Microscopic Colitis as a Diagnosis to Consider by Clinicians and Pathologists Together: A Case Series and Review of Literature

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Abstract

Background: Microscopic colitis is a term used to define some clinical-pathological entities characterized by chronic watery diarrhea, normal radiological and endoscopic appearance, and microscopic abnormalities.

Aim: In this article we report a personal case series of microscopic colitis in patients with different age and sex and symptomatic seriousness to better represent the heterogeneous clinical presentation of this type of disease, and a review of available literature data.

Method: A definitive diagnosis of microscopic colitis is only possible by histological analysis. Specific histopathological findings, such as morphologically mild or moderate inflammation in the lamina propria, combined with either thickening of the sub-epithelial collagenous or lymphocytic attack on the surface epithelium, can be used to further classify these clinical entities as collagenous colitis (CC), lymphocytic colitis (LC), or other conditions.

Result: We presented a review of the most recents studies about microscopic colitis and we presented a case serie of four lymphocitic colitis and one collagenous colitis in patients with different age and sex and symptomatic seriousness to better represent the heterogeneous clinical presentation of this type of disease, and we also performed a brief review of available literature data analyzing epidemiology, pathogenesis, clinical presentation and therapy strategies of these group of colitis.

Discussion: Clinicians should be able to provide elements of clinical suspicion to pathologists that could justify more specific histological evaluation that includes the assessment of the number of intraepithelial cells (IEL), and the thickness of the band of tissue collagen.

Keywords: Microscopic colitis; Clinician; Pathologist

Background

Microscopic colitis (MC) is a term used to define some clinical-pathological entities characterized by chronic watery diarrhea, normal radiological and endoscopic appearance, and microscopic abnormalities [1,2]. Specific histopathological findings can be used to further classify these clinical entities as collagenous colitis (CC), lymphocytic colitis (LC), or other conditions [1,2].

Both CC and LC exhibit inflammatory changes in the lamina propria and superficial epithelial damage. The term lymphocytic colitis has been proposed [3,4] to designate a syndrome characterized by chronic watery diarrhea and histological findings of increased intraepithelial lymphocytes and a chronic inflammatory infiltrate in the lamina propria. Collagenous colitis was first described in 1976 [5] as a separate subtype that differs from LC by the presence of a sub-epithelial collagen band that must be thicker than ≥ 10 mm and that may be combined with mild or moderate inflammation in the lamina propria.

In 1980, Read et al. [6] described microscopic colitis characterized by chronic diarrhea with normal endoscopic and radiologic findings, but with increased colonic mucosal inflammatory cells and epithelial lymphocytic infiltration on histological examination. Later, Levison et al. [7] emphasized that microscopic colitis covered all cases of colitis with normal colonoscopy, but abnormal histopathologic features.

Since a clinical context of diarrhea without endoscopic findings traditionally represents a diagnostic dilemma for clinicians, microscopic colitis and its clinical sub-entities constitute a diagnostic area in which clinicians and pathologists should always share a diagnostic path through dialog and the exchange of information. These elements are essential in order to reach an accurate diagnosis and avoid problems of misdiagnosis.

In this article we reported a personal case series of five cases of microscopic colitis in patients with different age and sex and symptomatic seriousness to better represent the heterogeneous clinical presentation of this type of disease, and we also performed a brief review of available literature data.

Definition of Disease

Clinically active LC and CC has been defined as ≥3 loose or watery stools/day [8]. The diagnostic criteria for LC are histological findings of increased ratio between Intra-Epithelial Lymphocytes (IELs) and

surface epithelial cells (≥20/100), in conjunction with surface epithelial cell damage and infiltration of lymphocytes in the lamina propria, but a normal collagen layer [9,10]. In CC, in addition to lymphocytic infiltration in the lamina propria and the epithelium, deposition of a sub-epithelial collagen layer of ≥10 mm is seen [11].

Case Series

	Case 1	Case 2	Case 3	Case 4	Case 5
	Female, 55 years old, social assistant	**Male, 24 years old, law student and amateur actor**	**67 year old, housewife**	**Female, 42 years old, entrepreneur**	**Female high school student, 18 years old**
Symptomatology	Progressive weight loss, daily episodes of diarrhea and cramps/abdominal pain (up to 30 bowel movements/day of semi-liquid stool;).	Abdominal pain and frequent diarrheal bowel movements (three/four movements/day of semi-liquid feces without blood, often with the presence of discrete quantities of mucus)	Diffuse cramps/abdominal pain associated with liquid normochromic stools in the absence of mucus and/or blood (about 10 bowel movements/day); hyporexia with a weight loss of about 15kg	Frequent intestinal movements (2-3 discharges of stools a day, sometimes liquid and sometimes semi-liquid; gradual abdominal distension and flatulence	Diarrhea, colicky abdominal pain in mesogastrium and lower quadrants of the abdomen, asthenia and laboratory findings of iron-deficiency anemia
Histological Findings	Large bowel biopsy: H&E staining shows a lymphogranulocytic and plasmacellular nflammatory infiltration in the lamina propria, and increased number of intraepithelial T lymphocytes (IELs),	Large bowel biopsy: H&E staining shows a moderate lymphogranulocytic and plasmacellular inflammatory infiltration in the lamina propri ; increase of the intraepithelial T lymphocytes (IELs), typical of lymphocytic colitis	Immunohistochemical analysis of T lymphocytes in LC with anti-CD3 antibody (DAKO). Colonic mucosa showing increased IELs in a patient with lymphocytic colitis.	Large bowel biopsy: H&E staining (on the left) shows a moderate lymphogranulocytic and plasmacellular inflammatory infiltration in the lamina propria. CD3 immunostaining (picture on the right) highlights the increase of the intraepithelial T lymphocytes (IELs).	Large bowel biopsy: H&E staining (on the left) shows a moderate lymphogranulocytic and plasmacellular inflammatory infiltration in the lamina propria. CD3 immunostaining (picture on the right) highlights the increase of the intraepithelial T lymphocytes (IELs). Marked thickening of the sub-epithelial collagen band (arrowhead

Table 1: Case series.

First case: female, 55 years old, social assistant (case 01)

Since the age of 54 years, the patient has complained of progressive weight loss despite no reduction in food intake. After a few months she reported the onset of daily episodes of diarrhea associated with cramps/abdominal pain (up to 30 bowel movements/day of semi-liquid stool; no blood nor mucus were in the stool).

Blood tests showed: WBC: 5050/mm^3, neut: 51%; LINF: 43.4%, monocytes 4.6%, eos: 0.8%, RBC: 3,860,000/mm^3, Hb: 11.9 g/dl, MCV: 93 fl, MCH: 30.8 pg, PLT: 187,000/mm^3, ESR: 2, CRP: 0.97 mg/dl. The feces culture and the parasitological examination of stools, and the search for fecal occult blood were negative, fecal calprotectin was negative.

Colonoscopy: Exploration extended to the last ileal loop (20 cm), whose mucosa appeared normal. The mucosa of the rectum, left and right colon, also appeared normal. In consideration of the diagnostic question biopsies were performed on the ileum, ascending, transverse, and descending colon. Six biopsy specimens were obtained using standard biopsy forceps. Immunohistochemical staining for T cells, CD3 (DAKO) and hematoxylin, and eosin staining, provided findings suggesting lymphocytic colitis (see Figure 1-2)
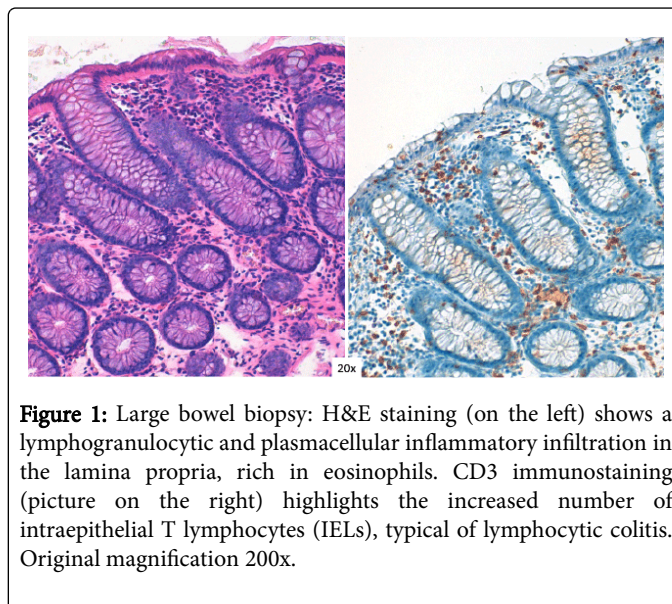

Figure 1: Large bowel biopsy: H&E staining (on the left) shows a lymphogranulocytic and plasmacellular inflammatory infiltration in the lamina propria, rich in eosinophils. CD3 immunostaining (picture on the right) highlights the increased number of intraepithelial T lymphocytes (IELs), typical of lymphocytic colitis. Original magnification 200x.
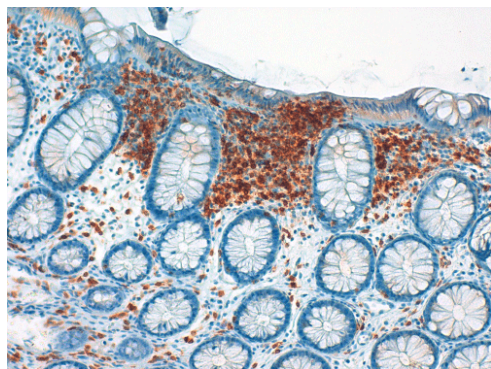
Figure 2: Immunohistochemical analysis of T lymphocytes in LC with anti-CD3 antibody (DAKO). Colonic mucosa showing increased IELs in a patient with lymphocytic colitis. Original magnification 200x.

Second case: male, 24 years old, law student and amateur actor (case 02)

At age of 18 he began suffering from symptoms such as abdominal pain and diarrhea. He also reported frequent diarrheal bowel movements (three/four movements/day of semi-liquid feces without blood, often with the presence of discrete quantities of mucus), until the age of 22. Since age 22 the abdominal symptoms gradually became more and more frequent (five/six movements/day of semi-liquid feces).

Blood tests: WBC: $9800/mm^3$, neut: 70%%, LINF: 24%, monocytes 4%, eos: 1%, RBC: $5,100,000/mm^3$, Hb: 13.5 g/dl, MCV 86 fl, MCH: 33 pg, PLT: $245,000/mm^3$, ESR: 19, CRP: 1.19 mg/dl. The remaining blood tests and thyroid hormones were normal. Feces culture and parasitological exam were negative. Fecal occult blood test: negative on three samples; fecal calprotectin: negative.

Colonoscopy: The colonoscopy examination revealed a normal mucosa but slight edema or erythema occurred. Six biopsy specimens were obtained using standard biopsy forceps.
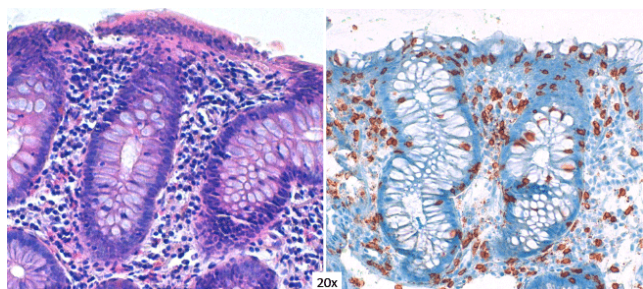

Figure 3: Large bowel biopsy: H&E staining (on the left) shows a moderate lymphogranulocytic and plasmacellular inflammatory infiltration in the lamina propria. CD3 immunostaining (picture on the right) highlights the increase of the intraepithelial T lymphocytes (IELs), typical of lymphocytic colitis. Original magnification 200x.

Immunohistochemical staining for T cells, CD3 (DAKO) and hematoxylin, and eosin staining suggested lymphocytic colitis (see Figure 3).

Third case: 67 year old, housewife (case 03)

At age 67 she reported the onset of diffuse cramps/abdominal pain associated with liquid normochromic stools in the absence of mucus and/or blood (about 10 bowel movements/day). She also reported hyporexia with a weight loss of about 15 kg. The patient came to our attention due to the persistence of the above symptoms. Blood tests showed laboratory abnormalities consistent with HCV-related chronic liver disease in evolution to cirrhosis: WBC: $3390/mm^3$, neut: 69.6%, LINF: 16.2, monocytes 13.9% , eos: 0.3%, RBC: $3,240,000/mm^3$, Hb: 10.9, MCV: 10^6 fl, MCH: 33.6 pg, PLT: $288,000/mm^3$, ESR: 41, CRP: 4.2 mg/dl. The remaining blood tests and thyroid hormones were normal except for the presence of hyperferritinemia.
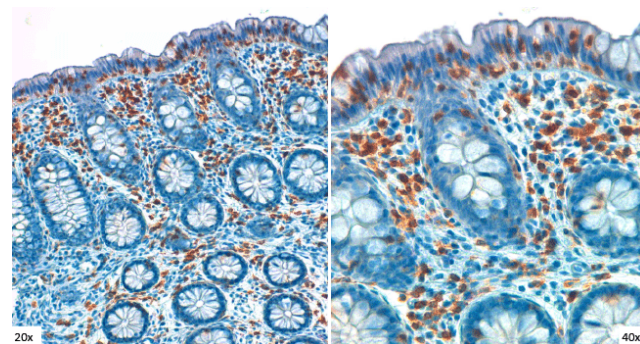

Figure 4: Immunohistochemical analysis of T lymphocytes in LC with anti-CD3 antibody (DAKO). Colonic mucosa showing increased IELs in a patient with lymphocytic colitis. Original magnification 200x; higher magnification, on the right 400x.
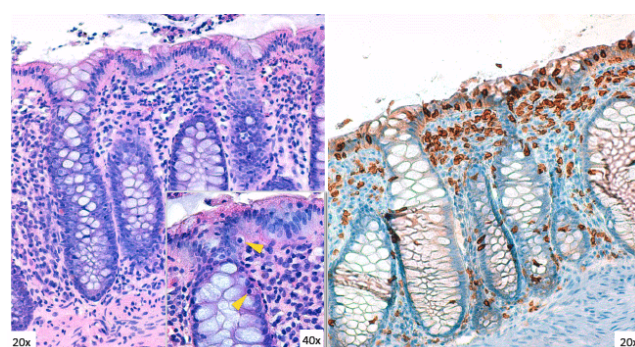

Figure 5: Large bowel biopsy: H&E staining (on the left) shows a moderate lymphogranulocytic and plasmacellular inflammatory infiltration in the lamina propria. Intraepithelial lymphocytes show a basophilic nuclear chromatin pattern, irregular nuclear outline, and clear perinuclear halo (inset, yellow arrowheads). CD3 immunostaining (picture on the right) highlights the increase of the intraepithelial T lymphocytes (IELs), typical of lymphocytic colitis. Original magnification 200x; inset 400x.

The ultrasounds of the abdomen showed liver with mild hypertrophy of the left lobe with dense and homogeneous echogenicity and thickening wall of the ascending colon. Feces culture and parasitological exam were negative.

The colonoscopy examination revealed a normal mucosa but slight edema or erythema occurred. Six biopsy specimens were obtained using standard biopsy forceps. Immunohistochemical staining for T cells, CD3 (DAKO) and hematoxylin and eosin staining suggested lymphocytic colitis (see Figure 4,5).

Fourth case: female, 42 years old, entrepreneur (case 04)

Since the age of 19 she reported frequent intestinal movements with the emission of two/three discharges of stools a day, sometimes liquid and sometimes semi-liquid. The above symptoms were also accompanied by a gradual feeling of abdominal distension and flatulence (not productive according to the patient). For these reasons she came to our attention.

Blood tests: WBC: 7800/mm^3, neut: 62%, LINF: 28%, monocytes 3%, eos: 2%, RBC: 4-970000/mm^3, Hb: 12.9 g/dl, MCV: 87 fl, MCH: 32 pg, PLT: 210,000/mm^3, ESR: 23, CRP: 0.97 mg/dl. The remaining blood tests and thyroid hormone were normal. Feces culture and parasitological exam were negative. The search for occult blood loss (three samples), and fecal calprotectin were negative.

The colonoscopy examination revealed a normal mucosa but slight edema or erythema occurred. Six biopsy specimens were obtained using standard biopsy forceps.

Immunohistochemical staining for T cells, CD3 (DAKO) and hematoxylin, and eosin staining suggested lymphocytic colitis (see Figure 6).
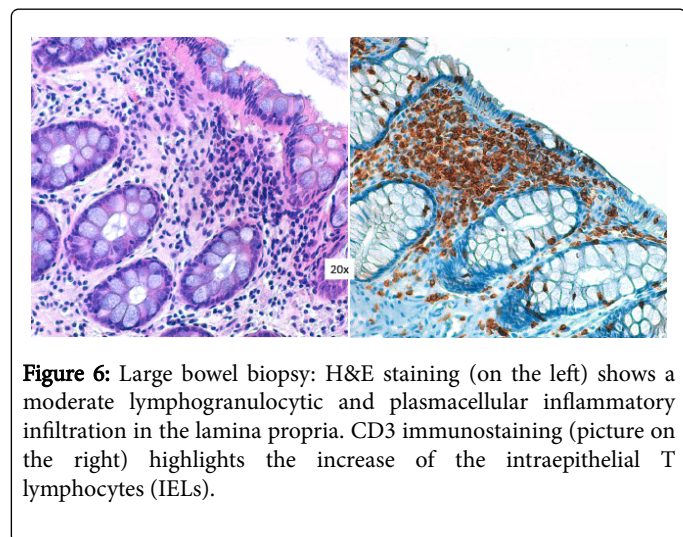

Figure 6: Large bowel biopsy: H&E staining (on the left) shows a moderate lymphogranulocytic and plasmacellular inflammatory infiltration in the lamina propria. CD3 immunostaining (picture on the right) highlights the increase of the intraepithelial T lymphocytes (IELs).

Fifth case: female high school student, 18 years old (case 05)

In October 2012, after the onset in the previous months of symptoms characterized by diarrhea, colicky abdominal pain in mesogastrium and lower quadrants of the abdomen, asthenia and laboratory findings of iron-deficiency anemia, she underwent biohumoral screening for celiac disease, and was positive for endomysial antibodies (EMA- IgA), anti-transglutaminase IgA, and anti-gliadin IgA and IgG. She also underwent esophagogastro-

duodenoscopy with multiple duodenal biopsies and histological examination that documented a framework compatible with celiac disease Marsh 3 (ratio of villus/crypt altered, marked reduction of the villi, hyperplastic crypts, low height enterocytes, intraepithelial lymphocytes >25/100). She was then diagnosed with celiac disease and since then the patient has started a gluten-free diet. During the last six months the patient reported the onset of colicky abdominal pain in the upper quadrants independent of meals and "dismotility-like" dyspepsia (feeling of fullness after meals, localized swelling of the upper quadrants, early satiety), despite the continuous and careful adherence to the gluten-free diet. She came to our observation for re-evaluation of the aforementioned abdominal and cutaneous signs and symptoms.

Blood tests: WBC: 7310/mm^3, neut: 42.4%, LINF: 44.6%, monocytes 8.3%, eos: 3.8%, RBC: 4150000/mm^3, Hb: 11.7 g/dl, MCV: 88.7 fl, MCH: 28.8 pg, PLT: 239,000/mm^3, CRP: 1.28 mg/dl.

We evaluated serum markers for celiac disease (anti-endomysial IgA, anti-gliadin and anti-transglutaminase antibodies), which were all negative. EGDS showed a picture compatible with celiac disease type 3 according to Marsh (mild and focal atrophy of the villi relationship with IEL/EC>25/100).

The colonscopy performed revealed a normal mucosa, and multiple biopsies were performed. Six biopsy specimens were obtained using standard biopsy forceps. Large bowel biopsy: H&E staining (on the left) showed a moderate lymphogranulocytic and plasmacellular inflammatory infiltration in the lamina propria. CD3 immunostaining (picture on the right) highlights the increase of the intraepithelial T lymphocytes (IELs). Marked thickening of the sub-epithelial collagen band (arrowhead). Original magnification 200x; inset 400x. (see Figure 7).
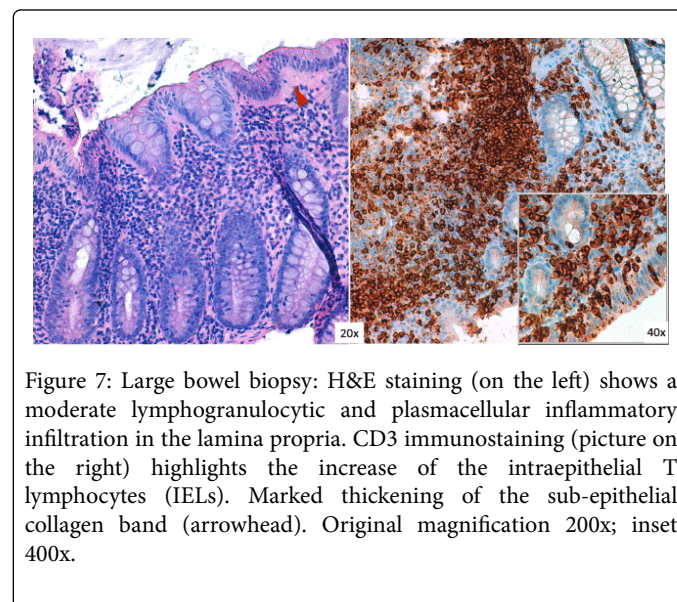

Figure 7: Large bowel biopsy: H&E staining (on the left) shows a moderate lymphogranulocytic and plasmacellular inflammatory infiltration in the lamina propria. CD3 immunostaining (picture on the right) highlights the increase of the intraepithelial T lymphocytes (IELs). Marked thickening of the sub-epithelial collagen band (arrowhead). Original magnification 200x; inset 400x.

Brief Review of Literature

Definition and diagnostic criteria

Anatomical-pathological pattern of microscopic colitis is featured by a mild or moderate inflammation has been described in the lamina propria, combined with either thickening of the sub-epithelial collagenous layer (collagenous colitis), or lymphocytic attack on the

surface epithelium (lymphocytic colitis) [3-6]. These rare diseases are of unknown etiology and the relation between the two forms is not clear; there has been much debate as to whether there are more than two forms of microscopic colitis.

Veress et al. [12] conducted a study collecting colorectal biopsy specimens from 30 patients with chronic watery diarrhea but normal endoscopic and radiographic findings. Three distinct groups of microscopic colitis were delineated by the analysis of the specimens: lymphocytic colitis, collagenous colitis without lymphocytic attack on the surface epithelium (seven patients), and a mixed form with both increased number of intraepithelial lymphocytes and thickening of the collagen plate.

Recent case reports and small series have shown that intraepithelial lymphocytosis may occur in other areas of the gastrointestinal tract in both LC and CC. For example, intraepithelial lymphocytosis has been described in the proximal small bowel and stomach in patients with LC or CC [13-17]. Nevertheless, both LC and CC primarily affect the colon. Involvement of the distal small intestine has not been systematically studied. On this basis [18] Sapp et al evaluated terminal ileal mucosal biopsies from 22 patients with LC, and 23 with CC, to assess the type and degree of intraepithelial lymphocytosis in the terminal ileum of these patients compared to 30 patients with inflammatory bowel disease, and 24 patients without colonic pathology as normal controls. Specimens were studied to estimate the number of intraepithelial lymphocytes (IEL) per 100 epithelial cells (EC) both in the villi and crypts. IEL count in LC and CC patients was significantly higher both in the crypts and in the villi than in inflammatory bowel disease patients and normal controls. Intraepithelial lymphocytes were CD3+, CD8+, CD20-, and LN3-HLA-DR-, indicating a suppressor T-cell phenotype and this pathway of inflammatory cell infiltration is similar to the reported findings in colonic mucosa of CC [1,2].

Moayyedi et al. [19] also evaluated the mean number of IELs in the duodenum of five patients with LC and four patients with CC. A normal-appearing villous architecture was described by these authors in all cases and a mean IEL count (per 100 epithelial cells) of 14 and 22 was found for LC and CC respectively. Fine et al. [14] evaluated the degree of lymphoplasmacytic infiltrate, the number of IELs, and the condition of the villi in distal duodenal biopsies from 37 patients with either LC or CC. The study of specimens showed that 70% of cases had a mild increase in the degree of mononuclear inflammation in the lamina propria and/or epithelium, which was associated with some degree of villous atrophy in 28% of cases.

It has been assessed that biopsy specimens from some patients with CC or LC contain certain histological features frequently observed in inflammatory bowel disease (IBD), such as Paneth cell metaplasia (PM), thus these overlapping features may cause diagnostic trouble. With the aim of clarifying this problem Ayata et al [20] described the prevalence of "so called" IBD-like morphologic features in colonic mucosal biopsies from patients with CC or LC. They evaluated 150 patients with clinically, endoscopically, and histologically confirmed LC (71 patients) or CC (79 patients), through the analysis of hematoxylin-eosin stained colonic mucosal biopsies. They considered some patho-gnomonic IBD-like histological features such as active crypt inflammation, Paneth cell metaplasia, surface ulceration, number of intraepithelial lymphocytes, crypt architectural irregularity, and thickness of the sub-epithelial collagen layer (CC only). The results have been compared between LC and CC and correlated with the clinical and endoscopic data. No case of clinical transition towards IBD was observed. While active crypt inflammation was a frequent finding in both groups, surface ulceration was not seen in LC subjects but was present in 2 of 79 (2.5%) CC patients, Paneth cell metaplasia was common in both groups and significantly more frequent in CC compared to LC patients, and crypt architectural irregularity was observed in 7.6% of patients with CC, and 4.2% of LC patients. The presence of Paneth cell metaplasia in patients with CC was related to more severe disease with a higher prevalence of bowel movements (>3 bowel movements/day) and abdominal pain (p=0.06). Thus these findings could indicate to pathologists that they should be conscious that some histological features normally associated with IBD such as crypt irregularity, neutrophilic cryptitis, and crypt abscesses can also be present in patients with LC or CC, and that the presence of one or more of these characteristics should not necessarily be interpreted as evidence against either of these diagnoses [20].

Histological findings

Histological findings of microscopic colitis				
Classic Lymphocytic Colitis	**Classic Collagenous Colitis**	**Atypical forms of microscopic colitis**		
Increased surface intraepithelial lymphocytes (IELs) with surface epithelial damage, normal crypt architecture with increased cryptal IELs, and (3) increased lamina propria inflammatory cell infiltrate including lymphocytes, plasma cells, and eosinophils.	Thickened sub-epithelial collagen band. This finding is not present in lymphocytic colitis and consists of a linear deposition of dense collagen in the sub-epithelial area with an additional finding of cells, fibroblasts, and capillaries entrapped in the collagen	Cryptal Lymphocytic Colitis the main feature of lymphocytic colitis is the intraepithelial lymphocytosis in surface epithelium	Paucicellular Lymphocytic Colitis increased lamina propria lympho-plasmacytic inflammation and increased surface intraepithelial lymphocytes alternating with foci or tissue fragments of normal mucosa	Microscopic Colitis With Giant Cells the main feature of lymphocytic colitis is the intraepithelial lymphocytosis in surface epithelium.
		Microscopic Colitis With Giant Cells atypical form of microscopic colitis characterized by the presence of multinucleated	Pseudomembranous Collagenous Colitis collagenous colitis associated with pseudomembranes	Microscopic Colitis with Granulomatous Inflammation atypical form of microscopic colitis with a

		giant cells in an otherwise classic microscopic colitis		conspicuous granulomatous reaction.

Table 2: Histological classification of microscopic colitis.

Classic Lymphocytic Colitis

Lymphocytic colitis is featured by: (a) increased surface intraepithelial lymphocytes (IELs) with surface epithelial damage, (b) normal crypt architecture with increased cryptal IELs, and (c) increased lamina propria inflammatory cell infiltrate including lymphocytes, plasma cells, and eosinophils.

The main characteristic of lymphocytic colitis is increased intraepithelial lymphocytes [21-25]. These cells have a basophilic nuclear chromatin pattern, irregular nuclear outline, and clear perinuclear halo on routine hematoxylin and eosin (H&E) stained section. The IELs belong to a unique T cell population interspersed between epithelial cells [24]. Phenotypically, IELs are predominantly CD3+ CD8+ T lymphocytes (commonly staining for CD45, CD3 and CD8 in the immunohistochemistry). In normal colonic mucosa the IEL count is about 5 IELs per 100 surface epithelial cells. In lymphocytic colitis this count increases to 20 or more IELs per 100 surface epithelial cells [16].

Classic Collagenous Colitis

In classic collagenous colitis the most typical histological aspect has been identified in a thickened sub-epithelial collagen band. This finding is not present in lymphocytic colitis and consists of a linear deposition of dense collagen in the sub-epithelial area with an additional finding of cells, fibroblasts, and capillaries entrapped in the collagen [25]. A collagen band thicker than 10mm is abnormal [26]. Other features of collagenous colitis include increased mononuclear inflammation in the lamina propria, increased intraepithelial lymphocytes, and normal crypt architecture.

Atypical forms of microscopic colitis

In addition to the classic forms, there have been increasing reports showing atypical or extraordinary histological patterns on colonic biopsies in patients with symptoms suggesting microscopic colitis.

Cryptal Lymphocytic Colitis

As described above, the main feature of lymphocytic colitis is the intraepithelial lymphocytosis in surface epithelium. Rubio and Lindholm [27] recently reported a series of six patients with a clinical pattern similar to those of lymphocytic colitis and an anatomical-pathological outline characterized by increased IEL count not in the surface epithelium but within the cryptal epithelium. Therefore, the authors proposed the name "cryptal lymphocytic colitis", as opposed to the classic "surface" lymphocytic colitis. In this series of patients the mean number of IELs was 46/100 cryptal epithelial cells and the mean number of lymphocytes recorded in the surface columnar cells was 7/100 surface epithelial cells. Similar to the classic form of lymphocytic colitis, all patients with cryptal lymphocytic colitis had long periods of watery diarrhea of unknown etiology. On endoscopy the colon showed either normal or mild patchy changes such as erythema.

Paucicellular lymphocytic colitis

Paucicellular lymphocytic colitis is a term used to describe a condition where the morphologic criteria of classic lymphocytic colitis are not fulfilled because of its paucity and lower density of surface IELs. Goldstein and Bhanot [28] have recently reported some cases with colonic biopsies showing foci of mildly increased lamina propria lympho-plasmacytic inflammation and increased surface intraepithelial lymphocytes alternating with foci or tissue fragments of normal mucosa.

Microscopic Colitis with Giant Cells

This is a recently described, rare and atypical form of microscopic colitis characterized by the presence of multinucleated giant cells in an otherwise classic microscopic colitis [29]. In these patients the clinical pattern was characterized by non-bloody diarrhea, colonoscopy was macroscopically normal and biopsies revealed the histopathological features of classic lymphocytic or collagenous colitis with scattered sub-epithelial multinucleated giant cells. There was no evidence of granulomas nor Crohn's disease.

Pseudomembranous collagenous colitis

Pseudomembrane formation in association with collagenous colitis has been reported by several groups [30-34]. These authors reported two cases of collagenous colitis associated with pseudomembranes. The stool analysis for C. difficile toxin and organism culture was negative in both patients. Both showed improvement with anti-inflammatory agents after diagnosis of collagenous colitis was achieved.

Microscopic Colitis with Granulomatous Inflammation

This term describes an atypical form of microscopic colitis with a conspicuous granulomatous reaction. Four such cases have been reported [35,36]. In all cases patients were female, the primary symptom was frequent watery diarrhea, and the only endoscopic finding was mild mucosal erythema. In three of the patients the symptoms began after antibiotic use or had worsened with antibiotic use.

Epidemiology

Different studies from various countries reported microscopic colitis rates between 4%-13% in the cohort of population with non-bloody diarrhea of unknown origin [47-40].

The incidence of collagenous colitis ranges from 0.6 to 5.2 cases per 105 person-years, and for lymphocytic colitis from 3.7 to 4.0 cases per 105 person-years [47,48]. Nevertheless, as diagnostic awareness of these entities continues to improve, both the incidence and prevalence of microscopic colitis have been shown to increase. Some studies from Sweden and Iceland reported higher prevalence of microscopic colitis [33,34,40,41].

In 1993 microscopic colitis was reported in 4% of patients with non-bloody chronic diarrhea in Sweden, but in 1998 this rate was reported as 10% [36]. The prevalence of collagenous colitis in Sweden between 1984 and 1988 was 0.8/105 inhabitants, but increased to 6.1/105 inhabitants between 1996-1998 [42].

Wickbom et al. [43] described an epidemiologic study of CC and LC from 1999-2008, as a follow-up of previous studies conducted by the same group in the 1980s. In this study CC was diagnosed in 96 patients (75 females) and LC in 90 patients (74 females). The mean annual age-standardized incidence (per 100,000 inhabitants) for MC, CC and LC was 10.2 (95% confidence interval: 8.7-11.7), 5.2 (4.2-6.3), and 5.0 (4.0-6.0) respectively. Age-specific incidence showed a peak in females older than 70 years. Prevalence (per 100,000 inhabitants) on December 31, 2008, was MC 123 (107.6-140.0), CC 67.7 (56.4-80.6), and LC 55.3 (45.2-67.1). This study revealed that after an initial rise during the 1980s and early 1990s, annual incidence of CC and LC has been stable during the last 15 years.

More recently Vigren et al. [44] reported that during the ten year period from 2001-2010, 198 CC patients were newly diagnosed with microscopic colitis in the southwest part of the country of Skåne in Sweden, with a female/male ratio of 2.8:1. The median age at diagnosis was 71 years (range 28-95, inter-quartile range 59-81), and the mean annual incidence was 5.4/105 inhabitants. According to these findings the authors concluded that the observed incidence of CC is comparable to previous reports from northern Europe and America. The incidence is stable but the female/male ratio seems to be decreasing.

Recently, some authors reported higher prevalence values from Iceland where the mean annual prevalence of CC was 5.2/105 inhabitants and the mean annual incidence of LC was 4.0/105 inhabitants in the period 1995-1999 [39]. According to various studies prevalence of CC and LC is 10-15.7/100000 and 14.4/100000, respectively [37].

In a study performed in Spain, 9.5% of patients were diagnosed with LC when they underwent colonoscopy because of chronic diarrhea during a period of 5 years [37]. In this study, the prevalence of LC was 3 times that of the prevalence of CC, female/male ratio in lymphocytic was 2.7/1, and in collagenous colitis 4.7/1. Female/male ratio was reported as 5/1 in Iceland [34] and 2.1 in Sweden [33,40].

In the reported series this ratio for CC was found as 4/1-20/1 [45] encountered in 13 LC and 1 CC in their 111 patients suffering from chronic-diarrhea with unexplained etiology. In another study of 132 consecutive patients who had undergone colonoscopy for chronic diarrhea and abdominal pain, LC and CC were found in 21 (16%) and 7 (5%) of patients, respectively [46]. In other studies, mean ages of the patients with LC were between 51-59 years and in CC between 64-68 years [3,4,9,10, 36,37,39,42,45].

Pathogenic Mechanisms

Etiology of LC is still not well understood. Gastrointestinal infections, autoimmune diseases and various drugs (ranitidine, carbamazepine, non-steroidal anti-inflammatory drugs, simvastatin, ticlopidine, flutamide etc) have been reported to be causative factors [41,47-49]. Some gastrointestinal inflammatory and rheumatologic disease-related disorders (celiac sprue, idiopathic pulmonary fibrosis, rheumatoid arthritis, uveitis, diabetes mellitus, pernicious anemia, autoimmune thyroiditis, etc.), and positivity for some autoantibodies, particularly antinuclear antibody (ANA), may be associated with both CC and LC [23,50-52].

Giardiello et al. [23] found 4 ANA positive patients in their 12 LC patients. Authors found only one case of ANA positivity in their patients, but none of them was associated with any of the disorders or conditions mentioned above.

Some patients with LC were reported to be effectively treated with medications used in inflammatory bowel disease such as 5-ASA and sulfasalazine. If this regiment fails, bismuth subsalicylate, corticosteroids, azathioprine and cyclosporine may be given [52,53]. In one study 5-ASA or sulfasalazine was used as first line treatment agents. Preliminary results have shown positive response in terms of symptom relief, but for the evaluation of long term outcome we should await the completion of the study.

Some pathogenic explanations have been postulated to explain the cause of the diarrhea in microscopic colitis. Bürgel et al. [54] employed an Ussing chamber technique to assess the diarrheal mechanism in CC, showing that a reduced Na^+ and Cl^- absorption accompanied by a secretory component of active chloride secretion could be involved in this subtype of microscopic colitis. Other studies [55] analyzing fecal electrolytes also confirmed the existence of a secretory mechanism.

Another possible pathogenic mechanism could be the inflammatory mechanisms. Others authors reported that the intensity of the inflammation in the lamina propria and not the thickness of the collagenous band is linked to the severity of diarrhea, sustaining a preeminent inflammatory origin [37,56]. Furthermore, clinical observations [57] indicate that fasting can reduce diarrhea and could suggest an osmotic component. On the basis of these results watery stools in MC could be driven by a combination of osmotic and secretory components. Using selenium-labeled homocholic acid-taurine (SeHCAT) some authors showed a concurrent bile acid malabsorption (BAM), frequently prevalent in patients with CC [58,59].

The potential linkage of colitis with human leukocyte antigens promotes further speculation about the cause and pathogenesis of MC. HLAB27 has not been correlated to the occurrence of MC in humans, but other HLA antigens have been [60-62]. One study indicates that HLA-AI increased and HLA-A3 decreased in frequency in patients with LC, but not in patients with CC [24]. Another study has suggested that HLA-DR antigens are anomalously expressed by colon epithelial cells [14]. A recent study reported HLA-DQ2 and DQI3 as associated to the occurrence of both LC and CC [62]. These antigens are of special concern because they are more closely correlated to celiac sprue than to MC. These data suggest that similar immune mechanisms may be behind both celiac sprue and MC.

A relationship with the immune system is also sustained by the coexistence of MC and various rheumatologic disorders [63]. However, attempts to find direct evidence of autoimmunity as a basis for the development of microscopic colitis have been disappointing. The linkages of MC to particular HLA alleles (such as DQ2) that have also been seen in patients with autoimmune disorders (such as sjogren's syndrome, insulin-dependent diabetes mellitus, and rheumatoid arthritis) indicate a common underlying pathogenesis. An alternative theory is that MC is caused by some of the treatments used for rheumatologic diseases. Most attention has focused on non-steroidal anti-inflammatory drugs [64-66]. These drugs have been correlated with exacerbations of ulcerative colitis [67,68], but their relationship to MC is not firmly established and remains controversial [69,70].

Although infection has also been suggested [71], a possible correlation between CC and NSAID consumption [72] as well as the role of abnormal pericryptal myofibroblasts in the production of the thickened collagen plate [73,74] have also been suggested. Because of the anatomic-pathological aspect of LC and mixed MC, we believe that an immunological mechanism is the primary cause of these diseases. The IEL in the two types of MC are of T cell origin according to findings reported by Armes et al. [75], and they are mainly CD8 suppressor cells [76]. These lymphocytes recognize class I linked antigens of endogenous origin.

It can be speculated that T lymphocytes are stimulated by a luminal antigen that cross-reacts with an endogenous antigen expressed by epithelial cells. Kumawat et al. [77] studied the T helper (Th) cell and cytotoxic T lymphocyte (CTL) mucosal cytokine pattern and protein levels in patients with MC. Authors observed that mucosal mRNA but not protein levels of IFN-γ and IL-12 were significantly up-regulated in CC, LC as well as UC patients compared to controls. Transcription of the Th1 transcription factor T-bet significantly increased in CC but not LC patients. The mRNA levels for IL-17A, IL-21, IL-22 and IL-6 were significantly up-regulated in CC and LC patients compared to controls, albeit less than in UC patients. Significantly increased IL-21 protein levels were noted in both CC and LC patients. IL-6 protein and IL-1β mRNA levels were enhanced in CC and UC but not LC patients. Enhanced mucosal mRNA levels of IFN-γ, IL-21 and IL-22 were linked with higher clinical activity, recorded as the number of bowel movements per day, in MC patients. Neither mRNA nor protein levels of IL-4, IL-5 or IL-10 were significantly changed in any of the colitis groups. LC-HR and especially CC-HR patients had normalized mRNA and protein levels of the above cytokines compared to LC and CC patients. No significant differences were found between LC and CC in cytokine production/expression. On the basis of these findings authors concluded that LC and CC patients showed a mixed Th17/Tc17 and Th1/Tc1 mucosal cytokine pattern.

Previous retrospective data [78,79] and a post hoc analysis from a randomized controlled trial observed that a possible overlap between overall symptomatology of MC and irritable bowel syndrome (IBS) symptoms could exist. To test these previous data [80] more recently Abboud et al studied a cohort of patients prospectively evaluated. They gave a symptom questionnaire to a cohort of patients with biopsy-proven MC and determined the proportion of patients who met various definitions for IBS; then the clinical characteristics of those with IBS criteria were compared to those without these criteria. In 38% to 58% of the recruited patients the diagnostic criteria for IBS were met. These patients tended to be younger and more likely female than those who did not meet IBS criteria. Thus subjects with microscopic colitis frequently meet the diagnostic criteria for IBS. Nevertheless, the presence of these criteria are not specific enough to permit clinicians to exclude "a priori" a diagnosis of MC, so this symptom pathway seems to suggest the diagnostic justification to perform a colonoscopy with multiple mucosal biopsy even in the presence of mucosal macroscopic integrity in subjects with watery diarrhea suggesting MC.

A dysfunctional regulation of T cells with aberrant T cell activation is thought to be a major contributor to inflammatory colitis. Furthermore an inappropriate T cell activation can be due to improper selection of autoreactive T cells and/or impaired education of regulatory T cells (TR cells) [80].

In contrast to ulcerative colitis and Crohn's disease are considered driven by aberrant CD4+ T lymphocyte responses, MC presents with heavy infiltration of CD8+ IELs [81-83]. Using immunohistochemistry, a significant increase in the amount of CD8+ T lymphocytes was found in the epithelium in both LC and CC patients compared to controls, with the most pronounced increase found in LC. In contrast, the amount of CD4+ T cells was markedly reduced in the lamina propria of both LC and CC patients compared to controls. The expression of the activation/memory marker CD45RO, found on CD4+ as well as CD8+ T cells, and the transcription factor Foxp3, involved in differentiation of CD4+ and CD8+ regulatory T cells (Tregs), was more abundant in lymphocytes in the epithelium, as well as in the lamina propria of both LC and CC compared to controls.

CD4+25+ TR cells originate in the thymus subsequently exporting to the periphery at which they exert their suppressive function [84], thus very recently the importance of thethymic cortex to the generation of TR cells been suggested. Faubion et al [85] tested the hypothesis that treatment of colitis restores thymic capability to generate regulatory CD4+25+ TR reporting how treatment of inflammatory colitis prevents destruction of the thymus of adult transplanted tgε26 mice and sustains production of TR cells. This study confirmed a negative impact of colitis on TR development in the thymus with profound implications for the pathogenesis inflammatory colitis.

Foxp3+ Treg are known to play an important role in intestinal homeostasis and some studies reported that transfer of Treg inhibits experimental colitis induced in immunodeficient mice and react to the intestinal flora [85].

Although some studies underlined on thymically imprinted natural Treg action in regulation of gut homeostasis, there is also evidence that the intestine with its associated lymphoid tissue is a site for induction of Foxp3+ Treg from naïve precursors. For instance, has been reported a role of dendritic cells (DC) that are essential in antigen presentation as having an important role in Treg generation [86] with specialized intestinal DC expressing integrin CD103 strictly involved in Treg development [87,88]. Furthermore CD103+ DC can also induce Foxp3+ Treg in an antigen-specific manner, through a mechanism depending on transforming growth factor-b (TGF-b) and retinoic acid [87,88]. Several factors have been identified to be critical for Treg function, including IL-10, IL-2, TGF-b and cytotoxic T lymphocyte antigen-4 (CTLA-4) [89-94] and IL-10 is required for Treg to prevent colitis in an innate model of intestinal inflammation, suggesting that IL-10 not only controls pathogenic T cells, but can act on other immune cells in the intestine [95]. Moreover, other mechanisms such as a lacking signal transducer and activator of transcription-3 (STAT3), an essential mediator of IL-10 signalling, specifically in macrophages and neutrophils develop colitis [96].

The finding of increased Foxp3 expression in MC may in the light of this seem paradoxical. Increased frequencies of Foxp3+ CD4+ Tregs have also been reported in Crohn's disease and ulcerative colitis [97]. We have demonstrated in a mouse model of colitis that whereas the suppressive function of FoxP3+ Tregs was similar between wild-type mice and mice with colitis, CD4+ effector T cells from colitic mice were much less suppressed by Tregs irrespective of if they were derived from colitic or wild-type mice [98]. Therefore, the problems seemed to be confined to the effector T cells being resistant to Treg-mediated suppression.

Clinical Features

The principal symptom of MC is chronic diarrhea. Diarrhea is continuous, although with some fluctuation. Weight loss is not

prominent, and when it occurs, happens early in the course of the illness. Progressive weight loss suggests a diagnosis other than MC. Diarrhea could be sometimes accompanied by cramps/abdominal pain, but the latter is not typically prominent. The diarrhea is watery, but stool consistency can vary depending on diet and other factors. Rectal bleeding may be present, caused by hemorrhoids or anal fissures. Analysis of stool may reveal fecal leukocytes, but passage of true pus or mucus is rare [99,100].

Fecal electrolyte concentration profile is usually typical of a secretory diarrhea with a low fecal osmotic gap. Steatorrhea is not common, unless there is coexisting small-bowel disease. Previous studies observed that LC affects men and women equally [100,101], and that CC predominantly occurred in women [45,102]. More recent studies on larger numbers of patients have reported a similar female predominance of about 7:1 in both forms of the disease [103]. Most patients with either disease are over 40 years of age, with a mean age of onset in the sixth decade [104]. However, some patients will present in their 20s and 30s, and even children have been diagnosed with MC.

Treatment

Most important is a careful evaluation of concomitant drug use and dietary factors such as excess use of alcohol, caffeine and dairy products that might worsen the condition. Concomitant celiac disease or bile acid malabsorption should be considered. In the patient with mild symptoms, loperamide or cholestyramine are recommended as the first step of treatment.

Budesonide represents the best-documented treatment and significantly ameliorates clinical symptoms and the patient's quality of life. Studies suggesting that budesonide is effective for the treatment of CC have been small and differed in efficacy measures. Mesalamine has been proposed as a treatment option for CC, although its efficacy has never been verified in placebo-controlled trials.

Very recently some authors [105] have conducted a phase 3, placebo-controlled multicenter study to assess budesonide and mesalamine as short-term treatments for CC. In this study patients with active CC were randomly assigned to groups given pH-modified release oral budesonide capsules, mesalamine granules, or placebo for 8 weeks. Results of this study indicated a greater percentage of patients in the budesonide group were in clinical remission after 8 weeks than the mesalamina and placebo group. Budesonide significantly improved stool consistency and mucosal histology, and relieved abdominal pain.

To assess the outcomes of corticosteroid-treated MC in a population-based cohort, and to compare these outcomes in patients treated with prednisone or budesonide, Gentile et al. [106] performed a study on a historical cohort of Olmsted County, Minnesota residents diagnosed with CC or LC. Of 315 patients with MC, 80 (25.4%) were treated with corticosteroids. Authors observed that patients treated with budesonide had a higher rate of complete response than those treated with prednisone (82.5 vs. 52.9%) and were less likely to relapse than those treated with prednisone.

Furthermore, a systematic review and meta-analysis [107] on the short- and long-term efficacy of corticosteroids in treatment of MC analyzed 8 randomized trials in which the drug used was budesonide in 7 trials and prednisolone in 1 trial. This meta-analysis indicated that budesonide was significantly more effective than placebo for short-term clinical response and long-term clinical response and that anatomic-pathologic improvement was seen with both short- and long-term budesonide. Symptom relapse occurred in 46%-80% of patients within 6 months of treatment cessation.

However, diarrhea recurred frequently when budesonide was tapered and a few patients became budesonide intolerant. On this basis a recent study [108] assessed the effect of mercaptopurine (MP) and azathioprine (AZA) retrospectively in patients with chronic, active MC, indicating that the majority of chronic, active MC patients were intolerant to AZA, leading to cessation of treatment. However, further studies are needed to evaluate the efficacy, acceptance, tolerance, and safety of MP in patients with chronic, active MC refractory to budesonide.

Conclusions

Chronic diarrhea, reported in 4%-5% of individuals in Western populations, is a common reason for consulting a physician in general practice or internal medicine, and for referral to a gastroenterologist [109]. MC is an increasingly common cause of chronic diarrhea. MC may be defined as a clinical syndrome of unknown etiology, consisting of chronic watery diarrhea, with no alterations in the large bowel on the endoscopic and radiologic evaluation. Therefore, a definitive diagnosis is only possible by histological analysis. Given the normal colonoscopic and radiologic findings, the important role of the pathologist in diagnosing MC is evident. Nevertheless, equally important is the role of the clinician through a careful process of differential diagnosis and through careful clinical evaluation. This way clinicians should be able to provide elements of clinical suspicion to pathologist that could support the justification for a more specific histological evaluation that includes the assessment of the number of intraepithelial cells (IEL), and the thickness of the band of tissue collagen, which because of the additional costs in terms of time and technical material used cannot be regarded as routine but must be reserved only for those patients whose symptoms make clinical diagnostic suspicion very likely. In this setting the role of the clinician is crucial in preparing and alerting the pathologist by providing the right information to perform a thorough evaluation of the inflammatory state of the mucosa and lamina propria.

In this clinical setting a question possibly difficult to answer is which patients should undergo biopsies of mucosa that appears normal. It seems unreasonable to obtain biopsies of colorectal mucosa in all patients with chronic diarrhea and macroscopically normal colon, owing to the significant added expense of an indiscriminate biopsy policy. Taking biopsies from patients with chronic watery diarrhea suggesting MC, whether lymphocytic or collagenous, is advisable, especially when associated with nocturnal bowel movements, weight loss or increased inflammatory markers such as sedimentation rate or C-reactive protein.

Further studies on the pathogenesis and characterization of the full spectrum of this heterogeneous entity will aid in further defining this disease, minimizing confusion in terminology, and improving communication between the pathologist and the clinician. Furthermore, although CC and LC are considered rare conditions, increasing awareness of these entities among pathologists and clinicians should result in more frequent diagnosis and perhaps reveal them as the tip of the iceberg.

Conflicts of Interest

No conflict of interest.

Funding

Acknowledgments

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