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Original contribution

Topographical distribution of microscopic colitis and the importance of orientation of paraffin-embedded biopsies☆,☆☆

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Summary The diagnosis of microscopic colitis (MC) relies on specific histopathological findings in colon biopsies. The number of biopsies needed to diagnose MC remains disputed. The aim of the study was to determine the number and site of biopsies necessary for the diagnosis and the effect of perpendicular orientation when embedding the biopsies. This retrospective multicenter European study included 42 patients with a consensus diagnosis of collagenous colitis (CC), 51 patients with lymphocytic colitis (LC), and three patients with incomplete LC (LCi). The number of individual diagnostic biopsies from each patient was determined. The diagnostic rate of 744 individual biopsies from 96 patients with MC was 69.5% for the specific MC subgroup, 79.4% for MC and 93.4% for MC plus incomplete MC (MCi). The risk of missing a diagnosis of the specific subgroup of MC when analyzing four biopsies was 0.87%, decreasing to 0.18% for MC and 0.0019% for MC plus MCi. More biopsies from the right colon were diagnostic of the specific MC subgroup (76.3% vs. 64.0%, p = 0.0014).

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Perpendicular orientation of biopsies increased the diagnostic rate of the specific MC subgroup (73.1% vs. 65.0%, p = 0.0201). Histological changes diagnostic of MC were present in almost all biopsies from the right colon, with orientated biopsies more often being diagnostic of the specific MC subgroup. The results of this study indicate that four biopsies from the colon, rectum excluded, are sufficient to diagnose MC.
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1. Introduction

Microscopic colitis (MC) is a common cause of chronic, nonbloody diarrhea. The clinical diagnosis relies on specific histopathological findings in biopsies from the colonic mucosa and comprises the main subtypes: lymphocytic colitis (LC) and collagenous colitis (CC). Lately, incomplete MC (MCi) consisting of incomplete LC (LCi) and incomplete CC (CCi) has been accepted as a separate entity [1,2]. Although the histological criteria have been agreed on, controversies remain relating to the number of biopsies, distribution of histological changes along the colon, and biopsy orientation.

The European Microscopic Colitis Group (EMCG) was founded in 2010 with the primary objectives of creating awareness on MC among patients, general practitioners, gastroenterologists, surgeons, and pathologists and of promoting scientific collaborations in clinical trials and basic science. In 2014, a prospective data registry was established under EMCG named the European PRO-MC collaboration. Concurrent accumulation of clinical and pathological data from several European centers provides a unique possibility to extend knowledge and consensus on the diagnostic criteria.

The first PRO-MC pathology study was recently published describing the diagnostic approach, validating the original histological diagnosis, and assessing the interobserver agreement among pathologists. The study identified considerable differences among centers with regard to strategies for sampling biopsies, choice of stains, and the minimum number of biopsies and segments required for diagnosing MC. Despite this, interobserver agreement between the participating centers and expert pathologists as well as among the latter was substantial [3].

LC is most often reported to have a uniform distribution throughout the colon although the number of intraepithelial lymphocytes (IELs) can vary [4]. CC is reported to be patchy with wide variation in the thickness of the collagenous band through the colon [5−13]. The number of biopsies and colonic segments needed to diagnose MC remains unknown, and only few studies have dealt with this issue [14−16]. Correct orientation of the biopsies is crucial because evaluation of the collagenous band can only be made in areas with crypts perpendicular to the surface. Furthermore, counting of IELs is easier and more precise as there are no overlapping epithelial cells.

The present study aims to determine in a European cohort (1) the proportion of individual biopsies from patients with MC that show the characteristic histological changes of MC and MC subgroups and can thus be considered diagnostic; (2) the diagnostic sensitivity in biopsies from the right and left colon; and (3) the effect of a perpendicular orientation of biopsies on the diagnostic rate.

2. Materials and methods

2.1. Patients

All patients included fulfilled the clinical criteria of MC, ie, chronic watery diarrhea. The study included patients from two different sources. The first cohort consisted of biopsies from patients included in the prospective European PRO-MC registry. In brief, all eleven centers participating in the PRO-MC registry were invited to participate with material from their first 10 patients included in the registry [3]. The second cohort consisted of patients diagnosed at the Institute of Pathology, Brescia, Italy. This institute and its associated endoscopists use precut cellulose acetate filters for mounting the biopsies in the endoscopy room, attempting to achieve a correct perpendicular orientation of the biopsy specimens [17,18]. Furthermore, the most proximal biopsies are placed at one end of the filters signed by *clarinet beak-shaped cut* (Bio-Optica, Milan, Italy), and the most distal biopsies are placed at the opposite end, reducing the need for separate cassettes from each location and reducing the number of slides to prepare and evaluate.

2.2. Staining and scanning of slides

Supplementary slides of the paraffin-embedded tissue were cut at the local pathology department for all patients in the PRO-MC cohort. The unstained slides were sent to the Department of Pathology, Zealand University Hospital, Roskilde (Denmark), and stained with hematoxylin and eosin (H&E), Van Gieson, and CD3 as per instructions provided by the manufacturer. Only the newly cut tissue and stained slides were included in the present study. The slides were scanned and digitalized using a Nanozoomer

Table 1 Key histological features of collagenous and lymphocytic colitis, including incomplete forms.

Histological feature	CC	LC	CCi	LCi
Degree of inflammation in the lamina propria	Moderate/severe	Moderate/severe	Mild/moderate	Mild/moderate
Number of intraepithelial lymphocytes	Normal or increased	>20/100 epithelial cells	Normal or increased	>10 to ≤20/100 epithelial cells
Thickness of the subepithelial collagenous band	>10 μm	≤10 μm	>5 to ≤10 μm	≤5 μm

Abbreviations: CC, collagenous colitis; LC, lymphocytic colitis; CCi, incomplete collagenous colitis; LCi, incomplete lymphocytic colitis.

HT 2.0 scanner from Hamamatsu Photonics (Hamamatsu, Honshu, Japan) and viewed using Leica SlidePath Gateway LAN software (Leica Biosystems, Buffalo Grove, United States) [3]. In the Brescia cohort, H&E-stained slides and additional Masson Trichrome− or CD3-stained slides of patients with CC and LC, respectively, were scanned using an Aperio Scanscope Cs and viewed using Aperio ImageScope software (Leica Biosystems, Buffalo Grove, United States). All slides were anonymized.

2.3. Histopathological consensus diagnosis

A consensus diagnosis was compiled for the patients included in the primary PRO-MC pathology study based on individual diagnosis given by five pathologists. The diagnostic criteria used by the pathologists are shown in Table 1, and examples are shown in Fig. 1A−D. When a case presented with more than one MC subtype, the final diagnosis followed the hierarchical order of CC > LC > CCi > LCi > non-MC [3].

For the second cohort of patients, the entry criterion was a diagnosis of CC or LC given at the Institute of Pathology, Brescia, Italy. Fig. 1E and F illustrate the use of cellulose filters. Two pathologists assessed the biopsies individually and verified the diagnosis. In one case of disagreement, a third pathologist was included to obtain a consensus diagnosis.

2.4. Further histological analysis

Next, one pathologist rereviewed all slides. The number of biopsies and the location were registered. The right side was defined as the cecum, ascending colon, and transverse colon, whereas the left side was defined as the descending colon, sigmoid colon, and rectum. The number of individual biopsies with characteristic histological changes fulfilling the criteria for the consensus diagnosis was registered for each patient based on the location. Biopsies with a diagnosis different from the consensus diagnosis were classified with regard to existence of another MC subgroup or alternatively classified as nondiagnostic of MC/MCi.

2.5. Statistical analysis

Association between the number of diagnostic biopsies belonging to each subgroup and location as well as number of diagnostic biopsies with or without use of cellulose filters for embedding the biopsies was evaluated using a two-tailed Fisher's exact test. P values <0.05 were considered statistically significant. All statistical analyses were performed using GraphPad, QuickCalcs (GraphPad, San Diego, United States) (https://www.graphpad.com/quickcalcs/, accessed on June 16, 2020).

2.6. Ethics

The use of slides was permissible through local ethical approvals for the PRO-MC study. A further transfer agreement, stating the pathology departments' acceptance of participating in the pathology study, was signed by all the participating centers and by the Institute of Pathology, Brescia.

3. Results

3.1. Included patients

Six PRO-MC centers participated (two from Spain, two from Sweden, one from Lithuania, and one from Denmark), and the cohort thus consisted of biopsies from 60 patients. Only one center consequently used cellulose filters when embedding the biopsies, whereas the other centers carried out embedding of biopsies without previous attempt of orientation. The second cohort consisted of 41 patients diagnosed at the Institute of Pathology, Brescia, Italy, where all biopsies were routinely subjected to orientation on precut cellulose acetate filters before paraffin embedding.

Table 2 shows the consensus diagnosis. Forty-two, 51, and three patients were diagnosed with CC, LC, and LCi, respectively. No patients with CCi were included. Five patients had a consensus diagnosis of non-MC (this group consists of nonspecific changes and normal mucosa) and were excluded from further analysis, leading to a total number of 96 patients in the present study.

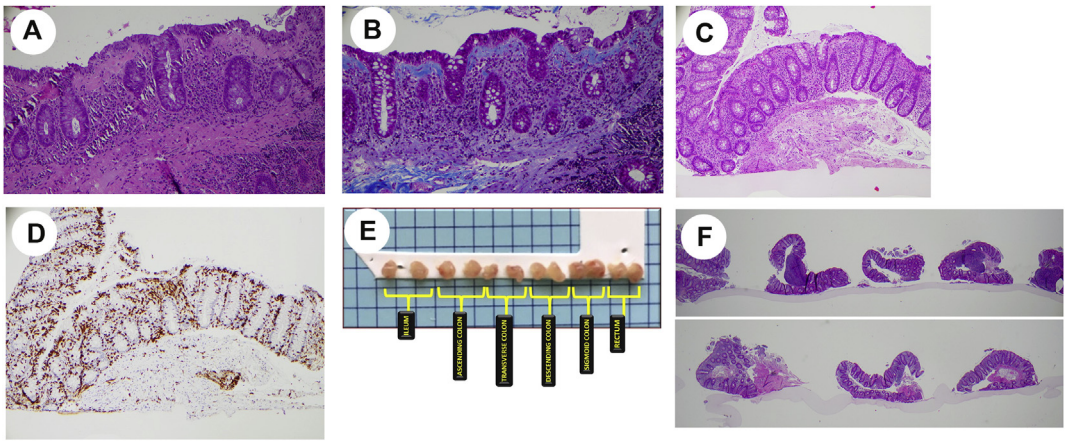

Fig. 1 A, Collagenous colitis: H&E-stained slide, magnification ×20. B, Collagenous colitis: Masson Trichrome–stained slide, magnification ×20. C, Lymphocytic colitis: H&E-stained slide, magnification ×10. D, Lymphocytic colitis: CD3-stained slide, magnification ×10. E, Biopsies mounted on precut cellulose filters. F, H&E-stained slides of biopsies mounted on cellulose filters, magnification ×4. H&E, hematoxylin and eosin.

Table 2 Consensus diagnosis as per the center.

Diagnosis	Center 1	Center 2	Center 3	Center 4	Center 5	Center 6	Brescia	Total
CC	0	5	7	3	4	4	19	42
LC	4	5	3	7	4	6	22	51
CCi	0	0	0	0	0	0	0	0
LCi	1	0	0	0	2	0	0	3
Non-MC	5	0	0	0	0	0	0	5
Total	10	10	10	10	10	10	41	101

Abbreviations: CC, collagenous colitis; LC, lymphocytic colitis; CCi, incomplete collagenous colitis; LCi, incomplete lymphocytic colitis, non-MC, nonmicroscopic colitis.

Table 3 Proportion of diagnostic biopsies as per the center and consensus diagnosis. The diagnosis of biopsies differing from the consensus diagnosis is also shown.

Diagnosis	Center 1	Center 2	Center 3	Center 4	Center 5	Center 6	Brescia	Total
CC	0/0	15/36 (41.7%)	25/47 (52.2%)	11/25 (44.0%)	15/29 (51.7%)	9/16 (56.3%)	99/172 (57.6%)	174/325 (53.5%)*
LC	12/32 (37.5%)	32/32 (100.0%)	24/27 (88.9%)	57/60 (95.0%)	18/27 (66.7%)	16/21 (72.2%)	178/204 (87.3%)	337/403 (83.6%)
LCi	2/7 (28.6%)	0/0	0/0	0/0	4/9 (44.4%)	0/0	0/0	6/16 (37.5%)
Total MC	14/39 (35.9%)	47/68 (69.1%)	49/74 (66.2%)	68/85 (80.0%)	37/65 (56.9%)	25/37 (67.6%)	277/376 (74.7%)	517/744 (69.5%)
Biopsies with LC in CC consensus	0	9	11	10	10	0	34	74
Biopsies with MCi in MC consensus	14	7	13	7	8	9	46	104
Biopsies with nondiagnostic, nonspecific findings in MC consensus	7	0	0	0	6	0	8	21
Nondiagnostic, unsuitable biopsies	4	5	1	0	4	3	11	28

Abbreviations: CC, collagenous colitis; LC, lymphocytic colitis; LCi, incomplete lymphocytic colitis; MCi, incomplete microscopic colitis; MC, microscopic colitis.
*$p < 0.0001$ for CC vs. LC.

Table 4 Proportion of diagnostic biopsies as per the consensus diagnosis and localization in the two cohorts.

Localization	CC	LC	LCi	Total MC
PRO-MC cohort				
Right colon	21/45 (46.7%)	46/54 (85.2%)	1/4 (25.0%)	68/103 (66.0%)
Left colon	20/45 (44.4%)	38/49 (77.55%)	3/5 (60.0%)	61/99 (61.6%)
Unknown	34/63 (57.1%)	75/96 (78.1%)	2/7 (28.6%)	111/166 (66.9%)
Brescia cohort				
Right colon	63/88 (71.6%)	94/104 (90.4%)	0	157/192 (81.8%)
Left colon	36/84 (42.9%)	84/100 (84%)	0	120/184 (65.2%)
Total cohort				
Right colon	84/133 (63.2%)	140/158 (88.6%)	1/4 (25.0%)	225/295 (76.3%)*
Left colon	56/129 (43.4%)	122/149 (81.9%)	3/5 (60.0%)	181/283 (64.0%)

Abbreviations: CC, collagenous colitis; LC, lymphocytic colitis; LCi, incomplete lymphocytic colitis; MC, microscopic colitis.
*p = 0.0014 for right vs. left.

Table 5 Proportion of diagnostic biopsies as per the embedding method.

Number of diagnostic biopsies	No use of cellulose filters	Use of cellulose filters	Total
No. of cases	45	51	96
No. of biopsies	331	413	744
Diagnostic biopsies from patients with CC	66/137 (48.2%)	108/188 (57.4%)	174/325 (53.5%)
Diagnostic biopsies from patients with LC	143/178 (80.3%)	194/225 (86.2%)	337/403 (83.6%)
Diagnostic biopsies from patients with a final LCi	6/16 (37.5%)		6/16 (37.5%)
Total	215/331 (65.0%)[a]	302/413 (73.1%)	517/744 (69.5%)

Abbreviations: CC, collagenous colitis; LC, lymphocytic colitis; LCi, incomplete lymphocytic colitis.
[a] p = 0.0201 for use of cellulose filters vs. no use of cellulose filters.

Biopsies from both the right and left colon were available from 68 patients, whereas the localization was unknown for 28 patients. The embedding technique with use of cellulose filters was used in biopsies from 51 patients. The PRO-MC cohort included only two rectal biopsies, whereas the Brescia cohort included a higher, but unspecified, number of biopsies from the rectum.

3.2. Diagnostic biopsies

In total, 744 biopsies were analyzed, 368 from cohort 1 and 376 from cohort 2, with three to 12 biopsies available from each patient and a mean number of 7.8 biopsies. Table 3 provides the distribution of diagnostic biopsies as per the center and consensus diagnosis. In cohort 1, 49.0%, 79.9%, and 37.5% of the total number of individual biopsies from patients with a consensus diagnosis of CC, LC, and LCi, respectively, was diagnostic of the specific MC subgroup. One center had a lower diagnostic rate. This center only included five cases (four LC and one LCi) as five cases were excluded owing to a consensus diagnosis of non-MC. In cohort 2, the diagnostic rate was 57.6% and 87.3% for patients diagnosed with CC and LC, respectively.

Overall, the diagnostic rate was 69.5% for the specific MC subgroup. In both the PRO-MC cohort and the Brescia cohort, significantly more biopsies were diagnostic of LC than of CC (Table 3). Biopsies from patients with CC not diagnostic of the consensus diagnosis fulfilled the criteria for LC in 9.9% of the cases. Biopsies from patients with CC or LC not diagnostic of the consensus diagnosis were diagnostic of MCi in 14.0% of the cases. Thus, 79.4% of the biopsies were diagnostic of MC, increasing to 93.4% when including MCi. This corresponds to a risk of missing a diagnosis of the specific subgroup of MC, which when analyzing four biopsies was 0.87% and decreased to 0.18% for MC and 0.0019% when including MCi. In all, only 21 biopsies were reported as not diagnostic of MC or MCi. Further 28 biopsies were not suitable for diagnostics owing to too small size, artifact, or too poor quality of the scanned slides.

3.3. Diagnostic biopsies based on the right or left side

When comparing the right and left side of the colon, statistically significantly more biopsies from the right side were diagnostic of the specific MC subgroup. The number of diagnostic biopsies for the specific subgroup of MC was 76.3% and 64.0% from the right and left colon, respectively. Table 4 specifies the numbers in each of the two cohorts. According to the local biopsy protocol in Brescia, it was recommended to take biopsies from all five segments, inclusive of the rectum if possible. The exact number of biopsies from individual patients from the rectum in this material is unknown but is much higher than in cohort 1.

The total number of patients with biopsies not diagnostic of the consensus diagnosis in either the right or the left colon was four, six, and two, with biopsies from the right side, left side, and unknown location, respectively. Left-sided biopsies were not diagnostic of the consensus diagnosis in six patients with CC (n = 4), LC (n = 1), and LCi (n = 1). However, in patients with CC in the right colon, left-sided biopsies were diagnostic of LC (three patients)

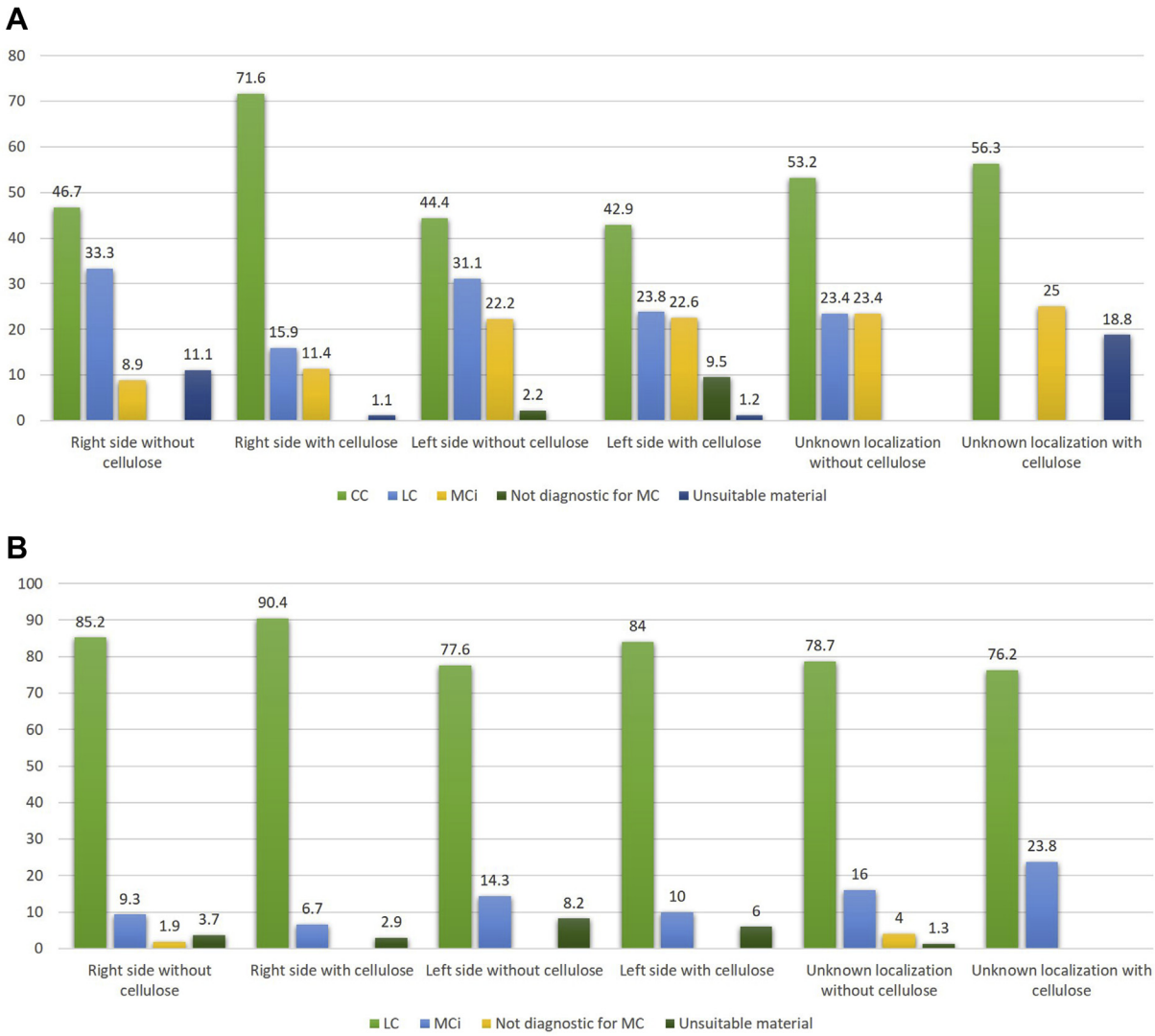

Fig. 2 A, Diagnosis in the percentage of individual biopsies from patients with a consensus diagnosis of CC based on the localization and use of cellulose filters (no. of patients: 19, no. of biopsies: 137 without cellulose filters and no. of patients: 23, no. of biopsies: 188 with cellulose filters). B, Diagnosis in the percentage of individual biopsies from patients with a consensus diagnosis of LC based on the localization and use of cellulose paper (no. of patients: 23, no. of biopsies: 178 without cellulose filters and no. of patients: 28, no. of biopsies: 225 with use of cellulose filters). CC, collagenous colitis; LC, lymphocytic colitis; MCi, incomplete microscopic colitis; MC, microscopic colitis.

and MCi (one patient), and the patient with LC had changes compatible with LCi. Only two left-sided biopsies were available from the patient with LCi, and both were without MC/MCi. Right-sided biopsies were not diagnostic of the consensus diagnosis in four patients with CC (n = 2), LC (n = 1), and LCi (n = 1). Again, in patients with CC in the left colon, right-sided biopsies were diagnostic of LC, and the patient with LC fulfilled the criteria of LCi. Two right-sided biopsies available from the patient with LCi were not diagnostic of MC/MCi. The two patients with biopsies of unknown location both had changes in the biopsies of another subtype of MC or MCi.

3.4. Diagnostic biopsies based on the method for embedding the tissue

The Brescia cohort and center 6 from cohort 1 orientated biopsy specimens on precut cellulose acetate filters. These groups were merged and compared with center 1–5 (Table 5). The diagnostic rate for orientated biopsies was 57.4% and 86.2% for CC and LC, respectively, compared with 48.2% and 80.3% in the group of not-orientated biopsies. Fig. 2A and B illustrate the percentage of diagnostic biopsies based on subgroup, localization, and embedding technique.

4. Discussion

The present study provides a meticulous evaluation of the diagnostic rate and the distribution of MC in colon biopsies from a representative European cohort and demonstrates that four biopsies oral to the rectum suffice for diagnosing MC in patients with chronic watery diarrhea. The diagnostic rate varied as per the MC subgroup, localization, and the method used for embedding and orientating the biopsies. The diagnostic rate was higher for the specific subgroup of MC from patients with LC, in right-sided biopsies, and with use of cellulose filters.

The overall diagnostic rate in individual biopsies as per the consensus diagnosis and specific subgroup was 69.5%, with an additional 9.9% of the biopsies showing features of any subgroup of MC and another 14% when including biopsies with MCi changes, resulting in almost 94% of the biopsies being diagnostic of MC including MCi.

The diagnostic rate was higher for LC than for CC. In cases of CC, the nondiagnostic biopsies were often diagnostic of LC or MCi and only rarely revealed nonspecific findings or normal mucosa. As the consensus diagnosis was defined by the hierarchical order of CC > LC when more than one subtype of MC was present, this excludes the possibility of a patient with a consensus diagnosis of LC having CC in biopsies not diagnostic of the consensus diagnosis. The higher diagnostic rate in patients with LC is in agreement with the previous literature, and in the context of the results of this study, a possible explanation could be that biopsies with suboptimal orientation make it impossible to diagnose CC. The diagnostic rate increased significantly when using cellulose filters to obtain a perpendicular orientation of the biopsies and was most pronounced for CC. Center 1 to 5 from the PRO-MC cohort only included two rectal biopsies, whereas the Brescia cohort included a higher number of biopsiesfrom this location. As rectal biopsies have proven to be insufficient for the diagnosis, we assume that our study even underestimates this difference [9,10,19–21].

The diagnostic rate of the specific MC subgroup was significantly higher in the right-sided biopsies, but only two patients with LCi would have been misdiagnosed as non-MC if only biopsies from one side were available. Several previous studies have compared the diagnostic rate of histological changes in right- and left-sided biopsies, but they all pooled biopsies from one segment and thus did not assess the individual biopsies. The studies including the highest number of patients with biopsies from both sides of the colon showed that 95–98% of the patients had characteristic histological changes of MC in biopsies from both sides [22–24]. Other studies including fewer patients reported similar high concordance, higher than or close to 90%, between left- and right-sided biopsies [8,25–33]. Conflicting studies including a small number of patients not examined using a strict protocol with biopsies from all segments have reported a smaller number of diagnostic biopsies from the left colon [9,10,19,34]. Although a full colonoscopy is recommended to rule out other diseases including malignancy, our results contribute to the total amount of data, indicating that biopsies from anywhere in the colon oral to the rectum would suffice to diagnose MC.

The number of biopsies needed to diagnose MC remains a matter of debate. American Gastroenterological Association recommends eight biopsies from both sides of the colon [35], whereas the Spanish guidelines recommend two biopsies from each examined segment [36]. These numbers correlate with those of a large study that reported the mean number of biopsies from patients with MC to be 8.4 [15]. A similar mean was reported in an additional study [16], and the result is close to the number in our study. A recent study suggested reduction of the number of biopsies to two from the ascending colon and two biopsies from the descending colon [14]. This study also assessed biopsies individually and found a slightly higher diagnostic rate than that in the present study. The authors showed that all cases would have been detected following their proposed protocol. The study was retrospective, with no clinical information, and did not use a standardized biopsy protocol with matched biopsies from the same segment of each patient. However, our results support the recommendation that the risk of missing a diagnosis of MC or MCi is less than one percent if four biopsies are examined. From a clinical point of view, discriminating between the subtypes is less important because recommendations for treatment are identical (Miehlke S et al., UEG journal, under review).

Another point of dispute is the number of biopsies with the characteristic histological changes required for diagnosis of MC. Histopathological changes diagnostic of CC or LC in only one or two biopsies are extremely uncommon when receiving a larger number of biopsies, and in that situation, we recommend considering differential diagnoses carefully. In the present study, we assessed all biopsies individually. Some of the biopsies categorized as nondiagnostic and unsuitable could possibly have been of some use in a routine setting wherein biopsies not strictly diagnostic by themselves owing to, ie, too small fragments or artifact can still add to the sum of changes in all the biopsies.

The described embedding technique using precut cellulose filters depends on the experience of the endoscopist and the endoscopy room nurse for a precise orientation of the biopsies but reduces the time for paraffin embedding of tissue, the number of sections to be cut and stained, and the time for microscopy. Introduction of a new technique is always challenging, but our study indicates that the use of cellulose filters would be of benefit in the end.

A limitation of our study is that the consensus diagnosis was reached by five pathologists for the patients in cohort 1 and only two pathologists in cohort 2. We hypothesize that a higher number of pathologists might result in a lower

number of cases with a diagnosis of CC. Cases with only borderline thickened subepithelial band often fulfill the criteria of LC, and more cases might have been classified as this subtype. If so, we would assume the diagnostic rate to be even higher in cohort 2.

5. Conclusions

In conclusion, the present study shows that histological changes diagnostic of MC are present in most biopsies and in both the right and left colon, although suboptimal orientation of the biopsies sometimes hinders assignment to a definitive subgroup, which is especially evident in CC. The use of cellulose filters for perpendicular orientation of the biopsies increases the diagnostic rate significantly. The results of this study indicate that four biopsies from the colon, with the rectum excluded, are sufficient to diagnose MC regardless of the embedding technique. This could reduce the workload of both endoscopists and pathologists significantly.

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