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ORIGINAL ARTICLE

Prevalence and natural history of potential celiac disease in adult patients

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Abstract

Objective. Potential celiac disease (PCD) is a form of CD characterized by positive endomysial/tissue transglutaminase antibodies and a preserved duodenal mucosa despite a gluten-containing diet (GCD); it can evolve into flat, active CD. This evolution is, however, not certain. Our aim was to retrospectively study the prevalence and the natural history of adult patients with PCD. ***Methods.*** The clinical notes of all 47 patients with PCD attending our clinic between September 1999 and October 2011 were retrospectively reevaluated. To study their clinical features, patients with active CD, randomly selected and matched for sex and date of birth, served as controls. Symptoms, associated diseases, familiarity, and laboratory data at diagnosis were compared. ***Results.*** Prevalence of PCD among all celiac patients directly diagnosed in our center was 42/187, (1/4.4, 18.3%, 95% confidence interval (CI) 13.3–23.4%). Age at diagnosis, laboratory data, prevalence of symptoms, associated diseases, and familiarity for CD did not differ between patients with PCD and those with active CD. Some patients with PCD maintained a normal duodenal mucosa for many years and their symptoms spontaneously improved despite maintaining a GCD. ***Conclusions.*** PCD is not a rare form of CD. Having found no difference at all in age at diagnosis and clinical features between PCD and active CD could suggest that PCD is not a prodrome of CD but is a separate entity that can only subsequently evolve into active CD.

Key Words: *disease progression, follow up studies, gliadins, intraepithelial lymphocytes, tissue transglutaminase*

Introduction

Celiac disease (CD) is a gluten-dependent chronic enteropathy characterized by specific serum antibodies and a variable degree of intestinal damage. In some celiac patients with positive antibodies, the intestinal lesions are, however, absent or extremely mild and consist of only an increased intraepithelial lymphocyte (IEL) count [1,2]. To distinguish this mild form of CD from active CD, characterized by a flat duodenal mucosa, this situation is defined as potential CD (PCD) [3,4].

PCD is well known to evolve into active CD, which definitely requires a gluten-free diet (GFD) [1,4–6]. However, this evolution is not certain, and in children, it was shown that PCD can last for many years despite a gluten-containing diet (GCD) [6]. The need for a GFD is therefore questionable and it is not clear whether it is preferable to start a GFD immediately after a diagnosis of PCD or only after an endoscopic follow-up has shown that frank villous atrophy actually occurred.

The prevalence of PCD among adult patients with CD is virtually unknown. Although it was considered

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to be rare, a recent Italian study performed in children with CD suggested that it is very high (20%) [6]. The reason for these scarce and contrasting data can probably be attributed to the fact that most patients with PCD are sent to large referral centers. Similarly, a considerable number of patients affected by active CD are also referred to these centers. While sending all these patients to such referral centers certainly makes it easier to study the disease, it also introduces an inevitable selection bias and thus makes an epidemiological study of PCD extremely difficult. Finally, it is unclear whether PCD is a prodrome occurring in every patient found to be affected by active CD or whether it is a distinct entity that can subsequently evolve into active CD.

The clinical pictures of PCD are definitely intriguing. Although some patients are diagnosed without clinical symptoms through serological screening of first-degree relatives or associated autoimmune conditions, others are diagnosed because of gastrointestinal symptoms suggestive of enteropathy, such as diarrhoea, abdominal pain, and anemia. How is it possible that these patients can develop these symptoms of malabsorption if their mucosal architecture is still preserved? We believe that the maturity of enterocytes can have an important role. We could hypothesize that, similarly to other conditions, in PCD the intestinal mucosa is maintained architecturally normal thanks to an increased enterocytic proliferation, which, however, will end up in a reduced enterocytic maturity and thus in a reduced absorptive capacity of the small bowel [7,8].

On the basis of this background, our research has several aims. First of all, we investigated the prevalence of PCD among patients with CD. We then performed a clinical study to investigate the clinical features of patients with PCD at diagnosis and compared them with the clinical features of patients with active CD. We also performed a follow-up study, investigating how and when these patients with PCD evolved into active CD. Finally, we performed a histological study to test whether in PCD the coexistence of normal intestinal morphology and symptoms suggestive of malabsorption is due to an increased enterocytic proliferation rate leading to a reduced maturity of the enterocytes themselves and thus a reduced absorptive capacity.

Patients and methods

The clinical notes of all the 47 adult patients affected by PCD (32 females, mean age 35 ± 16 years) attending our clinic between September 1999 and October 2011 were reevaluated. Patients were considered to be affected by PCD on the basis of positive endomysial antibodies (EMAs) (titre 1/5) [9] and a well-oriented duodenal biopsy (four samples) showing no evidence of villous atrophy at dissection microscopy and that pathologists had considered to be architecturally normal, being classified as either normal or grade A, while on a GCD [10].

Prevalence study

To calculate the prevalence of PCD, we reevaluated all the patients with either active CD (145) or PCD (42) directly found to be affected by CD in our clinic between September 1999 and October 2011. This means that all those patients who were diagnosed elsewhere and reached our center to obtain a certificate entitling them to gluten-free products through the Italian National Health Service or to have diagnostic confirmation were excluded. The prevalence of PCD was evaluated over the entire study period (12 years) and then analyzed per single year and per distinct periods of two and three years. Basic statistical concepts and methods (chi-square) were utilized to measure the prevalence and to test the possible differences [11].

Clinical picture study

For the purpose of the clinical study, we took into account only the patients with PCD directly diagnosed in our hospital. Patients that were found to be affected by PCD, thanks to both EMA and duodenal biopsies performed in other centers and then subsequently referred to us, were excluded. Since we cannot exclude that these patients were referred to us not only to confirm the diagnosis of PCD but also for clinical reasons, they could represent a selection bias. Similarly, only patients with active CD directly diagnosed in our centers served as controls. For each patient with PCD, two controls, or at least one, were randomly selected after matching for sex and date of birth (±3 years). Overall, we collected clinical data from 77 patients with active CD (56 females, mean age 34 ± 13 years). They had all been diagnosed on the basis of a duodenal biopsy showing frank villous atrophy (grade B1 or B2 [10]) and positive EMA. Age at diagnosis, prevalence of patients diagnosed because of different symptoms (classical/major, non-classical/minor, associated diseases, and familiarity [3]), and laboratory data at diagnosis were compared by means of Student's *t*-test or chi-square test as appropriate.

Follow-up study

Contrary to the epidemiological and clinical studies, we feel that the follow-up study cannot be affected by a selection bias. Therefore, we took into account all the 47 patients with PCD (32 females, mean age 35 ±

Table I. Laboratory tests in patients with PCD and controls, that is, patients with flat ACD.

	Hb (g/dl)	MCV (fl)	Iron (ug/dl)	Ferritin (ng/ml)	ESR (mm/h)	CRP (mg/L)
ACD (mean ± SD)	12.7 ± 2.1	85.1 ± 8.3	90.3 ± 42.3	28.1 ± 49.8	13.6 ± 12.4	0.3 ± 0.3
PCD (mean ± SD)	13.4 ± 1.5	87.1 ± 6.4	88.3 ± 31.7	57.6 ± 68.4	10.8 ± 6.2	0.2 ± 0.1
t-test (*p*)	0.08	0.26	0.83	**0.037**	0.33	0.24

The value in bold indicate stastistically significant $p < 0.05$.

16 years) we saw in our clinic and not only those directly found by us.

Whenever we see a new patient with PCD, we explain to him/her that PCD is an unusual condition and that it is not yet clear what the best management is. If there are no strong clinical reasons to start a GFD, such as dermatitis herpetiformis or severe gastrointestinal symptoms likely to be gluten sensitive, we offer the patients a choice between starting a GFD or a strict clinical and histological follow-up. We were thus able to retrospectively study both the clinical and histological evolution of these 47 patients with PCD. The curve of cumulative probability was calculated by means of the Kaplan and Meier method to study the mucosal flattening of PCD patients over time [12].

Histopathology study

To study the enterocytic proliferation pattern of PCD, we evaluated the immunohistochemical expression of antigen Ki-67, a nuclear protein that is associated with cellular proliferation, in a panel of intestinal biopsies obtained from 37 of the patients with PCD (26 females, mean age 35 ± 14 years) and 37 controls (24 females, mean age 45 ± 17 years) in whom CD had been excluded thanks to a normal duodenal biopsy and negative EMA. Paraffin-embedded duodenal sections from patients and controls were immunostained using an anti-human Ki-67 antibody (MIB-1, The Binding Site, Bristol, UK). The proportion of Ki-67$^+$ nuclei along both the villous column, divided equally into lower, middle and upper compartments, and the crypt was calculated [13].

Brush border of the enterocytes can be considered one of the most important markers of cellular maturity, and it is certainly the simplest one to study. The histological slides from the same PCD patients and controls were therefore stained with alcian blue–periodic acid–Schiff (PAS)–hematoxylin method to blindly evaluate the morphology of the brush border and to test whether it was possible to distinguish patients with PCD from controls.

The study was approved by the Ethics Committee of the Fondazione IRCCS Policlinico San Matteo.

Results

Prevalence study

Between September 1999 and October 2011, PCD was found in 42 patients (29 females, 37 ± 14 years). At the same time, active CD was found in 187 adult celiac patients (131 females, 35 ± 12 years). Overall prevalence is therefore 42/187 (1/4.4, 18.3%, 95% CI 13.3–23.4%). Although this prevalence tends to vary in each year, no significant differences were found (data not shown).

Clinical study

Age at diagnosis (37 ± 14 vs. 35 ± 12 years), prevalence of patients diagnosed because of diarrhea and/or weight loss (44% vs. 47%), anemia or minor symptoms of malabsorption (44% vs. 56%), associated diseases (47% vs. 41%), and familiarity for CD (31% vs. 25%) did not differ at all between patients with PCD and active CD. Tables I and II show the laboratory data. Only albumin and ferritin were slightly reduced in patients with active CD compared to PCD.

Follow-up study

The 47 patients with PCD were all on a traditional Italian GCD when we saw them in our clinic after the diagnosis of PCD. Symptoms leading to diagnosis were diarrhea/weight loss (20 patients), anemia (15 patients), familiarity (11 patients), and/or associated diseases (20 patients).

Table II. Laboratory tests in patients with PCD and controls, that is, patients with flat ACD.

	Ca^{++}(mEq/L)	K^+ (mEq/L)	Albumin (g/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
ACD (mean ± SD)	8.9 ± 1.0	3.9 ± 0.4	4.1 ± 0.5	165.2 ± 30.3	82.1 ± 84.8
PCD (mean ± SD)	9.3 ± 0.3	3.8 ± 0.3	4.4 ± 0.3	165.2 ± 36.3	64.7 ± 32.5
t-test (*p*)	0.11	0.44	**0.005**	0.99	0.13

The value in bold indicate stastistically significant $p < 0.05$.

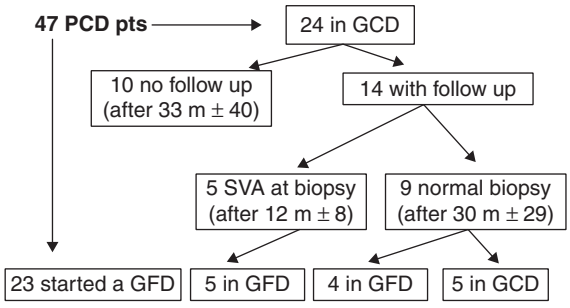

Figure 1. Results of the follow up study. PCD: potential coeliac disease, pts: patients, GCD: gluten-containing diet, GFD: gluten-free diet, m: month, SVA: subtotal villous atrophy.

Figure 1 shows the results of the follow-up study. A GFD was started in 23 of them: 14 patientts were suffering from gastrointestinal symptoms and refused the endoscopic follow-up, 5 were suffering from dermatitis herpetiformis, 2 from dilative cardiomiopathy, and 1 from multiple autoimmune diseases. The last two patients had been found to be affected by flat CD in the past. The 14 patients suffering from gastrointestinal symptoms and the 5 patients with dermatitis herpetiformis all improved after the start of a GFD.

The remaining 24 patients maintained a GCD and started a follow-up: 14 of them underwent at least one histological reevaluation that showed a flat duodenal biopsy in 5 of them (time between diagnostic and follow-up biopsy: 12 ± 8 months); they were then started on a GFD. The remaining nine patients still had a preserved mucosal architecture at the follow-up biopsy (30 ± 29 months after the diagnostic one); four of them decided to stop the follow-up and started a GFD; and the other five are still on a GCD and, 20 ± 15 months after diagnosis, are all in good clinical conditions, except for one patient who complains about symptoms of esophageal reflux disease. Finally, the 10 patients who have not yet undergone a follow-up biopsy are in good clinical condition, in spite of being on a GCD. At diagnosis, three of them were suffering from diarrhea that spontaneously improved; anemia was present in five of them and the associated diseases (two thyroid diseases, one alopecia, one rheumatoid arthritis) in four of them. The last one was diagnosed through family screening for CD.

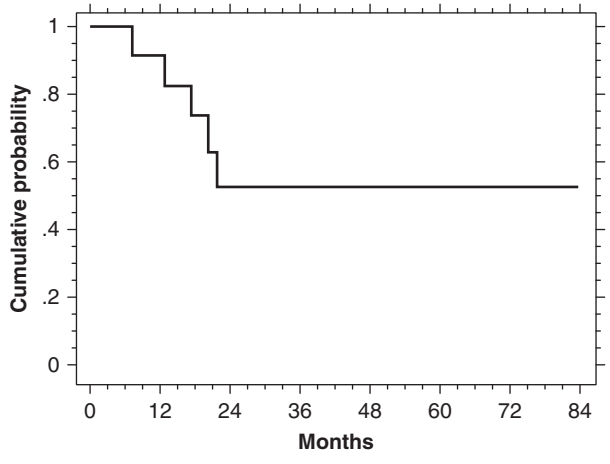

Figure 2. Kaplan Meier curve showing cumulative probability of mucosal flattening in patients with potential coeliac disease. 5 PCD patients had a follow up time longer than 24 months.

Figure 2 shows the cumulative probability of mucosal flattening over time. Although this was calculated on the basis of only 14 patients, it is evident that timing of mucosal flattening is rather unpredictable and, in some patients, can occur several years after the diagnosis of PCD.

Histopathology study

14.1% ± 18.1% of the enterocytes from patients with PCD and 19.8% ± 11.2% of the enterocytes from controls were Ki-67 positive. The difference was not statistically significant. Again, on the basis of the brush border PAS staining it was not possible to distinguish PCD patients from controls (data not shown).

Discussion

Although our study is limited by its retrospective design and the relatively small number of patients, we feel that the results are interesting. PCD is a particular form of CD, very different from flat, active CD in several aspects. Compared to active CD, PCD is characterized by a still preserved mucosal architecture, a reduced prevalence of DQ2, and a higher prevalence of HLA DQ8 [1,2,14]. Moreover, it is well known that mortality in patients with CD is increased compared to the general population and mortality in patients with dermatitis herpetiformis, a cutaneous gluten-sensitive condition invariably associated with CD, and very often with PCD [15], is not increased [16]. PCD can therefore be considered to be the mildest form of CD.

Although the prevalence of CD in the general population was the subject of several publications, probably because of the biases described in the Introduction section, the prevalence of PCD in adults has not been ascertained so far. However, the only study providing epidemiologic data suggests that, at least in children, PCD is very frequent [6]. Our study confirms that PCD is definitely very frequent also in adult patients with CD (~20%).

Practically, 50% (23/47) of the patients with PCD that we saw in our clinic between September 1999 and October 2011 were started on a GFD at the time of

the initial diagnosis of PCD: 19 of them were suffering from either gastrointestinal or cutaneous gluten-sensitive symptoms and/or refused the follow-up; 2 were affected by dilatative cardiomiopathy, a condition precluding repetitive gastroscopies; and 2 had been found to be affected by active CD in the past, who should actually be considered to be affected by latent CD rather than PCD [3]. The last one was suffering from multiple autoimmune conditions and we preferred to start a GFD. The remaining 24 patients maintained a GCD and started a follow-up. Although the cumulative probability of mucosal flattening is high (Figure 2), the timing of flattening is totally unpredictable. So, similarly to children with PCD [6], adult patients with PCD can maintain a normal duodenal mucosa for many years and their symptoms can spontaneously improve despite a GCD.

To understand whether GFD has a role in improving the gastrointestinal symptoms of PCD patients is a difficult task. Although this was the case not only in this study but also in two recent Finnish papers [4,5], an Italian pediatric study showed that a GFD was followed by a remission of symptoms in only 50% of the patients [6]. It is, therefore, very difficult to understand whether it is preferable to start a GFD immediately after a diagnosis of PCD or only after an endoscopic follow-up has shown that frank villous atrophy actually occurred.

In the clinical part of our study, we confirmed that symptoms of malabsorption (diarrhea and/or weight loss) can be very frequent in patients with PCD [17]. Moreover, as far as age at diagnosis, prevalence of presenting symptoms, and laboratory data are concerned, we were surprised to see that our clinical study could not find any difference between patients with PCD and controls, that is, randomly selected patients with active CD matched for sex and date of birth. We therefore think that PCD could not be a prodrome of CD occurring in all celiac patients, but that it could be a separate entity that can only subsequently evolve into active CD. This idea is further supported by the recent observation that, compared to active CD, PCD is characterized by an increased frequency of DQB1*0302 and a reduced frequency of DQB1*02 homozygosity [14]. On the other hand, the high prevalence of associated conditions in PCD (47%) clearly indicates that PCD cannot be underestimated.

As far as histopathology is concerned, we could not find any difference between Ki-67-positive cell distribution and brush border PAS staining in patients with PCD and controls. The gastrointestinal symptoms cannot therefore be advocated to immaturity of the enterocytes resulting from an increased proliferation to maintain mucosal architecture. The origin of these symptoms thus remains unclear.

As far as the diagnostic criteria for PCD are concerned, we feel that a diagnosis of PCD requires positive EMA. Since it has already been shown that an increased IEL count is not specific for CD and that an increased IEL count in an EMA-negative patient is unlikely to be related to CD [18,19], we think that an increased IEL count per se does not suffice for a diagnosis of PCD. Moreover, the relatively lower specificity of tissue transglutaminase antibody should discourage a diagnosis of PCD in a patient with positive tissue transglutaminase antibody but negative EMA [9].

In conclusion, although the pathogenesis of the gastrointestinal symptoms remains unclear, we have shown that PCD is a frequent form of CD, not only in children but also in adults. It is unlikely to be a prodrome occurring in all patients with CD.

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