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Serology in adults with celiac disease: limited accuracy in patients with mild histological lesions

Anna Licata · Maria Cappello · Andrea Arini · Ada M. Florena · Claudia Randazzo · Giuseppe Butera · Piero L. Almasio · Antonio Craxì

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Abstract Celiac disease (CD) is a gluten-triggered enteropathy, presenting with insidious clinical patterns. It can occasionally be diagnosed in asymptomatic subjects. Our aim was to define the relationship among symptoms at diagnosis, serological markers [tissue transglutaminase antibodies (tTGA), anti-endomysium antibodies (EMA) anti-actin antibodies (AAA)] and degree of mucosal damage. A total of 68 consecutive adult patients with CD were enrolled. Intestinal biopsies were scored according to the Marsh classification modified by Oberhuber: I–II minimal lesions or absent villous atrophy; IIIA partial villous atrophy; IIIB–C total villous atrophy (TVA). HLA-typing was done for all patients. No association between clinical presentation and severity of mucosal damage was found. Presence of EMA or tTGA was significantly associated with more severe mucosal damage ($P < 0.001$). Of 12 patients, 11 with AAA were also positive for TVA. The severity of mucosal damage is the main factor governing the detectability of serological markers of CD. The sensitivity of serological testing is questionable in patients with minimal lesions.

Keywords Celiac disease · Duodenal histology · Serology · Minimal lesions

A. Licata (✉) · M. Cappello · A. Arini · C. Randazzo · G. Butera · P. L. Almasio · A. Craxì
Sezione e U.O.C. di Gastroenterologia, Di.Bi.M.I.S., University of Palermo, Piazza delle Cliniche 2, 90127 Palermo, Italy
e-mail: annalisalicata@yahoo.com

A. M. Florena
Department of Pathology, University of Palermo, Palermo, Italy

Introduction

The prevalence of celiac disease (CD) in the general population varies widely around the world [1]. In Italy, about 1 in every 200 persons is estimated to have overt or latent CD [2]. CD may cause non-specific symptoms, and is often silent when found at screening of at-risk subjects. When symptomatic, its features may mimic those of other diseases, making the diagnosis challenging. Peculiar histopathologic changes of the small bowel mucosa are considered the gold standard of diagnosis [3], but random biopsies are not always diagnostic because of the variable expression and patchiness of lesions. The heterogeneity of the degree and the extension of mucosal damage are at least partially correlated with the clinical expression of CD [4]. The gluten-triggered inflammatory response leads to activation of the humoral pathway and production of autoantibodies [5, 6], such as those against tissue transglutaminase (tTGA) and endomysium (EMA), commonly used to screen patients for small bowel biopsy and to assess adherence to a gluten-free diet (GFD) [7, 8].

IgA tTGA and IgA EMA are considered the most sensitive and specific serologic tests for CD. In children, they are reported to have a sensitivity and specificity of above 95% [9, 10]. It is a well-established fact that increased EMA and tTGA correlate with abnormal small bowel histopathology [11]. Fewer data correlating serologic levels with degree of villous atrophy are available. Clemente et al. [12] found IgA antibodies against actin filaments (AAA) in 60% of children and 90% of adults with severe or moderate villous atrophy caused by CD. In their study, IgA AAA were strongly correlated with more severe degrees of intestinal villous atrophy.

The aims of this study were to characterize, in a prospective cohort of Italian adult patients with a diagnosis of

CD, the serological markers and to correlate them with the severity of mucosal damage and with symptoms at diagnosis.

Methods

Patients

A total of 68 adults consecutively seen as out- or inpatients at our unit between January 2004 and December 2008, and who received a final de novo diagnosis of CD, were enrolled. All subjects were 18 years or older, had no previous diagnosis of CD and were on a normal diet with no restrictions. Patients had been referred because of gastrointestinal symptoms (abdominal pain, diarrhea), systemic signs (iron-deficiency anemia, cryptogenic chronic liver damage, a family history of CD—any first-degree relative with CD) or a recent diagnosis of type I diabetes or other autoimmune disease. Patients were stratified into subgroups according to their clinical presentation of CD as follows: classic presentation of CD (C-CD) with gastrointestinal symptoms; atypical presentation (A-CD) with iron-deficiency anemia or high levels of aminotransferases; silent disease (S-CD), detected on screening because of a family history of CD. The final diagnosis of CD was based on the National Institutes of Health Consensus Criteria [13].

Histology

All patients underwent upper gastrointestinal endoscopy, with small bowel biopsies. At least three specimens were taken from the distal duodenum and oriented before fixation. Histological analyses of the biopsies were carried out by an expert pathologist (A.F.) and scored according to the Marsh classification [14] as follows: increase in intraepithelial lymphocytes (IEL, grade I), increase in IEL with crypt hyperplasia (grade II), mild villous flattening (grade IIIA), marked villous flattening (grade IIIB) and total villous flattening (grade IIIC). The severity of intestinal mucosa damage was graded according to the scale proposed by Oberhuber et al. [15] as follows: partial villous atrophy (PVA) for Marsh IIIA, subtotal villous atrophy (STVA) for Marsh IIIB and total villous atrophy (TVA) for Marsh IIIC. In this study, STVA and TVA were combined as a severe form of villous damage (TVA).

Patients with Marsh III lesions and negative serology were considered as having CD only if there was a clinical and histological response to a GFD and no alternative diagnosis to account for the histological abnormalities. Marsh I–II lesions were considered nonspecific, but possibly consistent with CD, if serology was positive or the patient responded to a GFD with improvement of the mucosal architecture.

Serology

Venous blood samples were collected at the time of endoscopy and tested for IgA EMA, IgA tTGA and IgA AAA. Total serum IgA was measured to exclude selective IgA deficiency. Serum IgA EMA was tested by indirect immunofluorescence (IF) on a monkey esophagus substrate (EUROIMMUN, Labordiagnostika, Lübeck, Germany). Serum IgA tTGA was tested by ELISA, using recombinant tissue transglutaminase as antigen (EUROIMMUN, Labordiagnostika, Lübeck, Germany). IgA AAA was tested by IF on rat intestinal epithelial cells (EUROSPITAL, Trieste, Italy).

HLA testing

Complete HLA-typing for DR and DQ alleles was performed on genomic DNA extracted from peripheral blood by polymerase chain reaction, with sequence-specific primers at low and high resolution. Allele, genotype and haplotype frequencies were studied. Three phenotype groups were considered: HLA-DQ2 (homozygous or heterozygous for DQ2 and without DQ8), HLA-DQ2/-DQ8 and HLA-DQ8 (homozygous or heterozygous for DQ8 and without DQ2).

Statistics

Continuous variables were summarized as mean ± standard deviation (SD) or median and range, and categorical variables as frequency and percentage. Comparison of continuous and categorical variables was made with the Student's t test, for normally distributed variables, the Mann–Whitney U test, for not-normally distributed variables, and the χ^2 test. Data were analyzed with the Statistical Package for Social Science (SPSS) version 13.0 for Windows. Differences were reported as statistically significant if the P value was <0.05.

Results

Demographic, clinical, laboratory and histological features of the patients are shown in Table 1. The majority of patients were women (77.9%) and the mean age at diagnosis was 40 years (range 18–80). Thirty-three patients (48.5%) had a classic presentation of CD (C-CD) with gastrointestinal symptoms (diarrhea, abdominal pain). Ten of them also had anemia, seven abnormal liver tests, and five a first-degree relative with CD. A total of 26 patients

(38.2%) had an atypical presentation (A-CD), with iron-deficiency anemia and/or high levels of aminotransferases. Nine patients (13.3%) had a silent disease (S-CD), detected upon screening because of a family history of CD. tTGA/EMA were positive in 49 (72.1%) CD patients, and AAA were present in 12 (17.6%). As much as 39 patients (57.4%) carried HLA-DQ2, 22 (32.3%) HLA-DQ8 and 7 (10.3%) HLA-DQ2/DQ8 (Table 1).

No association was found between clinical presentation and severity of mucosal damage (Table 2). tTGA/EMA and IgA AAA showed no correlation with the clinical presentation. The only variable statistically associated with the clinical pattern was the ferritin value ($P = 0.008$). When analyzed for the severity of intestinal mucosa damage, tTGA/EMA were positive in 4/5 (80.0%) patients with TVA (histopathology IIIC), in 29/33 (87.9%) patients with STVA (histopathology IIIB) and in 13/18 (72.2%) patients with PVA (histopathology IIIA). Three of 12 patients (25.0%) with minimal histological lesions (histopathology I–II) were tTGA/EMA positive. Among the ten patients with villous atrophy and negative serology, the diagnosis was made by integrating the histopatholic evaluation, clinical pattern and the presence of HLA-DQ2/DQ8. Seven Marsh I–II patients were diagnosed as suffering from CD on the basis of response to a GFD, and two patients had latent CD on the basis of family history.

tTGA/EMA positivity was much less frequent in patients with minimal lesions (Marsh I–II) as compared to PVA and TVA (25.0 vs. 72.2 vs. 86.8%, respectively; $P < 0.001$) (Table 3). IgA AAA were positive in 2/5 (40.0%) patients with TVA (histopathology IIIC), 9/33 (27.3%) patients with STVA (histopathology IIIB), 1/18 (5.5%) patients with PVA (histopathology IIIA) and in none of the 12 patients with minimal histological lesions (histopathology I–II). The association of positivity for tTGA/EMA and AAA did not increase the sensitivity for diagnosis of PVA and TVA.

Table 1 Main clinical, serological and histological data at diagnosis of 68 patients with celiac disease

Gender	
Male	15 (22.1%)
Female	53 (77.9%)
Age (years, mean ± SD)	40.0 ± 16.0
Hemoglobin (g/dL) (mean ± SD)	12.3 ± 1.9
Ferritin (ng/mL) (median, range)	17.5 (3–1,532)
ALT (UI/l) (median, range)	23 (9–183)
Pattern of clinical presentation	
C-CD	33 (48.5%)
A-CD	26 (38.2%)
S-CD	9 (13.3%)
Autoimmune disease	13 (19.1%)
Duodenal histology	
Minimal lesions	12 (17.6%)
PVA	18 (26.5%)
TVA	38 (55.9%)
IgA tTGA/EMA positive	49 (72.1%)
IgA AAA positive	12 (17.7%)
HLA class II	
DQ2	39 (57.4%)
DQ8	22 (32.3%)
DQ2/DQ8	7 (10.3%)

C-CD classic celiac disease, *A-CD* atypical celiac disease, *S-CD* silent celiac disease, *PVA* partial villous atrophy, *TVA* total villous atrophy, *tTGA* tissue transglutaminase antibodies, *EMA* anti-endomysium antibodies, *AAA* anti-actin antibodies

Discussion

The gold standard for establishing a diagnosis of CD is the presence of gluten-dependent intestinal histological lesions [16]. Major problems are usually related to patchy lesions or artifactual damage caused by the difficulty in handling or correctly orienting biopsy tissues [3]. For these reasons, serological assays are the primary screening for deciding to perform an intestinal sampling [17]. Nonetheless, adult patients with mild or moderate mucosal changes are more frequently seronegative for tTGA/EMA than children [18]. In both children and adults, high titers of EMA and tTGA are found in patients with severely abnormal small bowel mucosa [10, 17–22]. Data on lesser degrees of villous atrophy in relation to EMA and tTGA are discordant, making the role of serology unclear when duodenal biopsy is not reliable for diagnosing CD with a certain degree of confidence. Establishing a correlation between positive serology and small bowel histopathology has considerable clinical utility. We observe a significant correlation between Marsh III histopathology and positive tTGA/EMA serology, as reported by other studies [23]. Presence of EMA or tTGA in our study was significantly associated with more severe villous atrophy. Sensitivity of serological testing is questionable among patients presenting with PVA, thus reducing its effectiveness in clinical practice [12, 18, 19, 22]. Rostami et al. [23] show that EMA has a lower sensitivity in CD patients with milder intestinal damage. Tursi et al. [18] observe, in 119 adults, that the prevalence of tTGA seropositivity and the mean tTGA titer are higher in CD patients with more severe inflammation at biopsy. Hence, a finding of tTGA/EMA seropositivity at a high titer may predict severe villous atrophy in patients with a suspected diagnosis of CD, though negative serology does not exclude a diagnosis of CD.

In our series, only three patients with Marsh I–II were tTGA/EMA positive and had gastrointestinal complaints

Table 2 Epidemiological, clinical and serological data of patients with celiac disease according to clinical presentation

	C-CD ($n = 33$)	A-CD ($n = 26$)	S-CD ($n = 9$)	*P*
Gender				
Male	6 (18.2%)	6 (23.1%)	3 (33.3%)	0.6
Female	27 (81.8%)	20 (76.9%)	6 (66.7%)	
Age (years, mean ± SD)	41.9 ± 19.2	38.5 ± 13.6	40.4 ± 15.1	0.7
Hemoglobin (g/dL) (mean ± SD)	12.1 ± 2.1	12.0 ± 1.9	13.7 ± 0.9	0.06
Ferritin (ng/mL) (median, range)	22 (3–1.256)	9.5 (4–1.532)	68 (15–131)	0.008
ALT (UI/l) (median, range)	23 (9–90)	27.5 (9–183)	18 (13–35)	0.2
Autoimmune disease	8 (24.2%)	5 (19.2%)	0	0.3
Duodenal histology				
Minimal lesions	5 (15.2%)	4 (15.4%)	3 (33.3%)	0.5
PVA	8 (24.2%)	9 (34.6%)	1 (11.1%)	
TVA	20 (60.6%)	13 (50.0%)	5 (55.6%)	
IgA tTGA/EMA positive	23 (69.7%)	20 (76.9%)	6 (66.7%)	0.8
IgA AAA positive	7 (21.2%)	5 (19.2%)	0	0.3
Any combination of tTGA/EMA/AAA	24 (72.7%)	21 (80.8%)	6 (66.7%)	0.6

C-CD classic celiac disease, *A-CD* atypical celiac disease, *S-CD* silent celiac disease, *PVA* partial villous atrophy, *TVA* total villous atrophy, *tTGA* tissue transglutaminase antibodies, *EMA* anti-endomysium antibodies, *AAA* anti-actin antibodies

Table 3 Frequency of Ttga/EMA and AAA positivity according to severity of mucosal damage

	Minimal lesions ($n = 12$)	Mucosal histopathology		
		PVA ($n = 18$)	TVA ($n = 38$)	*P*
tTGA/EMA	3 (25.0%)	13 (72.2%)	33 (86.8%)	<0.001
AAA	0	1 (5.5%)	11 (28.9%)	0.02
Any combination of tTGA/EMA/AAA	3 (25.0%)	14 (77.7%)	33 (86.8%)	<0.001

PVA partial villous atrophy, *TVA* total villous atrophy, *tTGA* tissue transglutaminase antibodies, *EMA* anti-endomysium antibodies, *AAA* anti-actin antibodies

suggestive of early CD. According to a recent update, these could also be classified as potential CD [24]. It must be stressed, however, that the small bowel lesions of CD may be patchy [25], and that we had no way of measuring the extent of the lesions throughout the small bowel in our patients. In the case of positive tTGA/EMA with Marsh II or lesser lesions, higher grade pathology may have been missed by sampling error. Serology markers were positive not only in almost all CD patients with subtotal and total villous atrophy, but also in 72.2% of CD patients with PVA and in 3 out of 12 patients with minimal lesions.

Despite serologic, histopathologic and clinical data, the diagnosis may remain unclear in some patients. As expected in our cohort, there were negative serologies in 17.9% of patients with Marsh III lesions. Intermediate Marsh grades and PVA have been associated with negative serology [16]. Dickey et al. [25] show that one out of five EMA-negative CD patients have Marsh IIIA lesions. Villous atrophy in the context of negative serology may result from non-gluten-sensitive enteropathy disease [26]. False-positive biopsies may also result from overinterpretation of specimens or poor specimen orientation. Of 19 patients, 9 with negative serology had minimal lesions; these patients, despite HLA compatibility and response to GFD, would not be celiac according to Biagi et al. [27], but would be considered gluten-sensitive patients, according toVerdu et al. [28].

In the past few years, IgA AAA have been found in CD patients, and a close correlation has emerged between the presence of AAA and mucosal damage [29]. Our data indicate that 28.9% of patients with TVA are AAA seropositive. Although AAA cannot replace EMA and tTGA in the diagnostic algorithm of CD, testing for the presence of both tTGA/EMA and AAA in subjects at high risk of CD (such as first-degree relatives) and with CD-related symptoms might be a useful tool in the follow-up of patients with severe disease to monitor the response to GFD [30].

As much as 90% of CD patients carry the HLA-DQ2 molecule, while approximately 5% express HLA-DQ8. Although genetic and environmental factors involved in the development of CD are already understood, it still unclear what it is that determines whether patients develop the classic or atypical form of the disease. Patients who are homozygous for HLA-DQ2 have a higher risk of developing CD than those who are heterozygous The association between HLA-DQ2 homozygosity and more severe villous atrophy is observed in a Finnish cohort of adult CD patients, while in an Italian cohort there is no correlation

with clinical presentation and mucosal damage [31, 32]. The prevalence of -DQ2 and -DQ8 heterodimers (57.4 and 32.3%) in our study confirms the evidence of a strong genetic predisposition to CD. We find an influence of HLA-DQ2/-DQ8 status on the degree of villous atrophy, as previously suggested [33]. In our cohort there is a tendency toward a higher predominance of the HLA-DQ2/-DQ8 genotype in patients with TVA (data not shown).

Though it is conceivable that more severe intestinal damage is correlated with a symptomatic presentation, we are unable to find a link between the degree of villous atrophy and the disease, as shown in another study [33]. However, it must be stressed that CD mucosal lesions might be patchy [4], and that we had no way of measuring the extent of the lesions throughout the small bowel in our patients. The area of involved mucosa may be another major determinant of the clinical expression of CD and should be assessed by video capsule endoscopy studies [34].

In conclusion, our data show that IgA tTGA and EMA predict villous atrophy on biopsy. The sensitivity of serological testing is questionable among patients with minimal lesions. We believe that these results call for further validation in a larger series. Until then, duodenal biopsy remains the gold standard for confirmation of the diagnosis.

Conflict of interest None.

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