Serum transglutaminase antibodies do not always detect the persistent villous atrophy in patients with celiac disease on a gluten-free diet

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Background and aim Serum transglutaminase antibodies (tTGs) are used for celiac disease screening and to monitor celiac disease patients on a gluten-free diet (GFD). The need for histology of duodenal biopsies to assess mucosal healing after a GFD is still a matter of debate. We evaluated whether tTGs are adequate to detect the persistence of histological lesions of duodenal mucosa in celiac patients after a GFD.

Methods In total 253 patients with histological diagnosis of celiac disease according to Marsh criteria, both at the time of diagnosis (T0) and 18–24 months after starting a GFD (T2), were included. tTGs were evaluated both at T0 and T2; endomyosial antibodies (EMAs) only at T0.

Results At T0, 9.2% of patients had both tTG and EMA negative values, despite the evidence of duodenal lesions: 33.3% of Marsh 1, 14.3% of Marsh 2 and 5.2% of Marsh 3. At T2, tTGs were negative in 77.6% of patients: 82.2% of Marsh 0, 79.8% of Marsh 1, 70.0% of Marsh 2 and 59.1% of Marsh 3. At T2, approximately 60% of patients with the persistence of mucosal atrophy had negative tTGs. At T0, tTG median values were lower in patients with Marsh 1 and Marsh 2 than patients with Marsh 3 (P < 0.001), whereas no difference was found at T2 regardless of Marsh’s grade (P = 0.4).

Conclusions The results of our study highlight how histologic evaluation of duodenal biopsies remains the gold standard for both celiac disease diagnosis and the evaluation of mucosal recovery after 18–24 months of a GFD. Eur J Gastroenterol Hepatol XXX: 00–00

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Introduction
Celiac disease is a chronic autoimmune disorder that affects 1% of the general population [1–4]. In the last two decades, increase in the rate of celiac disease diagnosis was reported due to the availability of accurate diagnostic techniques [3–6]. Despite technological development, approximately two-thirds of patients (70–80%) are still not identified; this phenomenon is referred to as the submerged part of the celiac iceberg [4,5,7,8]. The follow-up of celiac patients is very important. A gluten-free diet (GFD) is the only available treatment for celiac disease [2,5], and the nonadherence to a GFD may lead to the development of complications (other autoimmune disorders and neoplasms) [2,3,9–13]. For these reasons, interest in both celiac disease diagnosis and follow-up remains very high [14]. Today, the diagnosis of celiac disease is based on the evaluation of clinical, serological, genetic and histological findings [2,5,6,15]. Concerning follow-up and GFD monitoring, the latest celiac disease guidelines recommend the evaluation of clinical findings and serology; upper gastrointestinal endoscopy (UGE) with biopsies is recommended only in cases of the relapse of symptoms despite a GFD [2,5]. However, the same guidelines reported that the serological normalization of antibodies after a GFD does not necessarily imply the recovery of the gut mucosa and that the histological evaluation is the only method to verify the mucosal healing [2,5]. The serology evaluation includes antitissue transglutaminase antibodies (tTGs), endomyosial antibodies (EMAs) and deamidated gliadin peptide antibodies (DGPs). The genetic evaluation includes typing for HLA-DQ2 and HLA-DQ8 haplotypes [5,16]. The histological evaluation assesses the tissue changes in the duodenal mucosa and is currently graded by Marsh criteria [5]. In consideration of the invasiveness and costs of UGE, a consensus on the need for UGE with duodenal biopsies for both celiac disease diagnosis and follow-up has not yet been achieved. The aim of this study was to investigate the concordance of serology and Marsh grade at the time of diagnosis and after 18–24 months of a GFD to confirm the necessity of UGE with duodenal biopsies both at the time of celiac disease diagnosis and in the follow-up after a GFD.

Materials and methods

Patients
The medical records of 328 consecutive adult patients with celiac disease followed at our Gastroenterology,
Hepatology and Nutrition Unit (n = 328) were retrospectively evaluated.

Of 328 celiac disease patients, 253 who had Marsh grade assessment both at the time of diagnosis (T0) and 18–24 months after diagnosis and GFD initiation (T2) and who had strictly adhered to the GFD were included in the study. The remaining 75 patients were excluded from the study because their laboratory or histologic assessment data were incomplete, they were not fully adhering to a GFD, or they were lost to follow-up.

The study was performed with the institutional review board’s approval (Prot. N.29169-2018) and the written informed consent of all patients. All clinical investigations were conducted according to the principles laid down in the Declaration of Helsinki.

The celiac disease diagnosis was made in the presence of elevated serological values of tTG IgA or EMA associated with specific histological alterations at duodenal biopsies, according to the Marsh criteria as follows: Marsh grade 1, increased intraepithelial lymphocytes (IELs); Marsh grade 2, increased IELs and crypt hyperplasia; Marsh grade 3, villous atrophy with increased IELs and crypt hyperplasia [5]. The histological examination of the biopsies of all celiac disease patients was assessed by two pathologists (G.C. and G.C.). In all seronegative patients, the celiac disease diagnosis was made in cases with a positive genetic test (HLA-DQ2 or HLA-DQ8 positivity) associated with the typical celiac disease duodenal lesions. In particular, in cases of Marsh 1 with a positive genetic test, but with negative serology (both tTG and EMA), the celiac disease diagnosis was confirmed only if at histological control after 18–24 months of GFD the histological changes passed from Marsh 1 to Marsh 0, that is, only if the normalization of the number of intraepithelial lymphocytes was observed. In all seronegative patients, other causes of increased IELs or villous atrophy were excluded such as Helicobacter Pylori infection (by gastric biopsies or Urea breath test or fecal test), other diseases of small intestine mimicking celiac disease (by duodenal biopsies and specific laboratory tests), food allergies, recent episodes of gastroenteritis and the use of intestinal-damaging drugs (e.g. nonsteroidal anti-inflammatory drugs, Olmesartan and chemotherapy drugs). Patients presenting with both tTG and EMA negativity and genetic test negativity (HLA-DQ2 or DQ8) were not diagnosed as celiac and were not included.

Adherence to a GFD was assessed by personal confirmation, patient interviews by a trained nutritionist and tTG values every 6 and 12 months, respectively. Patients reporting poor adherence were excluded.

### Collected data

Age, sex, the date of diagnosis, symptoms at diagnosis (including intestinal and extra-intestinal manifestations), the presence of HLA-DQ2 and HLA-DQ8 haplotypes, tTG IgA values and EMA positivity, and full blood count were collected. In all patients, at T0, total IgA was assessed to exclude IgA deficiency. Furthermore, in all patients, at T0, wheat allergy was excluded by serum-specific IgE antibody evaluation.

In the 253 enrolled patients, tTGs assessment was performed in 189 patients at T0 and in 201 at T2, EMA in 147 patients at T0. For tTG IgA values, we considered a threshold value of 10 U/ml. In some patients, DGP were also assessed.

Gastric and duodenal (two in the bulb and four in the second tract) biopsies, taken at T0 and T2, were assessed by hematoxylin and eosin and CD3 immunohistochemical staining.

### Statistical analysis

The Shapiro–Wilk test was performed to assess the normality of the distribution of the variables. The Kruskal–Wallis test was performed to evaluate the difference of tTG IgA values at T0 and T2 among the different Marsh’s grade. For the descriptive analysis, the median was used, associated with the maximum and minimum values. All differences were considered statistically significant when P was < 0.05. For all statistical analyses, the SAS software (version 9.4, Copyright © 2002-2012 by SAS Institute Inc., Cary, North Carolina, USA) was used.

### Results

Our cohort included 253 patients: 211 (83.4%) were female and 42 (16.6%) were male. The median age at celiac disease diagnosis was 32 (2–70) years. The complete demographic and clinical data of all celiac disease patients included in the study are summarized in Table 1. IgA deficiency or wheat allergy were ruled out in all the included patients.

At the time of diagnosis (T0), 9.9% of patients were classified as Marsh grade 1, 10.7% as Marsh grade 2 and 79.4% as Marsh grade 3. At T0, besides Marsh grade, tTG or EMA values were available in 218 patients. Of these 218, 11% of patients were classified as Marsh grade 1, 9.6% as Marsh grade 2 and 79.4% as Marsh grade 3 (Fig. 1). Out of 218 patients, 20 (9.2%) had both tTG and EMA negative values, despite the evidence of duodenal lesions: 33.3% of Marsh 1, 14.3% of Marsh 2 and 5.2% of Marsh 3 (Fig. 1).

At T0, besides Marsh grade, tTG value was available in 189 patients: 11.7% of patients were classified as Marsh grade 1, 10.1% as Marsh grade 2 and 78.2% as Marsh grade 3. tTGs were negative in 31/189 (16.4%) patients: 59.1% of Marsh 1, 31.6% of Marsh 2 and 8.1% of Marsh

| Table 1. Demographic and clinical data of patients with celiac disease included in the study |
|-------------------|-------------|
| Patients          | Total number: 253 |
| Male, n (%)       | 42 (16.6)    |
| Female, n (%)     | 211 (83.4)   |
| Age at diagnosis years, median (range) | 32 (2–70) |
| Gastrointestinal symptoms, n (%) | 230 (91.0) |
| Extra-intestinal symptoms, n (%) | 181 (71.5) |
| Gastrointestinal and extra-intestinal symptoms, n (%) | 172 (88.0) |
| Asymptomatic, n (%) | 12 (4.7)    |
| Marsh grade       | n (%) |
| Marsh 1, n (%)    | 25 (9.9) |
| Marsh 2, n (%)    | 27 (10.7) |
| Marsh 3, n (%)    | 201 (79.4) |
| HLA-DQ2 and DQ8   | Total number: 70 |
| HLA-DQ2*, n (%)   | 60 (85.7) |
| HLA-DQ8*, n (%)   | 30 (42.9) |
| HLA-DQ2* and DQ8*, n (%) | 20 (28.6%) |
Concordance between tTG and Marsh in celiac disease

Stefanelli et al.

At T0, the median value of tTGs were 8.0 U/ml (1.0–178.0), 22.5 U/ml (1.0–208.0) and 45 U/ml (1.0–609.0) in Marsh 1, Marsh 2 and Marsh 3, respectively. At T0, besides Marsh grade, EMA value was available in 147 patients: 10.8% of patients were classified as Marsh grade 1, 8.2% as Marsh grade 2 and 81% as Marsh grade 3. EMA was negative in 46/147 (31.3%): 81.2% of Marsh 1, 50% of Marsh 2, and 22.7% of Marsh 3 (Table 2).

The genetic haplotype was assessed in 70 (27.7%) patients, especially in all patients with negative tTG and EMA. HLA-DQ2 was present in 60 (85.7%) patients, HLA-DQ8 in 30 (42.9%) and both HLA-DQ2 and HLA-DQ8 in approximately 30% (Table 1).

After 18–24 months of a GFD (T2), 253 patients were revalued by UGIE with histology of duodenal biopsies: 23.3% patients were classified as Marsh grade 0, 60.9% as Marsh grade 1, 5.5% as Marsh grade 2 and 10.3% as Marsh grade 3. In 201 patients, tTG values were reassessed: 22.4% patients were classified as Marsh grade 0, 61.7% as Marsh grade 1, 5.0% as Marsh grade 2 and 10.9% as Marsh grade 3 (Table 2). tTGs were negative in 156/201 (77.6%) of patients: 82.2% of Marsh 0, 79.8% of Marsh 1, 70% of Marsh 2 and 59.1% of Marsh 3. At T2, the median values of tTGs were 4.0 U/ml (1.0–26.0), 4.0 U/ml (1.0–176.0), 4.5 U/ml (2.0–40.0) and 5.0 U/ml (1.0–190.0) in Marsh 0, Marsh 1, Marsh 2 and Marsh 3, respectively.

At T0, tTG values were lower in patients with Marsh 1 and Marsh 2 compared to those in patients with Marsh 3 (P < 0.001), whereas no difference was found at T2 regardless of Marsh’s grade (P = 0.4) (Fig. 2).

All patients who at T2 presented persistent histological lesions of the duodenal mucosa and negative tTG values, at T0 all had reported high tTG values, testifying that 18–24 months of a GFD had resulted in normalization of tTG levels, but without improving the histological lesions.

UGIE with biopsies was performed after 16.5 (SD 5.7) and 27.5 (SD 10.9) days from tTG and EMA assessment at T0 and T2, respectively.

Discussion

The diagnosis of celiac disease is based on the evaluation of serology, histology and sometimes genetic findings, in addition to clinical features [2,5,6]. In fact, celiac disease is usually identified by serological testing, including tTGs and EMA, and then it is confirmed by UGIE with histological assessment of duodenal biopsies [2,5,6]. Symptoms and signs of celiac disease are both intestinal and extra-intestinal [2,5,17,18], and many patients with celiac disease have no symptoms [2,5]. Regarding genetic predisposition, the HLA-DQ2 heterodimer is present in more than 95% of celiac patients, whereas HLA-DQ8 is present in the other 5% of celiac patients [2,5,16]. The
presence of HLA-DQ2 or HLA-DQ8 is necessary but not sufficient for celiac disease diagnosis, as HLA-DQ2 is present in 30–40% of the general population, whereas only 1% of subjects will develop celiac disease [2,5,16]. Then, in the diagnostic flow chart of celiac disease, the typing for HLA-DQ2 and HLA-DQ8 is very important for its high negative predictive value, as celiac disease is very unlikely in the absence of HLA-DQ2 and HLA-DQ8 [2,5,16,19]. Celiac disease probability in subjects with the positivity of tTGs and EMAs is very high; in this case, the UGIE with the histological evaluation of the duodenal biopsies is necessary to confirm the celiac disease in adult patients [2,5,6,15]. DGPs are less accurate in screening for celiac disease diagnoses [19,20], but they are useful in cases of IgA deficiency and children (age 0–2 years) with tTGs negativity [2,5]. In adult patients, histology of duodenal biopsies is necessary to confirm celiac disease: due to the patchy lesions of celiac disease, at least four biopsies from the second/third tract of the duodenum and one/two from the duodenal bulb are required [2,5]. On the other hand, in most pediatric patients, a UGIE with histology of duodenal biopsies is considered not necessary to confirm celiac disease diagnosis. In fact, the latest European Society of Pediatric Gastroenterology, Hepatology and Nutrition celiac disease guideline in 2020 reported that duodenal biopsies are not required for celiac disease diagnosis when the tTG IgA is >10 times the upper limit of normal values and EMA IgA is positive; in these cases, HLA-DQ2/HLA-DQ8 assessment and symptoms are not obligatory criteria [21,22]. This is due to a tight correlation between villous atrophy and a high tTG value (>10 times the upper limit of normal values) [21,23].

In our cohort, tTG values were lower in patients with Marsh 1 and Marsh 2 than patients with Marsh 3 at the times of diagnosis (P<0.001). On the other hand, no difference was found at T2 regardless of Marsh’s grade (P = 0.4) (Fig. 2).

In pediatric patients with celiac disease, Hawamdeh et al. [24] demonstrated a significant association between high values of antibodies and the severity of duodenal histological damage, although there was a non-negligible percentage of patients with Marsh grade 3 and low values of tTGs. Similarly, our data showed a non-negligible percentage of patients with negative tTG (16.4%) or EMA (31.3%), at the time of diagnosis (T0) despite the evidence of duodenal lesions at histological evaluation (Marsh grade of 1, 2 or 3) (Table 2). Furthermore, at T0, several patients (n=20/218, 9.2%) had both EMA and tTG negative values, but at histological evaluation, they showed mucosal lesions (Marsh grade 1, 2 or even 3) (Fig. 1). Recently, Farina et al. [25] reported that several seronegative adult celiac patients positively respond to a GFD. In all patients, 6–12 months after celiac disease diagnosis, a clinical evaluation is appropriate, and then every 12–24 months if there are no complications. At every clinical assessment, it is useful to verify adherence to a GFD and to obtain a complete blood count and tTG IgA values [2,5]. It is also useful to verify the possible development of autoimmune diseases (e.g. thyroiditis), and to check iron metabolism, vitamin D levels and bone densitometry measurements [2,5].

Concerning follow-up and GFD monitoring, the latest celiac disease guidelines recommend the evaluation of clinical findings and serology; UGIE with histology of duodenal biopsies is recommended only in cases with a lack of clinical response or a relapse of symptoms despite a GFD [2,5]. Serological markers are gluten-dependent: generally, they decrease after a few months of starting a GFD. Conversely, they increase in the case of gluten challenge, but serology is not sufficient to identify minor degrees of gluten contamination [2,5]. However, the same guidelines explain that normal values of tTGs after a GFD do not necessarily imply the recovery of the gut mucosa and that histological evaluation is the only method to verify mucosal healing [5]. They stated that more studies are required to identify tools to detect patient adherence to a GFD [5]. Furthermore, they underlined that follow-up with UGIE with duodenal biopsies 24 months after starting a GFD is reasonable to verify mucosal recovery, regardless of serology [5]. In fact, in our study, 18–24 months after celiac disease diagnosis and starting a GFD (T2), a high percentage of patients had negative tTG (n=119/201, 59.2%), although the persistence of the duodenal lesions as Marsh 1, 2 or 3 (Table 2). In recent years, several
studies have discussed the need for UGIE with biopsies to verify mucosal recovery after celiac disease diagnosis and starting a GFD [26,27]. Kaukinen et al. [26], in a cohort of 87 adult celiac patients on a GFD, demonstrated that among patients with persistent mucosal villous atrophy (n=27), a high percentage had negative tTG (n=16/27; 59%) or EMA (n=20/27; 74%) values. They concluded that duodenal biopsy is necessary to confirm the adherence to the GFD. Tursi et al. [27] concluded that tTG IgA has a poor correlation with histological damage and that it is not effective to verify mucosal recovery in the follow-up of celiac disease. Walker et al. [28] in their review, showed that the normalization of serology does not predict mucosal recovery. A lack of mucosal healing predicts the risk of refractory celiac disease and malignancies. The diagnosis of refractory celiac disease requires a combined clinical and histopathological approach. Silvester et al. [29] in a recent meta-analysis of 26 studies, investigated the role of tTGs and EMAs in detecting the persistence of villous atrophy despite a GFD, in both adult and children patients with celiac disease. They concluded that tTG IgA and EMA IgA negativity is poorly related to mucosal recovery: in fact, their negativity cannot be suggestive of mucosal recovery or adherence to a GFD (the sensitivity of tTG IgA and EMA IgA to identify villous atrophy was 0.50 and 0.45, respectively). However, tTG IgA and EMA IgA positivity suggest persistent villous atrophy (tTG IgA and EMA IgA specificity was 0.82 and 0.91, respectively). Therefore, in the follow-up of adults and children with celiac disease, it is necessary to verify mucosal recovery by UGIE with histology of duodenal biopsies.

In our cohort of celiac patients, at the time of diagnosis, a not negligible percentage (9.2%) of patients had negative serology (both tTGs and EMAs) despite the evidence of histological lesions as Marsh grade 1, 2 or 3 (Fig. 1). After 18–24 months of a GFD, a significant percentage (59.2%) of patients had negative tTGs, but with the persistence of histological alterations as Marsh grade 1, 2 or 3 (Table 2). A certain degree of concordance between antibody values and Marsh grade was found only at the time of celiac disease diagnosis: in fact, tTG values were lower in patients with Marsh 1 and Marsh 2 compared to those in then patients with Marsh 3 (P<0.001), whereas no difference was found at T2 regardless of Marsh’s grade (P=0.4) (Fig. 2). These data suggest that the negativity of tTGs is not adequate to verify the recovery of the duodenal mucosa. Therefore, histological evaluation of the duodenal mucosa is the only tool to ensure the identification of persistence of the mucosal damage. Then, at least one UGIE with histology of duodenal biopsies is necessary after 18–24 months of a GFD to verify mucosal recovery, independent of antibody negativity, and the absence of symptoms. Concerning the time for follow-up, we consider that histological evaluation 24 months after celiac disease diagnosis and GFD initiation is reasonable, but a definite time-lapse needs to be established [12]. Moreover, we suggest that a UGIE with duodenal biopsies is required in adult patients who had a celiac disease diagnosis in childhood without subsequent histological assessment. In conclusion, histological evaluation after starting a GFD is crucial to identify patients with low adherence to a GFD and persistence of histological mucosal lesions. These patients need to be informed about the potential risks of persistent mucosal damage and counseled on personalized nutrition.

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Conflict of interest
There are no conflicts of interest.

References


