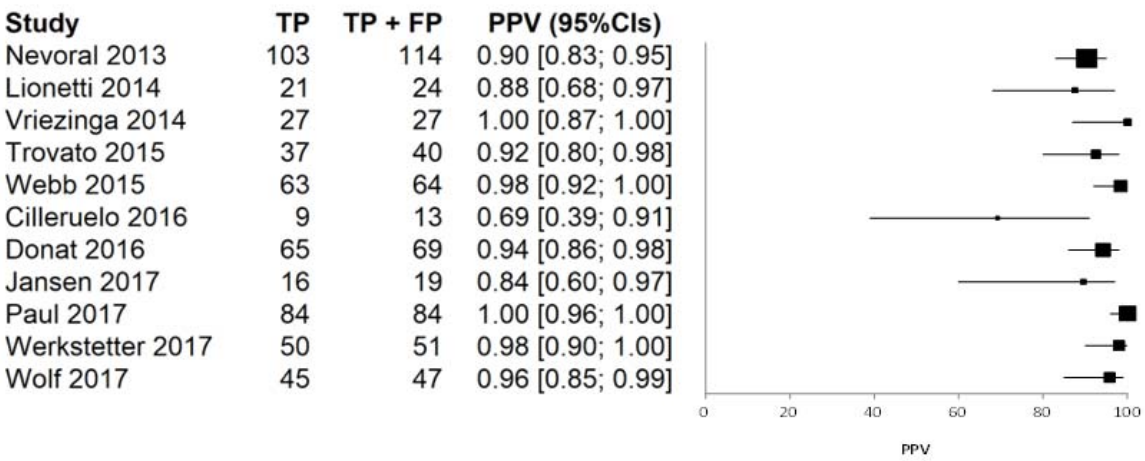


Fig. 2a) Forest plots for sensitivity and specificity for Question 4

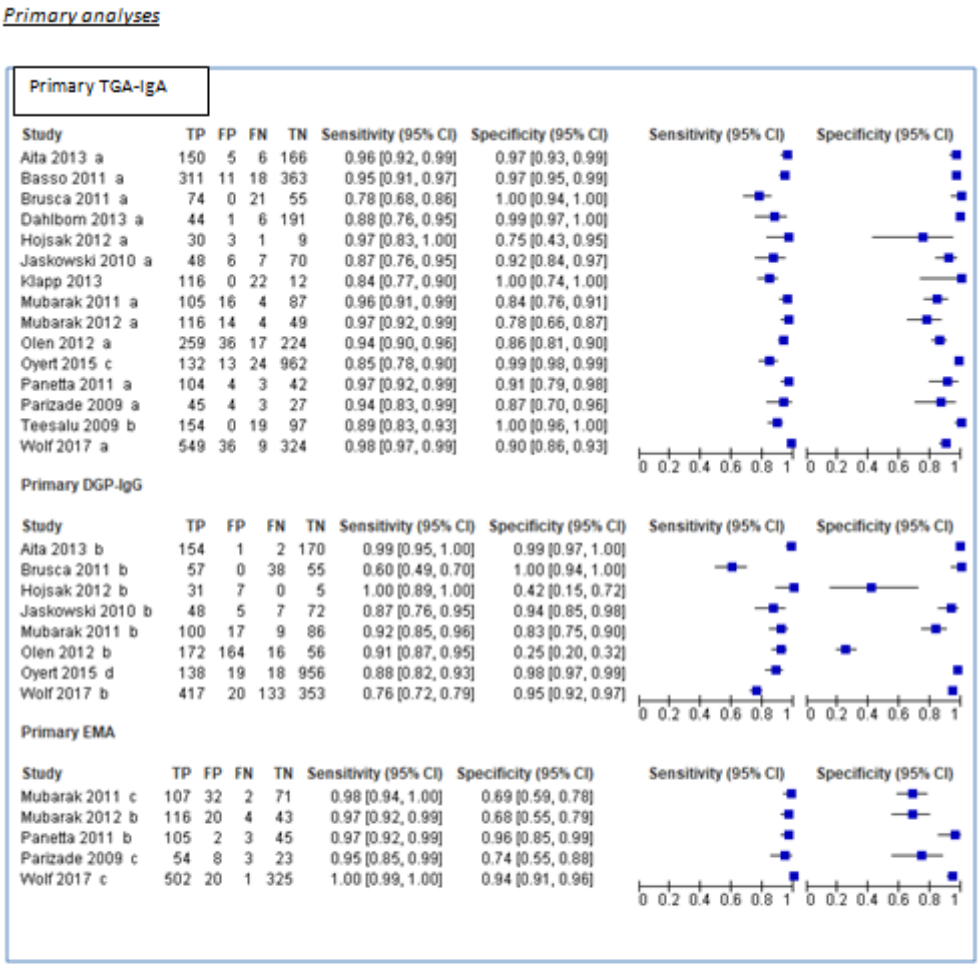


Fig. 2b) Meta-analysis for Question 4

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.

	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

Fig. 3A. Question 6: Positive predictive value of ≥10xULN TGA-IgA serum concentrations for coeliac disease

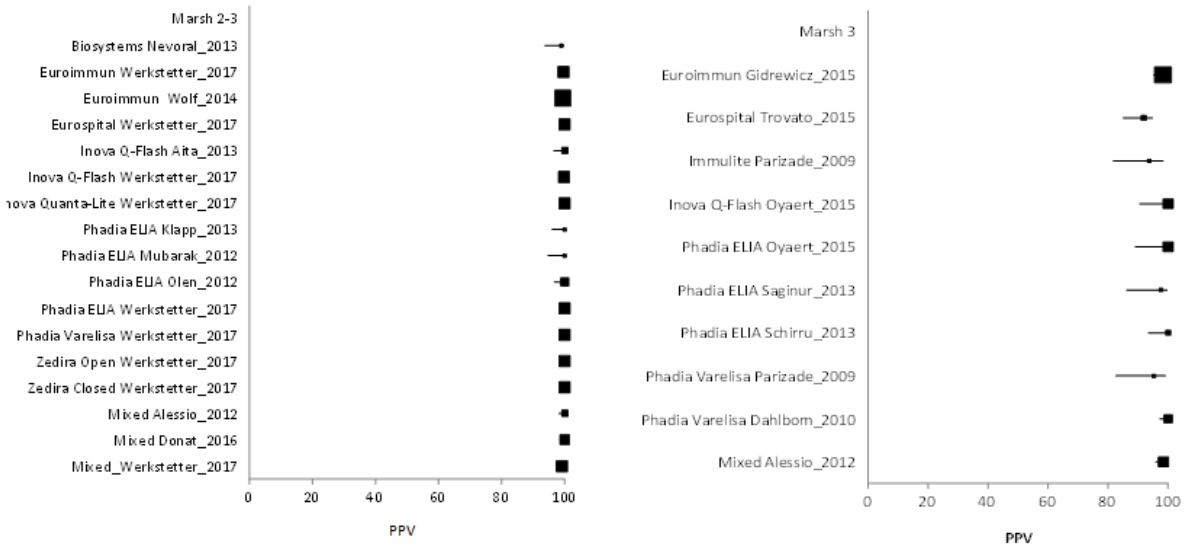


Fig. 3B. Question 6: Positive predictive values of at least 3-7.5xULN TGA-IgA serum concentrations for coeliac disease

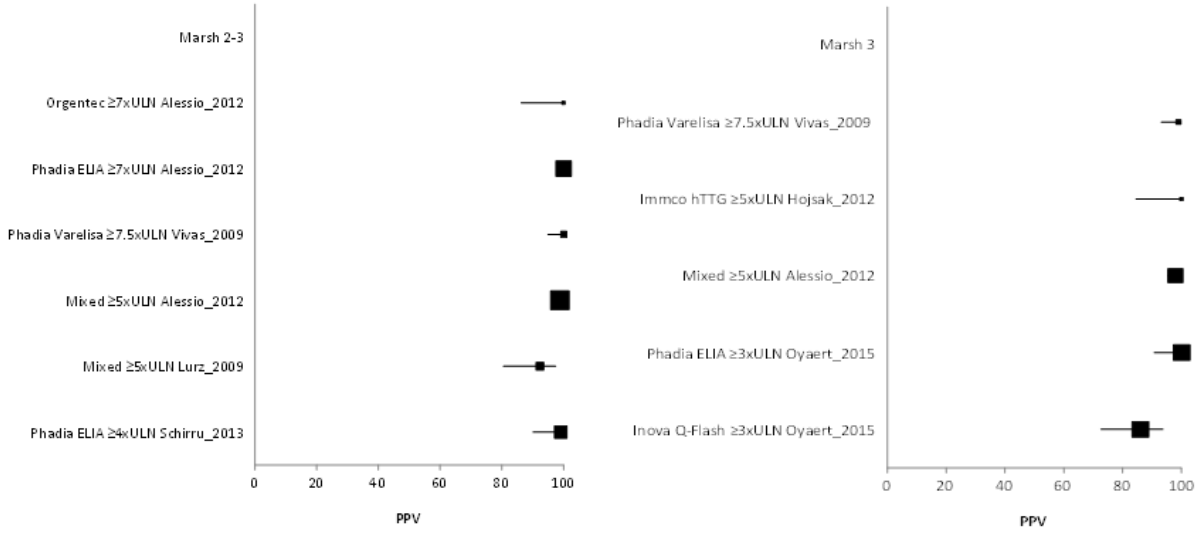
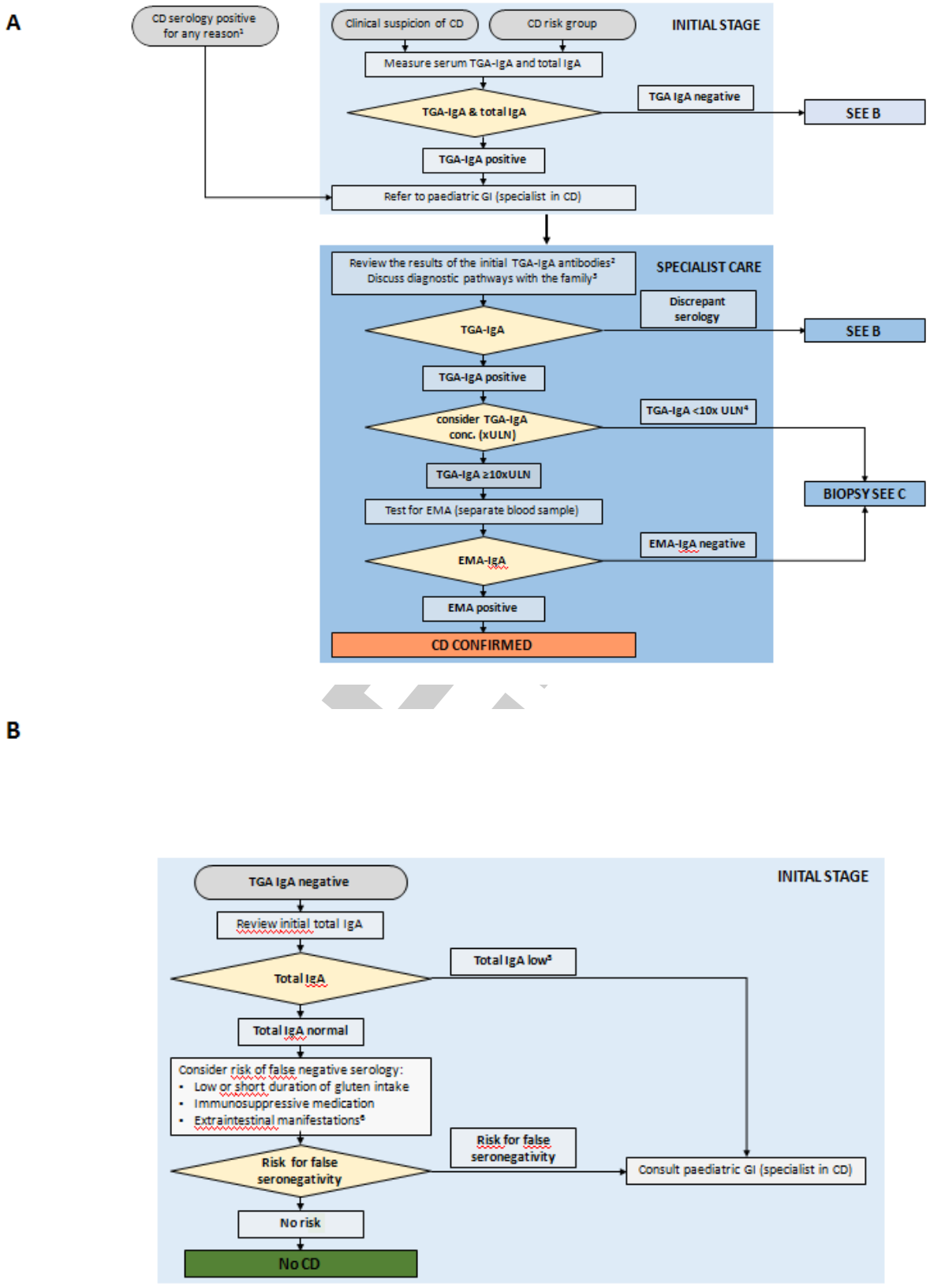
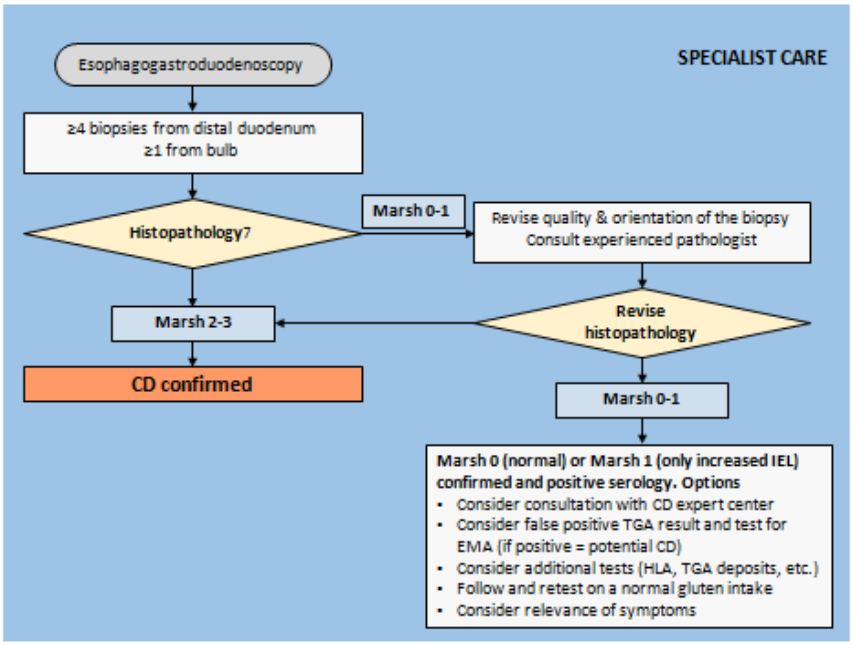


Fig. 4



C



Footnotes:

1. Other than TGA-IgA, including point-of-care tests (POCT) 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.

3. Convey the message that the diagnosis of coeliac disease with or without biopsy confirms the need for a life-long 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.

4. If TGA-IgA is only borderline positive confirm sufficient gluten intake and considerer re-testing of TGA-IgA and EMA

5. Low for age or <0.2 g/l above the age of 3 years

6. E.g. Dermatitis herpetiformis, in which serology is frequently negative

7. the cut-off for normal numbers of IEL is >25cells/100 enterocytes.

Table 1 Questions for the 2019 ESPGHAN criteria for the diagnosis of coeliac disease (CD)

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, mal-absorption, iron deficiency anaemia, oral aphthae as compared to 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 (≥10xULN) 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.	↑↑
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 one serological test be used and, if so, what should be the	We recommend testing for total IgA and TGA-IgA as initial screening in children with	↑↑

Question	Text	Recommendation	Grading (strength)
	sequence of testing?	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 (ULNx10, x7, x5)?	We recommend that for CD diagnosis without biopsies, TGA- IgA serum concentration of at least 10xULN should be obligatory. Only antibody tests with proper calibrator curve-based calculation, and having the 10xULN 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 without omission of biopsy?	We recommend that in children with TGA ≥10X 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 four biopsies from the distal duodenum and at least one 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 to Marsh 0 have a	We recommend before diagnosing potential CD to check the gluten content of the	↑

Question	Text	Recommendation	Grading (strength)
	different long-term outcome regarding diagnosis of CD in children with coeliac autoimmunity (positive TGA or EMA)?	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-gastro-duodenal) 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, one 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).

Table 2: Symptoms and signs suggesting coeliac disease (*) Common symptoms

Gastrointestinal	chronic or intermittent diarrhea* chronic constipation not responding to usual treatment chronic abdominal pain distended abdomen* recurrent nausea, recurrent vomiting
Extraintestinal symptoms	weight loss, failure-to-thrive*, stunted growth/ short stature* delayed puberty, amenorrhea 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 William's-Beuren syndrome IgA deficiency

Table 3: Major changes from 2012 to 2019 ESPGHAN guidelines

	2012 guidelines	2019 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 (≥10xULN) 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.
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 than2 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