Features and Progression of Potential Celiac Disease in Adults

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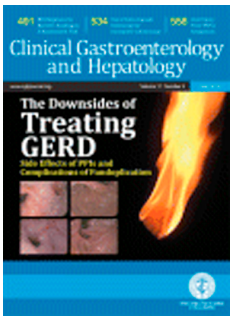

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Features and Progression of Potential Celiac Disease in Adults

Short title: Potential celiac disease in adults

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Abbreviations: ACD, active celiac disease; ANA, antinuclear antibodies; AU, arbitrary units; CD, celiac disease; EmA, endomysial antibodies; GCD, gluten containing diet; GFD, gluten free diet; HLA, histocompatibility leukocyte antigen; IBS, irritable bowel syndrome; IELs, intraepithelial lymphocytes; PCD, potential celiac disease; tTGA, tissue transglutaminase antibody; TG2, tranglutaminase 2.

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Abstract

Background & Aims: Individuals with potential celiac disease have serologic and genetic markers of the disease with little or no damage to the small intestinal mucosa. We performed a prospective study to learn more about disease progression in these people.

Methods: We collected data from 77 adults (59 female; median age, 33 years) diagnosed with potential celiac disease (based on serology and HLA type) at Bologna University, in Italy, from 2004 through 2013. The subjects had normal or slight inflammation of the small intestinal mucosa. Clinical, laboratory, and histologic parameters were evaluated at diagnosis and during a 3-year follow-up period.

Results: Sixty-one patients (46 female; median age, 36 years) showed intestinal and extra-intestinal symptoms, whereas the remaining 16 (13 female; median age, 21 years) were completely asymptomatic at diagnosis. All subjects tested positive for immunoglobulin (Ig)A endomysial antibody and tissue transglutaminase antibody, except for 1 patient with IgA deficiency; 95% of patients were carriers of HLA-DQ2. Duodenal biopsies from 26% patients had a Marsh score of 0 and 74% had a Marsh score of 1. A higher proportion of symptomatic patients had autoimmune disorders (36%) and antinuclear antibodies (41%) than asymptomatic patients (5% and 12.5%, respectively), and symptomatic patients were of older age at diagnosis ($P<.05$). Gluten withdrawal led to a significant clinical improvement in all 61 symptomatic patients. The 16 asymptomatic patients continued on gluten-containing diets and only 1 developed mucosal flattening; levels of anti-endoymsial and tissue transglutaminase antibodies fluctuated in 5 of these patients or became undetectable.

Conclusions: In a 3 year study of adults with potential celiac disease, we found most to have symptoms, but these improved upon gluten withdrawal. Conversely, we do not recommend a gluten-free diet for asymptomatic adults with potential celiac disease, since they do not tend to develop villous atrophy.

KEY WORDS: EmA, tTGA, autoimmunity, wheat.

Introduction

Celiac disease (CD) is an immune-mediated gluten-dependent systemic disorder characterized by a well-established serological and genetic profile along with small intestinal damage.[1] In most cases the enteropathy displays the classical *celiac* hallmark, i.e. villous atrophy consistent with active CD (ACD). However, an increasing number of patients show antibodies and genetic alleles as the only markers of the disease in the presence of a relatively normal or slightly inflamed non-atrophic small intestinal mucosa.[2] Potential CD (PCD) is the term coined for the non-atrophic variant of gluten-sensitive enteropathy,[3] which is characterized by serum endomysial (EmA) and tissue transglutaminase antibodies (tTGA), and a positive histocompatibility leukocyte antigen (HLA)-DQ2 and/or -DQ8 genotype. EmA are more specific than tTGA since an isolated positivity for tTGA at low titer can be also found in conditions other than CD.[4,5] In patients with PCD the intestinal mucosa may present a normal histology (Marsh 0) or an increased number of intraepithelial lymphocytes (IELs) (Marsh 1).[2] Regardless the status of the small intestinal mucosa, PCD patients can display both gastrointestinal and extra-intestinal symptoms or can be completely asymptomatic.[6]

Although diagnostic criteria are well established, PCD is still a gray area with many unsettled issues. So far, few studies dealing with PCD in children and adults have been published, and conflicting results have been reported.[7-12] In children, more than 80% of PCD patients are asymptomatic, and the remaining symptomatic patients display intestinal manifestations (e.g. malabsorption, chronic diarrhea, recurrent abdominal pain) more frequently than extra-intestinal ones (anemia, raised transaminases and short stature).[8,12] In contrast, the symptomatic phenotype was found to be prevalent in adults with more prominent extra-intestinal symptoms.[7,10,11] It is unknown whether PCD patients kept on a gluten-containing diet (GCD) will develop an overt gluten-dependent enteropathy over time.[7-12] Therefore, the need for a gluten-free diet (GFD) remains questionable in these patients, although the scientific community is eager to suggest GFD for symptomatic PCD patients. Asymptomatic patients are usually left on a GCD with a close

clinical, serological and histological follow-up.[7-12] Previous studies have reported a possible fluctuation of serological markers over time in both pediatric and adult PCD left on a GCD with variable progression to villous atrophy.[8,9,11-13]

Based on the controversial data regarding the natural history of PCD, the present study was designed to provide a prospective analysis of clinical, serological and histological features of adult PCD. Moreover, asymptomatic PCD patients were followed up in order to establish the percentage of patients progressing to villous atrophy consistent with ACD.

Patients and Methods

Study Protocol

In this prospective cohort study 77 adult patients (59 females; median age 33 years, range 14-66 years), with PCD diagnosed at the Celiac Disease Center of Bologna University from 2004 to 2013 were investigated. The diagnosis of PCD relied on the positivity of serology (EmA and tTGA), HLA typing (DQ2 and/or DQ8) and normal or slightly inflamed small intestinal mucosa (lesions 0-1 according to the Marsh-Oberhüber classification). Total serum IgA were normal in all but one patient with selective IgA deficiency (serum IgA < 5 mg/dL) who showed positivity for EmA and tTGA of IgG class. In order to determine the prevalence of PCD in the whole spectrum of CD, we calculated the total number of PCD and ACD patients diagnosed in our Center in the study period. CD cases diagnosed in other Centers and referred to our outpatient clinic were not enrolled.

PCD patients were subdivided in symptomatic and asymptomatic subgroups. Antibody prevalence with relative titers / activities of EmA and tTGA, HLA-DQ2 and/or -DQ8 genotype, duodenal histology, associated autoimmune disorders and markers of autoimmunity (antinuclear antibodies – ANA – detected by immunofluorescence on HEp-2 cells) as well as familiarity for CD were compared between symptomatic and asymptomatic PCD patients. PCD symptomatic patients were put on a GFD and underwent a periodic follow-up to assess their clinical and serological response to GFD. Patients without symptoms were left on a GCD and followed up every 6 months with a

clinical and serological assessment and every two years with histological evaluation. A thorough dietary survey was performed at each control visit to verify that asymptomatic PCD patients were still on GCD. Also, the asymptomatic PCD patients decided to adhere to a GCD and doctors checked that they actually continued to consume gluten in their diet. The follow-up of PCD patients lasted from 1 to 10 years (mean follow-up 3 years) (Figure 1).

Methods

Endomysial (EmA) and tissue transglutaminase antibodies (tTGA). Serum IgA EmA were measured by indirect immunofluorescence on 5-μm-thick frozen sections of human umbilical cord. A fluorescein-conjugated anti-human IgA (Dako, Copenhagen, Denmark) was used as a secondary antibody. Immunolabeling was assessed by a thin fluorescent network around smooth muscle fibers in the wall of the umbilical artery and vein. Sera were tested at an initial dilution of 1:5 and, when positive, titered up to the end point. IgA tTGA were assessed with an enzyme-linked-immunosorbent assay (ELISA) kit, based on human recombinant TG2 antigen (Eurospital, Trieste, Italy). A cut-off value of 16 arbitrary units (AU) was used. In the case of IgA deficiency, EmA and tTGA of the IgG class were detected.[5] All antibody tests were performed in our certified immunology laboratory.

HLA typing. HLA typing was performed at the laboratory of Immunogenetics of the St. Orsola-Malpighi Hospital. All 77 patients included in the present study were genotyped for HLA DQA1 and DQB1 alleles.[14] HLA-DQ2 and -DQ8 positivity were based on DQB1*02 and DQA1*05 and DQB1*0302 finding, respectively.

Duodenal biopsy. Six well-oriented duodenal biopsies (2 from the duodenal bulb and 4 from the distal duodenum) were taken during gastroduodenoscopy from all patients at the time of PCD diagnosis and during follow-up. Morphometric evaluation was performed using a careful orientation of the biopsies. IELs were counted using CD3 immunostaining and the worst average of and the villous height / crypt depth ratio was also assessed. Biopsies were evaluated by two pathologists blinded to the clinical history of the patients and were graded according to Marsh-Oberhüber.[15]

IgA anti-transglutaminase 2 (TG2) intestinal deposits. In 20 out of the 77 PCD cases, IgA anti-TG2 intestinal deposits were determined at PCD diagnosis on frozen small intestinal biopsies.[16]

Statistics. The 2-tailed Fisher's exact test was used to compare the prevalence of antibodies, genetic markers, duodenal histology and associated autoimmune disorders between symptomatic and asymptomatic PCD. The Mann-Whitney U test was used to evaluate statistical significance difference between symptomatic and asymptomatic PCD according to age of onset. The level of significance was set at $P<0.05$.

Ethics. The study protocol (119/2012/U/Tess) was approved by the Institutional Review Board of St. Orsola-Malpighi Hospital. All PCD and ACD patients signed an informed consent to enter the study.

Results

Prevalence and clinical features of PCD

Of the 735 consecutively diagnosed CD patients, 77 (10.5%) fulfilled the diagnostic criteria for PCD with an increasing prevalence of this clinical variant over years. PCD increased from 5% of the total number of CD diagnoses in 2004 to 18% in 2013. Both PCD and ACD were more frequent in the female than male gender (3.2 and 3.5, respectively), but PCD showed a slightly younger median age at diagnosis compared with ACD (33 vs. 36 years). Fourteen (18%) of PCD patients were first-degree relatives of CD patients.

Of the 77 PCD patients, 61 (47 females, median age 36 years) (79%), were symptomatic, whereas the remaining 16 (12 females, median age 21 years) (21%) did not complain of any symptom. Asymptomatic PCD differed from symptomatic PCD with a significantly younger age at onset ($P<0.05$) (Table 1). Among the 61 symptomatic cases, 10 (16%) had the classical phenotype characterized by diarrhea and weight loss, whereas the other 51 symptomatic PCD patients showed the non classical phenotype characterized by the occurrence of iron-deficiency (more frequently

than folic acid-deficiency) anemia, osteopenia, aphthous stomatitis, irritable bowel syndrome (IBS)-like symptoms, gastro-esophageal reflux disease and recurrent miscarriages (Figure 2).

Serology, genetics, duodenal histology, autoimmunity and CD-familiarity in symptomatic and asymptomatic PCD

No significant difference was found between symptomatic and asymptomatic PCD for serology, genetics, duodenal histology and CD-familiarity (Table 1). EmA and tTGA of IgA class were positive in all PCD cases except from one symptomatic patient with IgA deficiency who was positive for EmA and tTGA of IgG class. EmA titers and ELISA activities were both very low. Most of both symptomatic (95%) and asymptomatic (94%) PCD were HLA-DQ2+ with only 3 patients (2 symptomatic and one asymptomatic) being HLA-DQ8+. As for duodenal histology, symptomatic and asymptomatic PCD showed a higher prevalence of type 1 lesion (75% and 69%, respectively) vs. type 0 (25% vs. 31%, respectively). Eleven (18%) of the 61 PCD patients with symptoms and 3 (19%) of the 16 without symptoms had a first-degree relative with CD. Conversely, the prevalence of autoimmune disorders (i.e. Hashimoto thyroiditis, alopecia, psoriasis and type 1 diabetes mellitus) was significantly higher in symptomatic (36%) than in asymptomatic PCD (5%) (P<0.05). In addition, ANA were more frequently detected in symptomatic than in asymptomatic PCD patients (41% vs. 12.5%, P<0.05).

Follow-up

PCD patients put on a GFD

Sixty-one (79%) of the 77 adult PCD patients began a GFD because of symptoms (Figure 1). All the patients on GFD became negative for antibodies showing a significant clinical improvement. After gluten withdrawal the 25 patients with low levels of ferritin, with or without folic acid deficiency, normalized hemoglobin and oligoelements. Liver enzymes reverted to normal in 7 patients with hypertransaminasemia after 3-6 months of GFD. Three women with PCD and recurrent miscarriages improved dramatically and carried pregnancy to term following GFD. Patients with diarrhea and malabsorption (10 cases) showed a very good response to GFD as

confirmed by a marked increase in body weight. Aphthous stomatitis disappeared very quickly after GFD. Despite a partial improvement, the symptoms which periodically recurred in PCD patients on a GFD were those related to IBS and gastro-esophageal reflux disease. Overall, patients with symptomatic PCD experienced a significant relief of their symptoms after GFD.

PCD patients left on a GCD

Sixteen (21%) of the 77 PCD patients on a GCD remained asymptomatic. All of them were followed up from 1 to 10 years (mean 3-year follow-up). They received a clinical and serological evaluation every 6 months and, in cases with fluctuating or permanent antibody positivity, duodenal biopsies were taken every two years (Figure 3). Only one of the 16 PCD patients on GCD became symptomatic after a 2-year-follow-up, developing iron-deficiency anemia and diarrhea associated with a significant increase in antibody titer (1:160 for EmA and > 100 AU for tTGA). A small intestinal biopsy showed subtotal villous atrophy, confirming that the patient (a 43-year old woman) progressed to ACD. No CD-related symptoms were observed in the other 15 patients during follow-up. EmA and tTGA disappeared in four patients in the first 6 months. At diagnosis these four patients had very low EmA titer (1:5 or 1:10) as well as tTGA activities (< 2 times the cut-off). Another PCD patient displayed a marked serological fluctuation, i.e. alternating antibody positivity (EmA 1:40; tTGA > 2 times the cut-off) with negativization. In the remaining 10 PCD cases antibody titers / activities remained stable over time. EmA and tTGA variations in PCD patients on GCD during follow-up are reported in Figure 4. Small intestinal biopsy was repeated every 2 years in 10 of the 11 PCD patients with persistent or fluctuating antibody positivity (one patient was not re-biopsied because of late enrollment in the study). No significant histological changes were observed in the duodenal biopsies repeated during the follow-up.

IgA anti-TG2 intestinal deposits were tested in 20 of the 77 PCD cases, resulting positive in 12 (60%). Six PCD cases positive for IgA anti-TG2 intestinal deposits were asymptomatic (Figure 5 and supplementary Figure 1). None of them had developed villous atrophy at follow-up.

Discussion

The scant and conflicting information on adult PCD prompted us to plan this prospective cohort study aiming to shed light on the natural history of this nosologic variant of CD and to determine the best approach for its management.[7,10,11,17]

The first evidence emerging from our study is that the diagnosis of PCD has significantly increased in the latest years as result of common CD antibody screening applied to the general population.[18] The number of patients diagnosed as having PCD is becoming sizeable, since this condition is currently estimated to represent about one-fifth of the total number of CD diagnoses. While previous studies have shown that PCD in childhood is asymptomatic in about 80% of cases,[8,12] our study clearly highlights that the majority of adult PCD are predominantly symptomatic, showing both gastrointestinal and extra-intestinal manifestations. Notably, the mean age of the few adult asymptomatic PCD patients was significantly lower than that observed in the larger group of symptomatic patients. Also, a significantly higher prevalence of associated autoimmune disorders and autoimmune markers (e.g., ANA) was seen in symptomatic than in asymptomatic PCD. In contrast, serology, genetics, duodenal histology and familiarity for CD did not differ in symptomatic and asymptomatic patients. EmA and tTGA tested positive in PCD, although with lower titers / activities than in ACD.[6,19,20] There was no difference in terms of HLA-DQ2 and -DQ8 genotype in PCD and ACD suggesting that these conditions, although apparently distinct, can be viewed as two variants of the same disease. From a histopathological standpoint, the most common pattern of PCD was characterized by an increased number of IELs (Marsh 1) prevailing over normal mucosa (Marsh 0) in both symptomatic and asymptomatic PCD. In our series, the increased IELs were not an indicator of progression of PCD to ACD. Finally, familiarity for CD was a remarkable feature commonly identified in our cases of PCD, confirming previous data.[21] However, our data did not show any difference between symptomatic and asymptomatic patients in terms of familiarity for CD, which is apparently in contrast to the well known lack of

symptoms in familial cases of CD.[9] Further studies will be necessary to elucidate the high prevalence of symptomatic familial cases in PCD.

An open issue in the management of PCD pertains to the presence of symptoms that can be considered as indicators for prescribing GFD, regardless of the absence of severe small intestinal damage. Although no established guidelines are so far available, recent data suggest that a subset of symptomatic pediatric and adult PCD patients can benefit from GFD.[7,12] In recent studies, the percentage of children with symptomatic PCD showing a significant clinical improvement after GFD was quite high, ranging from 55% (11/20 cases) to 91% (32/35 cases).[8,12] In adults with symptomatic PCD, a positive response to GFD has been reported in all cases,[7] although another study showed the spontaneous disappearance of symptoms on a GCD.[11] In the Kurppa et al study, clinical manifestations showed a significant improvement in all PCD patients after one year of GFD. Notably, the group of PCD patients (n= 10) left on GCD experienced a dramatic worsening of the clinical manifestations.[7] The results emerging from our study demonstrating that gluten withdrawal leads to a significant improvement of both intestinal and extraintestinal manifestations in all symptomatic patients, clearly expand our previous understanding of this issue and support the concept that GFD should be prescribed to adult symptomatic PCD. Again, in line with Kurppa et al,[7] GFD normalized gynecological disorders, since women with recurrent miscarriages were able to carry pregnancies to term.

A great dilemma facing the scientific community is whether and to what extent adult asymptomatic PCD may evolve to ACD. Based on pediatric literature data, the majority of asymptomatic PCD cases developed neither symptoms nor villous atrophy during long term follow-up.[8,9,12] In a 3-, 6- and 9-year follow-up, the progression from PCD to ACD in children was observed in 14%, 27% and 33% of 175 asymptomatic cases, respectively.[12] Male gender, slight mucosal inflammation at onset and a particular genetic profile identified a subgroup of patients prone to develop severe intestinal damage over time. Compared to the previous study, the

percentage of patients evolving from PCD to ACD in a family study was much lower (5%).[9] Caution before commencing a GFD in asymptomatic children is supported by the spontaneous negativization or fluctuation of serological markers on a GCD. In a 2-year follow-up of asymptomatic infants born to CD parents, EmA and tTGA disappeared spontaneously in 18 (86%) and fluctuated in 2 (9%) out of 21 PCD patients on a GCD.[9] Two other studies demonstrated the disappearance of antibodies in 15-20% of cases and fluctuation in 33-37% during a mean 3-year follow-up.[8,12] Moreover, the majority of pediatric patients with stable antibody positivity did not develop severe mucosal damage in a long term (up to 9 years) follow-up.[12] However, studies on adult PCD on a GCD are scant, limited by short follow-up periods and indiscriminately include symptomatic and asymptomatic patients, making data difficult to interpret. In one study based on a small sample size (24 cases) and a very short follow-up (20 months) only one-fifth of PCD cases showed a progression to villous atrophy.[11] In a more recent study on 57 PCD cases followed up for 12 months, only 7% progressed to ACD despite the persistence of serological tests in 80% of cases.[17] Our data, based on a prospective design and a considerable follow-up (up to 10 years), showed that only one (6%) of 16 adults with asymptomatic PCD on a GCD developed villous atrophy. Clearly, a higher number of asymptomatic potential celiacs will be required in order to confirm the low progression of PCD to ACD. Antibody markers disappeared or fluctuated in 5 (31.5%) out of 16 asymptomatic PCD patients, a figure overlapping the results reported in children by Auricchio et al.[12] In the 4 cases with antibody negativization, both EmA and tTGA (positive at a very low titer at onset) disappeared in the first 6 months of follow-up. Finally, previous data raised the hypothesis that the positivity for IgA anti-TG2 deposits in the small intestinal biopsies may be a useful indicator predicting villous atrophy in children with PCD.[8] However, these findings were not confirmed in our study as none of our cases with positive IgA anti-TG2 intestinal deposits at onset progressed to ACD in the follow-up. Our findings, however, were based on a small sample size and further research is expected to shed light on the relevance of IgA anti-TG2 deposits for ACD progression.

In conclusion, the present study provides novel knowledge on the very debated topic of PCD. Firstly, we clearly demonstrated that GFD is highly recommendable for adult symptomatic PCD patients as gluten withdrawal is associated with a significant improvement of intestinal and extra-intestinal symptoms / manifestations. Secondly, asymptomatic patients should continue a GCD although under strict surveillance, i.e. clinical, serological and histological follow-up. Thirdly, our study highlights that only a small proportion of asymptomatic adult PCD patients progresses to ACD over time. Thus, our study expands our understanding of PCD to adult patients, and paves the way to research for those cases that are prone to develop overt CD.

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We wish to dedicate this manuscript to the beloved memory of our Colleague and dearest friend Dr. Ronny Cicola who devoted a lot of energy and sincere enthusiasm for the collection, processing and antibody testing of the sera obtained from all patients enrolled in this study.

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Figure Legends

Figure 1. Management of the 77 potential celiac disease (PCD) patients enrolled in the study. Symptomatic PCD (n= 61) patients commenced a gluten-free-diet (GFD) with a clinical and serological follow-up every 6 months to assess the response to GFD. Asymptomatic PCD (n= 16) patients were left on a gluten-containing diet (GCD) with a clinical and serological follow-up every 6 months and a histological evaluation every two years to assess the progression to active celiac disease (ACD).

Figure 2. Clinical features of potential celiac disease (PCD). Sixty-one (79%) of the 77 patients with PCD were symptomatic, whereas the remaining 16 (21%) were asymptomatic. Of the symptomatic patients, only 16% showed the classical phenotype (diarrhea and weight loss), whereas the majority (84%) had the non-classical phenotype characterized by anemia, osteopenia, aphthous stomatitis, hypertransaminasemia, irritable bowel syndrome (IBS)-like symptoms, gastro-esophageal reflux disease (GERD) and recurrent miscarriages.

Figure 3. Follow-up of the 16 cases with asymptomatic potential celiac disease (PCD) left on a gluten-containing diet (GCD) (3-year mean follow-up). One patient developed symptoms and raised antibody titers with duodenal biopsy confirming active celiac disease (ACD). The remaining 15 patients remained asymptomatic: 4 of them had antibody disappearance, 1 showed antibody fluctuation and 10 had stable antibody positivity. Repeated duodenal biopsies, performed every two years in cases with antibody persistence and fluctuation, did not reveal any progression to ACD.

Figure 4. Variation of anti endomysial antibody (EmA) titer and anti tissue transglutaminase antibody (tTGA) activity during a mean follow-up of three years in the 16 adults with potential celiac disease (PCD) on gluten containing diet (GCD).

Figure 5. Representative photomicrographs illustrating IgA anti-TG2 intestinal deposits in two cases of asymptomatic potential celiac disease (PCD). Positivity of such deposits is indicated by the

white arrows in both pictures. The green arrows show the presence of TG2, while the red ones indicate positivity for IgA. Calibration bars = 25 μm.

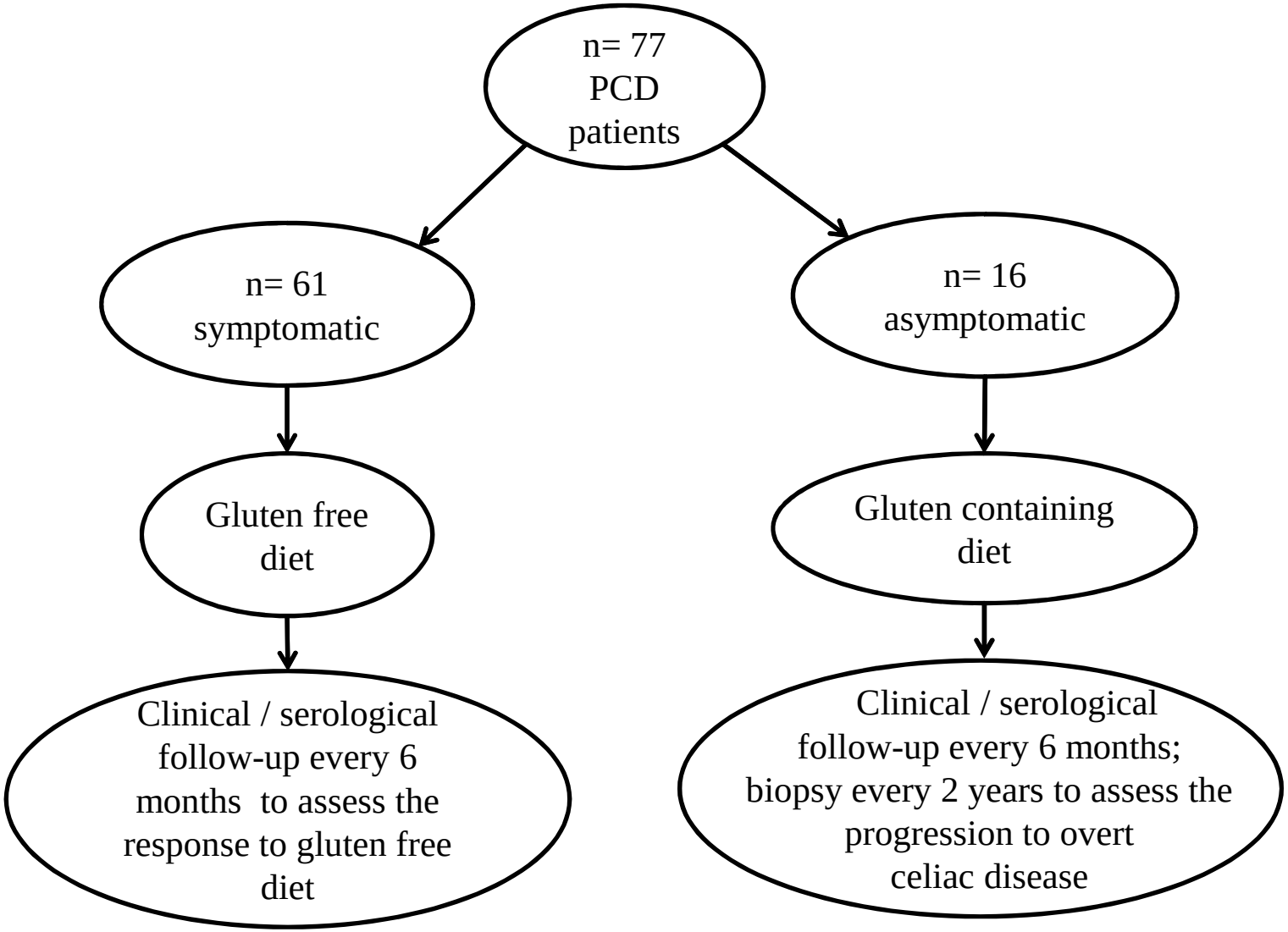
Table 1. Comparison between symptomatic and asymptomatic potential celiac disease (PCD).

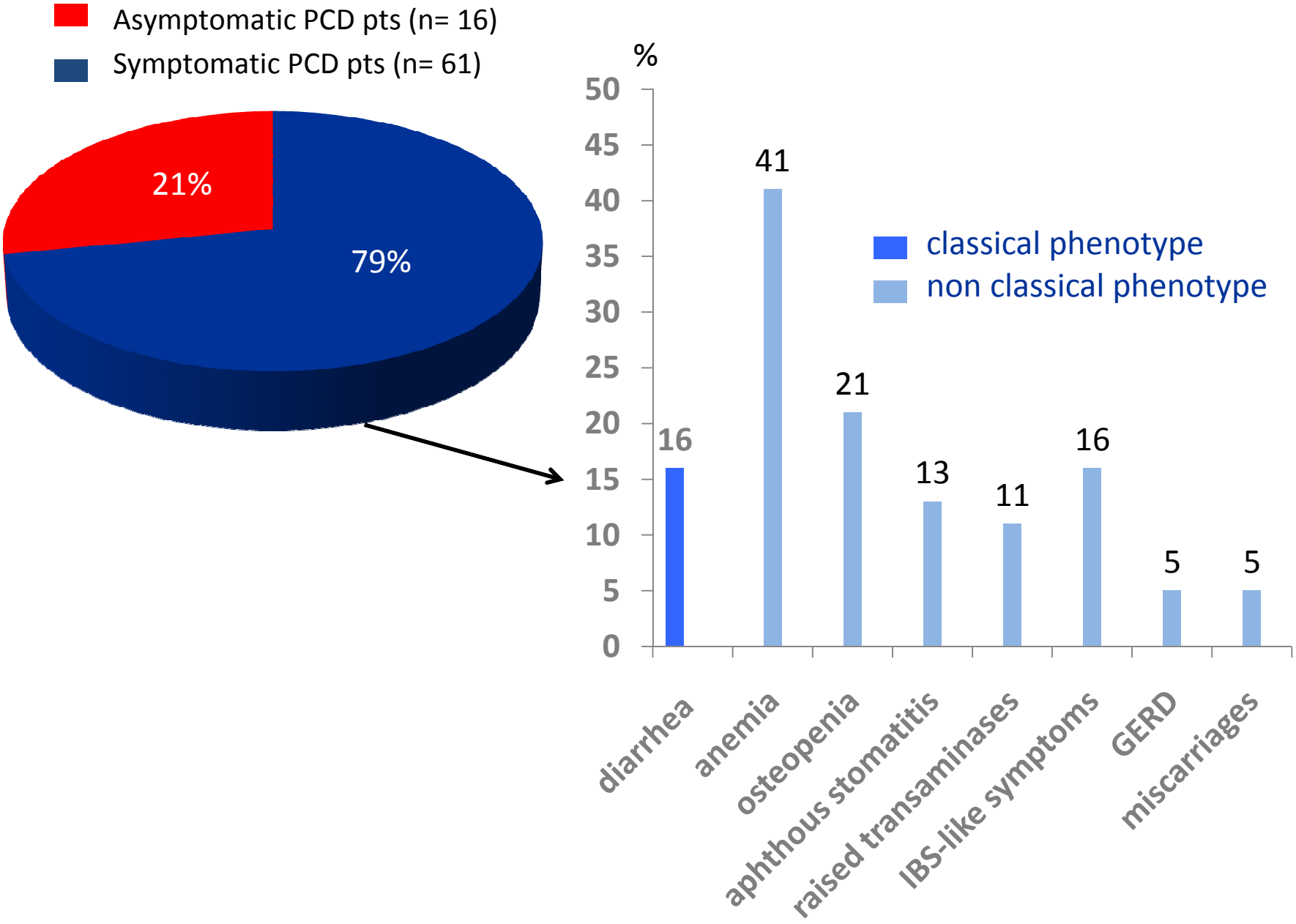
		Symptomatic PCD (61 cases)	Asymptomatic PCD (16 cases)	*P*
Age at diagnosis (median, range)		36 (14-66 years)	21 (14-58 years)	<0.05
Gender female		46 (75%)	13 (81%)	ns
EmA IgA		60 (98%)*	16 (100%)	ns
tTGA IgA		60 (98%)*	16 (100%)	ns
HLA	DQ2	59 (95%)	15 (94%)	ns
	DQ8	2 (5%)	1 (6%)	ns
Duodenal biopsy	M0	15 (25%)	5 (31%)	ns
	M1	46 (75%)	11 (69%)	ns
1st degree CD-relatives		11 (18%)	3 (19%)	ns
Autoimmune disorders**		22 (36%)	1 (5%)	< 0.05
ANA (HEp-2)***		25 (41%)	2 (12.5%)	<0.05

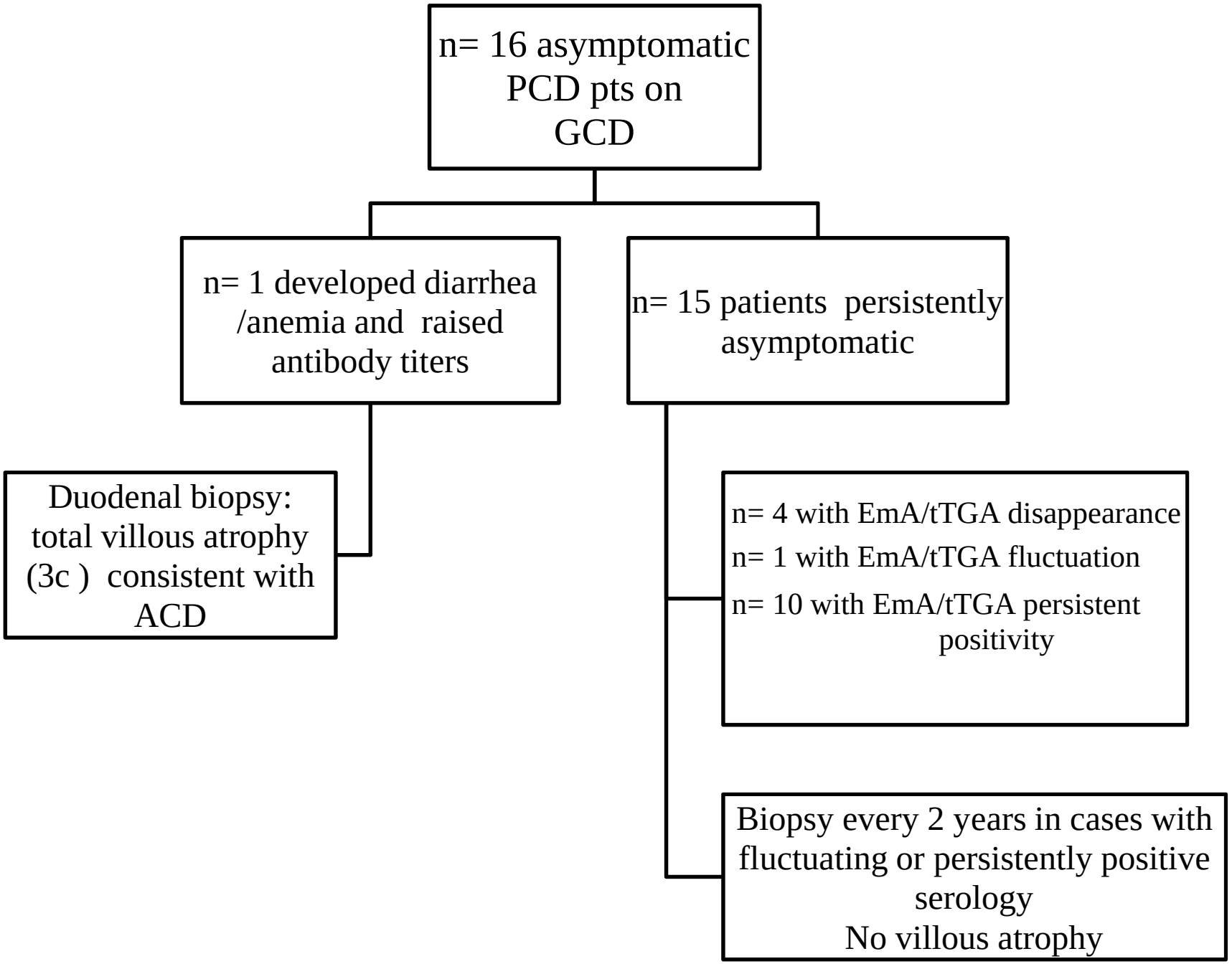
EmA: antiendomysial antibodies; EmA titer, mean 1:20 (range 1:5-1:40); tTGA: anti tissue transglutaminase antibodies; tTGA activities, mean 1.5 x cut-off (range 1.0-3.0 x cut-off);
*One symptomatic PCD with IgA deficiency positive for EmA and tTGA of IgG class;
**Autoimmune disorders including Hashimoto thyroiditis, alopecia areata, psoriasis, type 1 diabetes mellitus;
***ANA: anti-nuclear antibodies, ANA titer, mean 1:160 (range 1:80-1:640), predominant speckled pattern;
Statistical analysis: Mann-Whitney U test, 2-tailed Fisher's exact test; ns, not significant.

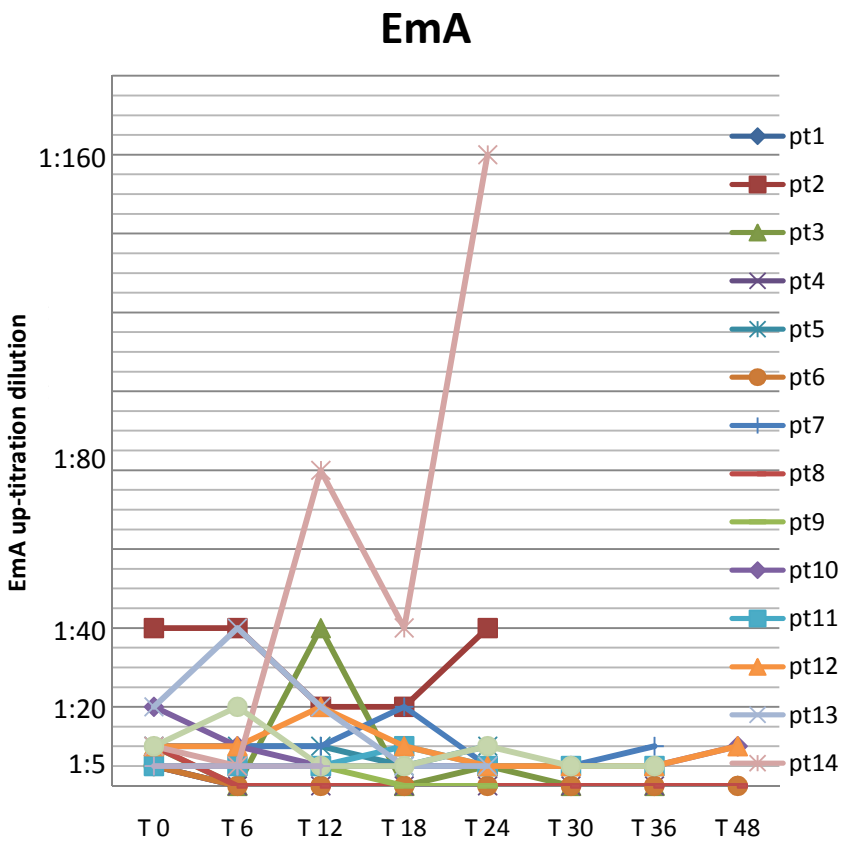
Supplementary Figure 1

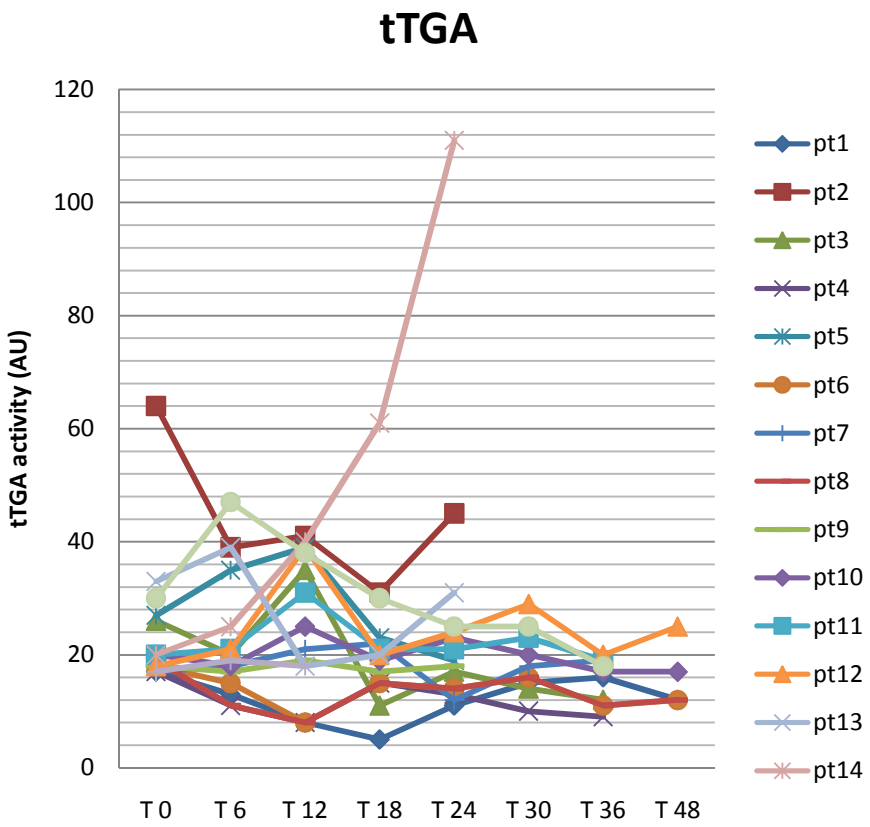
Another representative photomicrographs illustrating IgA anti-TG2 intestinal deposits (arrowheads, A), side-by-side with histological (H&E, B) and immunohistochemical (CD3, C) findings of a patient with potential CD. Note the dense CD3 immunostaining indicating an abundant lymphocytic infiltrate and increased intraepithelial lymphocytes. Calibration bar for A, B and C = 25 μm.

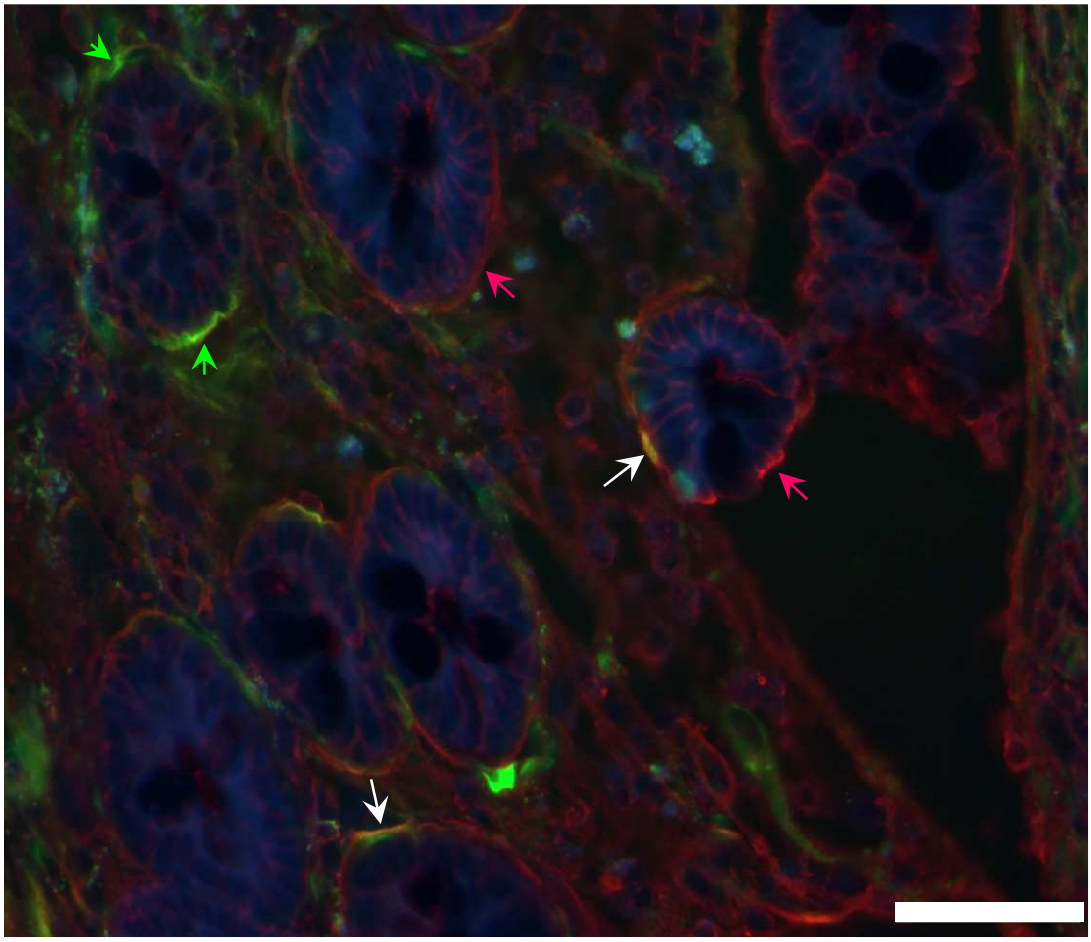


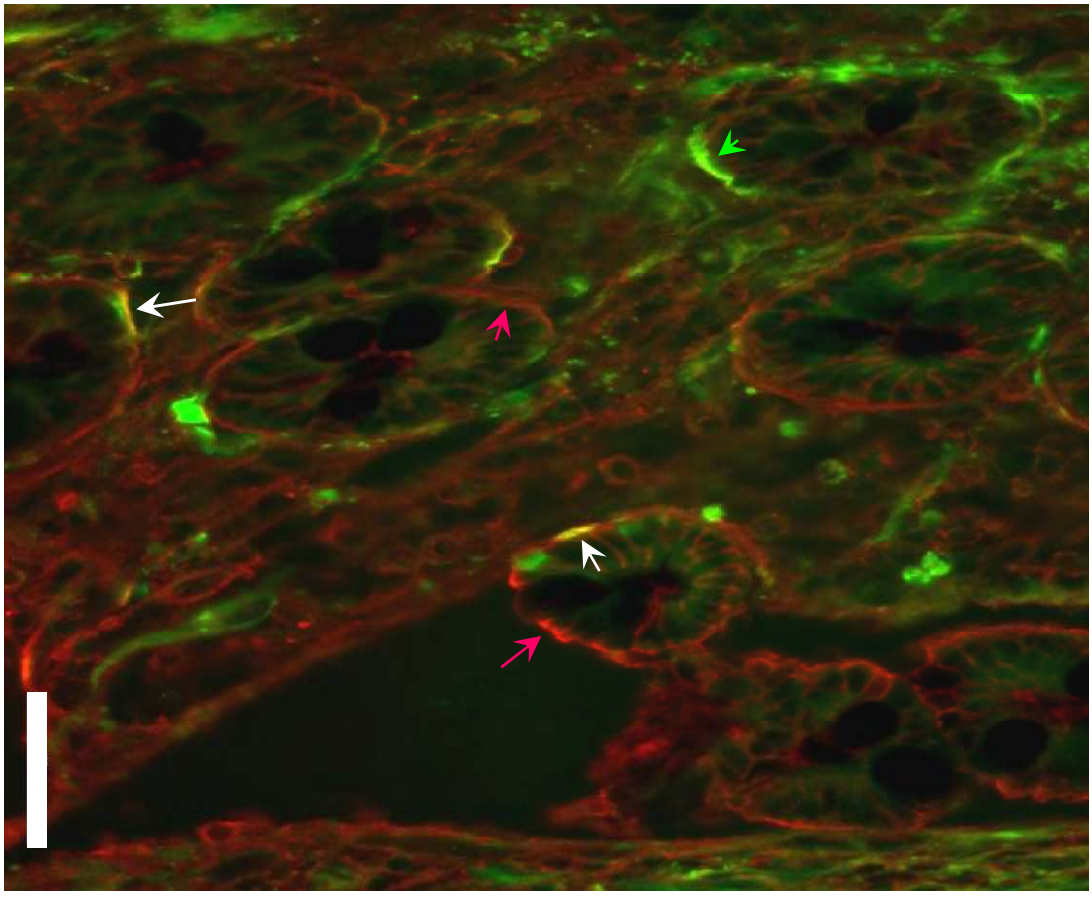

Asymptomatic PCD pts (n= 16)
Symptomatic PCD pts (n= 61)
21%
79%
%
50
45
40
35
30
25
20
15
10
5
0
16
41
21
13
11
16
5
5
classical phenotype
non classical phenotype
diarrhea
anemia
osteopenia
aphthous stomatitis
raised transaminases
IBS-like symptoms
GERD
miscarriages

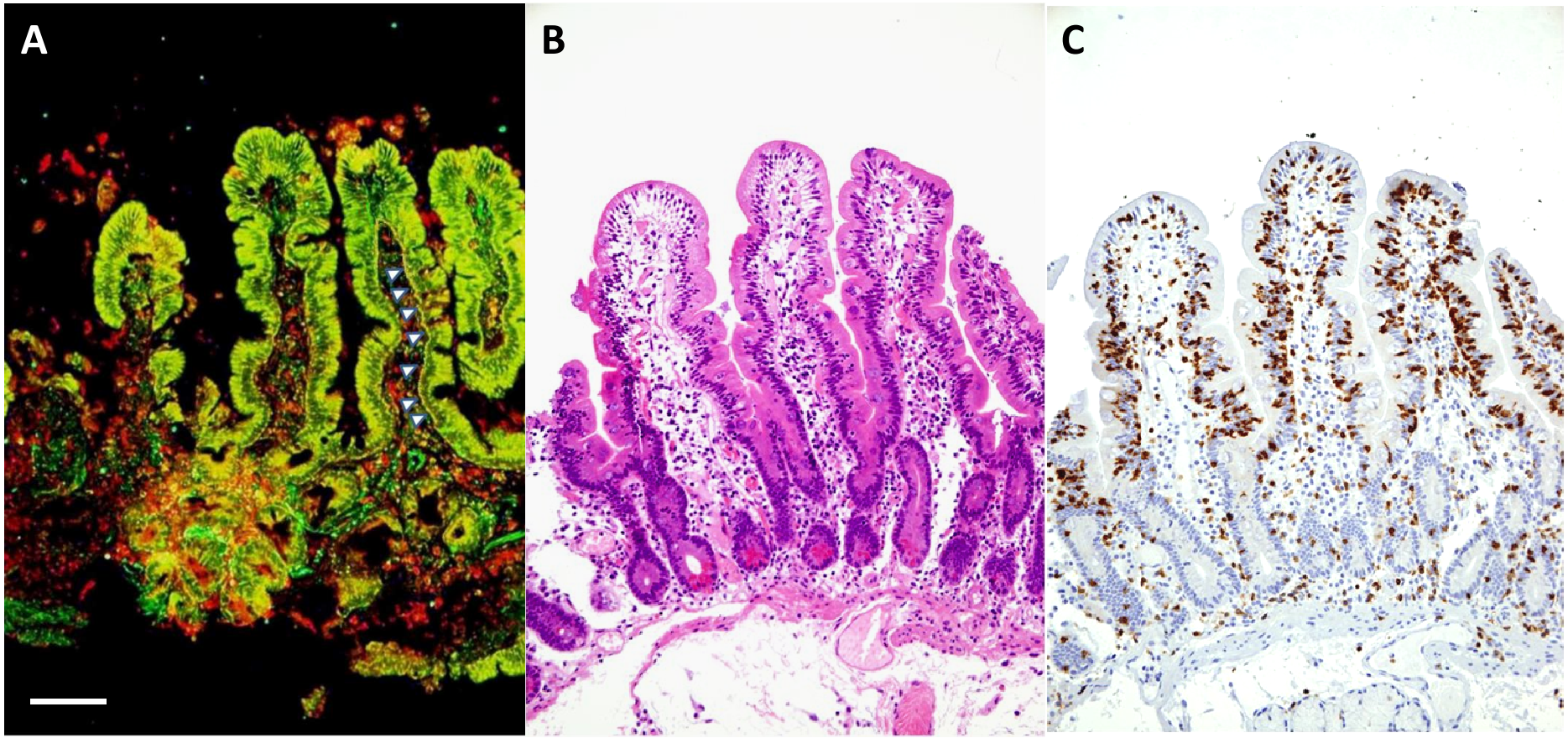



EmA
EmA up-titration dilution
1:160
1:80
1:40
1:20
1:5
T 0
T 6
T 12
T 18
T 24
T 30
T 36
T 48
pt1
pt2
pt3
pt4
pt5
pt6
pt7
pt8
pt9
pt10
pt11
pt12
pt13
pt14


tTGA
tTGA activity (AU)
120
100
80
60
40
20
0
T 0
T 6
T 12
T 18
T 24
T 30
T 36
T 48
pt1
pt2
pt3
pt4
pt5
pt6
pt7
pt8
pt9
pt10
pt11
pt12
pt13
pt14






A
B
C