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Original Article

Diagnostic accuracy of IgA anti-tissue transglutaminase for the diagnosis of coeliac disease

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ABSTRACT

INTRODUCTION. A no-biopsy approach has been suggested for diagnosing coeliac disease (CD) in adult patients. This approach is already well established in diagnosing children with CD. This study aimed to evaluate the accuracy of IgA anti-tissue transglutaminase (IgA anti-tTG) in predicting duodenal mucosal lesions diagnostic of CD in adult patients.

METHODS. We included all patients aged ≥ 18 years referred for CD diagnostics at our department in the period from 1 January 2019 to 31 December 2023 with raised IgA anti-tTG levels and in whom duodenal biopsies had been evaluated for CD-specific lesions. Data regarding IgA anti-tTG levels and duodenal histology evaluated by the modified Marsh classification were retrieved from the patient records.

RESULTS. A total of 235 adult patients had positive IgA anti-tTG levels and an available duodenal histology. High IgA anti-tTG levels (> 10 × upper limit of normal (ULN)) were associated with more severe enteropathy. The PPV of IgA anti-tTG for identifying Marsh ≥ 2 or 3 lesions increased when the serological cut-off was raised. The positive predictive value of IgA anti-tTG > 10 × ULN was 99.2% (95% CI: 95.8-100%) and 97.7% (95% CI: 93.4-99.5%) for predicting Marsh ≥ 2 and 3 lesions, respectively.

CONCLUSIONS. This study confirms that high titers of IgA anti-tTG may accurately identify adults with diagnostic duodenal mucosal lesions associated with CD. Our data support the use of a no-biopsy approach for diagnosing CD in adults with high IgA anti-tTG titers.

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Coeliac disease (CD) is a chronic small-intestinal, immune-mediated enteropathy caused by a complex immune response to gluten proteins present in wheat, rye and barley in genetically susceptible individuals. The prevalence of CD has increased over time and is approximately 1% of the population, yet many remain undiagnosed. The only efficient treatment for CD is a life-long gluten-free diet (GFD) [1].

Since the identification of tissue transglutaminase as a prominent autoantigen in CD, serum IgA anti-tissue transglutaminase antibody (IgA anti-tTG) has been used as a screening test in patients with suspected CD [1]. Demonstrating characteristic histopathological changes, such as intraepithelial lymphocytosis, crypt hyperplasia and villous atrophy in duodenal biopsies, is regarded as the gold standard in the diagnosis of CD in adults, and current guidelines recommend duodenal biopsies to confirm the diagnosis in adults irrespective of

even high-titer serology [2, 3]. However, duodenal biopsies can be challenging to interpret, and the histological abnormalities may be patchy and are not necessarily specific to CD. [4]. Endoscopy and biopsy sampling are costly and unpleasant for the patients. For these reasons, a non-invasive diagnostic approach for CD is highly desirable.

Mounting evidence demonstrates the high accuracy of IgA anti-tTG in diagnosing CD in adults [5, 6]. However, strict adherence to a life-long GFD can be challenging and negatively impact quality of life, making an accurate diagnosis of CD crucial. Therefore, additional research on the reliability of IgA anti-tTG in diagnosing CD is warranted.

This study aimed to assess the accuracy of IgA anti-tTG in predicting duodenal mucosal lesions diagnostic of CD in adult patients.

Methods

Study population

The study comprised a retrospective cohort established at the Department of Hepatology and Gastroenterology, Aarhus University Hospital, Denmark. The International Classification of Diseases, tenth version (ICD-10) code for CD (DK900) was used to identify patients with CD in the registries of the department. Patients were included if they had been referred for CD diagnostics in the period from 1 January 2019 to 31 December 2023. All included patients were 18 years or older at diagnosis and had a positive serum IgA anti-tTG. Patients had undergone the routine diagnostic workup for CD at our department. Patients had been referred on suspicion of CD by either primary care physicians, other hospital clinics or following demonstration of iron deficiency in the Danish Blood Donor Study [7]. The diagnosis of CD was made by the department's gastroenterologists, considering both serology, clinical presentation and small intestinal pathology according to clinical guidelines [2, 3].

Electronic patient records were used to obtain information on age, gender, symptoms, duodenal histology and biochemical data.

Serology

The serum IgA anti-tTG level was measured within four months before upper endoscopy. If upper endoscopy was performed first, the delay between obtaining biopsies and the blood sample was less than 30 days. For patients with more than one Iga anti-tTG test, the anti-tTG level closest to the upper endoscopy was considered.

The IgA anti-tTG level was measured by fluorescence enzyme immunoassay using human recombinant tissue transglutaminase, the ELiA Celikey IgA assay, on the Phadia 250 analyzer (Thermo Fischer, Freiburg, Germany). The results were expressed in U/ml. According to the manufacturer's guidelines, the upper limit of normal (ULN) for IgA anti-tTG is > 7 U/ml. A value higher than the ULN was considered a positive IgA anti-tTG test result. The upper limit for the assay is 128 U/ml. The same assay was used during the entire inclusion period.

Duodenal biopsies and histological evaluation

Each patient had at least four biopsies sampled from the duodenum. Patients already on a GFD had to undergo six to eight weeks of consistent gluten intake before upper endoscopy. These patients had an IgA anti-tTG measurement at the same time point as their upper endoscopy.

The modified Marsh classification system (Marsh-Oberhuber) [8] was used to grade mucosal lesions: Type 0: normal mucosa. Type 1: intraepithelial lymphocytosis, no crypt hyperplasia, no villous atrophy. Type 2: intraepithelial lymphocytosis, crypt hyperplasia, no villous atrophy. Type 3a: intraepithelial lymphocytosis, crypt hyperplasia, partial villous atrophy. Type 3b: intraepithelial lymphocytosis, crypt hyperplasia, subtotal

villous atrophy. Type 3c: intraepithelial lymphocytosis, crypt hyperplasia, total villous atrophy.

Statistical analysis

LibreOffice version 7.4 (2022) was used to calculate the positive predictive value (PPV) and the corresponding 95% CI. GraphPad Prism V.10 was used for graph construction and statistical analysis. Data without a normal distribution were presented as the median with the interquartile range. Categorical variables were presented as counts and percentage prevalence. To compare groups with categorical variables, the χ^2 test or Fisher's exact test was used. The Kruskal-Wallis and post hoc Dunn tests were performed to compare the Marsh score with the IgA anti-tTG level. A $p < 0.05$ was considered statistically significant. The concentration of IgA anti-tTG was expressed as median levels (U/ml) or as a multiple of ULN.

Ethics

The study was approved by the Aarhus University Hospital Institutional Review Board and did not require formal ethical approval because it was considered a quality-improvement study.

Trial registration: not relevant.

Results

Patient characteristics

A total of 302 patients were identified. Among these, 67 patients were excluded from analysis due to lacking consent for gastroscopy (n = 36); IgA deficiency (n = 9); start of GFD before the time of referral (n = 7); duodenal biopsies disqualified by the pathologist (n = 6); diagnosis of seronegative CD (n = 6); or because they received immunotherapy (n = 3). Overall, 235 patients had complete data on duodenal histology and IgA anti-tTG level and were included in the analysis. Data regarding gender distribution, age at diagnosis, symptoms and March classification are presented in **Table 1**.

TABLE 1 Demographic, clinical, biochemical and histological details of the cohort.

Gender, n (%)	
Men	64 (27.2)
Female	171 (72.8)
Total cohort	235
Age at diagnosis, median (IQR), yrs	29 (23-44)
Symptoms, n (%)	
Diarrhoea	110 (48.7)
Constipation	40 (17.7)
Weight loss	39 (17.3)
Bloating	126 (55.8)
No symptoms	42 (18.6)
Marsh-Oberhuber Classification, n (%)	
0	9 (3.8)
1	8 (3.4)
2	10 (4.3)
3a	57 (24.3)
3b	80 (34.0)
3c	71 (30.2)
IgA anti-tTG, n (%)	
< 10 × ULN	106 (45.1)
≥ 10 × ULN	129 (54.9)

anti-tTG = anti-tissue transglutaminase antibody; IQR = interquartile range; ULN = upper limit of normal.

A total of 226 patients had given information on symptoms, and 184 (81.4%) presented with at least one symptom. The most frequent symptom of CD was the experience of bloating, which 126 (55.8%) patients reported.

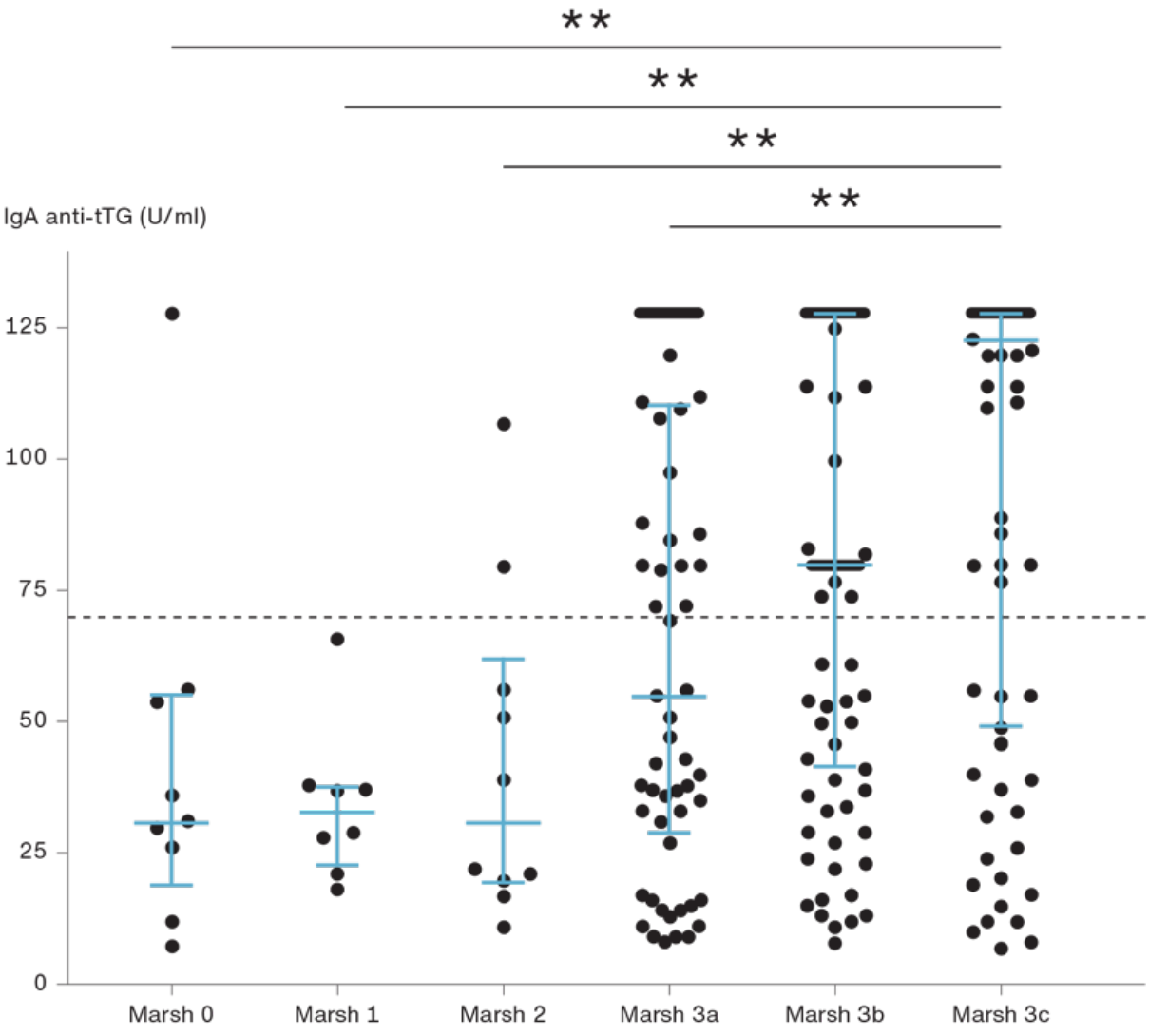
Among the 235 patients included, duodenal villous atrophy (Marsh type 3) was present in 208 (88.5%). Nine (3.8%) patients had a normal histology, 8 (3.4%) Marsh 1- and ten (4.3%) Marsh 2 lesions.

No association was found between different Marsh scores and either gender, symptoms or age. Gender had no relation to the presence of symptoms or age at diagnosis. There was no correlation between age at diagnosis and anti-tTG titer.

Median anti-tissue transglutaminase antibody level at various degrees of enteropathy

A wide variation was observed in IgA anti-tTG levels within groups of different degrees of enteropathy. The median IgA anti-tTG level for patients with a normal histology, Marsh type 1, 2, 3a, 3b, and 3c, were 31.0, 33.0, 30.5, 55.0, 80.0, and 123.0 U/ml, respectively. Severe Marsh scores were also observed in patients with low levels of IgA anti-tTG (**Figure 1**).

FIGURE 1 With increasing duodenal inflammation, graded using Marsh-Oberhuber classification, elevated anti-transglutaminase IgA titer was inc reasingly predictive of high Marsh score. Blue bars and whiskers represent the median with interquartile range. The black dotted line represents 10 × the upper limit of normal (70 U/ml). The comparison between groups was made with Kruskal-Wallis and the post hoc Dunn test.



**) p < 0.01.
anti-tTG = anti-tissue transglutaminase antibody.

Differences in the IgA anti-tTG levels were compared between different Marsh scores. The results showed significantly higher levels of IgA anti-tTG in patients with Marsh 3c lesions than in patients with either Marsh 0 ($p < 0.01$), 1 ($p < 0.01$), 2 ($p < 0.01$), or 3a ($p < 0.01$) lesions.

Only one patient with an anti-tTG level > 10 × ULN had normal duodenal biopsies. This patient was referred due to years of abdominal pain, irregular bowel habits and weight loss. The diagnosis of CD in this patient was based on the high IgA anti-tTG level, gastrointestinal symptoms, positive human leukocyte antigen (HLA)-DQ2 and response to a GFD with normalisation of the IgA anti-tTG level and resolution of symptoms.

Positive predictive value of different IgA anti-tissue transglutaminase antibody thresholds for identifying Marsh type ≥ 2 or Marsh type 3

The PPV for identifying patients, irrespective of symptoms, with Marsh ≥ 2 or Marsh 3 lesions increased with higher levels of IgA anti-tTG (**Table 2**). The PPV of different IgA anti-tTG thresholds for identifying Marsh type 3 and Marsh type ≥ 2, respectively, is presented in Table 2.

TABLE 2 The accuracy of different IgA IgA anti-tissue transglutaminase antibody thresholds for predicting duodenal mucosal lesions.

	Measured IgA anti-tTG concentration			
	> 1 × ULN	> 5 × ULN	> 7 × ULN	> 10 × ULN
IgA anti-tTG as a predictor of Marsh type 3				
Patients, n	235	174	149	129
Marsh type 3, n (%)	208 (89)	161 (93)	141 (95)	126 (98)
Positive predictive value (95% CI), %	88.5 (83.7-92.3)	92.5 (87.6-96.0)	94.6 (90.0-97.7)	97.7 (93.4-99.5)
IgA anti-tTG as a predictor of Marsh type ≥ 2				
Patients, n	235	174	149	129
Marsh type ≥ 2, n (%)	218 (93)	166 (95)	145 (97)	128 (99)
Positive predictive value (95% CI), %	92.8 (88.7-95.7)	95.4 (91.1-98.0)	97.3 (93.3-99.3)	99.2 (95.8-100)

anti-tTG = anti-tissue transglutaminase antibody; ULN = upper limit of normal.

Effect of symptoms on the positive predictive value of IgA anti-tissue transglutaminase antibody

The PPV of serology for different thresholds was determined depending on different symptoms (bloating, diarrhoea, constipation, weight loss) (**Table 3**). No significant difference in Marsh score was found when comparing symptomatic patients with asymptomatic patients at different anti-tTG titers (> 1 ×, > 3 ×, > 5 ×, > 7 ×, > 10 × ULN).

TABLE 3 Effect of clinical presentation on the positive predictive value of IgA anti-tissue transglutaminase antibody for diagnosing biopsy-proven coeliac disease (Marsh type ≥ 2).

	Patients presenting any symptom (N = 184)			Asymptomatic patients (N = 42)		
IgA anti-tTG concentration	Marsh type ≤ 1, n	Marsh type ≥ 2, n	PPV, %	Marsh type ≤ 1, n	Marsh type ≥ 2, n	PPV, %
> 1 × ULN	14	170	92.4	3	39	92.9
> 3 × ULN	11	139	92.7	2	37	94.9
> 5 × ULN	7	125	94.7	1	33	97.1
> 7 × ULN	4	106	96.4	0	32	100
> 10 × ULN	1	92	98.9	0	29	100

anti-tTG = anti-tissue transglutaminase antibody; PPV = positive predictive value; ULN = upper limit of normal.

Discussion

In this study, investigating the diagnostic accuracy of IgA anti-tTG in diagnosing CD in adult patients, we found a

very high PPV of IgA anti-tTG level > 10 × ULN of 97.7% and 99.2% for identifying patients with Marsh 3- and Marsh ≥ 2 lesions, respectively. If a threshold value of > 10 × ULN had been implemented as the only diagnostic criterion for CD in the included patients, upper endoscopy with duodenal sampling could have been omitted in 55% of the patients.

Our finding of high PPV of IgA anti-tTG > 10 × ULN aligns with the results of other retrospective studies that have evaluated the performance of IgA anti-tTG in diagnosing CD [9-14]. Using the same assay as in this study, Hill and Holmes demonstrated a PPV of 100% for diagnosing CD when IgA anti-tTG was elevated > 8 × ULN and reported a clear association between the degree of mucosal damage and anti-tTG level [11]. Another study showed that a diagnostic cut-off at > 5 × ULN was highly accurate for predicting duodenal villous atrophy with 100% specificity, demonstrating no false positive cases at this cut-off [12].

Most patients in the present study had a moderate to high suspicion of CD because, besides elevated IgA anti-tTG levels, they also experienced symptoms suggestive of CD, had a first-degree relative with CD or demonstrated biochemical signs of malabsorption such as iron deficiency. This probably explains why most of the patients presented with severe enteropathy in the form of Marsh 3 lesions. This might result in a high referral bias, potentially increasing the PPV. Hence, our results only support the use of a no-biopsy approach for diagnosing CD in patients who have been referred for evaluation based on a moderate to high suspicion of CD. A study demonstrated that the predictive value of the no-biopsy approach varies with the prevalence of biopsy-proven CD, showing a higher PPV in populations with a greater CD prevalence [5]. However, others have shown that high IgA anti-tTG levels can accurately diagnose CD even in low-prevalence populations [9, 14].

Patients in our cohort showed a broad spectrum of gastrointestinal symptoms. The most frequently reported symptoms were bloating and diarrhoea, experienced by 55.8% and 48.7% of the patients, respectively. Only a small percentage of patients (17.7%) reported weight loss. The prevalence of symptoms aligns with findings from a previous study [15].

Interestingly, 18.6% of the patients reported no symptoms at all. The variability in symptom presentation underscores that CD should also be considered in patients with atypical gastrointestinal complaints and those who are asymptomatic but at risk.

We found no increase in the diagnostic accuracy of IgA anti-tTG when comparing symptomatic with asymptomatic patients. This underlines that the absence of symptoms does not exclude CD.

In this study, only one type of assay for measuring IgA anti-tTG level was used. Variations between assays occur and could be problematic in standardising IgA anti-tTG test results. Efthymakis et al. [16] demonstrated that assays from different manufacturers had intrinsic differences in performance, as the PPV peaked differently, and optimal cut-off values varied. Due to the different assay methods, it has been recommended that the determination of a threshold value should be validated locally [13].

Important limitations apply to our study. Firstly, our study was retrospective, implying that pathologists were not blinded to the serological or clinical status of the patient. Among pathologists, there is interobserver variability when using the modified Marsh classification [17]. Thus, the histological diagnosis can vary even between expert pathologists. Secondly, time points for IgA anti-tTG measurement and obtaining biopsies varied. Between the time points, some patients may have reduced their gluten intake due to the recognised risk of potentially having CD. If so, the histopathological lesions could have improved in the waiting time for histology sampling, resulting in an underestimation of the PPV of anti-tTG.

A concern with the no-biopsy approach is the risk of missing conditions that could be detected by upper endoscopy. Previous studies reported no significant non-coeliac pathology at upper endoscopy and concluded

that it would be safe to omit [10]. A study reported that no malignancies or premalignant lesions were identified at upper endoscopy in 1,328 adult patients with suspicion of CD [18]. In the present study, most patients were young with a median age of 29 years, and we suggest that aberrant clinical courses, particularly in patients older than 50 years, should lead to more extensive diagnostics.

Importantly, the no-biopsy approach should be implemented only within a gastroenterology unit and is not recommended in primary care, even when high IgA anti-tTG levels are present. Duodenal biopsy remains necessary in cases with lower antibody levels or when alternative diagnoses are considered. Additionally, a dietitian should guide the initiation of a GFD, and follow-up in a gastroenterology unit is essential to assess dietary adherence and clinical response.

CONCLUSIONS

Our study supports a no-biopsy approach for diagnosing CD in adults presenting with high levels of IgA anti-tTG. Omitting upper endoscopy in selected patients could reduce the overall costs associated with the diagnosis of CD, reduce the diagnostic delay and avoid unnecessary patient discomfort [19].

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REFERENCES

1. Catassi C, Verdu EF, Bai JC, Lionetti E. Coeliac disease. Lancet. 2022;399(10344):2413-2426. https://doi.org/10.1016/S0140-6736(22)00794-2
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):1210-1228. https://doi.org/10.1136/gutjnl-2013-306578
3. Rubio-Tapia A, Hill ID, Semrad C, et al. American College of Gastroenterology guidelines update: diagnosis and management of celiac disease. Am J Gastroenterol. 2023;118(1):59-76. https://doi.org/10.14309/ajg.0000000000002075
4. Picarelli A, Borghini R, Donato G, et al. Weaknesses of histological analysis in celiac disease diagnosis: new possible scenarios. Scand J Gastroenterol. 2014;49(11):1318-1324. https://doi.org/10.3109/00365521.2014.948052
5. Shiha MG, Nandi N, Raju SA, et al. Accuracy of the no-biopsy approach for the diagnosis of celiac disease in adults: a systematic review and meta-analysis. Gastroenterology. 2024;166(4):620-630. https://doi.org/10.1053/j.gastro.2023.12.023
6. Ciacci C, Bai JC, Holmes G, et al. 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. Lancet Gastroenterol Hepatol. 2023;8(11):1005-1014. https://doi.org/10.1016/S2468-1253(23)00205-4
7. Erikstrup C, Sorensen E, Nielsen KR, et al. Cohort profile: The Danish blood donor study. Int J Epidemiol. 2023;52(3):e162-e171. https://doi.org/10.1093/ije/dyac194

8. 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):1185-1194. https://doi.org/10.1097/00042737-199910000-00019
9. Penny HA, Raju SA, Lau MS, et al. Accuracy of a no-biopsy approach for the diagnosis of coeliac disease across different adult cohorts. Gut. 2021;70(5):876-883. https://doi.org/10.1136/gutjnl-2020-320913
10. Hoyle A, Gillett P, Gillett HR, et al. No-biopsy strategy for coeliac disease is applicable in adult patients: a 'real-world' Scottish experience. Frontline Gastroenterol. 2023;14(2):97-102. https://doi.org/10.1136/flgastro-2022-102254
11. Holmes GKT, Forsyth JM, Knowles S, et al. Coeliac disease: further evidence that biopsy is not always necessary for diagnosis. Eur J Gastroenterol Hepatol. 2017;29(6):640-645. https://doi.org/10.1097/MEG.0000000000000841
12. Zanini B, Magni A, Caselani F, et al. High tissue-transglutaminase antibody level predicts small intestinal villous atrophy in adult patients at high risk of celiac disease. Dig Liver Dis. 2012;44(4):280-285. https://doi.org/10.1016/j.dld.2011.10.013
13. Beltran L, Koenig M, Egner W, et al. High-titre circulating tissue transglutaminase-2 antibodies predict small bowel villous atrophy, but decision cut-off limits must be locally validated. Clin Exp Immunol. 2014;176(2):190-198. https://doi.org/10.1111/cei.12249
14. Fuchs V, Kurppa K, Huhtala H, et al. Serology-based criteria for adult coeliac disease have excellent accuracy across the range of pre-test probabilities. Aliment Pharmacol Ther. 2019;49(3):277-284. https://doi.org/10.1111/apt.15109
15. Schøsler L, Christensen LA, Hvas CL. Symptoms and findings in adult-onset celiac disease in a historical Danish patient cohort. Scand J Gastroenterol. 2016;51(3):288-294. https://doi.org/10.3109/00365521.2015.1092576
16. Efthymakis K, Serio M, Milano A, et al. Application of the biopsy-sparing ESPGHAN guidelines for celiac disease diagnosis in adults: a real-life study. Dig Dis Sci. 2017;62(9):2433-2439. https://doi.org/10.1007/s10620-017-4672-1
17. Corazza GR, Villanacci V, Zambelli C, et al. Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease. Clin Gastroenterol Hepatol. 2007;5(7):838-843. https://doi.org/10.1016/j.cgh.2007.03.019
18. Stefanolo JP, Zingone F, Gizzi C, et al. Upper gastrointestinal endoscopic findings in celiac disease at diagnosis: a multicenter international retrospective study. World J Gastroenterol. 2022;28(43):6157-6167. https://doi.org/10.3748/wjg.v28.i43.6157
19. Shiha MG, Nandi N, Hutchinson AJ, et al. Cost-benefits and environmental impact of the no-biopsy approach for the diagnosis of coeliac disease in adults. Frontline Gastroenterol. 2023;15(2):95-98. https://doi.org/10.1136/flgastro-2023-102494

