Tissue transglutaminase levels above 100 U/mL and celiac disease: A prospective study

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Abstract

AIM: To investigate whether a tissue-transglutaminase antibody (tTGA) level \( \geq 100 \) U/mL is sufficient for the diagnosis of celiac disease (CD).

METHODS: Children suspected of having CD were prospectively included in our study between March 2009 and September 2011. All patients with immune globulin A deficiency and all patients on a gluten-free diet were excluded from the study. Anti-endomysium antibodies (EMA) were detected by means of immunofluorescence using sections of distal monkey esophagus (EUROMMUN, Luebeck, Germany). Serum anti-tTGA were measured by means of enzyme-linked immunosorbent assay using human recombinant tissue transglutaminase (ELIA Celikey IgA kit Phadia AB, Uppsala, Sweden). The histological slides were graded by a single experienced pathologist using the Marsh classification as modified by Oberhuber. Marsh II and III lesions were considered to be diagnostic for the disease. The positive predictive values (PPVs), negative predictive values (NPVs), sensitivity and specificity of EMA and tTGA along with their 95% CI (for the cut off values > 10 and \( \geq 100 \) U/mL) were calculated using histology as the gold standard for CD.

RESULTS: A total of 183 children were included in the study. A total of 70 (38.3%) were male, while 113 (61.7%) were female. The age range was between 1.0 and 17.6 years, and the mean age was 6.2 years. One hundred twenty (65.6%) patients had a small intestinal biopsy diagnostic for the disease; 3 patients had a Marsh II lesion, and 117 patients had a Marsh III lesion. Of the patients without CD, only 4 patients had a Marsh I lesion. Of the 183 patients, 136 patients were positive for EMA, of whom 20 did not have CD, yielding a PPV for EMA of 85% (95% CI: 78%-90%) and a corresponding specificity of 68% (95% CI: 55%-79%). The NPV and specificity for EMA were 91% (95% CI: 79%-97%) and 97% (95% CI: 91%-99%), respectively. Increased levels of tTGA were found in 130 patients, although only 116 patients truly had histological evidence of the disease. The PPV for tTGA was 89% (95% CI: 82%-94%), and the corresponding specificity was 78% (95% CI: 65%-87%). The NPV and sensitivity were 92% (95% CI: 81%-98%) and 97% (95% CI: 91%-99%), respectively. A tTGA level \( \geq 100 \) U/mL was found in 87 (47.5%) patients, all of whom were also positive for EMA. In all these 87 patients, epithelial lesions confirming CD were found, giving a PPV of 100% (95%CI: 95%-100%). The corresponding specificity for this cut-off value was also 100% (95% CI: 93%-100%). Within this group, a total of 83 patients had symptoms, at least gastrointestinal and/or growth retardation. Three patients were asymptomatic but were screened because they belonged to a group at risk for CD (diabetes mellitus type 1 or positive family history). The fourth patient who...
lacked CD-symptoms was detected by coincidence during an endoscopy performed for gastro-intestinal bleeding.

CONCLUSION: This study confirms based on prospective data that a small intestinal biopsy is not necessary for the diagnosis of CD in symptomatic patients with tTGA ≥ 100 U/mL.

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Key words: Celiac disease; Diagnosis, Serology; Anti-tissue-transglutaminase antibodies; Anti-endomysium antibodies

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INTRODUCTION

Celiac disease (CD) is an immune-mediated enteropathy affecting approximately 1% of the worldwide population[1,2]. The immune reaction occurs when genetically susceptible individuals ingest gluten, which is a storage protein in wheat and the related grain species barley and rye, and this reaction is completely reversible upon gluten withdrawal, which is currently the only available treatment for CD[3,4]. The gold standard for the diagnosis of CD has been considered to be a small intestinal biopsy since 1954[5]. However, a small intestinal biopsy is not only expensive, time-consuming and stressful for children and their parents but may also provide inconclusive or even false results, due to patchy disease or to inadequate quality or orientation of the biopsy specimen[6-8]. Therefore, there has long been research focused on finding non-invasive markers to diagnose CD. For this purpose, the disease-associated auto-antibodies, especially anti-endomysium antibodies (EMA) and anti-tissue-transglutaminase antibodies (tTGA), have proven to be highly sensitive and specific[9-11]. In fact, according to the new ESPGHAN guidelines for the diagnosis of CD, a confirmatory small intestinal biopsy is no longer necessary in genetically predisposed individuals who are symptomatic and who have a tTGA of at least 10 times the upper limit of normal, a positive EMA and a good clinical response to the gluten free diet[12]. However, these new guidelines for children are mainly based on retrospective data[13-14]. Because such study designs are subject to selection bias, and because the diagnosis of CD implies a lifelong gluten free diet, the diagnosis of CD should be based on serology only when the chance of a false positive result is close to zero. The aim of the present study was therefore to evaluate prospectively whether the new diagnostic approach in patients with high tTGA levels is justified.

MATERIALS AND METHODS

Study population

All patients who were referred to the Wilhelmina Children’s Hospital in Utrecht, the Netherlands with the suspicion of having CD were prospectively included in the study between March 2009 and September 2011. Patients were referred to us because of symptoms associated with CD (e.g., abdominal symptoms, growth retardation) or because they belonged to a group at risk for CD, e.g., patients with Down syndrome or Diabetes Mellitus and patients with a positive family history for CD. In this patient group, serology (both EMA and tTGA) was performed, and any patient with abnormal serology was biopsied, as were patients with negative serology but a high clinical suspicion of CD. Patients with immunoglobulin A (IgA) deficiency ($n=8$) and patients on gluten restriction during the diagnostic work-up were excluded from the study. The study was performed according to the guidelines of the local medical ethics board.

Serological assessment

IgA EMA values were detected by indirect immunofluorescence using sections of distal monkey esophagus mounted on glass slides (EUROIMMUN, Luebeck, Germany). Serum IgA tTGA values were measured using the ELiA Celikey IgA kit (Phadia AB, Uppsala, Sweden). As recommended by the manufacturer, serum samples containing an antibody titer greater than 10 U/mL were considered positive. Total IgA was measured in all patients, and a serum IgA concentration below 0.07 g/L was regarded as IgA deficiency.

Histological evaluation

Duodenal biopsies were obtained by upper gastrointestinal endoscopy. An average of 3.1 biopsies (range: 1-8 biopsies) per patient were taken from the distal duodenum. Starting at the end of 2009, duodenal bulb biopsies were also routinely obtained during endoscopy, as recent studies suggested that this region could be the only affected site in CD[15]. On average, 1.9 biopsies per patient were taken from this location with a range of 0 to 5.

Histological diagnosis for all patients was performed by a single experienced pathologist using the Marsh classification as modified by Oberhuber[20,21]. The pathologist had no knowledge of the serological results or of the clinical presentation of the patients. An increased number of intraepithelial lymphocytes (Marsh I) were considered not to be diagnostic for CD. By contrast, Marsh I combined with crypt hyperplasia (i.e., Marsh II) or findings with villous atrophy (Marsh III) were consid-


Table 1 Results of small-intestinal biopsy and serology n (%)  

| Biopsy data | Patients with CD (100.0) | Patients without CD (65.6) | Patients with normal histology n = 63 (34.4) |
|-------------|-------------------------|---------------------------|---------------------------------------------|
| IgA EMA     | Negative 47 (25.7)      | 4 (3.3)                   | 43 (68.3)                                   |
|             | Positive 136 (74.3)     | 116 (96.7)                | 20 (31.7)                                   |
| IgA tTGA >10 Negative 53 (29.0) | 4 (3.3) | 49 (77.8) |
|             | Positive 130 (71.0)     | 116 (96.7)                | 14 (22.2)                                   |
| IgA tTGA ≥10 Negative 96 (52.5) | 33 (27.5) | 63 (100)    |
|             | Positive 87 (47.5)      | 87 (72.5)                 | 0 (0.0)                                     |

CD: Celiac disease; IgA: Immunoglobulin A; EMA: Anti-endomysium antibodies; tTGA: Anti-tissue-transglutaminase antibodies.

Table 2 Sensitivity, specificity, positive predictive value and negative predictive value of anti-endomysium antibodies and anti-tissue-transglutaminase antibodies (%)  

| Test          | Sensitivity | Specificity | PPV | NPV |
|---------------|-------------|-------------|-----|-----|
| IgA EMA       | 97 (91-99)  | 68 (55-79)  | 85 (78-90) | 91 (79-97) |
| IgA tTGA >10  | 97 (91-99)  | 78 (65-87)  | 89 (82-94) | 92 (81-98) |
| IgA tTGA ≥10  | 73 (63-80)  | 100 (93-100) | 100 (95-100) | 66 (55-75) |

The 95% CI are given in parentheses. IgA: Immunoglobulin A; EMA: Anti-endomysium antibodies; tTGA: Anti-tissue-transglutaminase antibodies; PPV: Positive predictive value; NPV: Negative predictive value.

RESULTS
A total of 183 patients met the inclusion criteria of the study. Of those patients, 70 (38.3%) were male, and 113 (61.7%) female with an age range of between 1.0 and 17.6 years and a mean age of 6.2 years. A total of 120 (65.6%) patients had a biopsy diagnostic for CD, of whom only 3 patients had a Marsh II lesion. In the remaining 63 (34.4%) patients, the diagnosis of CD could be excluded. Of the patients without CD, only 4 patients had Marsh I histology.

Of the total study population, 138 patients had positive EMA and/or tTGA antibodies, while 45 patients were negative for both antibodies. The patients who were negative for both antibodies underwent a small intestinal biopsy because of a strong clinical suspicion of CD (CD-like symptoms). Three of these patients had a Marsh III lesion, and one patient had a Marsh II lesion, while the diagnosis of CD could be excluded in the remaining 41 patients.

A positive EMA was found in 136 (74.3%) patients; 20 (31.7%) of them did not meet the histological criteria for CD (Table 1), giving a specificity of only 68% (Table 2). The corresponding PPV was 85%. The specificity of tTGA was slightly better (78%), with 116 of 130 positive patients being correctly diagnosed (Table 1). The corresponding PPV was also better at 89% (Table 2).

EMA was undetectable in 47 (25.7%) patients, of whom 43 indeed showed normal histology (Table 1). Consequently, the sensitivity and NPV of EMA were high with values of 97% and 91%, respectively (Table 2). These values were equally high for tTGA, i.e., 97% and 92%, respectively. Illustratively, 49 of the 53 patients with negative tTGA did not have CD (Table 1).

A total of 42 patients (23.0%) had tTGA levels between 10 and 100 U/mL. Of those patients, only 28 (66.7%) had CD, while the diagnosis could be histologically excluded in 14 (33.3%) patients. Of the latter group, 3 patients had a Marsh I lesion. By contrast, the 87 patients with a tTGA level ≥ 100 U/mL all met the histological criteria for CD (Table 1), yielding a PPV of 100% (Table 2). All were also positive for EMA. Among these 87 patients, only 4 patients were asymptomatic. Three patients were screened because they belonged to a group at risk for CD (diabetes mellitus type 1 or a positive family history for CD). The fourth patient who lacked CD-symptoms was detected by coincidence during an endoscopy performed for gastro-intestinal bleeding. All other patients (n = 83) had typical symptoms (at least gastrointestinal symptoms and/or growth retardation). After the diagnosis of CD was made, all patients adhered to the gluten-free diet, and the vast majority showed clinical improvement.

DISCUSSION
In patients with high tTGA levels, there is increasing evidence that a small intestinal biopsy is not needed to confirm the diagnosis of CD, as these increased levels are highly suggestive of the disease. This conclusion was also stated in the new ESPGHAN guidelines for the diagnosis of CD in the pediatric population[13]. Briefly, these guidelines suggest that in symptomatic individuals who have tTGA levels of at least 10 times the upper limit of normal and who respond well to the gluten free diet, histological confirmation is unnecessary. However, prospective studies are needed to confirm the applicability of these guidelines in clinical practice.

The sole reliance on serology for the diagnosis of CD is appropriate only if the PPV is close to 100%. In this study, it was prospectively shown that 87/87 patients with a tTGA of at least 100 U/mL did indeed suffer from CD, giving a PPV of 100%. However, in this cohort, most of the patients had typical CD symptoms and responded well to the diet, while only 4 patients lacked any CD associated symptoms. Therefore, due to the under-representation of asymptomatic patients in this cohort, it can be questioned whether this perfect PPV will also be

CD: Celiac disease; IgA: Immunoglobulin A; EMA: Anti-endomysium antibodies; tTGA: Anti-tissue-transglutaminase antibodies.
observed in asymptomatic patients.

Comparable results were found in previous retrospective studies, showing that high tTGA levels are associated with histological lesions compatible with CD\(^{[16-18]}\). Barker \textit{et al}\(^{[22]}\) showed that 48 of 49 mostly symptomatic children with a tTGA level $\geq 100$ U/mL had at least Marsh II enteropathy. Comparably, Donaldson \textit{et al}\(^{[23]}\) showed that 38 of the 38 pediatric patients with tTGA $\geq 100$ U/mL had Marsh III histopathology. A subsequent retrospective study, also in a pediatric population, showed that all symptomatic patients with tTGA of at least 100 U/mL who responded well to the diet had CD ($n = 111$), thereby reaching a PPV of 100\(^{[16,24]}\).

Similarly, in a study conducted in a mixed adult/pediatric population, it was shown that a tTGA $\geq 100$ U/mL occurs almost exclusively in the setting of Marsh III (73 of 76 patients) and that the 3 patients without villous atrophy had either a Marsh II ($n = 2$) or a Marsh I ($n = 1$) lesion\(^{[25]}\). Likewise, a study performed in adults showed that 91 patients with a tTGA level of at least 10 times the upper limit all had at least Marsh II enteropathy\(^{[26]}\). By contrast, Freeman reported that 3 of 14 adult patients with tTGA $\geq 100$ U/mL did not have CD\(^{[27]}\). Notably, in the latter 3 studies, an exact description of the clinical presentation of the patients was lacking\(^{[16,25,26]}\).

To the best of our knowledge, only one other prospective study has been performed in a mixed pediatric and adult population. This study showed that 1 of the 72 patients with a tTGA of at least 11.4 times the upper limit of normal had a normal small intestinal biopsy, yielding a PPV of 98.6\%, which the authors considered to be insufficient for omitting a biopsy\(^{[27]}\). However, in this study, the presence of symptoms was not taken into consideration, which may influence the PPV. In fact, the patient with this high level of tTGA and a normal biopsy did have an excellent clinical and serological response to the diet, suggesting that CD may have been missed historically.

In conclusion, the current study shows that 87/87 patients with tTGA $\geq 100$ U/mL had CD, which confirms the new ESPGHAN guidelines and other retrospective studies. However, because almost all studied patients in this study were symptomatic, omitting a biopsy should only be considered in this group. By contrast, in asymptomatic individuals, a small intestinal biopsy should still be performed, at least until more studies become available studying this specific group.

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