INTRODUCTION
Celiac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamin in genetically susceptible individuals and characterized by the presence of a variable combination of gluten-dependent clinical manifestation, CD-specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes, and enteropathy (1). Different guidelines for CD diagnosis have been published so far by several societies, both for adults (2,3) and children (4). The first diagnostic criteria for CD in children were published in 1969 by the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) (5). At that time, three biopsies of the small bowel mucosa were necessary to establish the diagnosis of CD: the first one when the suspicion of CD was raised to demonstrate villous atrophy; the second one, after 1 year on a gluten-free diet (GFD), to show normalization of the intestinal mucosa; the third and last one after a gluten-challenge to prove unequivocally the causal relationship between gluten intake and mucosal relapse. These rules were used until 1990, when criteria were revised by...
European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (6). Based on these revised criteria, if the clinical history and antibody findings were suggestive of the disease, a single intestinal biopsy followed by a favorable response to a GFD was sufficient to confirm the diagnosis of CD. Anti-endomysial (EMA) and anti-tissue transglutaminase (anti-tTG) antibodies are the well-established serological marker; indeed, they are present at the moment of the CD diagnosis and vanish on a GFD diet, thus representing a true revolution in the field of diagnosis (7,8). A positivity of both anti-tTG and EMA antibodies has a sensitivity and positive predictive value for CD close to 100% (9,10).

Several studies (11–13) showed correlations between anti-tTG levels and histological features of CD in children: high titer's have always been associated with villous atrophy.

Given these studies, in 2012, ESPGHAN developed two different algorithms for CD diagnosis (1). The first algorithm can be applied to children and adolescents with signs and symptoms suggestive of CD. It is possible to omit the biopsy if patients are older than 2 years, have high-titer anti-TG antibodies (≥10 times upper limit of normal or ULN), show positivity for EMA and HLA DQ2 and/or DQ8. For diagnosis, the biopsy is necessary in asymptomatic patients and in patients with anti-tTG antibodies <10 times ULN. Omitting biopsies would reduce the burden of both endoscopy and general anesthesia, save costs, and avoid potential adverse effects of these procedures.

The aim of this retrospective study was to assess the accuracy of serological tests to diagnose CD in asymptomatic patients.

METHODS
We retrospectively reviewed data of 286 children and adolescents (median age: 8.3 years; age range: 10 months to 17 years) who had received a CD diagnosis, in accordance with 1990 ESPGHAN criteria (6), at the Pediatric Gastroenterology and Liver Unit of “Sapienza—University of Rome” between January 2007 and December 2013. All patients (95 boys and 191 girls) were positive for anti-tTG and EMA and underwent upper gastrointestinal endoscopy and small bowel biopsies for the CD diagnosis. According to ESPGHAN guidelines (1), diarrhea, weight loss, failure to thrive, anorexia, abdominal distention, abdominal pain, short stature, flatulence, irritability, increased titers of liver enzymes, constipation, and anemia were considered as symptoms of CD. In our study, asymptomatic children received screening for CD if they were first-degree relatives of CD patients or included in at-risk groups. For analysis purpose, we divided patients in four groups according to both the anti-tTG antibody titer and symptoms: “high-titer” (≥10 times ULN) symptomatic, “high-titer” asymptomatic, “low-titer” (<10 times ULN) symptomatic, and “low-titer” asymptomatic children. Histological lesions were graded according to the Marsh–Oberhuber (MO) criteria (in brief: MO 0=normal mucosa; MO 1=increased number of intraepithelial lymphocytes; MO 2=crypt hyperplasia; MO 3a=partial villous atrophy; MO 3b=subtotal villous atrophy; MO 3c=total villous atrophy) (14). IgA anti-tTG antibodies were assayed on ELISA commercially available kits from Eurospital (Trieste, Italy: cutoff value >9 UA/ml for anti-tTG).

RESULTS
The age of the examined patients ranged from 10 months to 17 years (median 8.3 years). Figure 1 summarizes study design and results. Out of 286 patients, only 26 underwent HLA typing for research purpose (7 HLA DQ8 and 19 HLA DQ2) as it was not required by the standard-of-care diagnostic protocol at the time of diagnosis. Only 196 patients, all EMA positive and with anti-tTG antibodies ≥10 times ULN were included for statistical analysis. Among them, a group of 156 (79.59%) had symptoms suggestive of CD (namely “high-titer” symptomatic children). The distribution of symptoms at the diagnosis was shown in Table 1. In this group, two patients with type-1 diabetes mellitus have been included in symptomatic patients because of chronic diarrhea. Diarrhea, recurrent abdominal pain, and constipation were the most common symptoms. Iron deficiency anemia was present in 15 patients. Among the 156 “high-titer” symptomatic children, 142 (91.02%) showed severe lesion degree (3a, 3b, and 3c MO).

On the contrary, 40 out of 196 (20.40%) children were asymptomatic (namely “high-titer” asymptomatic children): 14 patients had been screened as they were first-degree relatives of CD patients and 37 (92.5%) showed severe lesion degree (3a, 3b, and 3c MO).

Anti-tTG antibodies <10 times ULN and EMA positive were found in 90 patients (31.47%). Out of these, 74 (82.22%) can be defined as “low-titer” symptomatic children and 52 (70.28%) showed severe lesion degree (3a, 3b, and 3c MO). On the contrary, 16 out of 90 (17.78%) children were asymptomatic (namely “low-titer” asymptomatic children): 13 (81.25%) showed severe lesion degree (3a, 3b, and 3c MO). Representation of these data is summarized in Figure 1.

No difference was found between “high-titer” symptomatic children and “high-titer” asymptomatic children with regards to histological damage (Fisher exact test P=1.000), as it is shown in Table 2.

DISCUSSION
According to the new ESPGHAN guidelines it is possible to omit biopsy in children with clear symptoms of CD (recurrent abdominal pain, failure to thrive, diarrhea, distended abdomen, and anemia) showing high levels of anti-tTG antibodies, EMA, and HLA-DQ2/DQ8 positivity.

However, our results indicate that the absence of symptoms in children with anti-tTG antibody titer ≥10 times ULN and positive
EMA antibodies does not theoretically undermine a “biopsy-sparing” CD diagnostic approach; a “biopsy-sparing” protocol, indeed, might be suitable both for symptomatic and asymptomatic patients with anti-tTG antibody titer ≥10 times ULN and EMA and HLA-DQ2/DQ8 positive.

In support of our hypothesis, in 2005, a systematic review reported that EMA IgA and anti-tTG IgA antibodies have sensitivity and specificity for the diagnosis of CD above 90% in both children and adults (15). In 2007, Donaldson et al. (11) established a correlation between quantitative serology and small bowel histopathology, thus concluding that greatly increased antibody levels will predict villous atrophy on biopsy. Our data also agree with a previous study (16), which showed that a biopsy is not required when anti-tTG is ≥10 times ULN, as the positive predictive value for CD of anti-tTG ≥10 times ULN is 100%, irrespective of symptoms. These data have also been confirmed by Singh (17) demonstrating that the higher the titer of anti-tTG antibodies is, the more severe villous abnormalities are detected. In this study, this trend was showed in the presence of symptoms such as diarrhea or anemia, and the positive predictive value for the presence of villous atrophy was 100%.

The central role of the high level of anti-tTG was investigated by Tortora et al. (18): their study showed how the diagnosis of CD could be performed in adults without the need for histology. In our study, we have demonstrated that the percentage of atrophy in the group of “high-titer” symptomatic children and in the one of “high-titer” asymptomatic children overlaps. For this reason, the key role of symptoms in separating two different algorithms might be downsized. In support of our results, in 2008, Donaldson (13) showed that IgA tTG >100 units were nearly exclusively associated with Marsh 3 lesions, giving a positive predictive value of 96% in this subgroup. Previously, the same result has been obtained in a pediatric setting by Barker et al. (19).

Some attempts at evaluating ESPGHAN 2012 guidelines through retrospective analyses have been published so far. In 2013, Klapp et al. (20) conducted a study about the applicability of the new ESPGHAN criteria but they enrolled only symptomatic patients, thus not representing the whole population affected by

### Table 1. Symptom of CD in “high-titer” symptomatic patient

| Symptoms                  | Number of patients | % Of patients |
|---------------------------|--------------------|---------------|
| Diarrhea                  | 39                 | 25            |
| Failure to thrive         | 34                 | 21.8          |
| Recurrent abdominal pain  | 34                 | 21.8          |
| Iron-deficiency anemia    | 15                 | 9.6           |
| Constipation              | 14                 | 9             |
| Flatulence                | 5                  | 3.2           |
| Fatigue                   | 4                  | 2.6           |
| Recurrent aphthosis       | 3                  | 1.9           |
| Vomiting                  | 3                  | 1.9           |
| Dermatitis herpetiformis  | 1                  | 0.6           |
| Dysphagia                 | 1                  | 0.6           |
| Neurological symptoms     | 1                  | 0.6           |
| Precocious puberty        | 1                  | 0.6           |
| Celiac crisis             | 1                  | 0.6           |
| **Total**                 | **156**            |               |

CD, celiac disease.
CD. Another study (21) showed that symptom positivity is essential in order to omit biopsy, because in symptomatic patients the specificity of antibody titer was close to 99%, but it reaches only 85% in asymptomatic patients. These data do not match with our results, where the two groups (high-titer asymptomatic and high-titer symptomatic patients) overlap based on our statistical evaluation. However, a recent retrospective study from Canada has shown that in a population of 263 children with IgA anti-tTg >10 times ULN and EMA positive, 40 of 43 asymptomatic patients had biopsies conclusive for CD, thus calling for further studies exploring possibilities for a “non-biopsy” protocol also when symptoms are lacking (22).

In relation to our results, we confirm that the most important predictor factor for intestinal damage is the titer of anti-tTG antibodies. In most of the previous studies, the correlation between anti-tTG antibody titer and grade of intestinal damage was calculated without considering symptoms. According to our data, extending the biopsy sparing protocol to “high-titer” asymptomatic children would reduce the cost and the burden of biopsy and anesthesia (23,24).

We are aware that occasionally asymptomatic patients might hardly accept the definitive diagnosis of CD without histological confirmation and the following GFD. On the other hand, these patients may need a more strict follow-up for monitoring GFD adherence, but probably this does not outweigh avoiding unnecessary biopsies. Asymptomatic patients should be with GFD as strictly as symptomatic ones, in order to prevent other autoimmune diseases (25) and enteropathy-associated T-cell lymphoma (26).

All patients from this study are routinely followed up at our center; in the vast majority of cases (94.4%), anti-tTG and EMA have decreased during the follow-up, and symptoms improved until resolution.

If confirmed in large multicenter prospective studies, the “biopsy-sparing” protocol could definitely be recommended to both symptomatic and asymptomatic patients with anti-tTG antibody titer ≥10 times ULN and EMA and HLA-DQ2/DQ8 positive.

### CONFLICT OF INTEREST

**Guarantor of the article:** Chiara Maria Trovato, MD.

**Specific author contributions:** Chiara Maria Trovato: principal investigator, wrote the paper, contributed to the study design. Monica Montuori, Caterina Anania, and Maria Barbato: involved in patient recruitment and clinical management. Anna Rita Vestri: involved in statistical evaluation and data interpretation. Sofia Guida: involved in evaluation of antibodies. Salvatore Oliva: performed endoscopies and biopsies. Fabrizio Mainiero: revised the paper critically. Salvatore Cucchiara: designed the research study. Francesco Valitutti: reviewed the paper, contributed to the study design. All authors approved the submitted version of the manuscript.

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### Table 2. Statistical evaluation

| Anti-Tg value | MO score | Total |
|---------------|----------|-------|
|               | 1+2      | 3a+3b+3c |
| <10 Times ULN |          |        |
| Symptoms      |          |        |
| Silent        | 3        | 13     | 16    |
| Atypical+typical | 22      | 52     | 74    |
| Total         | 25       | 65     | 90    |
| ≥10 Times ULN |          |        |
| Symptoms      |          |        |
| Silent        | 3        | 37     | 40    |
| Atypical+typical | 14      | 142    | 156   |
| Total         | 17       | 179    | 196   |

| Anti-Tg value | Value | d.f. | Two-ways asymptotic significance | Two-ways exact significance | One-way exact significance |
|---------------|-------|------|---------------------------------|----------------------------|----------------------------|
| <10 Times ULN |       |      |                                 |                            |                            |
| Pearson χ²   | 0.363 | 1    | 0.547                           |                            |                            |
| Continuity correction | 0.075 | 1    | 0.784                           |                            |                            |
| Likelihood ratio | 0.379 | 1    | 0.538                           |                            |                            |
| Fisher’s exact test | 0.745 |      | 0.404                           |                            |                            |
| Linear-by-linear association | 0.359 | 1    | 0.549                           |                            |                            |
| Valid cases   | 90    |      |                                 |                            |                            |

| ≥10 Times ULN |       |      |                                 |                            |                            |
| Pearson χ²   | 0.087 | 1    | 0.768                           |                            |                            |
| Continuity correction | 0.000 | 1    | 10.000                          |                            |                            |
| Likelihood ratio | 0.090 | 1    | 0.764                           |                            |                            |
| Fisher’s exact test | 1.000 |      | 0.529                           |                            |                            |
| Linear-by-linear association | 0.087 | 1    | 0.768                           |                            |                            |
| Valid cases   | 196   |      |                                 |                            |                            |

Anti-Tg, anti-tissue transglutaminase; MO, Marsh–Oberhuber criteria; ULN, upper limit of normal.

*One cell (25.0%) has expected count <5. Minimum is 3.92.

*Valid for a 2×2 table only.

*One cell (25.0%) has expected count <5. Minimum is 3.47.

Bold values are relevant results.
Study Highlights

WHAT IS CURRENT KNOWLEDGE
✓ European Society of Pediatric Gastroenterology, Hepatology, and Nutrition 2012 guidelines can be applied only in symptomatic patients with ≥10 times upper limit normal (ULN) anti-transglutaminase (anti-tTG) and HLA compatibility.
✓ Biopsy is necessary to diagnose celiac disease (CD) in asymptomatic patients.
✓ Biopsy is an invasive and expensive procedure.

WHAT IS NEW HERE
✓ We retrospectively reviewed data of 286 children and adolescents and then divided them into four groups according to both the anti-tTG antibody titer and symptoms: “high-titer” (≥10 times ULN) symptomatic, “high-titer” asymptomatic, “low-titer” (<10 times ULN) symptomatic, and “low-titer” asymptomatic children.
✓ We investigated if the biopsy-sparing protocol can be applied in asymptomatic children with ≥10 times ULN anti-tTG.
✓ Our results show that there is no significant difference between “high-titer” symptomatic children and “high-titer” asymptomatic children with regards to histological damage.

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