

## Utility of Anti-actin Iga Antibody in Combination with the New ESPGHAN Guidelines for Coeliac Disease Diagnosis

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### ABSTRACT

**AIM:** An option to reduce the number of duodenal biopsies in the diagnosis of coeliac disease (CD) has recently been reported by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition. New criteria showed that the duodenal biopsy may be avoided in presence of symptoms, high anti-transglutaminase type 2 antibody (anti-TG2) levels, anti-endomysial antibodies (EMA) and at-risk HLA, whilst biopsy still remains mandatory for individuals with moderate and low anti-TG2 levels. In this study, we considered the addition of serum measurement of anti-actin IgA antibody (AAA-IgA) to the new criteria, with the aim of further reducing the number of duodenal biopsies.

**METHODS:** One hundred and forty consecutive symptomatic CD children and 78 controls were studied. All subjects were classified according to the new criteria with the addition of AAA-IgA levels and results were compared with the outcome of duodenal biopsy.

**RESULTS:** The biopsies from the sixty-four individuals (out of 218) identified by the new criteria, presence of symptoms, anti-TG2 levels >10 times upper limit of normal (ULN), positive EMA and at-risk HLA, showed CD with a Marsh 3 lesion. In the remaining individuals, the addition of AAA-IgA allowed the detection of further 20 CD patients with a Marsh 3 damage when moderate (4 to 10 times ULN) but not low anti-TG2 levels were present.

**CONCLUSIONS:** Our study confirms that the new criteria may avoid

the duodenal biopsy in many CD patients. Moreover, although our finding needs to be confirmed, positivity for AAA-IgA may further reduce the number of duodenal biopsies in moderate anti-TG2 levels.

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**Key words:** Celiac disease; ESPGHAN; AAA-IgA; anti-TG2

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### INTRODUCTION

Coeliac disease (CD), is an immune-mediated systemic disease triggered and maintained by dietary gluten in genetically predisposed individuals, characterized by a variable small intestinal villous damage and by different clinical manifestations<sup>[1]</sup>.

In the last 15 years, the knowledge of the disease mechanisms has increased with the discovery of the autoantigen in CD, tissue transglutaminase type 2, and its antibody (anti-TG2) and of a multitude of different genes by Genome Wide Association Studies (GWAS) with the human leukocyte antigen HLA-DQ2 and HLA-DQ8 being the most strongly associated<sup>[2]</sup>.

Recently, a synopsis summarizing some of the evidence statements and recommendations of the guidelines in CD diagnosis for use in clinical practice has been formulated by a working group within the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN)<sup>[3]</sup>.

An important statement of these guidelines is the development of two new algorithms for CD diagnosis which consider as starting point (1) the presence of symptoms and signs suggestive of CD in children and adolescents (algorithm 1) and (2) the absence of symptoms and signs in persons at genetic risk for developing CD (algorithm 2).

We have considered in the present work the most interesting of the algorithms, algorithm 1: it allows the diagnosis of CD without performing the duodenal biopsy for those children and adolescents with symptoms and signs suggestive of CD, anti-TG2 levels >10

times upper limit of normal (ULN), positive confirmation tests of anti-endomysium-IgA antibodies (EMA) and with the presence of at-risk HLA-DQ2 or -DQ8.

If all these requirements were fulfilled, the diagnosis of CD is confirmed, gluten free diet is started and the patient is studied for improvement of symptoms and decline of autoantibodies. A later gluten challenge in these patients is not required<sup>[3]</sup>.

However, it has been established that symptomatic CD patients with elevated degrees of intestinal damage may also be found among those with anti-TG2 levels lower than 10 times ULN<sup>[4,5]</sup>. Therefore, a high number of symptomatic CD patients with anti-TG2 levels lower than 10 times ULN, not enclosable within algorithm 1, still necessitate a duodenal biopsy.

Since anti-actin IgA antibodies (AAA-IgA) directed against actin filaments are strongly correlated with total or subtotal intestinal atrophy<sup>[6,7]</sup>, we hypothesized that serum measuring of this autoantibody could contribute in increasing the number of patients who could avoid the duodenal biopsy.

This study considered the addition of serum measurement of AAA-IgA to algorithm 1, with the aim of further reducing the number of duodenal biopsies.

## MATERIALS

### Patients

Our group consisted of 150 consecutive Sardinian CD patients (115 females, 35 males, ratio females/males 3.3, mean age at diagnosis 8 years, range from 2 to 18 years); 140 presented symptoms suggestive of CD (Table 1) whilst 10 were not included in the study because asymptomatic and for this reason the algorithm 1 of Husby *et al* was not applicable. In addition, 5 patients with IgA deficiency, a well-known condition complicating the interpretation of the serological pattern of CD, were not included in the study. All patients were diagnosed according ESPGHAN criteria<sup>[8]</sup> and were recruited from a larger number of 5680 subjects attending the ambulatory of the Pediatric Gastroenterological Unit in Cagliari, Italy between 2005-2011. A further group of 78 individuals with persistent significant gastrointestinal symptoms, already characterized by upper digestive endoscopy and small bowel biopsy<sup>[7]</sup>, were used as non-CD subjects. All 218 individuals were characterized for histopathology, anti-TG2, EMA, HLA typing and AAA-IgA.

**Table 1** Prevalence of the symptoms suggestive of CD in our cohort of children and adolescents.

Symptoms	CD patients, n (%)
Diarrhoea	39 (27.8%)
Iron deficiency anaemia	23 (16.4%)
Short stature/growth failure	23 (16.4%)
Abdominal pain	21 (15%)
Weight loss	9 (6.4%)
Chronic fatigue	8 (5.7%)
Constipation	7 (5.0%)
Vomiting	4 (2.9%)
Increased level of liver enzymes	4 (2.9%)
Irritability	2 (1.4%)

### Anti-TG2

Anti-TG2 was determined using the ELIA commercial kits ImmunoCAP (Phadia, Milan, Italy) after serum dilution when necessary. Results were expressed in times upper limit of normal with a cut-off of 7 U/mL.

### EMA

EMA was determined by immunofluorescence (Delta

Biologicals, Rome, Italy). Results were expressed as intensity of immunofluorescence from 0 to 4. With the aim of reducing false positives, only strong immunofluorescence intensity (fluorescence of 2 or higher) was considered positive.

### HLA typing

To type for HLA-DRB1 and -DQB1 alleles<sup>[9]</sup>, HLA Olerup SSP Molecular Typing Kits (Roche, Sweden) were used according to the manufacturer's instructions. The identification of the various DRB1, DQA1, DQB1 haplotypes in CD patients was performed following the segregation of HLA haplotypes in families<sup>[10]</sup>. In this study, the two forms of HLA-DQ2, termed DQ2.5 and DQ2.2 respectively, were considered separately as the risk of DQ2.2 molecule for coeliac disease is lower than DQ2.5, unless it is expressed together with DQ2.5<sup>[11,12]</sup>.

### AAA-IgA

AAA-IgA is an immunofluorescence-based serological test developed in our laboratory, which has been validated in a multicenter study<sup>[6,7]</sup> as a useful marker of an elevated grade of intestinal damage associated with CD. Results were expressed by immunofluorescence intensity from 0 to 4. To reduce false positives, only strong fluorescence intensity (2 or higher) was considered positive.

### Histopathology

For each patient, 2 or more biopsies were taken from the second/third portion of the duodenum (at least 4 samples), and at least 1 biopsy was taken from the duodenal bulb<sup>[3,13]</sup>. Intestinal villous atrophy has been graded according to the Marsh classification<sup>[14]</sup> modified by Oberhuber in type 3c, 3b and 3a 2, 1 and 0<sup>[15]</sup>, always by the same board-certified pathologists.

### Statistical analysis

The frequencies of outcomes were analyzed using the chi square test where appropriate. A *p* value <0.05 was considered significant.

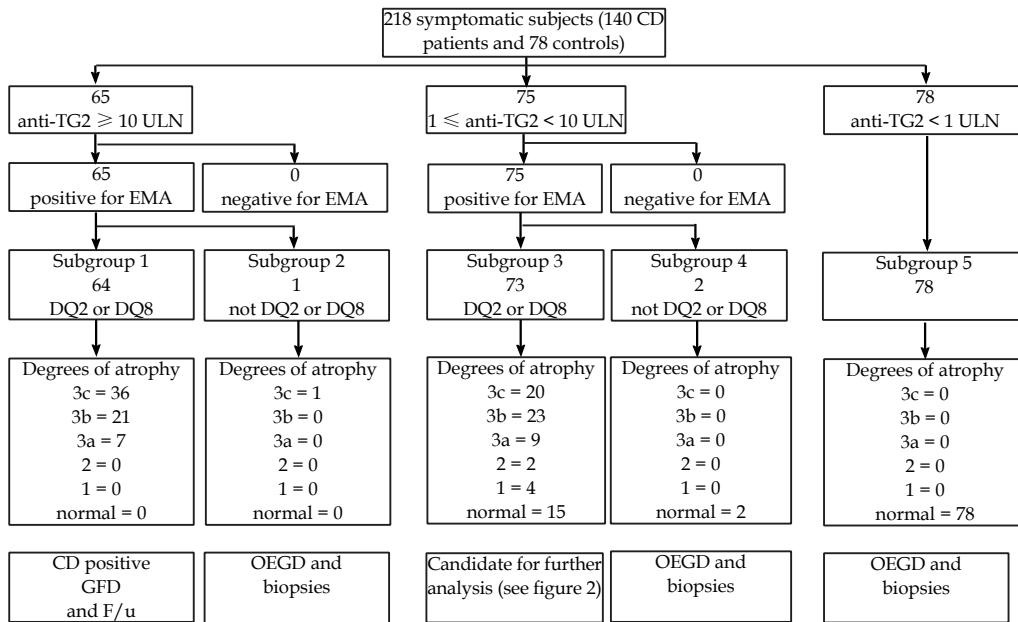
## RESULTS

The one hundred and forty CD patients and 78 controls were classified according to the presence of symptoms and signs suggestive of CD, anti-TG2 levels, positivity for EMA and for HLA-DQ2 (in *cis* or in *trans*) or -DQ8 as recently reported by Husby *et al*<sup>[3]</sup> (Figure 1). Among the CD patients, the histopathology according to Marsh-Oberhuber classification<sup>[14,15]</sup> showed a type 3c lesion in 57, type 3b in 44, type 3a in 16, type 2 in 2, type 1 in 4 and type 0 in 17 potential CD patients, whilst in all 78 controls with persistent significant gastrointestinal symptoms the duodenal histopathological signs associated with CD were not found.

The application of algorithm 1 to our cohort of patients and controls generated 5 different subgroups. The 5 subgroups and the degrees of intestinal damage associated to them are showed in figure 1.

We found that out of the 218 individuals studied, all 64 with symptoms and signs suggestive of CD, anti-TG2 levels >10 times ULN, positivity for EMA and for HLA-DQ2 (in *cis* or in *trans*) or -DQ8, showed a coeliac disease with a Marsh 3 atrophy (Subgroup 1 of figure 1).

As far as the HLA associated risk was concerned, three out of the 218 were found to express the DQ2.2 molecule without DQ2.5. One of them had anti-TG2 level higher than 10 times ULN and fell in the subgroup 2, whereas the remaining two patients, considering their anti-TG2 level lower than 10 times ULN, were included in the subgroup 4 (Figure 1). The patient of subgroup two showed Marsh 3c histopathology, whilst the two of the subgroup 4 showed Marsh 0 and



**Figure 1** Distribution of 140 CD patients and 78 controls according to the algorithm 1 proposed by Husby *et al.* For each of the 5 subgroups the degree of intestinal atrophy is also illustrated. CD: coeliac disease; anti-TG2: anti-transglutaminase type 2 antibody; ULN: upper limit of normal; EMA: anti-endomysial antibodies; HLA: human leukocyte antigen; 3a, 3b, 3c, 2, 1 and 0 indicate the grade of intestinal damage according to the Marsh-Oberhuber classification<sup>[14,15]</sup>; GFD: gluten free diet; OEGD: oesophagogastroduodenoscopy; F/u: follow-up; AAA-IgA: anti-actin IgA antibody.

were thus classified as potential CD (Figure 1).

Among the 218, seventy-three coeliac disease patients were included in the subgroup 3, that differed from the subgroup 1 only for the anti-TG2 levels lower than 10 times ULN (Figure 1).

The remaining subgroup five was constituted by the seventy-eight controls (Figure 1).

Based on these data, the association of AAA-IgA with severe intestinal damage was evaluated in the subgroups one, three and five. Subgroups two and four were not considered further because the low number of patients included in these groups did not consent to draw conclusions.

As expected, since AAA-IgA is a marker of severe intestinal damage, a higher frequency of AAA-IgA positive individuals was found in subgroups one and three that included the CD patients compared to subgroup five that included controls (Table 2). However, we found that in subgroup one, the addition of AAA-IgA to the outcome of the algorithm 1 did not offer any further useful information as only 76.5% of the CD patients, already defined by algorithm 1, were AAA-IgA positive (Table 2).

**Table 2** Distribution of positive AAA-IgA among subgroups obtained with the algorithm 1 of Husby *et al.*<sup>1</sup>.

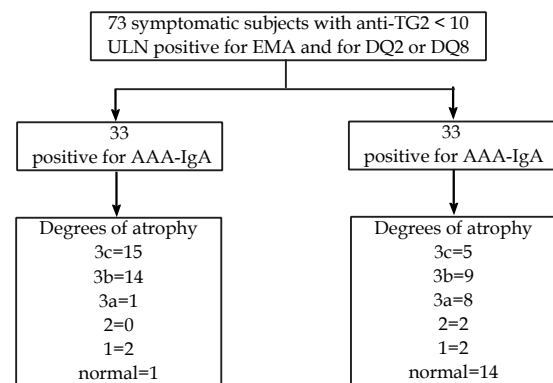
Intestinal atrophy according to the Marsh/Oberhuber classification	Subgroup 1	Subgroup 3	Subgroup 5
	AAA-IgA+/Alg. 1 (%)	AAA-IgA+/Alg. 1 (%)	AAA-IgA+/Alg. 1 (%)
3c	30/36 (83.3)	15/20 (75)	/
3b	18/21 (85.7)	14/23 (60.8)	/
3a	1/7 (14.2)	1/9 (11.1)	/
0-1-2	/	3/21 (14.2)	3/78 (3.8)
Total	49/64 (76.5)	33/73 (45.2)	3/78 (3.8)

<sup>1</sup> Subgroups two and three were not considered because the low number of patients

Conversely, in subgroup three the positivity for AAA-IgA correlated significantly (OR=10.45;  $p < 0.05$ ) with Marsh 3 histopathology (Figure 2). This association became even more elevated when increasing the cut-off level of anti-TG2. In fact, an unequivocal association between positivity for AAA-IgA and Marsh 3 histopathology was observed in patients with an anti-TG2 level

less than 10 times ULN but higher than 4 times ULN (Figure 3). Using this strategy, further 20 CD patients of subgroup three were confirmed to have a Marsh 3 histopathology (Figure 3).

Finally, in subgroup five only 3 out of 78 controls were AAA-IgA positive (Table 2).

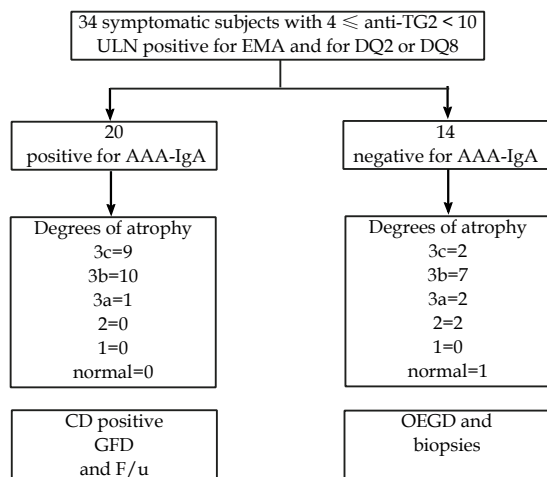


**Figure 2** Measurement of AAA-IgA among the 73 subjects of subgroup 3. CD: coeliac disease; anti-TG2: anti-transglutaminase type 2 antibody; ULN: upper limit of normal; EMA: anti-endomysial antibodies; HLA: human leukocyte antigen; 3a, 3b, 3c, 2, 1 and 0 indicate the grade of intestinal damage according to the Marsh-Oberhuber classification<sup>[14,15]</sup>; AAA-IgA: anti-actin IgA antibody.

## DISCUSSION

The new algorithm 1 recently reported by ESPGHAN suggested that in the diagnosis of CD, the duodenal biopsies could be avoided in children and adolescents with CD associated symptoms, high anti-TG2 levels, positivity for EMA and at-risk HLA. The aims of the present study were to confirm the reliability of algorithm 1 and to reduce further the number of duodenal biopsies by adding the serum measurement of another marker of intestinal damage (AAA-IgA)<sup>[7,16,17]</sup>.

In our cohort we found that the new algorithm would have avoided the duodenal biopsy in 64 out of 140 CD patients (Subgroup 1 of figure 1). We also found that all the 64 CD patients had a Marsh 3 grade of intestinal damage. This finding confirms, as previously



**Figure 3** Distribution of AAA-IgA in the 34 subjects of subgroup 3 with anti-TG2 levels comprised between 4 and 10 ULN. CD: coeliac disease; anti-TG2: anti-transglutaminase type 2 antibody; ULN: upper limit of normal; EMA: anti-endomysial antibodies; HLA: human leukocyte antigen; 3a, 3b, 3c, 2, 1 and 0 indicate the grade of intestinal damage according to the Marsh-Oberhuber classification<sup>[14,15]</sup>; GFD: gluten free diet; OEGD: oesophagogastroduodenoscopy; F/u: follow-up; AAA-IgA: anti-actin IgA antibody.

suggested, that anti-TG2 level > 10 times ULN is strongly associated with the most severe intestinal damages<sup>[18,19]</sup>. On the other hand, we noticed that the algorithm 1 was not able to detect with a sufficient accuracy a Marsh 3 damage when the values of anti-TG2 were lower than 10 times ULN. In fact, among the 73 subjects of subgroup 3 (differing from subgroup 1 only for anti-TG2 levels less than 10 times ULN) a variable degree of intestinal damage ranging from a Marsh 3 lesion to only an isolated increment in the number of intraepithelial lymphocytes was found.

Since numerous studies have reported a strong association of AAA-IgA with a severe CD intestinal damage<sup>[7,16,17]</sup> and since the likelihood of CD is unequivocal when a villous atrophy of Marsh 3 is found<sup>[14,15]</sup>, we measured the AAA-IgA in the subgroups 1, 3 and 5, with the aim to identify subjects with Marsh 3 intestinal histopathology. The most important result comes from the measurement of AAA-IgA in subgroup 3 in which positivity for AAA-IgA and a Marsh 3 lesion was restricted to subjects having a level of anti-TG2 antibody between 4 and 10 times ULN (Figure 3). Conversely, the addition of AAA-IgA to subjects with anti-TG2 level > 10 times ULN (subgroup 1) did not increase the number of patients already identified by algorithm 1 (Table 2). We also noticed that AAA-IgA was not unequivocally associated with a Marsh 3 histopathology in individuals with anti-TG2 levels less than 4 times ULN. Indeed, three CD patients of subgroup 3 (one with grade Marsh 0; two with Marsh 1) and three controls (Subgroup 5) were found AAA-IgA positive.

In total, adding AAA-IgA in the algorithm allowed the identification of 20 additional CD patients with a Marsh 3 lesion (Figure 3), thus increasing from 64 (45% of sensitivity and 100% of specificity of the algorithm 1) to 84 (60% of sensitivity and 100% of specificity of the algorithm 1 plus serum AAA-IgA measurement) the total number of patients in whom duodenal biopsies could have been avoided.

Although a wide range of sensitivity and specificity values of AAA-IgA have been reported, questioning its utility in the screening for CD<sup>[20]</sup>, our data suggest that the best manner of using AAA-IgA measurement in the diagnosis of CD is not as an isolated test, neither when anti-TG2 antibody has a titer higher than 10 times ULN (the

association with a Marsh 3 lesion is complete and no other test should be requested) nor when anti-TG2 has a titer lower than 4 times ULN (it has been shown that low values of anti-TG2 positivity are found also in non-CD patients affected by other autoimmune pathologies as well as infections, tumors, myocardial damage, liver disorders, and psoriasis<sup>[21,22]</sup>), but in the restricted number of patients having moderate values comprised between 4 and 10 times ULN.

Recently, strong positive Red Blood Cells - anti-TG2 (RBC-TG2ab) values have been shown to correlate very well with EMA and human recombinant anti-TG2 (Hr-TG2ab) and with positivity of DQ2/8, making it possible to establish the diagnosis of CD in 94% of both children and adults belonging to at-risk groups<sup>[23]</sup>. In contrast, moderately positive RBC-TG2ab showed poor correlation with the other tests, and the diagnosis of CD was formulated in only 69% of children and 86% of adults<sup>[23]</sup>. Our findings suggest that AAA-IgA could also be helpful in improving the diagnosis of CD in this setting: children and adults belonging to at-risk groups with moderately positive RBC-TG2ab levels.

In conclusion, our findings confirm that the duodenal biopsy may be omitted in a significant number of CD patients with the application of algorithm 1 as suggested by Husby *et al.*<sup>[3]</sup>. In addition, we show that positivity for AAA-IgA in children and adolescents with anti-TG2 antibody comprised between 4 and 10 times ULN may further reduce the number of duodenal biopsies.

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