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MICROSCOPIC COLITIS

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Microscopic colitis

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my daughters Julia, Tilda and Sara

ABSTRACT

Microscopic colitis (MC) is an inflammatory bowel disease (IBD) and a common cause of chronic non-bloody diarrhoea, especially in elderly women. There are two main subtypes, lymphocytic colitis (LC) and collagenous colitis (CC) which are clinically indistinguishable and can be separated only by their characteristic histopathological features. The colonoscopy is usually macroscopically normal although subtle mucosal changes have been reported. The aetiology of MC is unknown and the genetic factors are poorly investigated.

This thesis aims to describe MC in a large urban cohort and compare LC and CC regarding clinical and endoscopic features, both at diagnosis and at follow-up (F-U), and to observe the occurrence of coeliac disease, ulcerative colitis (UC) and Crohn's disease (CD). We also reported the histological change of the MC phenotype over time and patients' MC medication at last F-U. Further, we tested immune-related genes, known to impact several autoimmune diseases, for their potential CC-predisposing role. Finally, this thesis aims to report on chromoendoscopic findings in MC.

A retrospective study of 795 patients showed that the clinical features of LC (n=451) and CC (n=344) were similar, though watery diarrhoea occurred at a lower frequency in LC (43%) than CC (55%) as did nocturnal diarrhoea, LC (18%) and CC (28%). The mean age at diagnosis was lower in LC, at 59 years compared 63 years in CC. Subtle endoscopic mucosal findings were frequently reported at a higher rate in CC (37%) than LC (25%). Our study confirms MC's strong association with coeliac disease, which occurred in 6% of the patients. UC and CD occurred in 2.1% of the patients.

In the MC cohort, 687 patients had a clinical F-U after a mean time of 2.89 years at which 64% were in clinical remission. The cumulative clinical remission was higher in LC. About half of the patients had received medical treatment for MC at last F-U and about a quarter of them were on steroids; both these parameters were lower in patients with LC. The mean time for the first F-U colonoscopy (n=187) was 3.33 years and the histological remission was 44% and the cumulative histologic remission was higher in LC. Histological change of phenotype over time was not uncommon and was observed in 12 % of the patients (10 CC to LC, 13 LC to CC).

Three independent CC and control cohorts were genotyped with Immunochip and 42 markers gave rise to significant genome-wide associations signals, all within the HLA region of chromosome 6. The most pronounced risk effects were observed for 8.1 haplotype alleles including DQ2.5. The HLA genotype is associated with CC and indicates immune-driven pathogenesis.

Previously, two case reports described that chromoendoscopy in CC patients showed uneven surface. We reported additional chromoendoscopic findings in 13 MC patients that showed continuous mucosal changes. Our study supports the fact that chromoendoscopy can reveal mucosal changes in MC and therefore might be diagnostically useful.

LC and CC are similar but not identical, since LC has a milder clinical presentation and a better prognosis than CC. Conversions between subtypes and between MC and UC or CD exist. It is not uncommon with macroscopic changes of the colonic mucosa in patients with MC which are more manifest with chromoendoscopy. Specific HLA alleles are associated with CC, indicating an autoimmune role.

LIST OF SCIENTIFIC PAPERS

I. Marie-Rose Mellander, Anders Ekbom, Rolf Hultcrantz, Robert Löfberg, Åke Öst, Jan Björk.
Microscopic colitis: a descriptive clinical cohort study of 795 patients with collagenous and lymphocytic colitis.
Scandinavian Journal of Gastroenterology. 2016; 51(5):556-62.

II. Marie-Rose Mellander, Anders Ekbom, Rolf Hultcrantz, Robert Löfberg, Åke Öst, Jan Björk.
Microscopic colitis: a clinical and endoscopic follow-up study.
Manuscript.

III. Helga Westerlind, Marie-Rose Mellander, Francesca Bresso, Andreas Munch, Ferdinando Bonfiglio, Ghazaleh Assadi, Joseph Rafter, Matthias Hubenthal, Wolfgang Lieb, Henrik Källberg, Boel Brynedal, Leonid Padyukov, Jonas Halfvarson, Leif Törkvist, Jan Björk, Anna Andreasson, Lars Agreus, Sven Almer, Stephan Miehlke, Ahmed Madisch, Bodil Ohlsson, Robert Löfberg, Rolf Hultcrantz, Andre Franke, Mauro D´Amato.
Dense genotyping of immune-related loci identifies HLA variants associated with increased risk of collagenous colitis.
Gut. 2017 Mar;66(3):421-428.

IV. Gaku Suzuki, Marie-Rose Mellander, Akiko Suzuki, Carlos Rubio, René Lambert, Jan Björk, Peter Thelin Schmidt.
Usefulness of colonoscopic examination with indigo carmine in diagnosing microscopic colitis.
Endoscopy. 2011 Dec;43(12):1100-4.

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LIST OF ABBREVIATIONS

BAM	Bile acid malabsorption
CC	Collagenous colitis
CD	Crohn's disease
DNA	Deoxyribonucleic acid
ECCO	European Crohn's and Colitis Organisation
EIRA	Epidemiological Investigation of Rheumatoid Arthritis
ESP	European Society of Pathology
F	Female
F-U	Follow-up
GWAS	Genome-wide association study
HDTV	High-definition television
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IL	Interleukin
LC	Lymphocytic colitis
M	Male
MC	Microscopic colitis
MHC	Major histocompatibility complex
NS	Not significant
NSAID	Non-steroidal anti-inflammatory drug
OR	Odds ratio
PPI	Proton-pump inhibitor
QC	Quality control
SeHCAT	Selenium homocholic acid taurine
SNP	Single nucleotide polymorphism
SSRI	Selective serotonin reuptake inhibitor
TNFa	Tumour necrosis factor alpha
UC	Ulcerative colitis

1 INTRODUCTION

Microscopic colitis (MC) is an inflammatory bowel disease (IBD) clinically characterised by chronic non-bloody diarrhoea, predominantly presenting in female elderly patients. Macroscopically, the colonic mucosa has been described as normal or nearly normal, with characteristic histological findings differentiating between its two major subtypes, lymphocytic colitis (LC) and collagenous colitis (LC). The cause of MC is unknown and is most likely multifactorial. Budesonide is an effective treatment but the relapse rate is high after withdrawal.

CC was first described by the Swedish pathologist Lindström in 1976 (1), the same year that Freeman et al. reported two patients suffering from the same condition (2). In 1989, Lazenby et al. (3) described LC and in 1993, a French and an American research group suggested the use of MC as an umbrella term covering any form of colitis with normal endoscopic and radiological examination characterised by histological changes (4, 5).

1.1 EPIDEMIOLOGY

MC is a common disease and is mainly reported in Europe and North America but it is seen worldwide (6-13). Epidemiological studies in Europe, the United States and Canada show its rising incidence over the past two decades (12, 14, 15) but a recent Swedish study shows stabilising incidence rate of 5/100 000 inhabitants for each subtype (16) which is at the same level as the pooled incidence rates from North American and European studies (17). A recent nationwide study in Denmark (2002-2011) reported an annual MC incidence of 24.7/100 000 inhabitants in 2011, 9.8/100 000 for LC and 14.9/100 000 for CC (18) which aligns with a recent from United States study covering almost the same period (14). In the Danish study (18), there was almost a twofold increase over time in the number of endoscopies from which colon biopsies were taken, which partly explains the rising incidence – a result of increased awareness of MC; this is supported by another study (19). The prevalence of MC in Sweden is 123/100 000 (16) and 219/100 000 in United States (14) which is approaching the same prevalence as of classical IBD in Europe, 213/100 000 for Crohn's disease (CD) and 294/100 000 for ulcerative colitis (UC) (20).

The prevalence of chronic diarrhoea is 4–5 % in Western populations (21). Patients with chronic non-bloody diarrhoea undergoing a colonoscopy are diagnosed with MC in 10–14% of cases and in about 20% of cases in males over 70 years of age and females over 50 years of ages (22, 23).

MC is most seen in elderly individual but it can be diagnosed in patients of all ages including children (24-27). It has been reported that 25% of patients diagnosed with CC are younger than 45 years of age (28). The mean age at diagnosis is 61.1 ± 6.5 years, with no significant differences between the subtypes (29). Furthermore, MC is more common in females (16) and the female: male ratio is more pronounced in CC than LC, at about 7:1 (7, 28) and 2–3:1 (13, 30) respectively.

1.2 AETIOLOGY AND PATHOPHYSIOLOGY

The aetiology of MC is unknown, but it is probably multifactorial. The prevailing theory is that it is a specific immune response to unknown luminal agents in genetically predisposed individuals with a disturbed barrier function which leads to uncontrolled inflammation in the colonic mucosa. The theory is based on the fact that MC is associated with autoimmune diseases and has familial clustering. The theory of luminal agents in the pathogenesis of MC is supported by the observation that faecal stream diversion results in regression of colonic inflammation which reappears after the reconstruction of bowel continuity in CC (31). Whether or not LC and CC are the same disease remains an unanswered question and a matter of debate (32-35). In addition, conversions between the subtypes have been reported (35, 36). In the following section, some proposed factors are discussed.

1.2.1 Autoimmunity

Concomitant autoimmune disorders are common in MC patients, 30–50% have at least one autoimmune disease (37), such as type 1 diabetes mellitus, thyroid disorders, rheumatoid arthritis or Sjögren's syndrome. Coeliac disease is the most common concomitant autoimmune disease, seen in 2–20% MC patients (29). Furthermore, a United States study found that 4.3 % of a large coeliac disease patient cohort was diagnosed with MC (38). There have also been reports of concomitant or interchange between UC or Crohn's disease and MC (39, 40). However, the number of cases is small and histological changes typical of UC and CD (Paneth cell metaplasia and crypt architectural distortion) are occasionally seen in MC (41). A diagnostic autoantibody has yet to be found.

1.2.2 Genetic factors

Family occurrence has been described in MC (42-46). Studies have shown an association of MC with the HLA (HLA-DQ2), TNFα, IL-6 and serotonin transporter promoter gene polymorphisms as well as with allelic variation of the matrix metalloproteinase-9 (47-52). Coeliac disease is strongly associated with HLA-DQ2 (53) suggesting some similarities in pathogenesis. MC's HLA association, the high occurrence of immune-mediated diseases and the response to corticosteroid treatment in MC suggest an autoimmunity (48). However, genetic MC studies are scarce and underpowered and large-scale genome-wide association studies are lacking.

1.2.3 Drugs

There are several reports of drug-induced MC (54, 55). NSAIDs, PPIs and SSRIs were identified as the three drugs with highest likelihood to cause MC (56, 57). A direct cause-and-effect relationship has not been established for any of these drugs. Furthermore, most of the drugs that have been associated with MC are known to have diarrhoea as a side-effect (58) and therefore increase the likelihood for these patients to undergo endoscopy. However, after adjusting for more frequent endoscopies only the association between PPIs and CC and between SSRIs and LC remained significant (59). The pathophysiology can be explained by a

direct pharmacological effect on the colon, altered intestinal bacterial flora or idiosyncratic hypersensitivity reaction (60).

1.2.4 Smoking

A study by Vigren et al. showed that 37% of CC patients smoked, compared to 17% of controls. In younger individuals (16-44 years), 75% of CC patients were smokers versus 15% of controls. Active smokers develop the disease 10 years earlier than non-smokers (61). Further studies have confirmed that smoking is a risk factor for MC (62, 63).

1.2.5 Bile acid malabsorption

Patients with bile acid malabsorption (BAM) have diarrhoea. BAM has been diagnosed using SeHCAT in up to 60% of patients with LC and 44% of patients with CC (64, 65). Morphological changes in terminal ileum in MC such as villous atrophy, inflammation and collagen deposit have also been described in MC patients (34, 66). Similar to LC, colonic infusion of bile acids in animal models causes colitis (67). Bile acid binding treatment has been effective in CC, improving symptoms even in patients with normal SeHCAT tests (64, 66). This might be due to that this treatment may binds other unknown luminal agents (68). Although bile acid binders can be clinically effective, they do not improve the histological changes in CC (69).

1.2.6 Infections/Brainerd diarrhoea

Some MC patients have acute onset of symptoms similar to gastroenteritis and have been reported to respond to antibiotic therapy (28). Onset of MC after infections with Yersinia enterocolitica, Campylobacter jejuni or Clostridium difficile have occasionally been reported (70-72). A seasonal pattern of LC onset may support infectious origin (30). However, stool cultures are negative in most cases of MC with acute onset. Furthermore, LC resembles “Brainerd diarrhoea”, which is characterised by chronic acute onset watery diarrhoea with long duration and mucosal lymphocytosis in colonic biopsies. The cause is thought to be infectious and respond to antibiotics but no causative infectious agents have been identified (73, 74).

1.2.7 Diarrhoea mechanism

CC patients were found to have abnormal epithelial barrier function and increased paracellular and transcellular mucosal permeability (75, 76). The precise mechanism of diarrhoea in MC is not clearly known. Burge et al. describe the triple mechanism of diarrhoea in CC: (I) malabsorption due to collagen bands and defective transporters, (II) secretory due to active chloride secretion and (III) leakage flow secondary to alterations to the epithelial barrier due to the down-regulation of epithelial tight junctions and passive leak of water and ions into the lumen (77). On the other hand fasting seems to decrease diarrhoea supporting an osmotic component (78). MC patients do not seem to have anal dysfunction or rectal hypersensitivity (79).

1.2.8 Role of eosinophil granulocytes/mast cells

Eosinophil granulocyte infiltrates are seen in CC patients and these cells are activated in active CC (80), and they are regarded as pro-inflammatory cells that can release cytotoxic proteins. In one study of 12 CC patients, two eosinophil-derived cytotoxic proteins were found in the faeces and both decreased after budesonide treatment (81). The mast cells are also present in CC patients (82). A case report of a patient with MC with increased mast cells in the lamina propria improved on antihistaminic therapy (83). Mast cells may also mediate abnormal collagen deposition (84) and moreover, patients with mastocytosis often have fibrosis (85).

1.2.9 Abnormal collagen metabolism

The production and deposition of basement membrane collagen is regulated by peri-cryptal fibroblasts which seem to be activated in CC leading to increased collagen production (86). However, one study that measured messenger RNA levels found no increase collagen production (87). The findings are contradictory and difficult to draw any conclusions from. Since similar alterations in peri-cryptal fibroblasts in CC have been documented in the fibrotic form of UC (88) it is suggested that these changes in the subepithelial collagen layer are secondary to inflammation rather than a primary pathogenic element.

1.3 CLINICAL FEATURES

1.3.1 Symptoms

The main symptom is chronic or recurrent watery non-bloody diarrhoea (89), and despite some patients' severe diarrhoea, serious dehydration is rare. Nocturnal diarrhoea (up to 60%), diffuse abdominal pain (50%) moderate weight loss (up to 50%), fatigue (50–60%), faecal urgency (70%) and incontinence (40%) are often associated symptoms (37, 90, 91). Active MC may significantly impair patients' quality of life and the burden seems to be caused by faecal consistency, rather than faecal frequency (92, 93). The onset of MC is often insidious but it can mimic a sudden onset of enteric infection (28). There is symptomatic overlap between MC and IBS-D (94), and up to 58% of MC patients met the IBS criteria in a recent prospective study (95). The histopathological assessment separates MC from for IBS with a predominance of diarrhoea, since the colonoscopy is usually macroscopically normal. The clinical symptoms of LC and CC are similar and cannot be separated on clinical grounds (33, 91, 96), and the differentiation is based on the histopathological features. It is not entirely clear whether LC and CC are one or two diseases. There are only a few comparative studies on both subtypes in the same population and most have been based on small sample sizes.

1.3.2 Diagnosis

Routine laboratory test are non-diagnostic and pathogenic microorganisms are generally not found in stool analyses. Faecal calprotectin does not appear to be useful in MC and research has been conducted for alternative biomarkers such as faecal eosinophil protein X, faecal eosinophil cationic protein and secretoneurin (81, 97).

MC diagnosis is based on histopathological examination of mucosal biopsies taken at colonoscopy in combination with patient's clinical history of non-bloody chronic diarrhoea (98). Colonoscopy with random biopsies is required from the entire colon and rectum examined separately, as this allows the pathologist to know the origin of the biopsies. There are contradictory data about its topographical distribution, but it is generally accepted that the prevalence of microscopic findings of MC are highest in the right and transverse colon (37). Microscopic changes can be patchily distributed in MC (99) and the presence of inflammatory cells in normal colonic mucosa is more prominent in the right colon (100). MC is characterised by macroscopically normal colonic mucosa, or nearly normal with subtle mucosal findings such as erythema, oedema or abnormal vessel patterns (28, 30, 101). Even mucosal tears can be seen at colonoscopy in MC patients (102-106).

The histology is also necessary to differentiate between the two major subtypes, LC and CC. The characteristic histopathological features of LC include an increased number of intraepithelial lymphocytes (≥ 20 lymphocytes per 100 surface epithelial cells) in the colonic mucosa, mixed inflammation in the lamina propria with or without surface epithelial damage, and a subepithelial collagen layer <10 μm thick (107). The characteristic histopathological features of CC are a thickened subepithelial collagen layer (≥10 μm) in the colonic mucosa, mixed inflammation in the lamina propria with or without surface epithelial damage and intraepithelial lymphocyte infiltration (107).

Two case reports described chromoendoscopic findings in CC patients that showed uneven surfaces (108, 109). If further studies can confirm that macroscopic lesions could be more pronounced by using indigo carmine staining, targeted biopsies may be possible.

1.3.3 Treatment

Therapy primarily aims to achieve and maintain clinical remission and improve patient's quality of life since no curative treatment for MC exists.

Concomitant BAM and coeliac disease should be considered in MC patients, but so should smoking cessation and withdrawal from drugs that may induce diarrhoea.

Budesonide is the best-studied MC treatment, having been investigated in three randomised placebo-controlled induction studies in CC patients (110-112) and two in LC patients (113, 114), which were confirmed by Cochrane meta-analysis (115). In summary, budesonide was superior to placebos for inducing clinical remission in about 80% of MC cases; in fact, it may even be better than prednisolone (116) and has fewer side-effects. There is a high risk of relapse after budesonide cessation (60–80%) (110-112). Three randomised placebo-controlled studies showed that low-dose budesonide (6-4.5 mg) for six to twelve months can maintain clinical remission in the majority of CC patients (117-119). After ceasing budesonide maintenance therapy, there was no difference between budesonide and placebo in the median time to relapse (117).

Budesonide is recommended as a first treatment option for inducing and maintaining clinical remission, and the dose should be tapered to a minimum. Other treatment options are poorly investigated, though antidiarrheal medication and cholestyramine can be effective in patients with mild symptoms. If budesonide treatment is not sufficient, MC should be reconfirmed. Patients intolerant of or not responding to budesonide are a clinical challenge. In steroid-dependent or refractory patients, thiopurines may be effective as a maintenance therapy (120), and mercaptopurine seems better tolerated than azathioprine (121). Anti-TNF drugs have been tested in a few patients who are refractory to budesonide and immunomodulators (122-124) and suggest that anti-TNF drugs could be an alternative to colectomy, which is the last resort in patients who are refractory to medical treatment.

1.3.4 Clinical course and prognosis

The information on MC's natural course is limited and it often dates back to the time before budesonide treatment was available. MC has variable manifestations, from a single episode lasting a few months to persisting episodes or alternations between activity and remission. Two relatively recent studies show that most patients remain symptom-free or suffer only sporadic symptoms (125, 126). It is suggested that LC has a milder course than CC and a greater tendency towards spontaneous remission (127). Placebo response rates of 48% and 12% were reported in LC and CC patients (113), respectively. In LC patients, diarrhoea resolution and histology normalisation were described in 80% of the patients (128). In another study, LC was manifested as a single episode in 63 % of cases (22).

CC patients in remission (without diarrhoea) complain about abdominal pain more often compared to matched controls (93), suggesting that IBS like symptoms may be secondary to residual or past inflammation in the mucosa as seen in quiescent IBD (129).

MC is a benign disorder but there have been reports of both spontaneous and post-colonoscopy colon perforations that may be related to the presence of mucosal tears seen at colonoscopy (130) and this in turn reflecting NSAIDs use (131). Unlike other IBD forms, there is no evidence that MC is associated with increased risk of colonic cancer and therefore there is no need for screening colonoscopies (132, 133). Recent studies reported even smaller numbers of colonic polyps in MC patients compared to subjects with normal colon (134), even when including any history of adenoma and adenocarcinoma in earlier colonoscopies (133). Conversions between the subtypes have been observed over time (135-138), but it is unclear whether LC and CC are two separate entities or whether the two subtypes are one single disease. This issue is still a matter of debate (32-35). However, clinical practice management today is not influenced by the subtype.

2 AIMS

1. To clarify if there are any differences between LC and CC with regard to the demographic data and the clinical, endoscopic and histopathological features, both at onset and at follow-up, in a large, well-defined urban cohort.

2. To identify any associations between MC and coeliac disease, UC and CD, and to observe if any conversions between the subtypes occur over time.

3. To clarify the association between immune-related genes and CC.

4. To evaluate whether MC can be diagnosed macroscopically using chromoendoscopy.

3 PATIENTS AND METHODS

The LC and CC diagnoses were based on histopathological criteria in paper I and II whereas in paper III and IV the diagnoses were based on clinical and histopathological criteria and the clinical symptoms lasted at least three weeks.

The histopathology criteria in LC patients included an increased number of intraepithelial lymphocytes (≥ 20 lymphocytes per 100 surface epithelial cells) in the colonic mucosa; mixed inflammation in the lamina propria, with or without surface epithelial damage; and a subepithelial collagen layer <10 μm thick. The CC histopathology included a thickened subepithelial collagen layer (≥10 μm) in the colonic mucosa and mixed inflammation in the lamina propria, with or without surface epithelial damage.

3.1 PATIENTS AND CONTROLS

3.1.1 Paper I and II

All patients (n=818) diagnosed with MC at Karolinska University Hospital and Sophiahemmet Hospital in Stockholm, Sweden, from 1 January 1980 to 31 May 2010 were identified. Ten cases with MC were not subtyped and were thus re-examined by a pathologist; seven of these were CC cases, two were LC cases, and one did not fulfil the criteria of MC and was excluded. All histological reports of the LC patients were re-evaluated and 22 patients were then excluded, as they did not fulfil the histological criteria of LC. Thus, 795 patients were considered for this study.

A validation of the histological diagnosis of MC showed an accuracy of 92%. All clinical data were evaluated retrospectively. We reviewed medical charts and recorded demographic data, as well as clinical and endoscopic features, including the occurrence of coeliac disease, UC and CD. Twenty patients with IBD were identified. After re-evaluating the endoscopic and histopathological reports, three patients with previous IBD diagnoses were excluded, as they did not fulfil the IBD criteria. The date of diagnosis was defined as the year and month when MC was confirmed histologically.

As a control colorectal neoplasia population, we considered 745 individuals in the normal population, aged 19–70 years who were randomly invited to undergo a colonoscopy in Stockholm County as part of a study of Forsberg et al. (139).

The follow-up study reported that 687 of the 795 individuals underwent a clinical follow-up during the study period, which ranged between 1980-2010. A total of 108 patients with MC had only an index visit and were not included. All clinical, endoscopic and histological data were evaluated retrospectively by reviewing medical charts.

3.1.2 Paper III

Patients diagnosed with CC (n=321) were recruited at gastroenterology clinics in Hamburg, Germany (112, 140) and Stockholm, Malmö and Linköping in Sweden. Ninety-one German

and 163 Swedish patients were included in the discovery analyses, and 67 Swedish patients were included in the replication analyses. Exclusion criteria included history of IBD, coeliac disease, clinical/endoscopic signs of gastrointestinal infection, ischemic colitis or neoplastic disease. A diagnosis of coeliac disease was further ruled out in the majority of patients by anti-tissue transglutaminase serology.

For the controls, we used data from three sets of controls representative of the German and Swedish general populations. German PopGen discovery controls (n=2018) were from a community-based sample, recruited through population registries and the University Hospital Schleswig-Holstein, Campus Kiel, blood bank (141). The Swedish discovery controls (n=2046) were healthy individuals from the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) (142). The Swedish replication controls (n=330) were from study PopCol's population-based colonoscopy study, which included 745 individuals (143). Written informed consent was obtained from all individuals included in the studies.

3.1.3 Paper IV

Since 2007, endoscopists at Karolinska University Hospital have used chromoendoscopy in patients with chronic diarrhoea. Between 2007 and 2010, 13 patients with chronic diarrhoea and abnormal chromoendoscopic findings were identified including three men and 10 women; 10 patients had CC and three had LC. Eight of the 13 patients had been previously diagnosed with MC (n=8), and had undergone a new colonoscopy due to recurrent diarrhoea.

3.2 GENOTYPING AND QUALITY CONTROL

Genotyping with Immunochip beadarrays was carried out at the Institute of Clinical Molecular Biology genotyping facility in Kiel and the Genome Institute of Singapore. Signal intensity value data for all cases and controls were retrieved and combined at the Institute of Clinical Molecular Biology genotyping facility in Kiel for genotype clustering and calling, using the optiCall algorithm. After quality control (QC) measures were applied, 4216 individuals and 110 634 Immunochip markers common to the Swedish and German data sets remained and were included in the combined analysis of the discovery cohorts. Upon association testing, the individual genotype raw intensity (cluster) plots were manually inspected for all markers showing suggestive association signals with $p<1\times10^{-5}$ (n=207) and poorly clustering SNPs were removed from subsequent analyses (n=19). The rs2187668 (DQ2.5) and rs7454108 (DQ8) markers were genotyped in the replication cohort (both cases and controls) with Tagman SNP Genotyping Assays according to the manufacturer instructions.

3.3 CHROMOENDOSCOPY

The colonoscopies were carried out by three well-trained endoscopists, who had each performed more than 2000 colonoscopies. The colonoscopes were equipped with high-definition television and close focus, but not with high magnification. Chromoendoscopy with indigo carmine 0.2 – 0.5 % was used. The mucosa was judged normal if the

chromoendoscopy showed normal innominate grooves (144). The endoscopists noticed three abnormal macroscopic appearances after dying with indigo carmine; mosaic pattern, an uneven surface and a nodular surface. The chromoendoscopic appearance was categorised accordingly in consensus.

3.4 STATISTICAL ANALYSIS

3.4.1 Paper I

The results are expressed as means and as proportions. Student's t-tests were used to analyse unpaired parametric variables, and either the chi-squared method or Fisher's exact test was used to compare categorical variables. All statistics were performed using the Prism and Statistica software.

3.4.2 Paper II

Results are expressed as means, ranges and proportions. Histological and clinical remission at follow-up was analysed using Kaplan-Meier estimates, followed by comparing the curves using the log-rank test. Statistical significance was assumed by a p-value less than 0.05 (two-tailed analysis). Chi-squared test was used to compare categorical variables. All statistics were performed using the Prism and Statistica software.

3.4.3 Paper III

Quantile-quantile (q-q) plots were generated to highlight where the strong Immunochip association results came from. Case-control association testing was performed using logistic regression to adjust for age, sex and origin. HLA variants were imputed. In total, 100 imputed HLA alleles were tested through logistic regression analysis adjusting for age, sex and country of origin. Association testing versus replication controls was performed with age/gender-adjusted logistic regression using rs2187668 and rs7454108 Immunochip genotype data from the PopCol population-based cohort. Combined analyses of all data sets were run again with logistic regression adjusting for age, sex and origin. A forest plot of risk effects across CC cohorts was produced. The SNP risk effect concordance between IBD/CD/UC and CC were compared using SNP effect concordance analysis (SECA). We obtained IBD GWAS and Immunochip meta-analysis data from the IIBDGC (ibdgenetics.org) and used these as first input data-set. CC Immunochip summary results were used as the second input data-set. SECA then performed statistical tests to determine whether there is an excess of SNPs where the effect directions (OR) are concordant across *data set 1* and *data set 2*. Fisher's exact statistical tests were performed and a p-value was calculated for the observed number of clusters with significant concordance across data-sets.

3.4.4 Paper IV

The demographic data results are expressed as means, medians and ranges. The endoscopic and chromoendoscopic appearance results are expressed as categorical variables.

3.5 ETHICAL APPROVAL

The regional ethical committee at the Karolinska Institutet approved studies I, II and IV. Study III was approved by regional ethical committee at Karolinska Institutet, Stockholm; Linköping University Hospital, Linköping, Sweden; Skånes University Hospital, Malmö/Lund, Sweden; and Biobank Popgen & Ethik-Komission der Medizinischen Fakultät, Universitätsklinikum Schleswig-Holstein, Kiel.

4 RESULTS

4.1 PAPER I

4.1.1 Demographics

A total of 795 patients with MC patients were included, 43% (n=344) CC patients and 57% (n=451) LC patients. Of these MC patients, 76% (n=601) were females (F:M ratio 3.1:1), including 79% (n=271, F:M ratio 3.7:1) CC patients and 73% (n=330, F:M ratio 2.7:1) of LC patients. In CC, the mean age at diagnosis was 63.2 years (range 17–97 years): 62.5 years for females and 66.0 years for males (p=0.07). In LC, the mean age at diagnosis was 59.2 years (range 13–95 years): 59.8 years for females and 57.7 years for males (p=0.26). Sixteen per cent of MC (n=130) patients were diagnosed before the age of 45; 11% of CC patients (n=38) and 20% of LC patients (n=92). Smoking habits were recorded in 230 patients in CC patients, including 33% current smokers and 24% former smokers. Among the 304 LC patients who recorded smoking habits, 32% were current smokers and 20% were former smokers.

4.1.2 Clinical features

At onset, 94% of the MC patients had diarrhoea: information on stool frequency was available for 63% (n=472) of these patients.

CC: Diarrhoea (97%) with watery consistency (55%), weight loss (38%) with a mean of 7.2 kg, abdominal pain (28%), nocturnal diarrhoea (28%) and faecal urgency (23%), see Figure 1. Stool frequency (n=224): 53% had four to nine stools per day, 34% had ten or more stools per day and 13% had three or less stools per day.

LC: Diarrhoea (91%) with watery consistency (43%), weight loss (33%) with a mean of 5.9 kg, abdominal pain (30%), faecal urgency (21%) and nocturnal diarrhoea (18%), see Figure 1. Stool frequency (n=248): 52% had four to nine stools per day, 31% had ten or more stools per day and 16% had three or less stools per day.

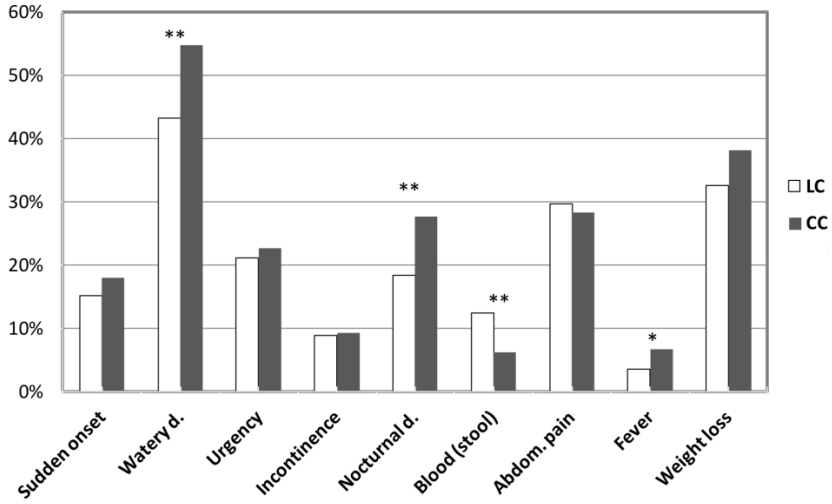

*Figure 1. Symptoms at diagnosis. *p-value <0.05, **p-value <0.01.*

4.1.3 Endoscopic findings

A complete colonoscopy was performed at diagnosis for 307 patients (89%) of CC patients and 403 patients (89%) in LC patients. At index colonoscopy, 37% of CC patients and 26% of LC patients showed subtle changes, such as erythema, oedema or abnormal vessel patterns. There were 14 adenomas found in 12 of the CC patients (3.5%) and 36 adenomas found in 30 of the LC patients (6.7%). None of the MC patients had colorectal cancer at the time of the index colonoscopy.

4.1.4 Coeliac disease and inflammatory bowel disease

Coeliac disease occurred in 6 % (n=48) and IBD occurred in 2.1% (n=17) of the MC patients.

CC: Coeliac disease was present in 17 patients (4.9%), including 15 females and two males. Twelve patients had a prior diagnosis, one at the same time and four after CC diagnosis. IBD was present in eight patients (2.3%), all diagnosed prior to CC. Three females and three males were previously diagnosed with UC and in CD, both patients with prior diagnoses were females.

LC: Coeliac disease was present in 31 patients (6.9%), including 20 females and 11 males. Twenty-two patients had a previous diagnosis, six at the same time and three after MC diagnosis. IBD was present in nine patients (2.0%), including seven diagnosed prior to LC diagnoses and two diagnosed after LC diagnoses, both had CD. Two females and two males were diagnosed with UC, and two females and three males were diagnosed with CD.

4.1.5 Collagenous colitis versus lymphocytic colitis

Significant differences were found between the two subtypes. The Age at diagnosis was significantly higher among CC patients (63 years) than LC patients (59 years) (p=0.005). Clinical symptoms were similar in both entities, but chronic diarrhoea was significantly more common in CC patients (97%) than LC patients (91%) (p=0.0012), as was watery diarrhoea (p=0.0014), nocturnal diarrhoea (p=0.002), and fever (p=0.042). Blood in the stool was significantly more common in LC patients (p=0.0029). Subtle mucosal findings at the index colonoscopy were significantly more frequent in CC patients (37%) than in LC patients (26%) (p=0.0011).

4.2 PAPER II

4.2.1 Clinical follow-up

Of the 795 MC patients, 108 had only a clinical index visit with no follow-up during the study period (63 LC patients and 45 CC patients). Eighty-six per cent (n=687) of the patients had at least one follow-up visit during the study period, including 86% (n=388) of LC patients and 87% (n=299) of CC patients. The mean time from diagnosis to last follow-up was 2.89 (0.01–23.87) years: 2.51 (0.01–18.65) years for LC patients and 3.39 (0.03–23.87) years for CC patients. Sixty-four per cent (n=441) were in clinical remission at last follow-up, including 66% (n=257) of LC and 62% (n=184) of CC patients (NS). The significant

difference between LC and CC in course over time is illustrated by the Kaplan-Meier survival curves (p=0.0013) in Figure 2. For seven patients there was no information about the clinical status at follow-up.

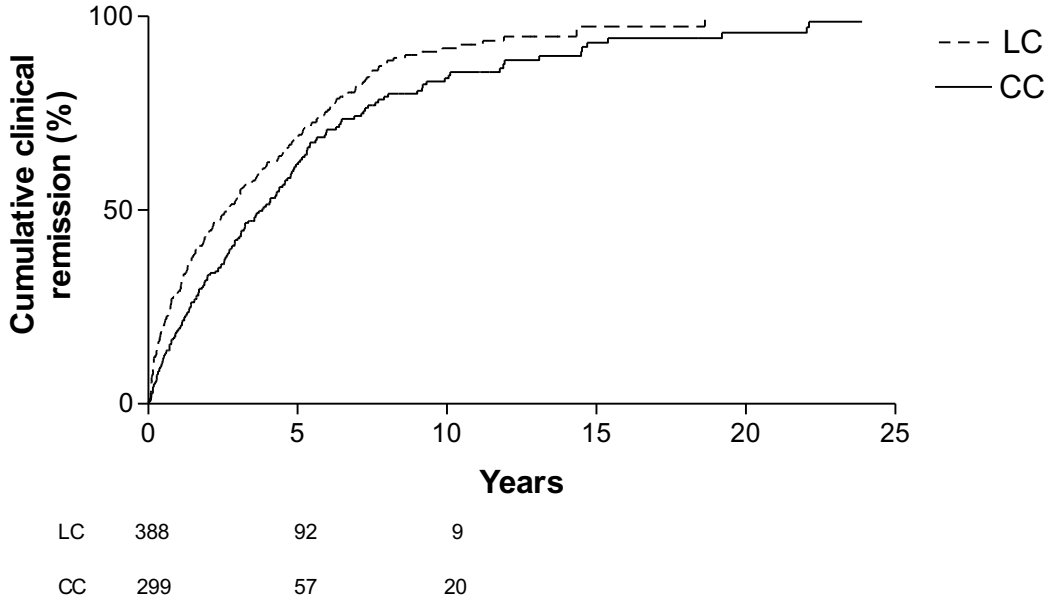

Figure 2. Kaplan-Meier curves show the difference in clinical remission between CC and LC patients at last of follow-up, p=0.0013.

4.2.2 Medication use

Overall, 49 % (n=336) were on medical treatment for MC at last clinical follow-up (45% for LC (176/388) and 54% (160/299) for CC (p=0.0377)). Steroid use at follow-up was recorded for 24% (164/687) of MC patients, including 21% (81/388) of LC and 28% (83/299) of CC patients (p=0.0382). Other treatments such as anti-diarrhoeal agents and cholestyramine were used by 9% and 8 % respectively. Of those in remission, 47% (208/441) were on medication for MC, compared with 54% (128/239) of those who were not in clinical remission (NS).

4.2.3 Endoscopic follow-up

A total of 187 MC patients (100 LC, 87 CC) had at least one follow-up colonoscopy. In LC patients, 22% (100/451) had a follow-up colonoscopy, compared to 25% (87/344) CC patients. The mean time from index colonoscopy to first follow-up colonoscopy was 3.33 (0.02–15.2) years: 3.17 (0.02–15.2) years for LC patients and 3.53 (0.08–13.1) years for CC patients. Endoscopic follow-up of those in clinical remission (n=52) was indicated by histological evaluation (n=17), unspecific gastrointestinal symptoms (constipation, rectal bleeding and abdominal pain) (n=13), polyp surveillance (n=3) and unknown (n=16). There were no colorectal cancer incidents reported at follow-up, though adenomas were found in 5% (n=10). Subtle mucosal abnormalities, such as edema, erythema or abnormal vessel patterns were described in 30% (n=56) of MC patients, including 27% (n=27) of LC and 33%

(n=29) of CC patient colonoscopies. Thirty-nine per cent (n=22) of these patients, including 48% (n=13) of LC and 31% (n=9) of CC patients, were in histological remission.

Histological remission was recorded at the follow-up colonoscopy for 44% (82/187) of MC patients, including 52% (n=52) of LC and 34% of CC patients (n=30) (p=0.018). The cumulative LC and CC histological rates are illustrated in Figure 3 (p=0.0098).

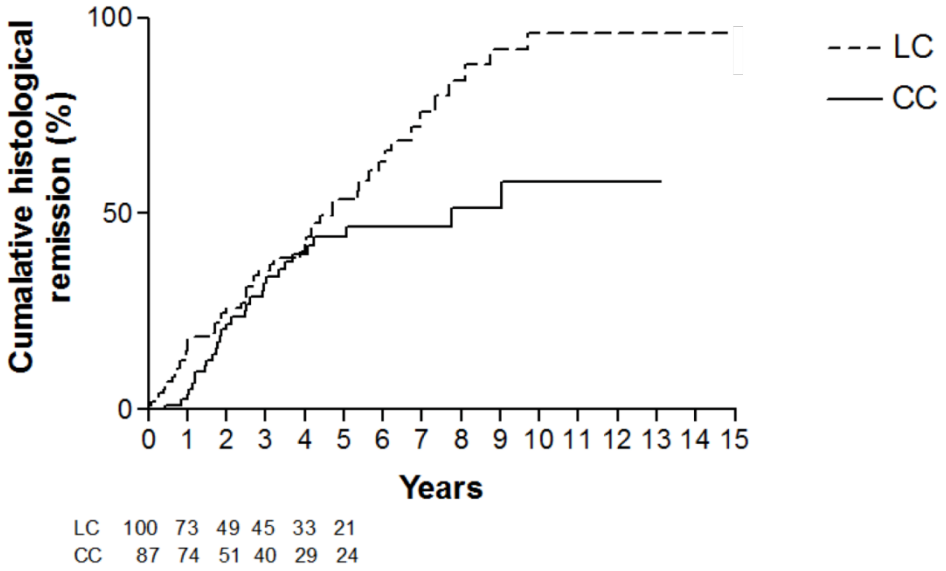

Figure 3. Kaplan-Meier curves show the differences in histological remission between CC and LC at the follow-up colonoscopy, p=0.0013.

Although a substantial number of patients (n=23) in histological remission were not in clinical remission, the association between histological and clinical remission was high (p=0.0001). Patients in histological and clinical remission were significantly more steroid-free compared to those only in clinical remission (p=0.0311). Ten per cent (n=19) of the MC patients changed histological phenotype between index colonoscopy and first endoscopic follow-up, including 11 LC to CC and 8 CC to LC cases. Out of these 19 patients, 12 were not in clinical remission, two were in clinical remission and five had unknown clinical status. Furthermore, one patient experienced a change histological phenotype from LC to UC and one had LC and Crohn's ileitis concomitantly. A total of 44 patients underwent a second follow-up colonoscopy, and four more patients with a changed histological phenotype were recorded, including two LC to CC and two CC to LC cases: none of these had changed phenotype at the first follow-up colonoscopy. Thus, in total 12% (23/187) experienced a histological change of MC phenotype during follow-up.

4.3 PAPER III

We genotyped 254 patients with CC from Sweden (n=163) and Germany (n=91), and compared their genotypes with Immunochip data from 2046 healthy Swedish controls from the EIRA study and 2018 healthy German controls from the PopGen cohort.

After quality control of genotype data, 110 634 Immunochip SNP markers and 4216 individuals were included in the association tests performed with logistic regression adjusting for age, sex and origin. The analysis resulted in the identification of 188 markers with association $p<1\times10^{-5}$, which were all but one contained within the major histocompatibility complex (MHC) region on chromosome 6, see Figure 4. Forty-two markers gave rise to significant genome-wide ($p<5\times10^{-8}$) association signals, all contained within the HLA region on chromosome 6 (best $p=4.2\times10^{-10}$ for SNP rs4143332), see Figure 4.

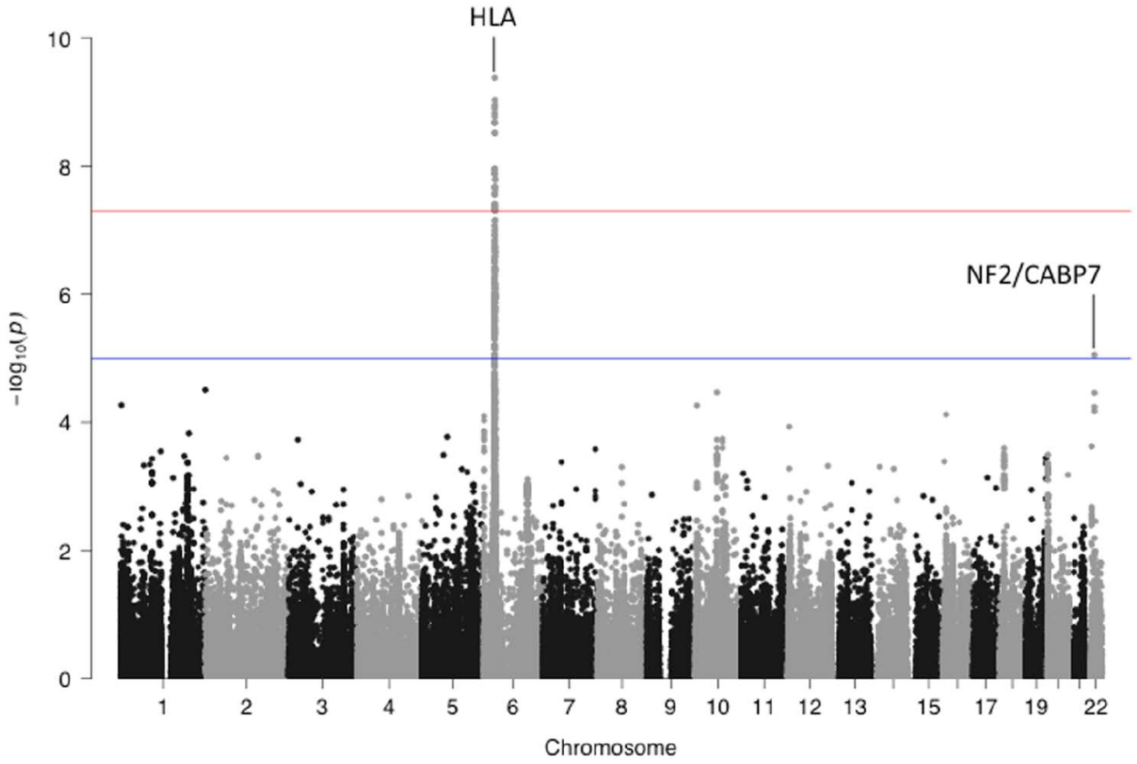

Figure 4. Manhattan plot showing –log 10 p values of association for all Immunochip single nucleotide polymorphism markers passing quality control and manual cluster inspection. Genome-wide and suggestive significance levels are indicated with red and blue lines, respectively. HLA, human leucocyte antigen.

There were significant differences in age and sex distribution between cases and controls, but after two additional tests, a strong MHC association signal was again detected in both analyses. We imputed classical HLA alleles and conducted an association test; a multiple HLA variants showed a significant association with diverse effects on CC risk. The strongest signals were detected for six HLA alleles associated with increased risk for CC which are all known to be part of the 8.1 ancestral haplotype (145). We could further confirm the association signal to be dependent on the 8.1 haplotype. The DQ2.5 molecule is carried by the 8.1 haplotype and this molecule is also found in the majority of patients with coeliac disease (146). DQ2.5 was targeted and confirmed in the replication data-set showing a significant association with strong effect on CC risk ($p=2.3\times10^{-11}$; OR=2.06; 95% CI (1.67 to 2.55) in the combined analysis).

We inspected CC Immunochip association signals in correspondence with DNA sequence variations known to impact IBD risk (>25 000 IBD risk SNPs). Concordant risk effects were significantly enriched in all three disease comparisons, namely IBD versus CC ($p<0.001$), CD versus CC ($p<0.001$) and UC versus CC ($p<0.01$), thus suggesting genetic overlap between CC and IBD at immune-related risk loci. We also looked at known non-HLA IBD risk loci (n=162), which could be done for 99 regions. Eight out these 99 loci showed associations with nominal significance in CC patients.

4.4 PAPER IV

Thirteen patients with chronic diarrhoea and abnormal chromoendoscopic findings were identified, 10 women and three men with mean age of 64.9 years (median 62, range 35-90) and mean duration of symptoms of 53.9 months (range 1-156). Ten had CC and three had LC. Endoscopic findings without indigo carmine staining were patchy and were seen in 10 of the thirteen cases. Endoscopic findings included diminished vascular patterns, increased light reflection, and increased vascular patterns. Continuous mucosal abnormalities were found at colonoscopy after indigo carmine staining in all thirteen cases, namely the disappearance of innominate grooves or presence of innominate grooves with a nodular surface. A swollen mosaic pattern was seen in five CC cases and in all three LC cases. An uneven surface (a rough surface without apparent nodules) was seen in four CC cases, one CC case in remission and one of LC case. A nodular surface (nodules of 1−3 mm) was recorded in five CC cases, but in no LC cases.

In one CC patient, chromoendoscopy showed an uneven surface; however, histopathological examination showed normal mucosa.

In another CC patient, chromoendoscopy showed edematous mucosa with mosaic changes in the sigmoid colon, but no pathologic findings were recorded in that region.

A third CC patient had a normal rectal mucosa appearance at chromoendoscopy but microscopic assessment showed a thick subepithelial collagenous band.

5 DISCUSSION

Study I and II are descriptive studies of all patients diagnosed with MC from two hospitals, spanning a period of nearly 30 years. We used the same histological criteria used in earlier, well-known Swedish MC studies from Örebro (28, 30) so that we could compare our results. The histopathological criteria of MC were recently reviewed by an elaboration of the European Crohn's and Colitis Organisation (ECCO) and the European Society of Pathology (ESP) (98), and the histological criteria we used are almost identical.

We validated the histological diagnosis of MC early in the process. Since the study population was so large, we randomly selected 50 cases, of the first 198 MC cases gathered, for histological reassessment. The accuracy was high at 92%, and therefore we refrained from re-examining the whole study population.

The strengths of these two studies are the large number of patients collected during 30 years and the geographic-restricted catchment area. The studies are retrospective and there was a large amount of missing data which we interpreted as no findings. This strategy may have underestimated the true frequency of, for example, symptoms and current medications presented at follow-up. The lack of information on prior colonoscopies may underestimated the true frequency of adenomas since polypectomies might have been performed before entering the study. Moreover, the therapy strategy for MC has changed during the study period in favour of Budesonide which could have an impact both on clinical and histological remission rates at follow-up. A number of patients (14%) did not have any clinical follow-up within the study period. This was due to several factors, such as follow-up contact first after the defined study period or geographic migration and may lead to an underestimation of the true frequency of clinical remission. There may also be a selection bias regarding the follow-up colonoscopies, since not all patients did an endoscopic control, and patients who submitted to colonoscopy might have had more persistent symptoms.

5.1 CLINICAL AND ENDOSCOPIC FEATURES

5.1.1 Demographics

MC is often seen in elderly patients and the mean age of diagnosis of MC (61 years) was similar to earlier reports which also showed no significant differences between the subtypes (11, 147, 148). However, as other Swedish studies have reported (22, 30, 149), we could show significant differences with lower mean ages in LC patients (59 years) than CC patients (64 years) at diagnosis. This supports the theory that LC may be an early stage of CC (150, 151). Old age is a risk factor for developing MC (16, 33, 152), but 16% of the patients in our study were <45 years of age at the time of diagnosis and in a previous study on CC this figure was 25% (28). MC has even found among children (24). Another risk factor for developing MC is smoking (62, 63) and the majority of our patients were smokers (54%), in accordance with pooled clinical studies analyses (33).

5.1.2 Symptoms

One of the aims was to characterise the clinical features of MC, and therefore only histopathological criteria were used. This allowed us to analyse the whole spectrum of clinical symptoms in cases with histologically proven MC. In our study, 4%, including a majority of LC patients, had no diarrhoea, which is same proportion reported in a Swedish study of 199 LC patients (30). In another Swedish study, 10% of the patients with histological features of MC – a majority of them CC patients – did not report chronic watery diarrhoea (153); other studies have reported similar findings (154, 155).

In our study, the main MC symptom was watery diarrhoea with a stool frequency of four to nine per day, often associated with abdominal pain, nocturnal diarrhoea, urgency and weight-loss, which is already described in earlier studies (60, 156). We confirmed that the symptoms of LC and CC are indistinguishable, as found in several previous studies (33, 89). However, we showed that the frequency of symptoms differed between the subtypes. There were small but significantly higher occurrences of chronic diarrhoea, watery diarrhoea and nocturnal diarrhoea in CC patients than in LC patients; this has been described in few previous studies as well (90, 91, 127).

5.1.3 Associated diseases

Autoimmune diseases are associated with MC in up to 50% of the patients (37). The overall prevalence of coeliac disease in Sweden is 3% (157), and 3–23% in the MC population (15, 158, 159). A large population study showed a strong association between MC and coeliac disease with disease concomitance being 50 times higher than expected in general population (15). In our study, we reported the prevalence of coeliac disease of 6%, higher in LC (6.9%) than in CC (4.9%) (NS). These figures fit well with the pooled results of 14 studies showing coeliac disease in 7% of LC and 5% of CC patients (33). There have been reports of MC progressing to IBD and vice versa (90, 160-164) and 2.1% of our MC patients had UC or CD. This is in the same range found in a previous Swedish study, 2.5% (28). At the same time, a recent large study concluded that there is no increased risk of developing IBD, only 2 of 547 cases established the diagnosis of UC or CD prior to the diagnosis of MC (165). In our study, the majority had established the IBD diagnosis prior to the MC diagnoses, and this may complicate the histological distinction between these diagnoses. MC biopsies can contain neutrophils with active cryptitis (41), although acute inflammation should not dominate the inflammatory infiltrate.

5.1.4 Endoscopic findings

Despite the name microscopic colitis, the colonic mucosa is not always normal. Subtle or nonspecific mucosal changes such as oedema, erythema, abnormal vessel patterns, nodular surface, linear tears and scratches, have been observed (101, 166, 167).

Subtle macroscopic mucosal changes in the colon have been described in other Swedish studies (28, 30), and such findings were seen in 37% of CC and 26% of LC patients;

however, we found a significant difference between the subtypes. Our study also reports low rates of neoplastic lesions at the index colonoscopy, despite the patients' high age, and this is significantly lower than in the previous population based on Forsberg et al.'s endoscopic study (139). This is supported by two studies showing that MC was negatively associated with the risk of colorectal adenomas, and in one study even including earlier colonoscopies (133, 134). In our study, we lack information on earlier colonoscopies and on NSAIDs/aspirin use which can prevent colorectal neoplasia (168).

5.1.5 Clinical and endoscopic follow-up

The long-term prognosis of MC is generally good, but most studies conducted to-date examine small sample sizes or are only limited in sample follow-ups.

In our data, 64% of the MC patients were in clinical remission at the last clinical follow-up at a mean time of 2.9 years after diagnosis. Two studies with much smaller sample sizes but a mean follow-up time of 6–9 years showed that only a minority of MC patients have symptoms at follow-up (125, 126), and other studies showed remission rates 48-70% (91, 93, 137, 169, 170). We could also show that the histological remission rate was 44% at the first follow-up colonoscopy and the histological remission rate might even be higher in our study since most asymptomatic patients did not undergo a follow-up colonoscopy. Comparative histological follow-up studies in MC are scarce (110, 111).

Our data, which studied the two MC subtypes in the same large, well-defined urban cohort, found that clinical and histological remission over time were better in LC patients who needed less treatment – specifically, lower steroid use – than CC patients. LC seems to be a milder disease with better prognosis than CC, as has been concluded in a few studies (113, 127). With regard to LC, one study showed that 80% of patients had no diarrhoea and showed normalised mucosal histology follow-up at a mean of three years (128), while another showed 73% histological remission already after six weeks in LC cases (113). One study showed that 63% of LC patients described the course of LC as one single outbreak (30), compared to 2% in CC cases (28). In case of CC, clinical studies showed relapse in 30% of patients, while prospective, placebo-controlled trials reported an 80% relapse rate (91, 117).

Changes in histological phenotype over time were not uncommon. According to our data, 12% changed subtype during follow-up. Four previous Swedish studies reported a total of 23 conversion cases between the subtypes, with a majority (n=19) shifting from LC to CC (30, 35, 93, 171). If this is true conversions between subtypes or rather reflects diagnostic difficulties or natural fluctuations of same disease is controversial.

5.1.6 Genetics

The aetiology of MC is still unknown, but the high frequency of autoimmune diseases, the high frequency among women, familial occurrence and the response to corticosteroid therapy support an autoimmune origin. Earlier genetic studies, though are sparse, small and underpowered, have shown an association between HLA-DQ and MC, as well as an

association between MC and TNFα, metalloproteinase-9, IL-6 and serotonin transporter promoter genes polymorphisms (47, 48, 50-53).

No large-scale genetic study of MC has been done before to determine whether genetics have a role in MC and, if so, identify the genetic variants associated with MC. We genotyped around 300 CC patients and 42 markers gave significant association signals, all in the HLA region on chromosome 6. This supports the idea of a genetic role in CC and a HLA related immune mechanism may be involved in CC pathogenesis. The small sample size is a major limitation in our study, limiting the statistical power and ability to detect other associations. However, Roda et al. recently conducted a study in the United States, which was in parallel to us and which appeared only in abstract form. The study examined a discovery cohort of similar size (N=295), used the same methodology (Immunochip) (172), and reported findings similar to ours. Roda et al. that same year, conducted a genome-wide association study (GWAS) of 450 CC patients and the preliminary results showed that 10 loci were significantly associated with CC, and that the most significant association signal was near HLA-DQ (173).

MC and coeliac disease are associated. The strongest genetic risk factor for coeliac disease is linked to the HLA-gene encoding HLA-DQ2 (146). In our study, in which patients with history of diagnosis of coeliac disease were excluded and the majority of the study population were screened for coeliac disease, we confirmed the association between CC and DQ2.5. The results suggest that they have a genetic overlap and similar pathogenesis mechanisms.

A similar genetic study using Immunochip in LC patients did not show any association with the HLA genotype (174). However, the study cohort was small, and it is known in general that LC is a heterogeneous disease where drugs, gastrointestinal infections or another gastrointestinal disease can cause lymphocyte infiltration in colonic mucosa epithelium (175). A small study also shows an increase of HLA A1 in LC patients (49). Still, this suggests that the LC and CC may have different pathogeneses and thus may be two different diseases, which aligns with our results and other studies showing that LC has a more benign clinical presentation and a better prognosis, and that concomitant autoimmune disease is less common in LC patients than CC patients (30, 91, 127). However, as said before, this remains controversial.

Conversions between MC and IBD have been observed in our descriptive studies (I and II), as well as in other studies (61, 171). We studied CC Immunochip associations signals at >25 000 IBD risk SNPs (176) and found concordant risk suggesting a genetic overlap between CC and IBD, which is supported by the findings of the genome-wide association study of CC (173).

5.1.7 Endoscopic diagnosis

Grooves deep enough to retain barium and give a radiographic pattern were found in the surface of the normal colon. These innominate grooves of colonic mucosa were first

described radiologically by using the air double-contrast method (177). Later, they were seen during colonoscopy, though only by using the dye spray method (144). Only two MC case reports described their appearance at colonoscopy with indigo carmine, showing uneven surface texture in CC patients (108, 109). A retrospective study of colonoscopies without chromoendoscopy in patients with diarrhoea reported finding mosaic patterns in MC patients (178). Our study was a case series where indigo carmine staining was used at colonoscopy as a diagnostic tool for MC in 13 patients. We reported subtle mucosal abnormalities shown in patients with MC using chromoendoscopy. We recognised that a mosaic mucosal pattern was seen in all LC patients and in half of CC patients, and a nodular mucosal pattern was observed only in CC patients, and uneven surface in both subtypes; however, these appearances seem to overlap with one another.

In addition, our study showed that chromoendoscopy revealed continuous mucosal changes in 13 MC cases. The continuous changes were also seen in the histopathology. This contrast previous studies that have reported patchy histological distribution (179, 180). In three CC patients, the correlation between the chromoendoscopy findings and the histopathology in the rectosigmoid was poor and may illustrate a limitation of this method in the left colon. Moreover, inflammatory changes of CC cases are less apparent in the left colon (179). However, studies on biopsies taken from the rectosigmoid showed that the diagnosis of MC could be confirmed in as much as 70–95% of cases (90, 181, 182). The data conflicts, and until further evidence is available, colonic biopsies from both right and left colon should remain the golden standard (183).

Only 3 of 13 MC patients had no mucosal abnormalities detected by white light endoscopy. This contrast the common belief, but these changes were considered as minor and are often overlooked in routine endoscopic examinations. Endoscopist bias must also be considered, as from the onset, the endoscopists knew that the majority of cases had previously been diagnosed with MC.

6 CONCLUSIONS

- LC and CC are similar but not identical.
- LC tends to occur earlier in life, and it has a milder clinical presentation and a better prognosis.
- Colonic mucosa in MC is not always normal, despite the name ‘microscopic’.
- There is a strong association between MC and coeliac disease.
- Conversions between MC and UC/CD and between LC and CC over time are not uncommon.
- Specific HLA alleles are associated with CC, which indicates an autoimmunity.
- Chromoendoscopy may improve detection of macroscopic lesions in MC patients and could help select patients for biopsy, as well as direct the biopsies.
- LC and CC might be two different entities, but further large prospective studies are needed to clarify the clinical and histological courses of the two subtypes. Further GWAS-based MC studies are warranted to detect genetic differences between the subtypes.

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8 REFERENCES

1. Lindström CG. 'Collagenous colitis' with watery diarrhoea--a new entity? Pathologia Europaea. 1976;11(1):87-9.

2. Freeman H, Weinstein W, Shnitka T, Wensel R, Sartor V. Watery diarrhea syndrome associated with a lesion of the colonic basement membrane-lamina propria interface. Ann R Coll Phys Surg Can. 1976;9:45.

3. Lazenby AJ, Yardley JH, Giardiello FM, Jessurun J, Bayless TM. Lymphocytic ("microscopic") colitis: a comparative histopathologic study with particular reference to collagenous colitis. Human pathology. 1989;20(1):18-28.

4. Flejou JF, Bogomoletz WV. [Microscopic colitis: collagenous colitis and lymphocytic colitis. A single concept?]. Gastroenterologie clinique et biologique. 1993;17(2 Pt 3):T28-32.

5. Levison DA, Lazenby AJ, Yardley JH. Microscopic colitis cases revisited. Gastroenterology. 1993;105(5):1594-6.

6. Gu HX, Zhi FC, Huang Y, Li AM, Bai Y, Jiang B, et al. Microscopic colitis in patients with chronic diarrhea and normal colonoscopic findings in Southern China. International journal of colorectal disease. 2012;27(9):1167-73.

7. Pardi DS, Loftus EV, Jr., Smyrk TC, Kammer PP, Tremaine WJ, Schleck CD, et al. The epidemiology of microscopic colitis: a population based study in Olmsted County, Minnesota. Gut. 2007;56(4):504-8.

8. Rubio-Tapia A, Martinez-Salgado J, Garcia-Leiva J, Martinez-Benitez B, Uribe M. Microscopic colitides: a single center experience in Mexico. International journal of colorectal disease. 2007;22(9):1031-6.

9. Garg PK, Singh J, Dhali GK, Mathur M, Sharma MP. Microscopic colitis is a cause of large bowel diarrhea in Northern India. Journal of clinical gastroenterology. 1996;22(1):11-5.

10. Agnarsdottir M, Gunnlaugsson O, Orvar KB, Cariglia N, Birgisson S, Bjornsson S, et al. Collagenous and lymphocytic colitis in Iceland. Digestive diseases and sciences. 2002;47(5):1122-8.

11. Williams JJ, Kaplan GG, Makhija S, Urbanski SJ, Dupre M, Panaccione R, et al. Microscopic colitis-defining incidence rates and risk factors: a population-based study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2008;6(1):35-40.

12. Fernandez-Banares F, Salas A, Esteve M, Pardo L, Casalots J, Forne M, et al. Evolution of the incidence of collagenous colitis and lymphocytic colitis in Terrassa, Spain: a population-based study. Inflamm Bowel Dis. 2011;17(4):1015-20.

13. Simondi D, Pellicano R, Reggiani S, Pallavicino F, David E, Sguazzini C, et al. A retrospective study on a cohort of patients with lymphocytic colitis. Revista espanola de enfermedades digestivas : organo oficial de la Sociedad Espanola de Patologia Digestiva. 2010;102(6):381-4.

14. Gentile NM, Khanna S, Loftus EV, Jr., Smyrk TC, Tremaine WJ, Harmsen WS, et al. The epidemiology of microscopic colitis in Olmsted County from 2002 to 2010: a population-based study. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2014;12(5):838-42.

15. Stewart M, Andrews CN, Urbanski S, Beck PL, Storr M. The association of coeliac disease and microscopic colitis: a large population-based study. Aliment Pharmacol Ther. 2011;33(12):1340-9.

16. Wickbom A, Bohr J, Eriksson S, Udumyan R, Nyhlin N, Tysk C. Stable incidence of collagenous colitis and lymphocytic colitis in Orebro, Sweden, 1999-2008: a continuous epidemiologic study. Inflamm Bowel Dis. 2013;19(11):2387-93.

17. Tong J, Zheng Q, Zhang C, Lo R, Shen J, Ran Z. Incidence, prevalence, and temporal trends of microscopic colitis: a systematic review and meta-analysis. Am J Gastroenterol. 2015;110(2):265-76; quiz 77.

18. Bonderup OK, Wigh T, Nielsen GL, Pedersen L, Fenger-Gron M. The epidemiology of microscopic colitis: a 10-year pathology-based nationwide Danish cohort study. Scandinavian journal of gastroenterology. 2015;50(4):393-8.

19. Masclee GM, Coloma PM, Kuipers EJ, Sturkenboom MC. Incidence of microscopic colitis in relation to the number of colonoscopies over time. Am J Gastroenterol. 2015;110(8):1246-7.

20. Stewart MJ, Seow CH, Storr MA. Prednisolone and budesonide for short- and long-term treatment of microscopic colitis: systematic review and meta-analysis. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2011;9(10):881-90.

21. Thomas PD, Forbes A, Green J, Howdle P, Long R, Playford R, et al. Guidelines for the investigation of chronic diarrhoea, 2nd edition. Gut. 2003;52 Suppl 5:v1-15.

22. Olesen M, Eriksson S, Bohr J, Järnerot G, Tysk C. Microscopic colitis: a common diarrhoeal disease. An epidemiological study in Örebro, Sweden, 1993-1998. Gut. 2004;53(3):346-50.

23. Guagnozzi D, Lucendo AJ, Angueira-Lapena T, Gonzalez-Castillo S, Tenias Burillo JM. Prevalence and incidence of microscopic colitis in patients with diarrhoea of unknown aetiology in a region in central Spain. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2012;44(5):384-8.

24. El-Matary W, Girgis S, Huynh H, Turner J, Diederichs B. Microscopic colitis in children. Digestive diseases and sciences. 2010;55(7):1996-2001.

25. Gremse DA, Boudreaux CW, Manci EA. Collagenous colitis in children. Gastroenterology. 1993;104(3):906-9.

26. Vanderhoof JA, Goble K, Young RJ. Collagenous colitis in a 4-year-old child: response to budesonide. Journal of pediatric gastroenterology and nutrition. 2010;50(6):688-90.

27. Liu X, Xiao SY, Plesec TP, Jiang W, Goldblum JR, Lazenby AJ. Collagenous colitis in children and adolescents: study of 7 cases and literature review. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 2013;26(6):881-7.

28. Bohr J, Tysk C, Eriksson S, Abrahamsson H, Järnerot G. Collagenous colitis: a retrospective study of clinical presentation and treatment in 163 patients. Gut. 1996;39(6):846-51.

29. Fernandez-Banares F, Casanova MJ, Arguedas Y, Beltran B, Busquets D, Fernandez JM, et al. Current concepts on microscopic colitis: evidence-based statements and recommendations of the Spanish Microscopic Colitis Group. Aliment Pharmacol Ther. 2016;43(3):400-26.

30. Olesen M, Eriksson S, Bohr J, Järnerot G, Tysk C. Lymphocytic colitis: a retrospective clinical study of 199 Swedish patients. Gut. 2004;53(4):536-41.

31. Järnerot G, Tysk C, Bohr J, Eriksson S. Collagenous colitis and fecal stream diversion. Gastroenterology. 1995;109(2):449-55.

32. Fernandez-Banares F, Gisbert JP. Letter: are lymphocytic colitis and collagenous colitis really the same disease? Aliment Pharmacol Ther. 2012;36(6):606.

33. Rasmussen MA, Munck LK. Systematic review: are lymphocytic colitis and collagenous colitis two subtypes of the same disease - microscopic colitis? Aliment Pharmacol Ther. 2012;36(2):79-90.

34. Veress B, Löfberg R, Bergman L. Microscopic colitis syndrome. Gut. 1995;36(6):880-6.

35. Vigren L, Olesen M, Benoni C, Sjöberg K. Are collagenous and lymphocytic colitis different aspects of the same disease? Scandinavian journal of gastroenterology. 2012;47(12):1448-53.

36. Nyhlin N, Bohr J, Eriksson S, Tysk C. Systematic review: microscopic colitis. Aliment Pharmacol Ther. 2006;23(11):1525-34.

37. Bohr J, Wickbom A, Hegedus A, Nyhlin N, Hultgren Hörnquist E, Tysk C. Diagnosis and management of microscopic colitis: current perspectives. Clinical and experimental gastroenterology. 2014;7:273-84.

38. Green PH, Yang J, Cheng J, Lee AR, Harper JW, Bhagat G. An association between microscopic colitis and celiac disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2009;7(11):1210-6.

39. Aqel B, Bishop M, Krishna M, Cangemi J. Collagenous colitis evolving into ulcerative colitis: a case report and review of the literature. Digestive diseases and sciences. 2003;48(12):2323-7.

40. Pokorny CS, Kneale KL, Henderson CJ. Progression of collagenous colitis to ulcerative colitis. Journal of clinical gastroenterology. 2001;32(5):435-8.

41. Ayata G, Ithamukkala S, Sapp H, Shaz BH, Brien TP, Wang HH, et al. Prevalence and significance of inflammatory bowel disease-like morphologic features in collagenous and lymphocytic colitis. The American journal of surgical pathology. 2002;26(11):1414-23.

42. Abdo AA, Zetler PJ, Halparin LS. Familial microscopic colitis. Canadian journal of gastroenterology = Journal canadien de gastroenterologie. 2001;15(5):341-3.

43. van Tilburg AJ, Lam HG, Seldenrijk CA, Stel HV, Blok P, Dekker W, et al. Familial occurrence of collagenous colitis. A report of two families. Journal of clinical gastroenterology. 1990;12(3):279-85.

44. Järnerot G, Hertervig E, Granno C, Thorhallsson E, Eriksson S, Tysk C, et al. Familial occurrence of microscopic colitis: a report on five families. Scandinavian journal of gastroenterology. 2001;36(9):959-62.

45. Kong SC, Keogh S, Carter MJ, Lobo AJ, Sanders DS. Familial occurrence of microscopic colitis: an opportunity to study the relationship between microscopic colitis and coeliac disease? Scandinavian journal of gastroenterology. 2002;37(11):1344-5.

46. Phull PS, Vijayan B, Bisset WM, Murray GI. Familial collagenous colitis involving a 6-year old child. Journal of Crohn's & colitis. 2012;6(5):606-9.

47. Koskela RM, Karttunen TJ, Niemela SE, Lehtola JK, Bloigu RS, Karttunen RA. Cytokine gene polymorphism in microscopic colitis association with the IL-6-174 GG genotype. Eur J Gastroenterol Hepatol. 2011;23(7):607-13.

48. Koskela RM, Karttunen TJ, Niemela SE, Lehtola JK, Ilonen J, Karttunen RA. Human leucocyte antigen and TNFalpha polymorphism association in microscopic colitis. Eur J Gastroenterol Hepatol. 2008;20(4):276-82.

49. Giardiello FM, Lazenby AJ, Yardley JH, Bias WB, Johnson J, Alianiello RG, et al. Increased HLA A1 and diminished HLA A3 in lymphocytic colitis compared to controls and patients with collagenous colitis. Digestive diseases and sciences. 1992;37(4):496-9.

50. Fernandez-Banares F, Esteve M, Farre C, Salas A, Alsina M, Casalots J, et al. Predisposing HLA-DQ2 and HLA-DQ8 haplotypes of coeliac disease and associated enteropathy in microscopic colitis. Eur J Gastroenterol Hepatol. 2005;17(12):1333-8.

51. Madisch A, Hellmig S, Schreiber S, Bethke B, Stolte M, Miehlke S. Allelic variation of the matrix metalloproteinase-9 gene is associated with collagenous colitis. Inflamm Bowel Dis. 2011;17(11):2295-8.

52. Sikander A, Sinha SK, Prasad KK, Rana SV. Association of Serotonin Transporter Promoter Polymorphism (5-HTTLPR) with Microscopic Colitis and Ulcerative Colitis. Digestive diseases and sciences. 2015;60(4):887-94.

53. Fine KD, Do K, Schulte K, Ogunji F, Guerra R, Osowski L, et al. High prevalence of celiac sprue-like HLA-DQ genes and enteropathy in patients with the microscopic colitis syndrome. Am J Gastroenterol. 2000;95(8):1974-82.

54. Beaugerie L, Pardi DS. Review article: drug-induced microscopic colitis - proposal for a scoring system and review of the literature. Aliment Pharmacol Ther. 2005;22(4):277-84.

55. Fernandez-Banares F, Esteve M, Espinos JC, Rosinach M, Forne M, Salas A, et al. Drug consumption and the risk of microscopic colitis. Am J Gastroenterol. 2007;102(2):324-30.

56. Keszthelyi D, Penders J, Masclee AA, Pierik M. Is microscopic colitis a drug-induced disease? Journal of clinical gastroenterology. 2012;46(10):811-22.

57. Verhaegh BP, de Vries F, Masclee AA, Keshavarzian A, de Boer A, Souverein PC, et al. High risk of drug-induced microscopic colitis with concomitant use of NSAIDs and proton pump inhibitors. Aliment Pharmacol Ther. 2016;43(9):1004-13.

58. Chassany O, Michaux A, Bergmann JF. Drug-induced diarrhoea. Drug safety. 2000;22(1):53-72.

59. Bonderup OK, Fenger-Gron M, Wigh T, Pedersen L, Nielsen GL. Drug exposure and risk of microscopic colitis: a nationwide Danish case-control study with 5751 cases. Inflamm Bowel Dis. 2014;20(10):1702-7.

60. Munch A, Aust D, Bohr J, Bonderup O, Fernandez Banares F, Hjortswang H, et al. Microscopic colitis: Current status, present and future challenges: statements of the European Microscopic Colitis Group. Journal of Crohn's & colitis. 2012;6(9):932-45.

61. Vigren L, Sjöberg K, Benoni C, Tysk C, Bohr J, Kilander A, et al. Is smoking a risk factor for collagenous colitis? Scandinavian journal of gastroenterology. 2011;46(11):1334-9.

62. Fernandez-Banares F, de Sousa MR, Salas A, Beltran B, Piqueras M, Iglesias E, et al. Impact of current smoking on the clinical course of microscopic colitis. Inflamm Bowel Dis. 2013;19(7):1470-6.

63. Yen EF, Pokhrel B, Du H, Nwe S, Bianchi L, Witt B, et al. Current and past cigarette smoking significantly increase risk for microscopic colitis. Inflamm Bowel Dis. 2012;18(10):1835-41.

64. Ung KA, Gillberg R, Kilander A, Abrahamsson H. Role of bile acids and bile acid binding agents in patients with collagenous colitis. Gut. 2000;46(2):170-5.

65. Fernandez-Banares F, Esteve M, Salas A, Forne TM, Espinos JC, Martin-Comin J, et al. Bile acid malabsorption in microscopic colitis and in previously unexplained functional chronic diarrhea. Digestive diseases and sciences. 2001;46(10):2231-8.

66. Einarsson K, Eusufzai S, Johansson U, Lofberg R, Theodorsson E, Veress B. Villous atrophy of distal ileum and lymphocytic colitis in a woman with bile acid malabsorption. Eur J Gastroenterol Hepatol. 1992;4(7):585-90.

67. Chadwick VS, Gaginella TS, Carlson GL, Debongnie JC, Phillips SF, Hofmann AF. Effect of molecular structure on bile acid-induced alterations in absorptive function, permeability, and morphology in the perfused rabbit colon. The Journal of laboratory and clinical medicine. 1979;94(5):661-74.

68. Tremaine WJ. Collagenous colitis and lymphocytic colitis. Journal of clinical gastroenterology. 2000;30(3):245-9.

69. Ung KA, Kilander A, Nilsson O, Abrahamsson H. Long-term course in collagenous colitis and the impact of bile acid malabsorption and bile acid sequestrants on histopathology and clinical features. Scandinavian journal of gastroenterology. 2001;36(6):601-9.

70. Erim T, Alazmi WM, O'Loughlin CJ, Barkin JS. Collagenous colitis associated with Clostridium difficile: a cause effect? Digestive diseases and sciences. 2003;48(7):1374-5.

71. Makinen M, Niemela S, Lehtola J, Karttunen TJ. Collagenous colitis and Yersinia enterocolitica infection. Digestive diseases and sciences. 1998;43(6):1341-6.

72. Perk G, Ackerman Z, Cohen P, Eliakim R. Lymphocytic colitis: a clue to an infectious trigger. Scandinavian journal of gastroenterology. 1999;34(1):110-2.

73. Bryant DA, Mintz ED, Puhr ND, Griffin PM, Petras RE. Colonic epithelial lymphocytosis associated with an epidemic of chronic diarrhea. The American journal of surgical pathology. 1996;20(9):1102-9.

74. Osterholm MT, MacDonald KL, White KE, Wells JG, Spika JS, Potter ME, et al. An outbreak of a newly recognized chronic diarrhea syndrome associated with raw milk consumption. Jama. 1986;256(4):484-90.

75. Munch A, Söderholm JD, Wallon C, Ost A, Olaison G, Strom M. Dynamics of mucosal permeability and inflammation in collagenous colitis before, during, and after loop ileostomy. Gut. 2005;54(8):1126-8.

76. Barmeyer C, Erko I, Fromm A, Bojarski C, Allers K, Moos V, et al. Ion transport and barrier function are disturbed in microscopic colitis. Annals of the New York Academy of Sciences. 2012;1258:143-8.

77. Burgel N, Bojarski C, Mankertz J, Zeitz M, Fromm M, Schulzke JD. Mechanisms of diarrhea in collagenous colitis. Gastroenterology. 2002;123(2):433-43.

78. Bohr J, Järnerot G, Tysk C, Jones I, Eriksson S. Effect of fasting on diarrhoea in collagenous colitis. Digestion. 2002;65(1):30-4.

79. Walter SA, Munch A, Ost A, Strom M. Anorectal function in patients with collagenous colitis in active and clinically quiescent phase, in comparison with healthy controls. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2010;22(5):534-8, e118.

80. Wagner M, Lampinen M, Sangfelt P, Agnarsdottir M, Carlson M. Budesonide treatment of patients with collagenous colitis restores normal eosinophil and T-cell activity in the colon. Inflamm Bowel Dis. 2010;16(7):1118-26.

81. Wagner M, Peterson CG, Stolt I, Sangfelt P, Agnarsdottir M, Lampinen M, et al. Fecal eosinophil cationic protein as a marker of active disease and treatment outcome in collagenous colitis: a pilot study. Scandinavian journal of gastroenterology. 2011;46(7-8):849-54.

82. Flejou JF, Grimaud JA, Molas G, Baviera E, Potet F. Collagenous colitis. Ultrastructural study and collagen immunotyping of four cases. Archives of pathology & laboratory medicine. 1984;108(12):977-82.

83. Baum CA, Bhatia P, Miner PB, Jr. Increased colonic mucosal mast cells associated with severe watery diarrhea and microscopic colitis. Digestive diseases and sciences. 1989;34(9):1462-5.

84. Molas GJ, Flejou JF, Potet F. Microscopic colitis, collagenous colitis, and mast cells. Digestive diseases and sciences. 1990;35(7):920-1.

85. Brunning RD, McKenna RW, Rosai J, Parkin JL, Risdall R. Systemic mastocytosis. Extracutaneous manifestations. The American journal of surgical pathology. 1983;7(5):425-38.

86. Hwang WS, Kelly JK, Shaffer EA, Hershfield NB. Collagenous colitis: a disease of pericryptal fibroblast sheath? The Journal of pathology. 1986;149(1):33-40.

87. Aigner T, Neureiter D, Muller S, Kuspert G, Belke J, Kirchner T. Extracellular matrix composition and gene expression in collagenous colitis. Gastroenterology. 1997;113(1):136-43.

88. Balazs M, Egerszegi P, Vadasz G, Kovacs A. Collagenous colitis: an electron microscopic study including comparison with the chronic fibrotic stage of ulcerative colitis. Histopathology. 1988;13(3):319-28.

89. Pardi DS. Diagnosis and Management of Microscopic Colitis. Am J Gastroenterol. 2017;112(1):78-85.

90. Bjornbak C, Engel PJ, Nielsen PL, Munck LK. Microscopic colitis: clinical findings, topography and persistence of histopathological subgroups. Aliment Pharmacol Ther. 2011;34(10):1225-34.

91. Madisch A, Miehlke S, Bartosch F, Bethke B, Stolte M. [Microscopic colitis: clinical presentation, treatment and outcome of 494 patients]. Zeitschrift fur Gastroenterologie. 2014;52(9):1062-5.

92. Hjortswang H, Tysk C, Bohr J, Benoni C, Vigren L, Kilander A, et al. Health-related quality of life is impaired in active collagenous colitis. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2011;43(2):102-9.

93. Nyhlin N, Wickbom A, Montgomery SM, Tysk C, Bohr J. Long-term prognosis of clinical symptoms and health-related quality of life in microscopic colitis: a case-control study. Aliment Pharmacol Ther. 2014;39(9):963-72.

94. Roth B, Ohlsson B. Gastrointestinal symptoms and psychological well-being in patients with microscopic colitis. Scandinavian journal of gastroenterology. 2013;48(1):27-34.

95. Abboud R, Pardi DS, Tremaine WJ, Kammer PP, Sandborn WJ, Loftus EV, Jr. Symptomatic overlap between microscopic colitis and irritable bowel syndrome: a prospective study. Inflamm Bowel Dis. 2013;19(3):550-3.

96. Storr MA. Microscopic colitis: epidemiology, pathophysiology, diagnosis and current management-an update 2013. ISRN gastroenterology. 2013;2013:352718.

97. Wagner M, Stridsberg M, Peterson CG, Sangfelt P, Lampinen M, Carlson M. Increased fecal levels of chromogranin A, chromogranin B, and secretoneurin in collagenous colitis. Inflammation. 2013;36(4):855-61.

98. Magro F, Langner C, Driessen A, Ensari A, Geboes K, Mantzaris GJ, et al. European consensus on the histopathology of inflammatory bowel disease. Journal of Crohn's & colitis. 2013;7(10):827-51.

99. Aust DE, Münch A, Olesen M, Vieth M, Bonderup OK, Bohr J, et al. Su1183 topographical distribution of collagenous colitis-a pooled histological analysis of 2 European prospective multicenter trials. Gastroenterology. 2013;144(5):S-421.

100. Kirby JA, Bone M, Robertson H, Hudson M, Jones DE. The number of intraepithelial T cells decreases from ascending colon to rectum. Journal of clinical pathology. 2003;56(2):158.

101. Koulaouzidis A, Saeed AA. Distinct colonoscopy findings of microscopic colitis: not so microscopic after all? World journal of gastroenterology : WJG. 2011;17(37):4157-65.

102. Bohr J, Larsson LG, Eriksson S, Järnerot G, Tysk C. Colonic perforation in collagenous colitis: an unusual complication. Eur J Gastroenterol Hepatol. 2005;17(1):121-4.

103. Freeman HJ, James D, Mahoney CJ. Spontaneous peritonitis from perforation of the colon in collagenous colitis. Canadian journal of gastroenterology = Journal canadien de gastroenterologie. 2001;15(4):265-7.

104. Allende DS, Taylor SL, Bronner MP. Colonic perforation as a complication of collagenous colitis in a series of 12 patients. Am J Gastroenterol. 2008;103(10):2598-604.

105. Wickbom A, Lindqvist M, Bohr J, Ung KA, Bergman J, Eriksson S, et al. Colonic mucosal tears in collagenous colitis. Scandinavian journal of gastroenterology. 2006;41(6):726-9.

106. Cruz-Correa M, Milligan F, Giardiello FM, Bayless TM, Torbenson M, Yardley JH, et al. Collagenous colitis with mucosal tears on endoscopic insufflation: a unique presentation. Gut. 2002;51(4):600.

107. Bogomoletz WV. Collagenous, microscopic and lymphocytic colitis. An evolving concept. Virchows Archiv : an international journal of pathology. 1994;424(6):573-9.

108. Sato S, Benoni C, Toth E, Veress B, Fork FT. Chromoendoscopic appearance of collagenous colitis--a case report using indigo carmine. Endoscopy. 1998;30(7):S80-1.

109. Sato S, Matsui T, Tsuda S, Yao T, Iwashita A, Takagi Y, et al. Endosocopic abnormalities in a Japanese patient with collagenous colitis. Journal of gastroenterology. 2003;38(8):812-3.

110. Baert F, Schmit A, D'Haens G, Dedeurwaerdere F, Louis E, Cabooter M, et al. Budesonide in collagenous colitis: a double-blind placebo-controlled trial with histologic follow-up. Gastroenterology. 2002;122(1):20-5.

111. Bonderup OK, Hansen JB, Birket-Smith L, Vestergaard V, Teglbjaerg PS, Fallingborg J. Budesonide treatment of collagenous colitis: a randomised, double blind, placebo controlled trial with morphometric analysis. Gut. 2003;52(2):248-51.

112. Miehlke S, Heymer P, Bethke B, Bastlein E, Meier E, Bartram HP, et al. Budesonide treatment for collagenous colitis: a randomized, double-blind, placebo-controlled, multicenter trial. Gastroenterology. 2002;123(4):978-84.

113. Miehlke S, Madisch A, Karimi D, Wonschik S, Kuhlisch E, Beckmann R, et al. Budesonide is effective in treating lymphocytic colitis: a randomized double-blind placebo-controlled study. Gastroenterology. 2009;136(7):2092-100.

114. Pardi DS, Loftus EV, Tremaine WJ, Sandborn WJ. T1193 A randomized, double-blind, placebo-controlled trial of budesonide for the treatment of active lymphocytic colitis. Gastroenterology. 2009;136(5):A-519-A-20.

115. Chande N, MacDonald JK, McDonald JW. Interventions for treating microscopic colitis: a Cochrane Inflammatory Bowel Disease and Functional Bowel Disorders Review Group systematic review of randomized trials. Am J Gastroenterol. 2009;104(1):235-41; quiz 4, 42.

116. Lanyi B, Dries V, Dienes HP, Kruis W. Therapy of prednisone-refractory collagenous colitis with budesonide. International journal of colorectal disease. 1999;14(1):58-61.

117. Bonderup OK, Hansen JB, Teglbjaerg PS, Christensen LA, Fallingborg JF. Long-term budesonide treatment of collagenous colitis: a randomised, double-blind, placebo-controlled trial. Gut. 2009;58(1):68-72.

118. Miehlke S, Madisch A, Bethke B, Morgner A, Kuhlisch E, Henker C, et al. Oral budesonide for maintenance treatment of collagenous colitis: a randomized, double-blind, placebo-controlled trial. Gastroenterology. 2008;135(5):1510-6.

119. Munch A, Bohr J, Miehlke S, Benoni C, Olesen M, Öst Å, et al. Low-dose budesonide for maintenance of clinical remission in collagenous colitis: a randomised, placebo-controlled, 12-month trial. Gut. 2016;65(1):47-56.

120. Pardi DS, Loftus EV, Jr., Tremaine WJ, Sandborn WJ. Treatment of refractory microscopic colitis with azathioprine and 6-mercaptopurine. Gastroenterology. 2001;120(6):1483-4.

121. Munch A, Fernandez-Banares F, Munck LK. Azathioprine and mercaptopurine in the management of patients with chronic, active microscopic colitis. Aliment Pharmacol Ther. 2013;37(8):795-8.

122. Esteve M, Mahadevan U, Sainz E, Rodriguez E, Salas A, Fernandez-Banares F. Efficacy of anti-TNF therapies in refractory severe microscopic colitis. Journal of Crohn's & colitis. 2011;5(6):612-8.

123. Munch A, Ignatova S, Strom M. Adalimumab in budesonide and methotrexate refractory collagenous colitis. Scandinavian journal of gastroenterology. 2012;47(1):59-63.

124. Anderson RJ, Makins R. Successful use of adalimumab in patient with treatment-refractory microscopic colitis. BMJ case reports. 2016;2016.

125. Calabrese C, Gionchetti P, Liguori G, Areni A, Fornarini GS, Campieri M, et al. Clinical course of microscopic colitis in a single-center cohort study. Journal of Crohn's & colitis. 2011;5(3):218-21.

126. Sveinsson OA, Orvar KB, Birgisson S, Agnarsdottir M, Jonasson JG. Clinical features of microscopic colitis in a nation-wide follow-up study in Iceland. Scandinavian journal of gastroenterology. 2008;43(8):955-60.

127. Baert F, Wouters K, D'Haens G, Hoang P, Naegels S, D'Heygere F, et al. Lymphocytic colitis: a distinct clinical entity? A clinicopathological confrontation of lymphocytic and collagenous colitis. Gut. 1999;45(3):375-81.

128. Mullhaupt B, Guller U, Anabitarte M, Guller R, Fried M. Lymphocytic colitis: clinical presentation and long term course. Gut. 1998;43(5):629-33.

129. Halpin SJ, Ford AC. Prevalence of symptoms meeting criteria for irritable bowel syndrome in inflammatory bowel disease: systematic review and meta-analysis. Am J Gastroenterol. 2012;107(10):1474-82.

130. Hussain Z, Kelly S, Clarke A, Adams S, Miller G. Colonic perforation in collagenous colitis: a systematic review of a rare complication and guidance on management. Surgical endoscopy. 2010;24(12):2930-4.

131. Kakar S, Pardi DS, Burgart LJ. Colonic ulcers accompanying collagenous colitis: implication of nonsteroidal anti-inflammatory drugs. Am J Gastroenterol. 2003;98(8):1834-7.

132. Chan JL, Tersmette AC, Offerhaus GJ, Gruber SB, Bayless TM, Giardiello FM. Cancer risk in collagenous colitis. Inflamm Bowel Dis. 1999;5(1):40-3.

133. Yen EF, Pokhrel B, Bianchi LK, Roy HK, Du H, Patel A, et al. Decreased colorectal cancer and adenoma risk in patients with microscopic colitis. Digestive diseases and sciences. 2012;57(1):161-9.

134. Sonnenberg A, Genta RM. Low Prevalence of Colon Polyps in Chronic Inflammatory Conditions of the Colon. Am J Gastroenterol. 2015;110(7):1056-61.

135. Perri F, Annese V, Pastore M, Andriulli A. Microscopic colitis progressed to collagenous colitis: a morphometric study. The Italian journal of gastroenterology. 1996;28(3):147-51.

136. Giardiello FM, Lazenby AJ. The atypical colitides. Gastroenterology clinics of North America. 1999;28(2):479-90, x.

137. Fernandez-Banares F, Salas A, Esteve M, Espinos J, Forne M, Viver JM. Collagenous and lymphocytic colitis. evaluation of clinical and histological features, response to treatment, and long-term follow-up. Am J Gastroenterol. 2003;98(2):340-7.

138. Bowling TE, Price AB, al-Adnani M, Fairclough PD, Menzies-Gow N, Silk DB. Interchange between collagenous and lymphocytic colitis in severe disease with autoimmune associations requiring colectomy: a case report. Gut. 1996;38(5):788-91.

139. Forsberg AM, Kjellström L, Agreus L, Nixon Andreasson A, Nyhlin H, Talley NJ, et al. Prevalence of colonic neoplasia and advanced lesions in the normal population: a prospective population-based colonoscopy study. Scandinavian journal of gastroenterology. 2012;47(2):184-90.

140. Madisch A, Miehlke S, Eichele O, Mrwa J, Bethke B, Kuhlisch E, et al. Boswellia serrata extract for the treatment of collagenous colitis. A double-blind, randomized, placebo-controlled, multicenter trial. International journal of colorectal disease. 2007;22(12):1445-51.

141. Nothlings U, Krawczak M. [PopGen. A population-based biobank with prospective follow-up of a control group]. Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz. 2012;55(6-7):831-5.

142. Stolt P, Bengtsson C, Nordmark B, Lindblad S, Lundberg I, Klareskog L, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. Annals of the rheumatic diseases. 2003;62(9):835-41.

143. Kjellström L, Molinder H, Agreus L, Nyhlin H, Talley NJ, Andreasson A. A randomly selected population sample undergoing colonoscopy: prevalence of the irritable bowel syndrome and the impact of selection factors. Eur J Gastroenterol Hepatol. 2014;26(3):268-75.

144. Tada M, Katoh S, Kohli Y, Kawai K. On the dye spraying method in colonofiberscopy. Endoscopy. 1977;8(2):70-4.

145. Price P, Witt C, Allcock R, Sayer D, Garlepp M, Kok CC, et al. The genetic basis for the association of the 8.1 ancestral haplotype (A1, B8, DR3) with multiple immunopathological diseases. Immunological reviews. 1999;167:257-74.

146. Koning F. Celiac disease: caught between a rock and a hard place. Gastroenterology. 2005;129(4):1294-301.

147. Verhaegh BP, Jonkers DM, Driessen A, Zeegers MP, Keszthelyi D, Masclee AA, et al. Incidence of microscopic colitis in the Netherlands. A nationwide population-based study from 2000 to 2012. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2015;47(1):30-6.

148. Fernandez-Banares F, Salas A, Forne M, Esteve M, Espinos J, Viver JM. Incidence of collagenous and lymphocytic colitis: a 5-year population-based study. Am J Gastroenterol. 1999;94(2):418-23.

149. Bohr J, Tysk C, Eriksson S, Jarnerot G. Collagenous colitis in Örebro, Sweden, an epidemiological study 1984-1993. Gut. 1995;37(3):394-7.

150. Sylwestrowicz T, Kelly JK, Hwang WS, Shaffer EA. Collagenous colitis and microscopic colitis: the watery diarrhea-colitis syndrome. Am J Gastroenterol. 1989;84(7):763-8.

151. Jessurun J, Yardley JH, Lee EL, Vendrell DD, Schiller LR, Fordtran JS. Microscopic and collagenous colitis: different names for the same condition? Gastroenterology. 1986;91(6):1583-4.

152. Sonnenberg A, Genta RM. Lymphocytic and collagenous colitis: epidemiologic differences and similarities. Digestive diseases and sciences. 2013;58(10):2970-5.

153. Thorn M, Sjoberg D, Ekbom A, Holmstrom T, Larsson M, Nielsen AL, et al. Microscopic colitis in Uppsala health region, a population-based prospective study 2005-2009. Scandinavian journal of gastroenterology. 2013;48(7):825-30.

154. Gubbins GP, Dekovich AA, Ma CK, Batra SK. Collagenous colitis: report of nine cases and review of the literature. Southern medical journal. 1991;84(1):33-7.

155. Wang HH, Owings DV, Antonioli DA, Goldman H. Increased subepithelial collagen deposition is not specific for collagenous colitis. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 1988;1(5):329-35.

156. Tysk C, Wickbom A, Nyhlin N, Eriksson S, Bohr J. Recent advances in diagnosis and treatment of microscopic colitis. Annals of gastroenterology : quarterly publication of the Hellenic Society of Gastroenterology. 2011;24(4):253-62.

157. Lebwohl B, Ludvigsson JF, Green PH. Celiac disease and non-celiac gluten sensitivity. BMJ (Clinical research ed). 2015;351:h4347.

158. Freeman HJ. Collagenous colitis as the presenting feature of biopsy-defined celiac disease. Journal of clinical gastroenterology. 2004;38(8):664-8.

159. Koskela RM, Niemela SE, Karttunen TJ, Lehtola JK. Clinical characteristics of collagenous and lymphocytic colitis. Scandinavian journal of gastroenterology. 2004;39(9):837-45.

160. Rönnblom A, Holmstrom T, Tanghoj H, Wanders A, Sjöberg D. Celiac disease, collagenous sprue and microscopic colitis in IBD. Observations from a population-based cohort of IBD (ICURE). Scandinavian journal of gastroenterology. 2015;50(10):1234-40.

161. Chandratre S, Bramble MG, Cooke WM, Jones RA. Simultaneous occurrence of collagenous colitis and Crohn's disease. Digestion. 1987;36(1):55-60.

162. O'Beirne JP, Ireland A. Progression of collagenous colitis to Crohn's disease. Eur J Gastroenterol Hepatol. 2005;17(5):573-5.

163. Janczewska I, Stal P, Sandstedt B. [Transformation of microscopic colitis to inflammatory bowel disease]. Lakartidningen. 2007;104(20-21):1597-8.

164. Geboes K. Lymphocytic, collagenous and other microscopic colitides: pathology and the relationship with idiopathic inflammatory bowel diseases. Gastroenterologie clinique et biologique. 2008;32(8-9):689-94.

165. Kao KT, Pedraza BA, McClune AC, Rios DA, Mao YQ, Zuch RH, et al. Microscopic colitis: a large retrospective analysis from a health maintenance organization experience. World journal of gastroenterology : WJG. 2009;15(25):3122-7.

166. Fine KD, Seidel RH, Do K. The prevalence, anatomic distribution, and diagnosis of colonic causes of chronic diarrhea. Gastrointestinal endoscopy. 2000;51(3):318-26.

167. Narabayashi K, Murano M, Egashira Y, Noda S, Kawakami K, Ishida K, et al. Endoscopic and histopathological evaluation of collagenous colitis. Digestion. 2012;85(2):136-40.

168. Ruder EH, Laiyemo AO, Graubard BI, Hollenbeck AR, Schatzkin A, Cross AJ. Non-steroidal anti-inflammatory drugs and colorectal cancer risk in a large, prospective cohort. Am J Gastroenterol. 2011;106(7):1340-50.

169. Goff JS, Barnett JL, Pelke T, Appelman HD. Collagenous colitis: histopathology and clinical course. Am J Gastroenterol. 1997;92(1):57-60.

170. Bonner GF, Petras RE, Cheong DM, Grewal ID, Breno S, Ruderman WB. Short- and long-term follow-up of treatment for lymphocytic and collagenous colitis. Inflamm Bowel Dis. 2000;6(2):85-91.

171. Ung KA, Kilander A, Willen R, Abrahamsson H. Role of bile acids in lymphocytic colitis. Hepato-gastroenterology. 2002;49(44):432-7.

172. Roda G, Hu J, Zhang Z, Torres J, Chen A, Petras RE, et al. Sa1430 Immunochip Analysis Identifies Multiple Susceptibility Loci for Collagenous Colitis. Gastroenterology. 2016;150(4):S313.

173. Roda G, Torres J, Hu J, Hao K, Zhongyang Z, Chen A, et al. Genome-wide association identifies multiple collagenous colitis susceptibility loci. Gastroenterology. 2015;148:875.

174. Westerlind H, Bonfiglio F, Mellander MR, Hibenthal M, Brynedal B, Björk J, et al. HLA Associations Distinguish Collagenous From Lymphocytic Colitis. Am J Gastroenterol. 2016;111(8):1211-3.

175. Ohlsson B. New insights and challenges in microscopic colitis. Therapeutic advances in gastroenterology. 2015;8(1):37-47.

176. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012;491(7422):119-24.

177. Williams I. Innominate grooves in the surface of mucosa. Radiology. 1965;84:877-80.

178. Cimmino DG, Mella JM, Pereyra L, Luna PA, Casas G, Caldo I, et al. A colorectal mosaic pattern might be an endoscopic feature of collagenous colitis. Journal of Crohn's & colitis. 2010;4(2):139-43.

179. Offner FA, Jao RV, Lewin KJ, Havelec L, Weinstein WM. Collagenous colitis: a study of the distribution of morphological abnormalities and their histological detection. Human pathology. 1999;30(4):451-7.

180. Thijs WJ, van Baarlen J, Kleibeuker JH, Kolkman JJ. Microscopic colitis: prevalence and distribution throughout the colon in patients with chronic diarrhoea. The Netherlands journal of medicine. 2005;63(4):137-40.

181. Tanaka M, Mazzoleni G, Riddell RH. Distribution of collagenous colitis: utility of flexible sigmoidoscopy. Gut. 1992;33(1):65-70.

182. Zins BJ, Tremaine WJ, Carpenter HA. Collagenous colitis: mucosal biopsies and association with fecal leukocytes. Mayo Clin Proc. 1995;70(5):430-3.

183. Strobel S, Abreu MT. Update on idiopathic colitides. Current opinion in gastroenterology. 2013;29(1):60-5.