Serum anti-tissue transglutaminase IgA and prediction of duodenal villous atrophy in adults with suspected coeliac disease without IgA deficiency (Bi.A.CeD): a multicentre, prospective cohort study

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Summary

Background Whether coeliac disease in adults can be diagnosed with serology alone remains controversial. We aimed to evaluate the accuracy of serum anti-tissue transglutaminase IgA (tTG-IgA) in the diagnosis of coeliac disease.

Methods In this multicentre, prospective cohort study, adult participants (aged ≥18 years) with suspected coeliac disease without IgA deficiency who were not on a gluten-free diet and who had a local serum tTG-IgA measurement, were enrolled from Feb 27, 2018, to Dec 24, 2020, by 14 tertiary referral centres (ten from Europe, two from Asia, one from Oceania, and one from South America) to undergo local endoscopic duodenal biopsy. Local serum tTG-IgA was measured with 14 different test brands and concentration expressed as a multiple of each test's upper limit of normal (ULN), and defined as positive when greater than 1 times the ULN. The main study outcome was the reliability of serum tests for the diagnosis of coeliac disease, as defined by duodenal villous atrophy (Marsh type 3 or Corazza–Villanacci grade B). Histology was evaluated by the local pathologist, with discordant cases (positive tTG-IgA without duodenal villous atrophy or negative tTG-IgA with duodenal villous atrophy) re-evaluated by a central pathologist. The reliability of serum tests for the prediction of duodenal villous atrophy was evaluated according to sensitivity, specificity, positive predictive value, negative predictive value, and the area under the receiver operating characteristic curve (AUC) for categorical and continuous data.

Findings We enrolled 436 participants with complete local data on serum tTG-IgA and duodenal histology (296 [68%] women and 140 [32%] men; mean age 40 years [SD 15]). Positive serum tTG-IgA was detected in 363 (83%) participants and negative serum tTG-IgA in 73 (17%). Of the 363 participants with positive serum tTG-IgA, 341 had positive histology (true positives) and 22 had negative histology (false positives) after local review. Of the 73 participants with negative serum tTG-IgA, seven had positive histology (false negatives) and 66 had negative histology (true negatives) after local review. The positive predictive value was 93·9% (95% CI 89·2–98·6), the negative predictive value was 90·4% (85·5–95·3), sensitivity was 98·0% (95·3–100·0), and specificity was 75·0% (66·6–83·4). After central re-evaluation of duodenal histology in 29 discordant cases, there were 348 true positive cases, 15 false positive cases, 66 true negative cases, and seven false negative cases, resulting in a positive predictive value of 95·9% (92·0–99·8), a negative predictive value of 90·4% (85·5–95·3), a sensitivity of 98·0% (95·3–100·0), and a specificity of 81·5% (73·9–89·1). Either using the local or central definition of duodenal histology, the positive predictive value of local serum tTG-IgA increased when the serological threshold was defined at increasing multiples of the ULN (p<0·0001). The AUC for serum tTG-IgA for the prediction of duodenal villous atrophy was 0·87 (95% CI 0·81–0·92) when applying the categorical definition of serum tTG-IgA (positive [>1× ULN] *vs* negative [≤1× ULN]), and 0·93 (0·89–0·96) when applying the numerical definition of serum tTG-IgA (multiples of the ULN). Additional endoscopic findings included peptic gastritis (nine patients), autoimmune atrophic gastritis (three), reflux oesophagitis (31), gastric or duodenal ulcer (three), and Barrett's oesophagus (one). In the 1-year follow-up, a midgut ileum lymphoma was diagnosed in a woman on a gluten-free diet.

Interpretation Our data showed that biopsy could be reasonably avoided in the diagnosis of coeliac disease in adults with reliable suspicion of coeliac disease and high serum tTG-IgA.

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Research in context

Evidence before this study

Following the development of reliable serological tests to detect coeliac disease, which were initially used to screen for the condition, it was suggested that these tests might be used to establish diagnosis without recourse to small bowel biopsy. PubMed was searched for articles published from database inception up to Dec 30, 2022, without language restrictions, using the terms “coeliac disease”, “diagnosis”, “serology”, and “biopsy”. Research papers on the subject collected by authors until Dec 30, 2022, were also reviewed. Some early studies supported a no-biopsy approach in paediatric and adult practice. In the paediatric setting, on the basis of available evidence, the no-biopsy approach has been incorporated into guidelines for the diagnosis of coeliac disease in childhood. Previous retrospective studies have shown high accuracy of serology in predictive duodenal mucosa damage also in adults. However, many gastroenterologists in adult practice are awaiting further evidence before changing long-standing diagnostic practices that use small bowel biopsy.

Added value of this study

The results of this multicentre prospective study indicate that a no-biopsy approach for the diagnosis of coeliac disease is safe and reliable in adult patients without IgA deficiency and with serum tTG-IgA greater than the assay-specific upper limit of normal; a post-hoc analysis suggested that a threshold of 10 times the upper limit of normal could be appropriate. We found no evidence that important comorbidities would be missed by adopting a no-biopsy strategy.

Implications of all the available evidence

A no-biopsy approach reduces pressure on endoscopy clinics and aids with cost savings. Other benefits to patients include the avoidance of an invasive, uncomfortable procedure that often requires sedation and, rarely, a general anaesthetic. With the use of tTG-IgA in patients with known concentrations of total serum IgA, a diagnosis will be established more quickly and treatment with a gluten-free diet started earlier than with biopsy. Although small bowel biopsy has been regarded as the gold standard for diagnosis, interpretation of histology is not always straightforward, leading to diagnostic delay or even misdiagnosis. Future research should refine serological criteria and determine economic and clinical benefits of a no-biopsy strategy.

Introduction

Since the early 1950s, intestinal biopsy has been the gold standard for the diagnosis of coeliac disease, although it has substantial cost implications, especially in some regions.[1,2] In addition, despite being a safe procedure, endoscopy for obtaining biopsy samples is uncomfortable and requires sedation. Moreover, about 10% of specimens cannot be reliably interpreted because of poor-quality biopsies, inadequate preparation, incorrect orientation, and missing duodenal bulb biopsies.[3] Previous studies have reported various degrees of agreement among different pathologists, with Cohen’s kappa (κ) values ranging from 0·35 to 0·88.[4,5] Patchy lesions might further complicate the assessment, potentially leading to misdiagnosis of coeliac disease.[5–8] In 1983, IgA anti-endomysial antibodies (EMA-IgA) were detected in the serum of patients with coeliac disease, and their high sensitivity and specificity made serology an attractive and practical method to assist in detecting the disorder.[9] The recognition of tissue transglutaminase in 1997 as the antigenic target of EMA-IgA led to the development of a simple antibody test that could be run by multiple antibody detection technologies.[10,11] Studies by Hill and Holmes[12] and others[13–15] showed that a diagnosis of coeliac disease based only on serology was reliable and feasible. Indeed, evidence in children was deemed strong enough for the no-biopsy principle to be incorporated by the European Society for Paediatric Gastroenterology Hepatology and Nutrition into their 2012 and 2020 guidelines for diagnosing coeliac disease in children.[16,17] The no-biopsy strategy has not been accepted by the North American Society For Pediatric Gastroenterology, Hepatology and Nutrition, which, in its 2005 guidelines and a clinical report in 2016, recommends that the diagnosis of coeliac disease be confirmed by the demonstration of characteristic changes in the histology of the small intestinal mucosa.[18,19] A primary concern regarding the no-biopsy approach is the incorrect diagnosis of coeliac disease in children and the substantial consequences for these children and their families. However, a US study of 3555 paediatric participants indicated the feasibility of avoiding duodenal biopsy in the diagnosis of coeliac disease in children when substituted by the detection of high serum anti-tissue transglutaminase IgA (tTG-IgA).[20] Additional prospective paediatric studies have shown similar results[21,22] and a no-biopsy strategy has now been largely adopted in children by many health-care systems and clinicians, in particular in Europe. By contrast, although many investigations have provided evidence that biopsy can also be avoided in adults,[23–38] this approach has not been widely adopted in adult practice. Therefore, we aimed to evaluate the accuracy of serum tTG-IgA in the diagnosis of coeliac disease in adults with suspected coeliac disease.

Methods

Study design

This multicentre, prospective cohort study was originally proposed at the 2017 meeting of the European

Society for the Study of Coeliac Disease. 14 tertiary referral centres for coeliac disease participated in the study: ten from Europe, two from Asia, one from Oceania, and one from South America (appendix p 2). The study aimed to enrol adults with suspected coeliac disease consecutively admitted to these sites from Feb 27, 2018, to Dec 24, 2020. The study was approved first by the ethical committee of the University of Salerno (Campania, Italy; approval number 21, 15-02-2018) and later approved by the local ethical committees of all participating centres before the start of enrolment. The protocol is available online.

Participants

We included individuals aged 18 years or older with clinically suspected coeliac disease (high pretest probability) who had an available serum tTG-IgA measurement from the local laboratory. Included participants were required to have provided signed written consent to undergo an upper gastrointestinal endoscopy with duodenal biopsies, measurement by the local laboratory of *HLA-DQ2* (*DQ2.2* and *DQ2.5*) and *HLA-DQ8* gene haplotypes in the case of duodenal villous atrophy with a negative tTG-IgA test (≤1× the upper limit of normal [ULN]), and collection of an additional venous blood sample for tests at the central laboratory. Patients were referred to the tertiary centres on the basis of referring clinician discretion. The definition of suspected coeliac disease was confirmed by the tertiary centres in the presence of at least one of the following: weight loss, gastrointestinal symptoms (diarrhoea, constipation, vomiting, irritable bowel syndrome-like symptoms, dyspepsia, or meteorism), anaemia, any vitamin deficiency, fatigue, infertility, osteoporosis, depression, neurological problems, hypertransaminasaemia, Hashimoto's thyroiditis, type 1 diabetes, autoimmune liver disease, autoimmune atrophic gastritis, psoriasis, rheumatoid autoimmune disorders, or family history of coeliac disease. Regarding clinical presentation, patients were divided into three groups: those with classical presentation, defined by the presence of anaemia, weight loss, or diarrhoea (indices of malabsorption);[39] those with non-classical presentation, defined by the presence of symptoms other than the classical symptoms; and asymptomatic participants with suspected coeliac disease based only on a family history of coeliac disease or the presence of associated autoimmune diseases.

Exclusion criteria were IgA deficiency, previous coeliac disease diagnosis, treatment with a gluten-restricted diet, a current or previous diagnosis of cancer, absence of local data on serum tTG-IgA, absence or withdrawal of written informed consent for study participation, unreadable duodenal histology, or duodenal villous atrophy with negative serum tTG-IgA test and negativity for both haplotypes on a *HLA-DQ2/DQ8* test (ie, criteria excluding the presence of coeliac disease[40]).

Procedures

The study teams at the local centres recorded data on an online platform using a unique code to anonymise the data. The collection of local data included sex at birth, age, anthropometry (height and bodyweight), race, self-reported family history of coeliac disease, serum tTG-IgA, the normal range of the local assay for serum tTG-IgA measurements, and results of duodenal histology. 14 different tests were used; the relative normal ranges and ULN cutoffs for the assays and number of patients tested with each are provided in the appendix (p 1). In addition, local data were collected on serum EMA-IgA and serum anti-deamidated gliadin peptide IgG (DGP-IgG) if the centre included these tests in the diagnostic work-up for coeliac disease.

See **Online** for appendix

For the **study protocol** see https://www.researchgate.net/publication/371671969_Biopsy_in_Adult_CeD_BioACeD-the_ESsCD_Consensus_Description_of_the_study

The duodenal endoscopy biopsies comprised six duodenal specimens oriented on paper and fixed in formalin: two from the bulb and four from the second part of the duodenum. Local pathologists processed the specimens and determined the count of intraepithelial lymphocytes (IELs) by CD3 staining according to the Marsh[41] and Corazza–Villanacci[42] protocols and classifications. Histology was defined according to the Marsh classification as: Marsh type 0 (normal mucosa; ≤25 IELs per 100 enterocytes); Marsh type 1 (>25 IELs per 100 enterocytes); Marsh type 2 (>25 IELs per 100 enterocytes plus crypt hyperplasia); and Marsh type 3 (>25 IELs per 100 enterocytes plus crypt hyperplasia and villous atrophy). According to the Corazza–Villanacci system,[42] histology was defined as grade A (equivalent to Marsh 1 or Marsh 2) or grade B (equivalent to Marsh 3), with no grade for Marsh type 0 in the Corazza–Villanacci system.

The main study outcome was the reliability of serum tests for the diagnosis of coeliac disease, as defined by duodenal villous atrophy. After exclusion of participants with duodenal villous atrophy with negativity for the *HLA-DQ2/DQ8* haplotypes, duodenal villous atrophy (Marsh type 3 or Corazza–Villanacci grade B) was used to define a coeliac disease diagnosis.[39] All patients identified to have coeliac disease were started on a gluten-free diet and followed up, with the first follow-up visit scheduled at 6 months and the second at 1 year.

Local centres were retrospectively requested to report two sets of additional data, pertaining to the established complications of coeliac disease. These data included any possible clinically significant finding from upper endoscopy, and diagnosis in the year after initial enrolment of gastrointestinal adenocarcinoma, gastrointestinal lymphoma, or refractory coeliac disease.

For central assessment, two frozen serum aliquots prepared by the laboratory of each local centre from a venous blood sample were sent to Werfen (San Diego, CA, USA) for central laboratory testing, including measurement of serum tTG-IgA by a particle-based multianalyte technology (Aptiva system and Aptiva Celiac Disease IgA Reagent; Inova Diagnostics, San Diego, CA, USA).

On the basis of local test results, patients who were positive for serum tTG-IgA (concentration >1× ULN) without duodenal villous atrophy and patients who were negative for serum tTG-IgA with duodenal villous atrophy were defined as discordant cases. Duodenal histology images of discordant cases were re-evaluated by a central pathologist (VV) who was masked to the patient's clinical history and local results. Non-discordant cases were not re-evaluated.

Statistical analysis

Serum tTG-IgA was expressed as both a numerical variable and a categorical variable. For the numerical variable, tTG-IgA concentration was expressed as a multiple of the assay-specific ULN for comparability of laboratory results among the various centres. The categorical variable consisted of two values only: positive serology, defined as a value higher than the ULN (>1× ULN), and negative serology, defined as a value within the normal range (≤1× ULN). Local serology tests of EMA-IgA and DGP-IgG were expressed with use of a similar categorical variable (positive *vs* negative). The EMA-IgA assay is an immunofluorescence test and is expressed as positive or negative. DGP-IgG was defined as positive when higher than the ULN (>1× ULN). The reliability of serum laboratory tests for the prediction of duodenal villous atrophy was evaluated by analysing: true positive cases (patients with positive serology and with duodenal villous atrophy); false positive cases (patients with positive serology without duodenal villous atrophy); true negative cases (patients with negative serology without duodenal villous atrophy); false negative cases (patients with negative serology with duodenal villous atrophy); positive predictive value (true positive cases divided by the sum of true positive cases plus false positive cases); negative predictive value (true negative cases divided by the sum of true negative cases plus false negative cases); sensitivity (true positive cases divided by the sum of true positive cases plus false negative cases); and specificity (true negative cases divided by the sum of true negative cases plus false positive cases). The 95% CI of the percentage measure (P) was calculated as:

$$P \pm 1{\cdot}96\sqrt{\frac{P(1-P)}{n}}$$

where n is number of participants. The upper limit was defined as 100 when greater than 100.

The area under the receiver operating characteristic curve (AUC) for tTG-IgA for the prediction of duodenal villous atrophy was analysed with use of both the tTG-IgA categorical data (with positive coded as 1 and negative as 0) and the continuous numerical tTG-IgA data (multiples of the ULN). Paired comparisons of AUC values for serology tests in predicting local histology were done with the DeLong test (between tTG-IgA and EMA-IgA, between tTG-IgA and DGP-IgG, and between EMA-IgA and DGP-IgG, expressed as categorical variables). Multivariable logistic regression was used to analyse the relationship of duodenal villous atrophy to numerically expressed local serum tTG-IgA, sex, age, family history of coeliac disease, and clinical presentation. Subgroup analyses of positive predictive value were done along prespecified arbitrary strata of serum tTG-IgA expressed as multiples of the ULN (≤1× ULN, >1 to 5× ULN, >5 to 10× ULN, >10 to 15× ULN, and >15× ULN). Measures of reliability were also assessed at a post-hoc tTG-IgA threshold (>10× ULN). Prespecified subgroup analyses of AUC values were done by age, sex, family history of coeliac disease, and clinical presentation, and in subgroups with and without complete data on the additional serology tests (local EMA-IgA, local DGP-IgG, and central tTG IgA). Analyses were done first with the use of the original local definition of duodenal villous atrophy and second with the definition after central re-evaluation. For serum tTG-IgA measurement, the central laboratory data and local laboratory data were analysed separately; agreement between the two measurements was assessed with Cohen's κ statistic. Additional subgroup analyses were done post hoc to evaluate the possibility of bias due to a high prevalence of duodenal villous atrophy. Categorical variables are reported as counts and percentage prevalence and numerical variables as mean with SD. Comparisons among groups were done with χ^2 analyses for categorical variables and and trends and with one-way ANOVA for numerical variables.

A p value less than 0·05 was considered as statistically significant; the presence of overlap between 95% CIs was considered to indicate the absence of significant differences. Statistical analyses were done with SPSS Statistics (version 19.0) and Stata (version 11.2).

Role of the funding source

There was no funding source for this study.

Results

Of 515 participants screened, 79 were excluded, due to duplicate recording (n=6), absence or withdrawal of consent (n=6), IgA deficiency (n=3), previous coeliac disease diagnosis (n=2), treatment with a gluten-free diet (n=5), absence of local data on serum tTG-IgA (n=56), or unreadable histological images (n=1; figure 1). Thus, the main study cohort comprised 436 participants with complete local data for inclusion (305 from Europe, 64 from Asia, seven from Oceania, and 60 from South America; appendix p 2). In addition, data were available on centrally measured serum tTG-IgA for 280 patients, on locally measured serum EMA-IgA for 267 patients, and on locally measured serum DGP-IgG for 133 patients (figure 1). Median enrolment duration was 20 months (range 13–34), and follow-up duration was 12 months for all patients.

The main study cohort comprised 296 (68%) women and 140 (32%) men, and mean age was 40 years (SD 15;

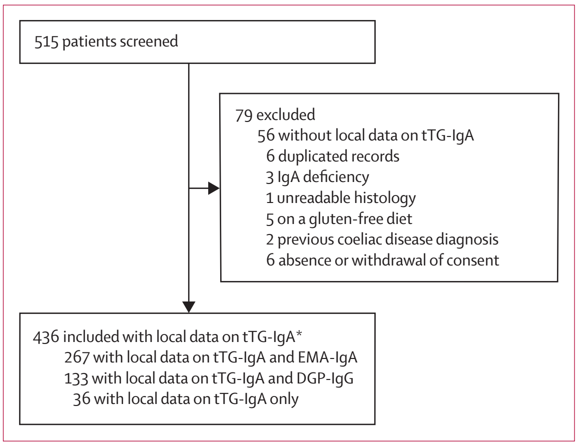

Figure 1: **Enrolment and antibody data collection**
Local data refers to measurement by the local laboratory. tTG-IgA=anti-tissue transglutaminase IgA. EMA-IgA=IgA anti-endomysial antibodies. DGP-IgG=anti-deamidated gliadin peptide IgG. *280 patients also had tTG-IgA measured by the central laboratory.

	Total cohort (with local data on serum tTG-IgA; n=436)	Subgroups with additional data		
		Central data on serum tTG-IgA (n=280)	Local data on serum EMA-IgA (n=267)	Local data on serum DPG-IgG (n=133)
Sex				
Female	296 (68%)	198 (71%)	176 (66%)	98 (74%)
Male	140 (32%)	82 (29%)	91 (34%)	35 (26%)
Race				
White	377 (86%)	278 (99%)	253 (95%)	132 (99%)
Asian	59 (14%)	2 (1%)	14 (5%)	1 (1%)
Family history of coeliac disease	74 (17%)	52 (19%)	51 (19%)	14 (11%)
Age, years	40 (15)	41 (15)	40 (15)	42 (16)
BMI, kg/m²	22·8 (4·5)	23·3 (4·4)	23·5 (4·6)	23·0 (4·7)
Clinical presentation				
Classical	156 (36%)	110 (39%)	101 (38%)	64 (48%)
Non-classical	232 (53%)	140 (50%)	130 (49%)	59 (44%)
Asymptomatic	48 (11%)	30 (11%)	36 (13%)	10 (8%)
Locally measured serum tTG-IgA >1 × ULN (positive serology)	363 (83%)	228 (81%)	225 (84%)	101 (76%)
Duodenal villous atrophy*				
Original local definition	348 (80%)	225 (80%)	221 (83%)	100 (75%)
After central re-evaluation	355 (81%)	225 (80%)	223 (84%)	100 (75%)

Data are n (%) or mean (SD). tTG-IgA=anti-tissue transglutaminase IgA. EMA-IgA=IgA anti-endomysial antibodies. DGP-IgG=anti-deamidated gliadin peptide IgG. ULN=upper limit of normal. *Marsh type 3 or Villanacci–Corazza grade B.

Table 1: **Descriptive statistics**

table 1). The pattern of missingness of additional serology data was at the centre level for both central data (serum tTG-IgA) and local data (serum EMA-IgA and serum DGP-IgG). Data on centrally measured serum tTG-IgA were missing due to the absence of authorisation to send serum samples abroad by the local ethics committee; missing data on locally measured serum EMA-IgA and serum DGP-IgG was due to the absence of these tests in the diagnostic work-up of some centres. In the subgroup of 280 participants with complete data on local and central tTG-IgA measurements, the prevalence of positive serum tTG-IgA (>1 × ULN) was highly concordant between local and central data (228 [81%] participants, Cohen's κ=0·952, p<0·0001). In the total cohort, 363 (83%) of 436 participants had positive serum tTG-IgA results and coeliac disease was diagnosed in 348 (80%) participants by local centre histology (figure 2). Based on local data, 29 (7%) of 436 participants had discordant results: 22 participants did not have duodenal villous atrophy but had positive serum tTG-IgA, and seven participants had duodenal villous atrophy but negative serum tTG-IgA. None of the seven participants with duodenal villous atrophy and normal serum tTG-IgA were *HLA-DQ2/DQ8* negative. After central histology re-evaluation of the discordant cases, seven of the 22 participants locally defined to be without duodenal villous atrophy were identified as having duodenal villous atrophy, and duodenal villous atrophy was confirmed in the seven participants locally identified to have duodenal villous atrophy (figure 2). Thus, 355 (81%) participants were diagnosed with coeliac disease after the central re-evaluation of discordant cases.

Analyses of the reliability of locally measured positive serum tTG-IgA (>1× ULN) for the prediction of duodenal villous atrophy defined by local histology indicated 341 true positive cases, 22 false positive cases, 66 true negative cases, and seven false negative cases, resulting in a positive predictive value of 93·9% (95% CI 89·2–98·6), a negative predictive value of 90·4% (85·5–95·3), a sensitivity of 98·0% (95·3–100·0), and a specificity of 75·0% (66·6–83·4). Indices of reliability showed slight improvement after central re-evaluation of duodenal histology in discordant cases, with 348 true positive cases, 15 false positive cases, 66 true negative cases, and seven false negative cases, resulting in a positive predictive value of 95·9% (92·0–99·8), a negative predictive value of 90·4% (85·5–95·3), a sensitivity of 98·0% (95·3–100·0), and a specificity of 81·5% (73·9–89·1).

Either with the use of the original local definition of duodenal villous atrophy or after central redefinition of discordant cases, the positive predictive value of local serum tTG-IgA increased along higher strata of serum tTG-IgA (table 2). In a post-hoc assessment, locally measured serum anti-tTG-IgA concentration was greater than 10 times the ULN in 162 patients (appendix p 1). Duodenal villous atrophy was present in 158 of these participants according to the local definition of histology, and in 161 after central re-evaluation of discordant cases, resulting in a positive predictive value of 97·5% (95% CI 93·4–99·2) with local histology, and 99·4% (96·0–100·0) after central re-evaluation (appendix p 3). After central histological evaluation, the one participant without villous atrophy had serum tTG-IgA greater than 10 times the ULN and Marsh type 2 (non-coeliac disease) lesions.

With the use of local data on histology and serology, the AUC for serum tTG-IgA for the prediction of duodenal villous atrophy was 0·87 (95% CI 0·81–0·92) when applying the categorical definition of serum tTG-IgA (positive [>1×ULN] *vs* negative [≤1×ULN]), and 0·93 (0·89–0·96) when applying the numerical values of serum tTG-IgA (multiples of the ULN). The slightly higher AUC for numerical tTG-IgA versus categorical tTG-IgA was consistent before and after central re-evaluation of histology and in subgroups with and without complete data on other serology tests (appendix p 5).

For the 280 participants with central data on serum tTG-IgA, the AUC for centrally measured tTG-IgA expressed as a continuous variable as a predictor of duodenal villous atrophy was the same when using the results of local histology and after the central re-evaluation of discordant cases (appendix p 4).

Analyses of the 95% CIs indicated that the AUC for local numerical or categorical serum tTG-IgA was similar to that in the subgroup with central data on serum tTG-IgA, in the subgroup without central data on serum tTG-IgA, in the subgroup with local data on serum EMA-IgA, in the subgroup without local data on serum EMA-IgA, in the subgroup with local data on serum DGP-IgG, and in the subgroup without local data on serum DGP-IgG (appendix p 5).

AUC values were also generally similar between different age strata (table 3). The AUC for local serum tTG-IgA expressed as multiples of the ULN was similar between men and women, and between individuals with and without family history of coeliac disease. The AUC was lower in patients with non-classical clinical presentation versus those with classical presentation (table 3).

In multivariable analysis including sex, age, family history of coeliac disease, and clinical presentation as covariates, the AUC for local serum tTG-IgA expressed as multiples of the ULN for the prediction of duodenal villous atrophy was 0·93 (95% CI 0·90–0·96). In the multivariable regression, sex, age, family history of coeliac disease, and clinical presentation did not relate to locally or centrally defined duodenal villous atrophy after controlling for local serum tTG-IgA expressed as multiples of the ULN (all Wald p>0·06; appendix p 6).

In the subgroup of 267 participants with complete local data on both EMA-IgA and tTG-IgA, the AUC for prediction of duodenal villous atrophy, as defined by local histology, was the same with use of the categorical variable of serum EMA-IgA (0·87 [95% CI 0·79–0·94]), and the categorical variable of serum tTG-IgA (0·87 [0·79–0·94]; appendix pp 5, 7). Pairwise comparison of the AUC values indicated similar diagnostic performance of the two serology tests (DeLong p=0·97). Data on the prevalence of positive serum EMA-IgA, measures of reliability (including positive predictive value), and AUC with and without central re-evaluation of duodenal histology are reported in the appendix (p 7).

In the subgroup of 133 participants with complete local data on both DGP-IgG and tTG-IgA, the AUC for prediction of duodenal villous atrophy, as defined by local histology, was the same with use of the categorical

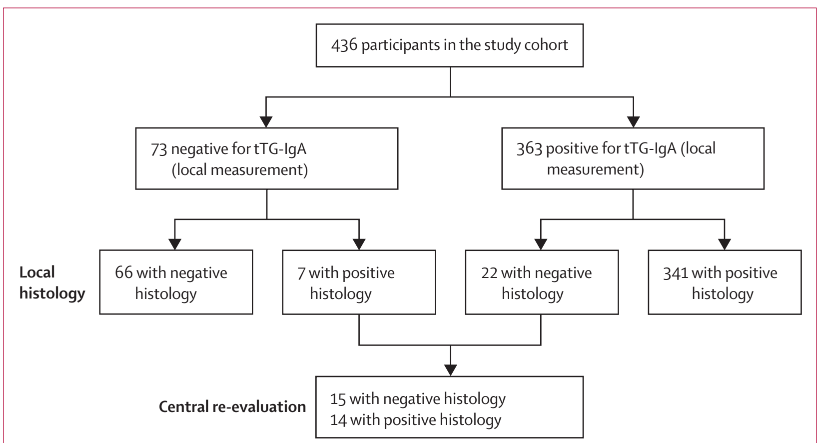

Figure 2: **Characterisation of the study cohort by serum antibodies and histological analyses**
Positivity for serum tTG-IgA was defined as a concentration of greater than 1 times the ULN. Positive histology was defined as the presence of duodenal villous atrophy. tTG-IgA=anti-tissue transglutaminase IgA.

	Locally measured serum tTG-IgA concentration					p value*
	≤1×ULN (normal)	>1 to 5×ULN	>5 to 10×ULN	>10 to 15×ULN	>15×ULN	
Original local definition of duodenal villous atrophy†						
Number of patients	73	109	92	28	134	..
Number with duodenal villous atrophy	7 (10%)	93 (85%)	90 (98%)	27 (96%)	131 (98%)	..
Number without duodenal villous atrophy	66 (90%)	16 (15%)	2 (2%)	1 (4%)	3 (2%)	..
Positive predictive value	9·6% (2·7–16·4)	85·3% (78·9–91·7)	97·8% (94·6–100·0)	96·4% (89·2–100·0)	97·8% (95·5–100·0)	<0·0001
After central re-evaluation of duodenal villous atrophy† in discordant cases						
Number of patients	73	109	92	28	134	..
Number with duodenal villous atrophy	7 (10%)	96 (88%)	91 (99%)	27 (96%)	134 (100%)	..
Number without duodenal villous atrophy	66 (90%)	13 (12%)	1 (1%)	1 (4%)	0	..
Positive predictive value	9·6% (2·7–16·4)	88·1% (81·7–94·5)	98·9% (96·7–100·0)	96·4% (89·2–100·0)	100·0% (98·5–100·0)	<0·0001

Data are n, n (%), or % (95% CI). tTG-IgA=anti-tissue transglutaminase IgA. ULN=upper limit of normal. *For trend along ULN strata by χ^2 test. †Marsh type 3 or Villanacci–Corazza grade B.

Table 2: **Serum tTG-IgA as a predictor of duodenal villous atrophy**

variable of serum DGP-IgG (0·84 [95% CI 0·75–0·94]) and the categorical variable of serum tTG-IgA (0·84 [0·75–0·94]; appendix pp 5, 8). Pairwise comparison of the AUC values indicated similar diagnostic performance of the two serology tests (DeLong p=0·98). Data on the prevalence of positive serum DGP-IgG, measures of reliability, and AUC with and without central histology re-evaluation are reported in the appendix (p 8). In the 105 participants with local data on both EMA-IgA and DGP-IgG, the AUC for prediction of local duodenal villous atrophy was 0·88 (0·81–0·95) for serum EMA-IgA and 0·86 (0·79–0·91) for serum DGP-IgG, and pairwise comparison of these values indicated similar diagnostic performance (DeLong p=0·67).

Given that the high prevalence of duodenal villous atrophy could have biased the results, additional subgroup procedures were designed post hoc to re-run analyses of the receiver operating characteristic curve for local serum tTG-IgA in subgroups with large differences in the prevalence of duodenal villous atrophy. On the basis of the data in table 3, women younger than 65 years, without a family history of coeliac disease, and with classical clinical presentation were selected for the low prevalence subgroup (n=89), and the remaining participants of the study cohort made up the reference subgroup (n=347). As expected, by definition, the two subgroups significantly differed in the prevalence of duodenal villous atrophy when using the local histology data (61 [69%] participants in the low prevalence subgroup *vs* 287 [83%] in the reference subgroup, χ^2 p=0·0029) and after central histology re-evaluation of discordant cases (61 [69%] *vs* 294 [85%], χ^2 p<0·0001). As shown by the 95% CIs, the AUC was larger for the low prevalence subgroup than for the reference group with use of the local definition of duodenal villous atrophy (0·99 [95% CI 0·98–1·00] *vs* 0·90 [0·85–0·95]) and after central re-evaluation of discordant cases (0·99 [0·98–1·00] *vs* 0·95 [0·91–0·97]).

Additional clinically relevant findings from upper endoscopy examination included *Helicobacter pylori*-related peptic gastritis in nine participants, autoimmune atrophic gastritis in three participants, mild reflux oesophagitis (Los Angeles grade A[43]) in 31 participants, Barrett's oesophagus in one participant, benign gastric ulcer in two participants, and benign duodenal ulcer in one participant. These findings were similarly distributed among patients with positive or negative serum tTG-IgA and the presence or absence of duodenal villous atrophy. Regarding possible coeliac disease-related disorders in the year after enrolment, a midgut ileum lymphoma was diagnosed when a 52-year-old woman was admitted to the emergency department for an intestinal obstruction a few weeks before the 1-year follow-up visit. The patient had been maintaining a strict gluten-free diet since diagnosis of coeliac disease at the initial endoscopy. This patient presented with classical symptoms (diarrhoea and weight loss) and had duodenal villous atrophy and high serum tTG-IgA. No other disorders were identified in the 1-year follow-up.

	Number of patients	Number with duodenal villous atrophy (%)	AUC (95% CI)
Original local definition of duodenal villous atrophy*			
Total cohort	436	348 (80%)	0·93 (0·89–0·96)
Sex			
Men	140	123 (88%)	0·94 (0·85–1·00)
Women	296	225 (76%)	0·92 (0·89–0·97)
Age group, years			
18–44	279	222 (80%)	0·92 (0·88–0·97)
45–64	127	100 (79%)	0·94 (0·87–1·00)
≥65	30	26 (87%)	1·00 (1·00–1·00)
Family history of coeliac disease			
Yes	74	63 (85%)	0·96 (0·90–1·00)
No	362	285 (79%)	0·92 (0·89–0·96)
Clinical presentation of coeliac disease			
Classical	156	115 (74%)	0·99 (0·98–1·00)
Non-classical or asymptomatic	280	233 (83%)	0·88 (0·82–0·94)
After central re-evaluation of duodenal villous atrophy* in discordant cases			
Total cohort	436	355 (81%)	0·96 (0·94–0·98)
Sex			
Male	140	124 (89%)	0·98 (0·95–1·00)
Female	296	231 (78%)	0·96 (0·93–0·98)
Age group, years			
18–44	279	228 (82%)	0·96 (0·93–0·99)
45–64	127	101 (80%)	0·97 (0·94–0·99)
≥65	30	26 (87%)	1·00 (1·00–1·00)
Family history of coeliac disease			
Yes	74	63 (85%)	0·96 (0·90–1·00)
No	362	292 (81%)	0·96 (0·94–0·99)
Clinical presentation of coeliac disease			
Classical	156	115 (74%)	0·99 (0·98–1·00)
Non-classical or asymptomatic	280	240 (86%)	0·93 (0·90–0·97)

Analyses with serum tTG-IgA expressed as multiples of the ULN as a numerical variable. AUC=area under the receiver operating characteristic curve. tTG-IgA=anti-tissue transglutaminase IgA. *Marsh type 3 or Villanacci–Corazza grade B.

Table 3: **Subgroup analyses of AUC for local serum tTG-IgA as a predictor of duodenal villous atrophy**

Discussion

This multicentre, international study reports data on the reliability of serum tTG-IgA as a predictor of duodenal villous atrophy in adults with suspected coeliac disease without IgA deficiency. Based on local data, positivity for serum tTG-IgA (>1× ULN) correctly predicted duodenal villous atrophy in 93·9% of participants. Normal serum tTG-IgA concentration was less reliable; duodenal villous atrophy was present in 10% of participants with normal serum tTG-IgA, with a positive predictive value of 9·6%. Results for the subgroups of participants with data on local serum EMA-IgA and DGP-IgG indicated that, overall, AUCs for the different serology tests were similar when concentrations were expressed as a categorical variable (positive or negative). Nevertheless, the negative predictive powers of EMA-IgA and DGP-IgG were lower than that of tTG-IgA, which could reflect several factors, including the observer-dependency of the EMA-IgA test,[44] the lower sample size in subgroup analyses, or differences in the modulation of the immune response against tissue transglutaminase and gliadin. The discordances in the histological evaluation of duodenal biopsy were in agreement with the idea that even at specialist coeliac disease centres, histology can represent a problematic area in the diagnosis of coeliac disease. The 1-year follow-up indicated that no other major complications were detected apart from one case of midgut ileum lymphoma that was not detectable by upper endoscopy at enrolment. Whether the lymphoma was already present or not at the time of endoscopy (and coeliac disease diagnosis) cannot be assessed, given that the evaluation of the intestinal mucosa does not go beyond the duodenum in upper endoscopy.

Study limitations are the absence of data on IgA-deficient participants, the low number of participants in some subgroup analyses, the absence of data for many ethnic groups and on participants from African and North American centres, the limited follow-up information, the limited assessments in the central laboratory, the limited data on EMA-IgA and DGP-IgG, and the high pretest probability (low number of participants without duodenal villous atrophy). The absence of data on IgA-deficient participants precludes the extrapolation of study results to the general population and to the specific subgroup with IgA deficiency (who comprise around 2% of the coeliac disease population[45]). The high pretest probability was the inevitable consequence of our selection criteria, focusing on participants with suspected coeliac disease, and could have increased the positive predictive value. Although post-hoc analyses indicated an AUC for serum tTG-IgA of greater than 0·9 in a subgroup with lower pretest probability, we cannot exclude the possibility that the predictive value could be lower in a wider setting, as reported by others.[23] We cannot exclude the possibility of true false positives and true false negatives in the non-discordant cases, given that their histology was not re-evaluated. Study strengths include the prospective design, the participation of 14 centres from different countries, the availability of data for different serology assays, the availability of central laboratory testing, and the histological re-evaluation of discordant cases.

The present results are in accordance with previous retrospective studies in adults,[23–38] with the guidelines of scientific societies in Finland,[46] Sweden,[47] and the UK,[48] and with a recent clinical study in Scotland.[49] The study has practical implications that are mainly applicable to European centres given the locations of participating centres. The post-hoc finding that serum tTG-IgA could correctly diagnose duodenal villous atrophy in 97·5% of patients with serum tTG-IgA concentration of greater than 10 times the ULN suggests that simplification of the diagnostic work-up is feasible with the use of identical tTG-IgA cutoffs in adults and children, given that the 10 times ULN threshold is recommended in paediatric guidelines to avoid biopsy.[17] However, local validation of the threshold is advised considering the variability of assays and the heterogeneity of patients with coeliac disease. Furthermore, the results indicated that the risk of an inappropriate prescription of a gluten-free diet in people with suspected coeliac disease and serum tTG-IgA of greater than 10 times the ULN but without duodenal villous atrophy was around 2·5%. If IgA status is unknown, clinicians should request a total IgA concentration to reduce the number of false negatives. Regarding the risk of missing diagnoses of other diseases assessable by upper endoscopy, there was little evidence to support this concern in our study and in another recently published study,[50] with benign lesions identified in our study. The risk of missed diagnoses and other hypothetical risks of a non-biopsy strategy should be counterbalanced not only by the elimination of the risks associated with an invasive procedure, but also by the reduced patient burden, economic costs, and time required for diagnosis of coeliac disease. For instance, a study in children estimated that costs could be reduced by 50% if a biopsy were omitted in the diagnosis of coeliac disease,[21] and similar savings are likely to apply in adult practice. An additional practical observation was that tTG-IgA measurements were markedly similar between local assays and central laboratory testing, with high concordance between these results (κ=0·952), suggesting that overall reliability did not differ substantially among various laboratory tests. The reliability of serum tTG-IgA assessment could potentially be improved by developing an international calibrator with a defined concentration of tTG-IgA for assay standardisation. Evidence for the reliability of serum tTG-IgA for coeliac disease diagnosis could be further strengthened by follow-up assessment of tTG-IgA, if such follow-up showed a reduction in tTG-IgA while on a gluten-free diet. However, available data suggest that the reliability of serum tTG-IgA could be limited in patients already on a gluten-free diet.[51,52]

Regarding the histological evaluation, our results were in accordance with the idea that histological diagnosis can vary, even when done by an expert pathologist and in accordance with specific guidelines.[53–56] This view is also supported by the observation that the single participant with very high serum tTG-IgA (>10×ULN) but without duodenal villous atrophy had Marsh type 2 histology. Furthermore, subgroup analyses in participants with positive or negative serum EMA-IgA and DGP-IgG did not indicate substantial differences in predictive power compared with serum tTG-IgA for determining duodenal villous atrophy. However, they might add value in cases of low tTG-IgA concentration and diagnostic uncertainty.

In conclusion, our results indicate that a serology-based coeliac disease diagnosis without biopsy is possible in adults with reliable suspicion of coeliac disease (high pretest probability).

Contributors

CC and FZ conceived and designed the study, analysed the findings, searched the literature, and wrote the manuscript. JCB and GH searched the literature and contributed to writing the manuscript. All authors recruited patients and collected patient data. GLN did the central laboratory analysis and contributed to writing the manuscript. VV re-evaluated discordant histological cases. All authors critically read and intellectually contributed to the manuscript and approved the submitted version. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. CC and FZ accessed and verified the data.

Declaration of interests

GLN is an employee of Werfen. All other authors declare no competing interests.

Data sharing

Data, analytical methods, and study materials are available to other researchers under specific request. The data collected for the study, including anonymous individual participant data and the data dictionary defining each field in the set, will be made available to others for scientific purposes. The data and related documents including analytical methods, and study materials will be available after the publication date on specific request to fabiana.zingone@unipd.it, with a signed data access agreement and restriction of publication without the authors' consent.

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