Perinuclear antineutrophil cytoplasmic antibodies in collagenous or lymphocytic colitis with or without celiac disease

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HJ Freeman. Perinuclear antineutrophil cytoplasmic antibodies in collagenous or lymphocytic colitis with or without celiac disease. Can J Gastroenterol 1997;11(5):417-420. Microscopic forms of colitis, including lymphocytic and collagenous colitis, have been observed in both those with and without celiac disease. Although perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) occur in most patients with ulcerative colitis, investigations in microscopic, particularly lymphocytic, colitis are still needed. In this study atypical p-ANCA was evaluated in 55 patients, including 27 with celiac disease alone, 13 with celiac disease and concomitant lymphocytic colitis, and 15 with microscopic forms of colitis, including lymphocytic and collagenous colitis. Nine patients (16.3%) had atypical p-ANCA, including six with celiac disease and three with a microscopic form of colitis alone. Although five of the six positive celiac disease patients had lymphocytic colitis, all three celiac disease patients with associated primary sclerosing cholangitis – a separate risk factor for a positive assay result – were serologically positive for atypical p-ANCA. These results indicate for the first time that this serological marker may occur in histologically defined celiac disease with or without concomitant lymphocytic colitis. Furthermore, these results suggest that the pathogenesis of ulcerative colitis differs from that of lymphocytic colitis and further emphasizes the heterogeneous nature of these newly recognized types of colonic inflammatory mucosal disorders.

Key Words: *Antineutrophil antibodies (p-ANCA), Celiac disease, Collagenous colitis, Inflammatory bowel disease, Lymphocytic colitis, Ulcerative colitis*

Anticorps cytoplasmiques antineutrophiles périnucléaires dans la colite collagénique ou lymphocytaire avec ou sans maladie cœliaque

RÉSUMÉ : Des formes microscopiques de colite, y compris la colite lymphocytaire et collagénique ont été observées chez les sujets atteints ou non de maladie cœliaque. Bien que les anticorps cytoplasmiques antineutrophiles périnucléaires (p-ANCA) s'observent chez la plupart des patients souffrant de colite ulcéreuse, il faut poursuivre la recherche sur la colite microscopique et particulièrement, lymphocytaire. Dans le cadre de cette étude, le dosage de p-ANCA atypiques a été effectué chez 55 patients, dont 27 atteints de maladie cœliaque seule, 13 atteints de maladie cœliaque et de colite lymphocytaire concomitante et 15 atteints de la colite microscopique, y compris de colite lymphocytaire et collagénique. Neuf patients (16,3 %) présentaient des p-ANCA atypiques, y compris six atteints de maladie cœliaque et trois souffrant de la forme microscopique de colite seule. Bien que cinq des patients atteints de maladie cœliaque positifs aient présenté une colite lymphocytaire, les trois patients atteints de maladie cœliaque associée à une cholangite sclérosante primaire, facteur de risque indépendant de résultats positifs au dosage, étaient séro-positifs à l'égard des p-ANCA atypiques. Ces résultats donnent à penser pour la première fois que ce marqueur sérologique puisse être présent dans la maladie cœliaque histologiquement définie, avec ou sans colite lymphocytaire concomitante. De plus, ces résultats suggèrent que la pathogenèse de la colite ulcéreuse diffère de celle de la colite lymphocytaire et souligne la nature hétérogène de ces troubles inflammatoires de la muqueuse du côlon de découverte récente.

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In 1980 'microscopic colitis' was coined to describe a chronic mucosal inflammatory process in the colon of patients with diarrhea (1). Later, histopathological descriptions (2-5) noted a predominance of intra-epithelial lymphocytes in the inflammatory infiltrate; as a result the term 'lymphocytic colitis' emerged. Although this form of colitis is a distinctive entity in patients with diarrhea, it shares some clinical and histopathological features with another form of microscopic colitis: collagenous colitis (4-8). Most intra-epithelial lymphocytes in both of these microscopic types of colitis stain with a T cell marker (eg, MT-1) (9).

Interestingly, both lymphocytic and collagenous colitis have been recognized in patients with celiac disease (9-13). Indeed, in our initially reported studies (9), lymphocytic colitis was recognized in 12 of 39 celiac disease patients (31%). Later, in an evaluation of 30 elderly celiac disease patients, lymphocytic colitis was recorded in 13 (43%) (14). Similar findings have been reported in gastric epithelium from patients with celiac disease (15) and recently in the bile duct epithelium from a patient with celiac disease and sclerosing cholangitis (16). Moreover, pathological studies by other investigators have reported that up to 40% of patients with collagenous colitis also have celiac disease (17).

While these newly recognized forms of microscopic colitis, along with ulcerative colitis, may represent similar mucosal immunopathological processes, their precise relationship is unknown. The pathophysiological and possible diagnostic roles of serological markers in both ulcerative colitis and Crohn's disease have recently been explored. In a prospective study of Canadian patients with inflammatory bowel disease, for example, detection of atypical perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) was reported in the majority of patients with ulcerative colitis, but only in a minority with Crohn's disease (18). To determine further whether this serological marker is also present in microscopic colitis, this investigation evaluated all recently diagnosed patients with lymphocytic or collagenous colitis with or without associated celiac disease.

PATIENTS AND METHODS

Patient groups: All patients in this investigation had histopathological features of celiac disease and/or microscopic types of colitis (ie, lymphocytic and collagenous colitis) as described earlier (8,9). All patients with celiac disease had colonoscopic biopsies to determine whether lymphocytic or collagenous colitis was present. Conversely, all patients with lymphocytic or collagenous colitis had a small intestinal biopsy to determine whether the histopathological features of occult untreated celiac disease were present.

Results of the 55 patients were evaluated on the basis of histopathological diagnosis. Table 1 lists the results of 40 patients with celiac disease, including 13 patients with celiac disease and microscopic (ie, specifically lymphocytic) colitis, and 27 patients with celiac disease and no colitis. Results of 28 patients with microscopic colitis (ie, lymphocytic or collagenous colitis) are shown in Table 2, including the same 13 patients with celiac disease and 15 patients with no celiac disease. Two patients from this latter group (with microscopic colitis but no celiac disease) were also administered high gluten diets to exclude latent celiac disease; these studies were previously reported (19).

All serological samples for this prospective study were collected in a consecutive fashion with no exclusions or re-

TABLE 1
Patients with celiac disease

Patient	Age/sex	p-ANCA	LC	Other
1	55/M	–	LC	Small bowel cancer
2	43/M	–	LC	
3	71/F	–	No colitis	T cell lymphoma
4	40/M	+	LC	
5	27/F	–	No colitis	
6	50/F	+	LC	PSC
7	47/F	–	No colitis	
8	44/F	–	No colitis	
9	42/F	–	No colitis	Duodenal stricture
10	65/F	–	LC	
11	84/M	–	No colitis	T cell lymphoma
12	41/F	–	No colitis	PBC
13	32/F	–	LC	
14	21/M	–	No colitis	
15	42/F	–	No colitis	
16	60/F	–	No colitis	
17	60/M	+	LC	T cell lymphoma, PSC, DH
18	15/F	–	LC	
19	52/M	+	No colitis	
20	24/F	–	No colitis	DH
21	52/M	–	No colitis	
22	35/F	–	No colitis	
23	78/M	+	LC	PSC
24	36/F	+	LC	
25	62/F	–	No colitis	T cell lymphoma
26	49/F	–	No colitis	B cell lymphoma
27	23/F	–	No colitis	
28	38/F	–	No colitis	
29	61/F	–	No colitis	
30	31/F	–	LC	
31	17/F	–	LC	
32	62/F	–	No colitis	Pancreatic insufficiency
33	83/F	–	No colitis	
34	60/M	–	No colitis	
35	29/F	–	No colitis	
36	75/F	–	No colitis	
37	41/F	–	No colitis	
38	41/F	–	No colitis	
39	37/F	–	LC	DH
40	65/F	–	No colitis	

DH Dermatitis herpetiformis; F Female; LC Lymphocytic colitis; M Male; p-ANCA Perinuclear antineutrophil cytoplasmic antibodies; PBC Primary biliary cirrhosis; PSC Primary sclerosing cholangitis

fusals; all samples were from out-patients rather than hospitalized patients.

Control groups: This study was completed using the same laboratory protocols previously reported by the author and co-workers (18). In that prior study, 194 of 500 consecutively studied patients with inflammatory bowel disease were positive for atypical p-ANCA versus none of 32 consecutive controls with either no detectable disease (infectious or ischemic colitis). In inflammatory bowel disease patients, 66.3% of 247 ulcerative colitis patients and 11.8% of 253 Crohn's disease patients were positive for atypical p-ANCA.

Laboratory studies: For each patient blood samples were collected into vacutainer glass tubes (Becton Dickinson, New Jersey) for hematological studies (hemoglobin, white blood cell count, platelet count), an erythrocyte sedimentation rate test, liver chemistry tests (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase), and serum protein, including serum albumin, and serum iron studies.

Blood samples were also collected into vacutainer glass tubes, allowed to clot at room temperature and used for detection of ANCA with ANCA indirect immunofluorescence; if atypical p-ANCA was detected, ANCA ELISA was done. As reported elsewhere with coded sera examined in a blinded fashion (18), excellent agreement was present between immunofluorescence and ELISA results.

ANCA immunofluorescence: ANCA immunofluorescence was performed using a standardized indirect fluorescence antibody detection method with a proprietary kit purchased from a commercial supplier (Inova Diagnostics Inc, California). Laboratory methods used were previously detailed (18).

ELISA assays: ANCA ELISA assays were performed using a standardized method (18) with commercial kits (QuantaLite MPO or PR3 ELISA, Inova Diagnostics Inc). The test kits use microtitration strips containing wells coated with proteinase-3 or myeloperoxidase. The laboratory methods used have been detailed (18).

RESULTS

Nine of the 55 patients (16.3%) were positive for atypical p-ANCA, including six of 40 (15%) celiac disease patients – with or without associated lymphocytic colitis – and three of 15 (20%) with microscopic colitis, either lymphocytic or collagenous colitis, but with no histological evidence for celiac disease. Thus, for both patient groups, the percentage of positive sera in the present investigation approximated the percentage of positive sera previously defined in patients with Crohn's disease rather than in ulcerative colitis (18).

Table 1 shows findings from 40 celiac disease patients. There were 30 females and 10 males, reflecting the previously reported overall female predominance in celiac disease (14). Average age of initial diagnosis of celiac disease was earlier for females than for males, ie, 44.8 years compared with 54.5 years, consistent with previous studies (14). Of the six patients with celiac disease (including two females and four males, average age 52.7 years) positive for atypical p-ANCA, five had a microscopic form of colitis, specifically lymphocytic colitis, including all three celiac disease patients with primary sclerosing cholangitis. Primary sclerosing cholangitis appears to be an independent risk factor associated with atypical p-ANCA (20). Three of the 37 remaining celiac disease patients (8.1%) were positive for this serological marker. Other disorders or complications of celiac disease, such as lymphoma, were not associated with positive atypical p-ANCA results.

TABLE 2
Patients with microscopic colitis

Patient	Age/ sex	p-ANCA	LC or CC	Celiac disease	Other
1	56/F	–	LC	None	
2	55/M	–	LC	Present	Small bowel cancer
3	38/F	–	CC	None	
4	43/M	–	LC	Present	
5	40/M	+	LC	Present	
6	67/M	–	LC	None	
7	64/F	–	LC	None	
8	56/F	–	LC	None	
9	56/F	–	CC	None	Spleen atrophy
10	50/F	+	LC	Present	PSC
11	55/F	–	CC	None	Giant cell arteritis
12	52/M	–	CC	None	
13	67/F	–	LC	None	
14	82/F	–	LC	None	
15	65/F	–	LC	Present	
16	84/M	–	LC	Present	T cell lymphoma
17	74/F	+	CC	None	
18	32/F	–	LC	Present	
19	60/M	+	LC	Present	T cell lymphoma, DH, PSC
20	15/F	–	LC	Present	
21	43/F	+	LC	None	
22	42/F	–	LC	None	
23	78/M	+	LC	Present	PSC
24	56/F	–	LC	None	
25	36/F	+	LC	Present	
26	31/F	–	LC	Present	
27	17/F	–	LC	Present	
28	36/F	+	LC	None	

CC Collagenous colitis; DH Dermatitis herpetiformis; F Female; LC Lymphocytic colitis; M Male; p-ANCA Perinuclear antineutrophil cytoplasmic antibodies; PSC Primary sclerosing cholangitis

Table 2 lists results from 28 patients with a microscopic form of colitis, either lymphocytic or collagenous colitis. Thirteen had a concomitant diagnosis of celiac disease and 15 had no detectable celiac disease using endoscopic small intestinal biopsies. Two of the 15 patients had also been treated with a high gluten diet but latent celiac disease was not detected (19). Overall, eight of 28 patients (28.6%) with microscopic colitis were positive for atypical p-ANCA.

Five of 13 patients (38.5%) with concomitant celiac disease were positive (including three patients with associated

primary sclerosing cholangitis). Three of 15 patients (20%) with no concomitant celiac disease were positive; in two of these patients with microscopic colitis and no celiac disease, there was a positive family history of documented ulcerative colitis, and one patient was positive for atypical p-ANCA. There was no familial history of inflammatory bowel disease in the celiac disease patients.

DISCUSSION

This study demonstrated that the previously recorded detection rate of atypical p-ANCA of almost 70% – in the majority of patients from our centre with ulcerative colitis (18) – was not observed in this prospective evaluation of celiac disease patients. Indeed, if celiac disease patients with primary sclerosing cholangitis were excluded from the analysis, only three of the remaining 37 patients (8.1%) were positive for this serological marker, approximating the percentage of patients with Crohn's disease (ie, about 10%) in our centre (18). The present results also appear to confirm the findings of Bansi et al (21), the only earlier study reporting results in celiac disease patients. In their report, however, the serological marker p-ANCA could not be detected in any of the 17 celiac disease or 10 dermatitis herpetiformis patients.

The present study also confirms that patients with primary sclerosing cholangitis are often positive for this marker (20); however, in the present investigation observations were extended to patients with primary sclerosing cholangitis and concomitant celiac disease.

Although this was the first study to evaluate patients with lymphocytic colitis for this serological marker (atypical p-ANCA) some prior studies have evaluated patients with collagenous colitis. Duerr and colleagues (22) initially recorded that five of 35 (14%) patients with collagenous colitis were positive for atypical p-ANCA, although diagnostic criteria for collagenous colitis and associated intestinal disorders, such as celiac disease, were not detailed. Similarly, Bohr et al (23) observed that four of 38 (11%) with collagenous colitis were positive for atypical p-ANCA. Although the frequency of concomitant celiac disease was not provided, 3% to 5% of sera from these collagenous colitis patients also had antibodies to endomysium and gliadin. Their results indirectly suggest – as do ours – that microscopic forms of colitis, even if associated with celiac disease, differ in immunopathogenesis from ulcerative colitis, a disorder with a very high detection rate of p-ANCA. Identification of the antigen(s) to which these autoantibodies are directed may facilitate understanding of the underlying immune response, not only in ulcerative colitis, but also in other forms of inflammatory bowel disease, including these microscopic forms of colitis.

This investigation also serves to emphasize further that microscopic forms of colitis represent a heterogeneous group of colonic inflammatory mucosal disorders. In some patients, celiac disease coexists, whereas in others, no clear relationship to celiac disease is evident. Future studies, using novel serological markers, may aid in further definition of these emerging entities.

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