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Alimentary Tract

Seronegative celiac disease: Shedding light on an obscure clinical entity

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ABSTRACT

Background: Although serological tests are useful for identifying celiac disease, it is well established that a minority of celiacs are seronegative.
Aim: To define the prevalence and features of seronegative compared to seropositive celiac disease, and to establish whether celiac disease is a common cause of seronegative villous atrophy.
Methods: Starting from 810 celiac disease diagnoses, seronegative patients were retrospectively characterized for clinical, histological and laboratory findings.
Results: Of the 810 patients, fourteen fulfilled the diagnostic criteria for seronegative celiac disease based on antibody negativity, villous atrophy, HLA-DQ2/-DQ8 positivity and clinical/histological improvement after gluten free diet. Compared to seropositive, seronegative celiac disease showed a significantly higher median age at diagnosis and a higher prevalence of classical phenotype (i.e., malabsorption), autoimmune disorders and severe villous atrophy. The most frequent diagnosis in the 31 cases with seronegative flat mucosa was celiac disease (45%), whereas other diagnoses were Giardiasis (20%), common variable immunodeficiency (16%) and autoimmune enteropathy (10%).
Conclusions: Although rare seronegative celiac disease can be regarded as the most frequent cause of seronegative villous atrophy being characterized by a high median age at diagnosis; a close association with malabsorption and flat mucosa; and a high prevalence of autoimmune disorders.

1. Introduction

Celiac disease (CD) is universally regarded as an immune-mediated enteropathy characterized by small intestinal villous atrophy occurring in genetically predisposed subjects [1,2]. In the last few decades, the identification of reliable serological biomarkers [3], i.e. anti-tissue transglutaminase (tTGA), endomysial (EmA) and deamidated gliadin peptide (DGP) antibodies, has progressively downgraded the prominent role of histology in CD diagnosis. In this respect, the ESPGHAN guidelines recommend skipping the duodenal biopsy in symptomatic children with high titer tTGA and positivity for genetic CD markers (i.e., HLA-DQ2 and/or -DQ8) [4]. Although CD-antibodies are detected in the vast majority of patients with CD (with an overall sensitivity ranging from 95% to 98%), a minority of CD patients may test negative for serology and in these cases the diagnosis is strictly dependent on the demonstration of villous atrophy at histopathology [5,6]. In these cases, HLA-DQ2 and/or -DQ8 positivity is a mandatory requirement to suspect the diagnosis of seronegative CD. Furthermore, both clinical and histological improvement should be proved after an adequate period of gluten-free diet (GFD). However, the finding of villous atrophy with negative CD serology is still a clinical challenge since severe small intestinal damage can be found in a variety of diseases other than CD, including parasitic infection (*Giardia lamblia*), autoimmune enteropathy, small intestine bacterial overgrowth (SIBO), common variable immunodeficiency (CVID), eosinophilic gastroenteritis, drug-induced enteropathy (mainly related to angiotensin II inhibitors), intestinal lymphoma, Crohn's disease, tropical sprue, human immunodeficiency virus (HIV) infection and Whipple disease [7–14]. Thus, prior to posing a firm diagnosis of seronegative CD, it is mandatory to rule out other causes of villous atrophy in order to avoid an unnecessary lifelong GFD.

Because of the paucity of data on seronegative CD, it is unclear whether this condition differs from CD with positive serology [15–17]. Also, the frequency of seronegative CD among the wide

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constellation of diseases associated with villous atrophy remains to be defined.

The aims of the present paper were threefold: (1) to define the prevalence of seronegative CD consecutively identified in a tertiary referral center; (2) to verify whether seronegative CD shows peculiar features, which sets it apart from the more commonly diagnosed seropositive CD; and (3) to establish the actual impact of seronegative CD amongst gluten unrelated disorders displaying villous atrophy.

2. Methods

2.1. Patients

During a 16-year-period (January 1998–January 2014), 810 CD patients (630 females, F/M ratio 3.5:1, median age at diagnosis 36 years, range 18–78 years) were diagnosed at the tertiary referral CD Center of St. Orsola-Malpighi University Hospital (Bologna, Italy). The diagnostic process adopted to confirm CD included duodenal histopathology, serology and HLA typing (when necessary). Small intestinal biopsy results of two samples taken from the duodenal bulb and 2 from the second portion of the duodenum were consistent with villous atrophy (mild, partial or total) in all the 810 patients [18]. Antibody testing was based on the identification of IgA tTGA and EmA; in cases with selective IgA deficiency, IgG tTGA were assayed. Serological tests have been always performed in the immunology laboratory of the St. Orsola-Malpighi University Hospital in Bologna. tTGA have been detected by ELISA using a home-made kit in the first three years (1998–2000), and a validated, standardized and reliable commercial kit (Eurospital, Trieste, Italy) in the remaining period (2001–2014). EmA detection was performed by indirect immunofluorescence on monkey oesophagus and human umbilical cord and the tests were always read by two blinded experts (U.V. and R. De G.). A detailed HLA typing including HLA-DQ2.5 (DQA1*0501, DQB1*0201), HLA-DQ8 (DQA1*03, DQB1*0302), HLA-DQ 2.2 (DQA1*0201, DQB1*0202) and HLA-DQ7.5 (DQA1*05, DQB1*0301) has been performed in cases with discrepancy between histology and serology. Among the 810 CD patients, we retrospectively focused our attention on seronegative CD cases, comparing their clinical, histopathological and genetic features to those of seropositive CD. The clinical phenotype of CD was defined as classical (diarrhoea with malabsorption), non-classical (gastrointestinal symptoms other than diarrhea and extraintestinal manifestations) and subclinical (fully asymptomatic or with symptoms below the threshold of detection) [19]. Essential requirements to confirm the diagnosis of seronegative CD were both the positivity for HLA-DQ2 and/or -DQ8 and the regrowth of small intestinal villi detected in a second duodenal biopsy after at least 1-year of GFD. The frequency of CD amongst all patients with seronegative villous atrophy of any origin was also established. Since patients were not individually identified, a simplified International Review Board approval by the Ethics Committee of our Hospital was obtained.

2.2. Statistical analysis

Statistical analysis was performed by applying the Mann–Whitney *U* test to compare the age of patients at diagnosis in seronegative *vs.* seropositive CD. Moreover, the Pearson Chi-square test was used to compare the classical phenotype, the presence of total villous atrophy and the association with autoimmune disorders in seronegative *vs.* seropositive CD patients. The level of significance was set at $P < 0.05$. Statistical evaluation was carried out using GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

Of the 810 CD patients, 796 (98.3%) were seropositive (780 had IgA tTGA and/or EmA and 16 with selective IgA deficiency tested positive for IgG tTGA). On the whole, only 14 patients (1.7%) fulfilled the criteria for seronegative CD. The median age at diagnosis was 49 years (range 19–75 years) with a female gender predominance (12 women). HLA typing disclosed positivity for DQ2 in 12 cases (of which 5 showed homozygosity), whereas the remaining 2 were DQ8-positive. Total villous atrophy was observed in 8 out the 14 patients, whereas the remaining 6 had partial ($n = 3$) and mild ($n = 3$) villous atrophy. All the 14 patients with seronegative CD had a classical phenotype characterized by diarrhoea and severe malabsorption with a significant weight loss. Of these 14 patients, 4 (29%) had at least one relative with seropositive CD. Seronegative CD patients displayed a frequent association with autoimmune disorders, which were found in 6 (43%) of them and included Hashimoto thyroiditis (2 cases), primary biliary cirrhosis (1 case), autoimmune gastritis (1 case), gluten ataxia (1 case) and peripheral neuropathy (1 case) (Table 1). Concerning autoantibody profile, 7 patients had antinuclear antibodies (ANA), 1 had anti-mitochondrial, 1 anti-smooth muscle, 1 anti-gastric parietal cell and 2 anti-neuronal antibodies. On the whole, 10 (71%) out of the 14 seronegative CD patients showed at least one autoantibody positivity. Although CD serological markers, i.e., EmA and tTGA, were negative, 4 out of the 14 patients tested positive for antibodies to native gliadin (AGA) of the IgG class (in one case associated with IgA positivity), nowadays no longer considered markers of CD (Table 1). The main differences between seropositive and seronegative CD are reported in Table 2. Compared to seropositive CD, seronegative CD showed a significantly higher median age at diagnosis (49 years *vs.* 36 years, $P < 0.005$) and a significantly more frequent classical phenotype (100% *vs.* 34%, $P < 0.001$). Both seronegative and seropositive CD were more frequent in female patients with a higher F/M ratio in the former *vs.* the latter group (F/M 6:1 *vs.* 3.5:1). Moreover, seronegative CD displayed total villous atrophy and co-association with autoimmune disorders more frequently than seropositive CD, although these differences did not reach statistical significance.

Globally, 31 cases of seronegative villous atrophy were identified. The most frequent cause of flat mucosa in this subset of patients was seronegative CD, found in 14 cases (45%). In the remaining cases the final diagnosis was Giardiasis in 6 (20%), CVID in 5 (16%), autoimmune enteropathy in 3 (10%), SIBO in 1 (3%), olmesartan enteropathy in 1 (3%) and eosinophilic enteritis in 1 (3%) (Fig. 1).

4. Discussion

The vast majority of CD patients display a wide array of serological biomarkers, namely tTGA, EmA and DGP, however CD can also occur in patients testing negative for CD serology [15–17,20]. The existence of seronegative CD strengthens the importance of duodenal biopsy since the finding of villous atrophy in these patients represents the first step towards a correct diagnosis [21,22]. According to established guidelines [5,23], patients with malabsorption and other related symptoms should always undergo a duodenal biopsy to rule out the occurrence of seronegative CD. The actual frequency of seronegative CD is still debated and discordant information is currently available on the clinical features of this CD subset [16]. Early studies found a high prevalence of seronegative CD associated with less severe intestinal damage and mild symptoms [6,20]. A more recent paper, however, reported that seronegative CD was rare, mainly diagnosed in elderly people and associated with a severe histological and clinical involvement [15]. Compared to previous studies, the latter one used a more sensitive