This article has been accepted for publication in FLGASTRO following peer review.
The definitive copyedited, typeset version is available online at
10.1136/flgastro-2025-103344

Anti-endomysial antibody detection is of no benefit as an adjunct to IgA anti tissue transglutaminase antibodies in the serological diagnosis of coeliac disease.

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Abstract

Objective

The anti-endomysial antibody immunofluorescence test (EMA) is recommended by some clinical guidelines as an adjunct to IgA anti-tissue transglutaminase antibody (TTG) in coeliac disease (CD) diagnosis. However, dual testing can complicate interpretation. We assessed the diagnostic value of EMA alongside TTG.

Method

We retrospectively analysed patients tested for TTG and EMA between 2018-2019 at a single laboratory. 587 patients were categorised into two groups: TTG 7-10 U/mL ('borderline') and TTG > 10 U/mL. CD was confirmed by duodenal biopsy; if unavailable, TTG ≥ 100 U/mL (10x upper limit of normal) was diagnostic, serologically.

Results

Biopsies were performed in all borderline cases (n=41). Among 23 EMA-negative patients, 17 (74%) had biopsy-confirmed CD. 12 of 18 (67%) EMA-positive patients had CD. EMA showed poor sensitivity (0.4138) and specificity (0.5000). Fisher's exact test found no association between EMA and biopsy results (p=0.7341), indicating EMA's limited diagnostic value in borderline cases.

In the TTG >10 U/mL group (n=546), 523 were EMA-positive (511 with CD, 98%). Of 23 EMA-negative patients, 14 (61%) had CD. Despite a statistically significant association with CD (p<0.0001), EMA added minimal clinical value (sensitivity 0.9733; specificity 0.4286).

Conclusion

EMA had low sensitivity in borderline cases and added no diagnostic value when TTG was positive. We recommend discontinuing EMA testing in clinical practice. The high rate of CD in borderline cases suggests current TTG diagnostic thresholds need reconsideration. Prospective, multi-centre replication using standardised assays is required to evaluate whether biopsies are warranted at lower TTG values than currently recommended.

What is already known on this topic

TTG is widely accepted as the primary serological test used to screen for CD due to its high sensitivity and specificity. Some clinical guidelines recommend the use of EMA testing alongside TTG, particularly in borderline cases. However, the interpretation of discordant TTG and EMA results can be clinically challenging, especially as the test characteristics for TTG depend on the assay platform used. There is limited evidence on the diagnostic value EMA adds when TTG is already positive.

What this study adds

This study demonstrates that EMA testing offers minimal diagnostic value in both borderline and positive TTG cases. In the borderline group, EMA had no significant association with biopsy-confirmed CD. In the positive TTG group, although EMA was statistically associated with CD, it added no clinical utility beyond TTG alone. This is one of few studies to quantify EMA's performance specifically in borderline TTG cases.

How this study might affect research, practice or policy

These findings suggest that the routine use of EMA in all diagnostic scenarios may warrant reconsideration. Our study indicates that EMA may be unnecessary in those with borderline TTG levels. Limiting the use of EMA to this borderline subgroup would reduce costs, simplify decision making and streamline the diagnostic pathway for CD. Additionally, redefining TTG positivity to include the borderline range may prompt earlier biopsy and diagnosis. Future research should focus on multi-centre validation of these findings across multiple assay platforms to inform current diagnostic guidance.

Introduction

Coeliac disease (CD) is a chronic gluten-sensitive enteropathy. It is characterised by histological changes in the small intestine, notably villous atrophy and the presence of chronic inflammatory epithelial lymphocytes (IELs) in the lamina propria and epithelium. Continuous exposure to gluten damages the intestinal mucosa resulting in its clinical symptoms: most typically, diarrhoea, abdominal pain and in children, failure to thrive. It is important that individuals with CD are diagnosed in a timely manner and accurately to reduce the risk of complications from the untreated disease. These complications include osteoporosis [1]and malignancies, such as enteropathy-associated intestinal T cell lymphoma (EATL)[2]. Similarly, risks of inappropriately diagnosing CD in its absence include the increased cost of a gluten free diet, negative psychosocial implications around eating and increased health anxiety.

Present guidelines (NICE, NG20) recommend a two-step algorithm for diagnosing CD[3]. Total immunoglobulin A (IgA) and IgA tissue transglutaminase (TTG) antibodies are tested on first presentation of symptoms. However, if IgA TTG is weakly positive, IgA endomysial antibodies (EMA) should be tested. If positive, individuals are then subjected to an endoscopic intestinal biopsy, the gold-standard method of diagnosis of CD [4]. However, a no-biopsy approach has been adopted by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[5], whereby children are diagnosed with CD if they have an IgA TTG ≥ 10 times the upper limit of normal (ULN) and a positive EMA on a separate blood sample. Negative HLA-DQ2/8 markers are indicative of a very low risk of CD.

In line with this shift to the no-biopsy approach, recent studies have explored whether this would be applicable to adults. A Finnish study demonstrated that a "triple criteria" of TTG ≥ 10x ULN, positive EMA and HLA DQ2/DQ8 genotype had a positive predictive value (PPV) of

100% for the serological diagnosis of CD, which was histologically confirmed regardless of pre-test probability [6]. Their findings suggest that 33% of individuals could have avoided an unnecessary invasive biopsy. Other paediatric studies [7,8] reported similar findings, supporting the no-biopsy approach by questioning the diagnostic reliability of duodenal biopsies due to false negatives, suboptimal practice [9]and patchy lesions [10].

Similarly, Hoyle et al (2023) [11]validated this no-biopsy approach in Scotland, showing a PPV of 99.37% when using TTG ≥ 10x ULN alone to predict CD. Another UK based study found that TTG ≥ 10x ULN had a 100% specificity at detecting Marsh 3 lesions using different assays [12]. An adult multicentre study [13] found that CD diagnostic accuracy improved with increasing TTG levels above the ULN, suggesting that biopsy could be avoided in adults with high pre-test probability and elevated TTG titres. This supports the British Society of Gastroenterology (BSG) interim guidance during the COVID-19 pandemic, which permitted a no-biopsy diagnosis in adults > 15 years if TTG ≥ 10x ULN[14]. Whilst current NICE and BSG guidance (non-COVID) still require a biopsy for confirmation, these recent studies challenge that position and clinical pathways need to be updated and reviewed.

Despite these developments regarding the non-biopsy approach, and predictive strength of TTG ≥ 10x ULN, in which our study followed, EMA testing remains a matter of clinical debate. EMA is regarded by some as the superior serological test due to its high specificity in adults [5,15–17]. However, the test is expensive, labour intensive and requires specialised expertise as it uses immunofluorescence with monkey oesophagus or human umbilical cord [18]. Penny and colleagues [12] further highlighted that the reliance on EMA may undermine the feasibility of the no-biopsy strategy. Other serological tests, including IgG anti-TTG and anti-DGP do not have these same limitations. They are cheaper and are possibly more objective. Therefore, whilst TTG ≥ 10x ULN is a strong sole indicator of CD, the overall optimal serological strategy to diagnose CD is yet to be determined.

This study aimed to evaluate the role of EMA testing as an adjunct to TTG in the serological diagnosis of CD, particularly in patients with a borderline or positive TTG result. It also aimed to assess the diagnostic performance of anti-TTG antibodies at the lower end of the diagnostic range.

Methods

We collected the results of 929 patients with both their TTG and EMA tested between January 2018 and December 2019 through a single laboratory at Addenbrooke's Hospital, Cambridge (Figure 1). Both tests were performed on the same blood sample. Of the 929 patients tested 328 were excluded from the primary diagnostic accuracy analysis because: (i) it was not possible to determine whether they were newly diagnosed at the time of their bloods being tested; (ii) insufficient information as Addenbrooke's is a regional centre for other local hospitals and General Practices; (iii) diagnosis made by another laboratory; (iv)

TTG <7 U/ml. 14 patients with TTG 7-10 U/mL were excluded post-hoc as they lacked a duodenal biopsy and were later identified as either IgA deficient or non-coeliac – IgG TTG and IgG EMA methods were not used in this study. No analyses were performed on these cases and their exclusion is acknowledged in the study limitations.

This retrospective observational cohort study included 587 patients (63.2%) in the primary analysis of whom all received the TTG and EMA index tests. Diagnosis of CD was confirmed by TTG > 100 U/mL, consistent with the BSG interim guidance of 10 x upper limit of normal (ULN) [19,20] or histological confirmation on duodenal biopsy. The diagnosis of coeliac disease was based on standard histological Marsh criteria (villous atrophy, crypt hyperplasia, increased lamina propria cellularity and intraepithelial lymphocytosis) in conjunction with clinical criteria. This criteria included symptoms and family history for cases classified as Marsh grades 1 and 2. Index tests and reference standard of biopsy were typically performed within a short time frame (< 3 months) as part of the initial diagnostic pathway. Due to the retrospective nature of this study, laboratory personnel were unaware of the clinical data or biopsy results when processing the tests. However, knowledge of the serology helped inform the clinical interpretation as per real world practice.

Serology

TTG was measured by EliA Celikey TTG IgA FEIA (Phadia AB, Thermofisher Scientific, Uppsala, Sweden) kit and platform, using human recombinant TTG as antigen: Manufacturer interpretation of results are as follows: Negative < 7 EliA U/mL, Equivocal 7-10 EliA U/mL and Positive > 10 EliA U/mL. We considered the equivocal 7-10 EliA U/mL as 'borderline' on our assay platform and serum samples with antibody titre > 10 U/mL were positive. 10 U/mL is established by the manufacturer as the ULN. EMA tests were carried out in our laboratory by trained operators who used immunofluorescence to identify anti-endomysial antibodies on the NOVALite monkey oesophagus (INOVA diagnostics, Werfen, Spain). The results are reported as negative, weak positive, positive or strong positive based on the intensity (brightness) of fluorescence relative to internal quality control samples. A positive EMA in this study encompassed weak positive, positive and strong positive.

Statistical Analysis

Data analysis for serological accuracy was performed using standard techniques including the Fisher's exact test on GraphPad Prism (version 10.6.1; GraphPad Software, 2023). No patient-identifiable data were collected for this analysis, and formal ethical approval was deemed unnecessary as it was a review of clinical practice.

Results

Sample Characteristics/Patient Demographics

929 patients were found on the Addenbrooke's database, of whom 587 were eligible to be included in the primary analysis. 554 of these 587 individuals were diagnosed with CD

(94.4%). Of those with CD, a total of 173 participants (31.2%) were < 18 years of age at diagnosis. Across all patients with CD, the mean age of diagnosis was 33.9 years, range 1-90 years and 118 patients (21.3%) were aged 55 years or over.

We collected data from 929 individuals and excluded 328 participants. 9 patients were deemed to not have enough information as they had a TTG > 10 U/mL but < 100 U/mL, with no supporting biopsy or clinical information to formally postulate a diagnosis.

Borderline TTG Range

All individuals in the TTG 7-10 U/mL group were subjected to biopsy (n = 41). Of these patients, 29 (70.7%) were diagnosed with CD. This included 17 of 23 individuals with a negative EMA and 12 of 18 with a positive EMA, suggesting a potentially high rate of true positivity (Figure 2). In this borderline range, EMA had a low sensitivity and specificity of 0.4138 (95% CI: 0.2551-0.5926) and 0.500 (95% CI: 0.2538-0.7462) respectively.

A Fisher's exact test showed no statistically significant association between EMA result and biopsy outcome (p = 0.7341), hypothesising that EMA offers limited diagnostic value in this TTG range. The odds ratio of 0.7059 (95% CI: 0.2081-2.437) implies a weak relationship between EMA positivity and CD diagnosis. A PPV of 0.6667 (95% CI: 0.4375-0.8372) was found. Further, there is a negative predictive value (NPV) of EMA of 0.2609 (95% CI: 0.1255-0.4647). This is suggestive that a negative EMA in the borderline range may fail to rule out CD.

TTG > 10 U/mL

In this group (n=546), 14 of the 23 who were negative for EMA had CD (60.9%), and 511 of 523 had CD (98%) in those who were EMA positive (Figure 3a). CD was confirmed in 317 patients by duodenal biopsy and assumed by TTG ≥ 100 U/mL in 208 patients. EMA in this positive TTG titre found a high sensitivity of 0.9733 (95% CI: 0.9557-0.9840) and PPV of 0.9771 (95% CI: 0.9603-0.9868). These values are greater than those observed in the borderline TTG group, indicating that EMA is more diagnostically accurate when TTG is elevated. However, there was low specificity and NPV of 0.4286 (95% CI: 0.2447-0.6345) and 0.3913 (95% CI: 0.2216-0.5921), respectively. All patients with a positive and strongly positive EMA were diagnosed with CD (Figure 3b). Amongst patients with TTG > 10 U/mL, EMA showed a statistically significant relationship with CD diagnosis ($p<0.0001$). Notably, 523 of the 546 patients in this cohort were EMA positive (95.8%, 95% CI: 93.9%-97.2%). This high concordance between EMA and TTG suggests that EMA is superfluous when TTG is already positive.

Across all cohorts with a negative EMA, the highest TTG recorded was 47 U/mL, in a patient who was diagnosed with CD on duodenal biopsy. There was a large range of TTG values in patients with a weak positive and positive EMA from 7 U/mL to >128 U/mL.

Discussion

There is considerable debate in the literature regarding the relative efficacy of different serological tests in the diagnosis of CD. Because various assays are available for detecting TTG, each with its own performance characteristics, there are no clear guidelines for interpreting borderline positive TTG results[21].

In the borderline TTG group, our findings indicate that EMA had limited additional diagnostic value, with an odds ratio of <1. Reliance on EMA in such cases could lead to significant underdiagnosis of CD [22], potentially exposing patients to further complications [23–25]. This will not only will have a significant impact on patient's overall health and quality of life [23], but will also pose a significant financial burden to the NHS due to increased healthcare use. Additional studies highlight the limited utility of EMA due to its low sensitivity [26,27] and high variability across different centres [28] and its lack of meaningful contribution to diagnosis [29]. Moreover, evidence from cases of EMA-negative CD further demonstrates its inferior sensitivity compared to the TTG test [30,31].

A meta-analysis by Sheppard et al. (2022)[32] reported that EMA had a specificity of 99.6%, while TTG demonstrated high sensitivity for diagnosing CD, 90.7% in adults and 97.7% in children. This suggests that EMA is a reliable indicator for CD when positive. However, it should be noted that EMA was tested across all TTG levels rather than being evaluated specifically within the borderline range. Consequently, its utility at specific TTG values remains unclear. Furthermore, there is insufficient evidence to support EMA as an add-on test for borderline TTG values, despite its current recommendation in NICE NG20 guidelines. Variability in clinical presentation and biopsy indications further complicates interpretation, as the prevalence of CD varied across the studies analysed. Given these limitations, we advocate for a duodenal biopsy when clinical suspicion of CD is high, even in cases with negative TTG[15].

In our cohort EMA performed better as a diagnostic test in TTG positive individuals than in those with borderline TTG levels. However, under NICE guidelines, these TTG positive individuals undergo duodenal biopsy regardless of EMA result, unless they meet the criteria for the no-biopsy approach[33]. The predictive value of TTG ≥ 10× ULN has gained increasing recognition, particularly following the COVID-19 pandemic when the BSG recommended treating patients < 55 years of age with clinical symptoms and TTG ≥ 10× ULN without requiring a biopsy[34]. Additionally, evidence suggests that at lower TTG levels, TTG still outperforms EMA in sensitivity [35,36]. Ciacci et al. (2023)[13] further reinforced TTG's reliability by demonstrating strong concordance between locally and centrally conducted TTG assays [37]. More recently, a study[29] found that 99.5% of children with TTG > 5× ULN were histologically confirmed to have CD, emphasising TTG's diagnostic strength, whilst EMA had no diagnostic value. There is sufficient evidence to show the discordance between the two markers [15,38–40], despite EMA and anti-TTG theoretically recognising the same antigen [41,42]. Extrapolating further, serology is a poor marker for detecting mucosal recovery in patients with established CD on a gluten-free diet [43,44], albeit inconsistently,

with the level of TTG at diagnosis positively correlating with the histological severity[43]. This incongruence between serological markers and mucosal damage suggests that we should critically reassess the reliability of these tests, particularly when using them to guide clinical decisions beyond initial diagnosis.

EMA testing is expensive, labour-intensive, and requires indirect immunofluorescence, leading to a risk of interobserver variability, longer turnaround times (10 days vs. 4 days for TTG), and increased susceptibility to false negatives and false positives at low titres[18]. As a result, many laboratories have discontinued EMA testing[41], with only 81.5% of centres performing TTG testing still offering on-site EMA testing[21].

Limitations

Our study is constrained by its retrospective design. A proportion of patients were excluded from the primary analysis due to incomplete diagnostic data or lack of biopsy confirmation. This may have introduced selection bias and limited the generalisability of our findings. Additionally, the relatively small number of patients with a negative TTG level (<7 U/mL) limits the comprehensive evaluation of the efficacy of EMA testing, albeit of limited importance as our study's focus was on EMA performance at borderline TTG values.

We must also consider whether the testing kit used in our study is applicable to those obtained by alternative assays on the market, developed by alternative suppliers as there is no international reference for calibration and references of titres[19,45,46]. Notwithstanding, the TTG and EMA assays used in this study are the majority methods used by participants in the UK External Quality Assurance scheme (UK NEQAS) for Coeliac Serology (53% of laboratories used the FEIA (Phadia) IgA TTG assay and 42% used the INOVA IgA EMA assay in the April 2025 distribution) [47]. This suggests our findings are relevant for a significant number of laboratories.

Other limitations to our design are that we have minimal information on whether serology and biopsy practice was optimal and whether patients were adherent to a gluten containing diet at the time of testing. This may contribute to potential false negative EMA results in the borderline group. Importantly however, our study reflects true "real world" settings, thus more likely to represent the true clinical effectiveness.

In our study biopsies were not taken of the duodenal bulb (D1) and it is currently thought that taking proximal duodenal biopsies from the bulb increases the sensitivity of histological diagnosis [48]. This omission highlights an important limitation of our study and the current gold-standard diagnostic approach, as even biopsy-confirmed diagnoses may be prone to false negatives due to suboptimal sampling. Therefore, the true sensitivity of TTG may be underestimated in our findings. Furthermore, the poor sensitivity of EMA at low TTG levels cannot be solely attributed to missed histological diagnoses but rather reflects the inherent limitations of the EMA test itself, which does not improve in sensitivity even when more accurate biopsy sampling is considered.

Conclusion

We must re-evaluate the necessity of EMA testing as an adjunct to TTG in borderline cases when diagnosing CD. Its use may hinder diagnosis, offer no clear additional value and potentially contribute to underdiagnosis due to its high false negative rate.

Although traditionally regarded as a highly specific test, our findings challenge EMA's efficacy, as its negative results demonstrate limited discriminatory power in diagnosing CD. Furthermore, the lack of standardisation and significant inter-laboratory variability across the UK further undermine its reliability in clinical practice.

We advocate for a reassessment of current diagnostic algorithms and propose discontinuing routine INOVA EMA testing in light of its limited diagnostic contribution in our cohort, prompting the need for further external validation. We propose that the borderline range in the FEIA (Phadia) TTG assay be considered positive serology due to the high rate of CD diagnosis. However, limitations, including verification bias and a single-centre design, warrant prospective validation and multi-centre replication using standardised assays. Despite known limitations of endoscopic duodenal biopsy which can contribute to the misdiagnosis of CD, we reaffirm the value of the histological approach in appropriate clinical contexts where TTG is positive but <10 x ULN. Concurrently we support the no-biopsy approach for individuals with TTG ≥ 10xULN in line with our study findings and the BSG COVID-19 interim guidance. Ultimately, clinical judgment must remain central to decision-making, ensuring timely and accurate diagnoses so that expert dietitians and clinicians can provide optimal patient care.

Conflict of interest

The authors declare that they do not have competing interests, financial or personal.

Ethics approval

Not applicable.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

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Figure 1. Flow chart demonstrating serology distribution.

Figure 2. Distribution of EMA results and CD diagnoses in patients with a borderline TTG.

Figure 3a (left). Distribution of EMA results and CD diagnoses in patients with a positive TTG value. Figure 3b (right). Individual TTG and EMA values of patients with a positive TTG.