

Is the Routine Histopathologic Diagnosis of Ulcerative Colitis Based on Cross-cut Sections or on Well-oriented Sections?

CARLOS RUBIO¹, CORINNA LANG-SCHWARZ² and MICHAEL VIETH²

¹Department of Pathology, Karolinska Institute and University Hospital, Stockholm, Sweden;

²Institute of Pathology, Friedrich-Alexander University Erlangen-Nuremberg, Klinikum Bayreuth, Bayreuth, Germany

Abstract. *Background/Aim:* For many years, it was empirically estimated that the majority of the routine colon biopsies in Swedish patients with ulcerative colitis (UC), exhibited cross-cut crypts. The aim of the present study was to assess the frequency of cross-cut crypts (CCC) and well-oriented crypts in routine colon biopsies in German patients with UC. *Patients and Methods:* In total, 447 colon biopsies: 376 with UC and 71 controls were investigated. *Results:* Out of 376 colon biopsies with UC, 73% exhibited $\geq 60\%$ CCC. Out of the 237 biopsies showing $\geq 80\%$ CCC, as many as 71% exhibited 100% CCC in individual biopsies. Similar percentages were found in control biopsies. *Conclusion:* The majority of the routine colon biopsies with UC, as well as control biopsies in German patients displayed CCC. Thus, an unnoticed, consequent, and systematic cutting technical hitch was introduced during the laboratory processing of colon biopsies. The reason(s) behind the similar histologic processing mode of colon biopsies at the two geographically disparate laboratories (Sweden and Germany) remains elusive. The cross-cutting mode influenced the narrative of biopsies in UC, inasmuch as some histological parameters listed among well-oriented colon sections were not present in sections displaying CCC.

Correspondence to: Carlos A. Rubio, MD, Ph.D., Gastrointestinal Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, 17176, Stockholm, Sweden. Tel: +46 851774527, Fax: +46 851774524, e-mail: Carlos.Rubio@ki.se

Key Words: Colon biopsies, ulcerative colitis, histology, cross-cut crypts.

©2023 International Institute of Anticancer Research
www.iiar-anticancer.org



This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND) 4.0 international license (<https://creativecommons.org/licenses/by-nc-nd/4.0>).

In 1859, a physician working at Guy's Hospital, London, described an inflammatory disease of the colorectum that he called "simple idiopathic colitis" (1). Since then, a wealth of literature has appeared concerning symptomatology, endoscopy, pathology, treatment, and final outcome of ulcerative colitis (UC) as the disease is currently being referred to (2-8). The disease was described by Sir Samuel Wilks in the middle of the XIX Century (1), its etiology remains unknown.

The histopathological judgement of colon biopsies is central to a definite diagnosis of UC. International consensus has been reached concerning the parameters required for a histological diagnosis of UC (9). These parameters are: *i)* Basal plasmacytosis between the base of the crypt and the *muscularis mucosae*, *ii)* Diffuse infiltration of chronic inflammatory cells (lymphocytes and plasma cells) in the *lamina propria*, *iii)* Cryptolytic granulomas, *iv)* Eosinophils in the *lamina propria* and *muscularis mucosae*, *v)* Active inflammation (cryptitis and crypt abscesses), *vi)* Mucin depletion of goblet cells in surface epithelium and in crypts, *vii)* Lymphoid aggregates in the mucosa and submucosa, *viii)* Surface flattening with metaplastic changes, *ix)* Surface erosions, *x)* Irregular mucosal pseudovillous in the surface, *xi)* Wide crypt orifices, *xii)* Inflammatory pseudo polyps, *xiii)* Crypt atrophy with shortening of the crypts, *xiv)* Paneth cell metaplasia, *xv)* Distortion of crypt architecture, *xvi)* Crypt branching (*i.e.*, fission), and *xvii)* Abnormal regeneration (2-8, 9).

In attempts to reset the normal number of crypts in crypt-free areas occupied by severe inflammation, bordering areas regenerate in an abnormal fashion. Crypt-free areas become occupied by crypts in symmetric and asymmetric branching (10, 11), some arranged as rings in tandem (12), and by not yet-dividing crypts, differing in length, width and/or shape.

In a diagnostic setting, the question arises as to whether the aforementioned histological parameters are present in all biopsies, only in well-oriented sections, or in cross-cut sections. These different modes of histologic arrangement of routine sections have received little attention but are a matter of concern. It is conceivable that some histologic parameters,

Table I. Number of colon biopsies with cross-cut crypts (CCC) in 376 colon biopsies in patients with ulcerative colitis (UC) and in 71 control patients (50 with infectious colitis and 21 with sigmoid diverticulitis).

Ad hoc grouping of the percentage of CCC in colon biopsies	No. of colon biopsies with CCC in UC (%)	No. of colon biopsies with CCC in controls (%)
0%	20 (5.3%)	3 (4.2%)
<20%	13 (3.5%)	3 (4.2%)
≥20%-<40%	19 (5.0%)	4 (5.6%)
≥40%-<60%	51 (13.5%)	4 (5.6%)
≥60%-<80%	36 (9.5%)	12 (16.9%)
≥80%-100%	237 (63%)	48 (67.6%)
	376 (100%)	71 (100%)

such as surface flattening with metaplastic changes, surface erosions, irregular mucosal pseudovillous in the surface, basal plasmacytosis (between the crypt base and *muscularis mucosae*), shortening of the crypts, and lymphoid aggregates in the submucosa, might not be found in cross-cut sections, as these parameters may become apparent in well-oriented, vertical sections.

In previous studies of rectal biopsies (13, 14) and colon biopsies in Swedish patients with UC (10, 15) we empirically estimated that the majority of the biopsies displayed cross-cut crypts. The pertinent question was: could the histological processing of routine sections be the result of a technical hitch while transferring the biopsy from the forceps to the fixation vial or by wax-bedding and tissue sectioning procedures used at the Swedish laboratory? This challenging question was explored by evaluating the arrangement of histologic sections in a diagnostic setting of colon biopsies, at a German laboratory.

The purpose of this study was to calculate the frequency of cross-cut tissue fragments in a relatively large cohort of colon biopsies with UC diagnosed at a German Department of Pathology. The rational was to investigate whether possible alternative routine procedures used by German laboratory personal, could influence the frequency of cross-cut tissue fragments in a diagnostic setting of biopsies from patients with UC. Another goal was to analyze whether some important parameters at the mucosal surface (cell flattening, metaplastic changes, erosions, irregular mucosal pseudovillous), and at the basal aspects of the mucosa: basal plasmacytosis, crypt shortening, and submucosal lymphoid aggregates, could be detected in cross-cut sections of routine colon biopsies with UC.

Patients and Methods

The material consists of 447 colon biopsies: 376 with UC and 71 controls with mucosal inflammation (50 with infectious colitis and 21 with sigmoid diverticulitis).

The material was retrieved from the electronic archive of the Institute of Pathology, Klinikum Bayreuth and DC Systeme,

Heiligenhaus, Germany. The biopsies were diagnosed at the Department of Pathology, Klinikum Bayreuth, Friedrich-Alexander-University Erlangen-Nuremberg, Bayreuth, Germany, on hematoxylin and eosin (H&E)-stained slides (4 mm sections). The preparations were subsequently scanned and digitalized with a Hamamatsu NanoZoomer Digital Pathology S360 (NDP, Hamamatsu, Herrsching am Ammersee, Germany) carrying a ×40 objective. Images were made available online to all authors.

The frequency of cross-cut crypts (CCC) present in diagnostic H&E stained sections were registered in an *ad hoc* group-listing of the percentages of biopsies with CCC (Table I): *i*) 0% biopsies with CCC (it implies that no CCC were found in those biopsies; all crypts were well-oriented, vertical, *ii*) <20% biopsies with CCC, *iii*) ≥20%-<40% biopsies with CCC *iv*) ≥40%-<60% biopsies with CCC, ≥60%-<80% biopsies with CCC, and *v*) ≥80%-100% biopsies with CCC.

The following parameters included in the histological diagnosis of UC in the literature (2-10) were search for in biopsies with CCC: surface cell flattening, metaplastic changes, erosions, irregular mucosal pseudovillous, basal plasmacytosis, crypt shortening, and submucosal lymphoid aggregates.

Ethical approval. Ethical approval was obtained from the Ethics Committee of Friedrich-Alexander University, Erlangen-Nuremberg, Germany (study number 175_20Bc).

Statistical analysis. The non-parametric Mann-Whitney *U* two-tailed test was applied to compare differences between two groups. Statistical significance was defined as $p < 0.05$.

Results

Frequency of CCC in UC. From the results presented in Table I, it may be deduced that out of 376 colon biopsies in patients with UC, 73% (n=273) exhibited ≥60% CCC. Table I also shows that 63% (n=237) of the colon biopsies had ≥80% CCC. Notably, out of the 237 biopsies with ≥80% CCC, as many as 71% (n=169) exhibited 100% CCC in individual biopsies.

Frequency of CCC in controls with mucosal inflammation. Since the frequency of CCC in infectious colitis (n=50) and

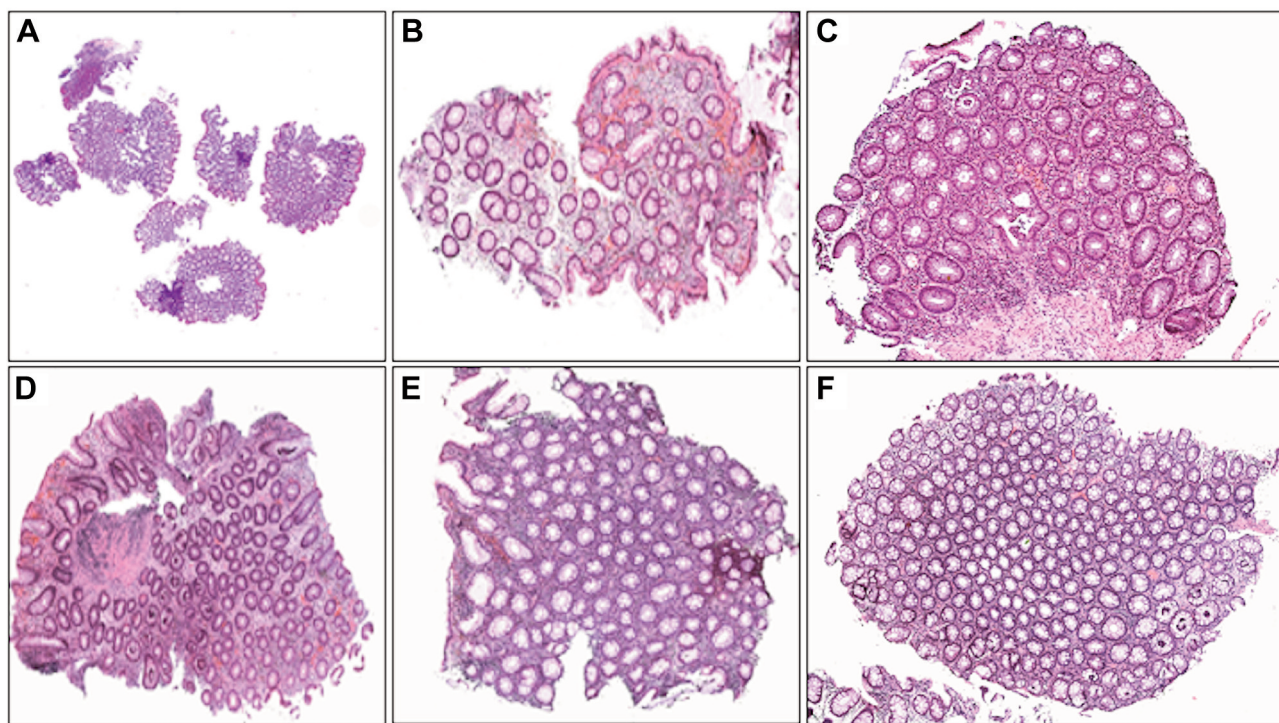


Figure 1. Example of fragments of colon biopsies displaying cross-cut crypts. A) Low power view showing cross-cut crypts in all mucosal fragments in a biopsy from a patient with ulcerative colitis (UC) (H&E, original $\times 4$). B, C, and D) Detail from biopsies in patients with UC showing cross-cut crypts (H&E, B and C, original $\times 16$; D H&E, original $\times 10$). E) Biopsy from a control patient with infectious colitis (H&E original $\times 16$). F) Biopsy from a control patient with sigmoid diverticulitis (H&E original $\times 10$).

sigmoid diverticulitis ($n=21$) was not significant ($p=0.4413$), the results in both inflammatory processes were pooled together.

Table I shows that out of the 60 control biopsies exhibiting $\geq 60\%$ CCC, CCC were found in 84.5%. Out of the 48 biopsies having $\geq 80\%$ CCC, as many as 29 (60%) had 100% CCC in individual biopsies (Figure 1).

Comparing the frequency of CCC between UC and controls
When the frequency of CCC found in the tissue fragments of 377 cases with UC was compared to that in 71 controls, the result was not significant ($p=0.328834$).

Relevant histological parameters in biopsies with CCC. In the literature (2-10), surface cell flattening, metaplastic changes, erosions, irregular mucosal pseudovillous, basal plasmacytosis, crypt shortening, and submucosal lymphoid aggregates are relevant parameters included in the histological diagnosis of UC.

We considered *a priori*, that due to the fact that the surface, base, and submucosal aspects of the mucosa were not included in the cross-cut histological sections, these parameters might not be present. That pitfall was confirmed in this survey, as none of these histologic parameters were

present in any of the mucosal areas having CCC in the 376 biopsies with UC.

Discussion

The results of this study showed that the majority (about two thirds) of the routine colon biopsies in German patients with UC displayed $\geq 80\%$ CCC. These findings substantiate the previous deep-rooted subjective impression that the majority of the routine biopsies in Swedish patients with UC (14, 15) displayed CCC. Since the vast majority of the routine control biopsies with inflammation also displayed CCC, it was suggested that an unnoticed, consequent, and systematic cutting technical hitch was introduced during the laboratory processing of colon biopsies at the two geographically disparate laboratories (Sweden and Germany).

This study also showed that some parameters included in the histologic diagnosis of UC: i) surface flattening with metaplastic changes, ii) surface erosions, iii) irregular mucosal pseudovillous in the surface, iv) basal plasmacytosis (between the crypt base and muscularis mucosae), v) shortening of the crypts, and vi) lymphoid aggregates in the submucosa, were not included in cross-cut sections. Thus,

the cross-cutting mode influenced the narrative of biopsies in UC, inasmuch as some histological parameters listed among well-oriented colon sections were not present in sections with CCC. From these deliberations, it becomes clear that the narrative of diagnostic biopsies with UC should include the histologic cutting mode at the respective laboratory, so clinicians can interpret why some histologic parameters listed in the diagnosis of UC, are missing in the pathological report.

In conclusion, a recent Google search and PUBMED search (2023-03-18) for “ulcerative colitis, pathology” revealed 5,450,000 hits and 14,240 publications, respectively. These results underscore the interest for the parameters involved in the microscopic assessment of UC. Nevertheless, no detailed information is in record regarding the significance of the histologic cutting mode in the narrative of the histologic parameters in colon biopsies in UC. This study highlights the possible cause(s) for that pitfall.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

CAR was responsible for the concept and design, the review of the scanned sections, analysis, and interpretation of data, and wrote the original draft. CL-S and MV scanned sections with a Nanozoomer S360, making them available online to all authors. CL-S and MV revised and finally approved the manuscript.

References

- Wilks S: Simple idiopathic colitis. In: Lectures on Pathological Anatomy, pp. 408, 1875.
- Caron B, Jairath V, D'Amico F, Al Awadhi S, Dignass A, Hart AL, Kobayashi T, Kotze PG, Magro F, Siegmund B, Paridaens K, Danese S and Peyrin-Biroulet L: International consensus on definition of mild-to-moderate ulcerative colitis disease activity in adult patients. *Medicina (Kaunas)* 59(1): 183, 2023. PMID: 36676807. DOI: 10.3390/medicina59010183
- Gajendran M, Loganathan P, Jimenez G, Catinella AP, Ng N, Umapathy C, Ziade N and Hashash JG: A comprehensive review and update on ulcerative colitis(). *Dis Mon* 65(12): 100851, 2019. PMID: 30837080. DOI: 10.1016/j.disamonth.2019.02.004
- Moum B, Ekblom A, Vatn MH and Elgjo K: Change in the extent of colonoscopic and histological involvement in ulcerative colitis over time. *Am J Gastroenterol* 94(6): 1564-1569, 1999. PMID: 10364026. DOI: 10.1111/j.1572-0241.1999.01145.x
- Seldenrijk CA, Morson BC, Meuwissen SG, Schipper NW, Lindeman J and Meijer CJ: Histopathological evaluation of colonic mucosal biopsy specimens in chronic inflammatory bowel disease: diagnostic implications. *Gut* 32(12): 1514-1520, 1991. PMID: 1773958. DOI: 10.1136/gut.32.12.1514
- DeRoche TC, Xiao SY and Liu X: Histological evaluation in ulcerative colitis. *Gastroenterol Rep (Oxf)* 2(3): 178-192, 2014. PMID: 24942757. DOI: 10.1093/gastro/gou031
- Ma C, Sedano R, Almradi A, Vande Castele N, Parker CE, Guizzetti L, Schaeffer DF, Riddell RH, Pai RK, Battat R, Sands BE, Rosty C, Dubinsky MC, Rieder F, Harpaz N, Abreu MT, Bryant RV, Lauwers GY, Kirsch R, Valasek MA, Crowley E, Sandborn WJ, Feagan BG, Pai RK and Jairath V: An international consensus to standardize integration of histopathology in ulcerative colitis clinical trials. *Gastroenterology* 160(7): 2291-2302, 2021. PMID: 33610533. DOI: 10.1053/j.gastro.2021.02.035
- Theodossi A, Spiegelhalter DJ, Jass J, Firth J, Dixon M, Leader M, Levison DA, Lindley R, Filipe I and Price A: Observer variation and discriminatory value of biopsy features in inflammatory bowel disease. *Gut* 35(7): 961-968, 1994. PMID: 8063225. DOI: 10.1136/gut.35.7.961
- Lang-Schwarz C, Agaimy A, Atreya R, Becker C, Danese S, Fléjou JF, Gähler N, Grabsch HI, Hartmann A, Kamarádová K, Kühl AA, Lauwers GY, Lugli A, Nagtegaal I, Neurath MF, Oberhuber G, Peyrin-Biroulet L, Rath T, Riddell R, Rubio CA, Sheahan K, Tilg H, Villanacci V, Westerhoff M and Vieth M: Maximizing the diagnostic information from biopsies in chronic inflammatory bowel diseases: recommendations from the Erlangen International Consensus Conference on Inflammatory Bowel Diseases and presentation of the IBD-DCA score as a proposal for a new index for histologic activity assessment in ulcerative colitis and Crohn's disease. *Virchows Arch* 478(3): 581-594, 2021. PMID: 33373023. DOI: 10.1007/s00428-020-02982-7
- Rubio CA and Schmidt PT: Crypts in asymmetric fission in endoscopic biopsies from Swedish patients with inflammatory bowel disease. *Anticancer Res* 41(7): 3511-3517, 2021. PMID: 34230146. DOI: 10.21873/anticancer.15138
- Rubio CA, Lang-Schwarz C and Vieth M: Crypts in asymmetric fission in endoscopic biopsies from German patients with inflammatory bowel disease. *Anticancer Res* 41(9): 4401-4405, 2021. PMID: 34475060. DOI: 10.21873/anticancer.15245
- Rubio CA, Vieth M and Lang-Schwarz C: Novel histological repertoire of crypt-associated anomalies in inflamed colon mucosa. *J Clin Pathol*, 2022. PMID: 35273118. DOI: 10.1136/jclinpath-2022-208152
- Rubio CA and Kock Y: A digital quantitative method of estimating inflammation in the rectal mucosa. I. The normal limits in asymptomatic patients. *Scand J Gastroenterol* 16(6): 731-735, 1981. PMID: 6976614. DOI: 10.3109/00365528109180997
- Rubio CA, Johansson C and Kock Y: A quantitative method of estimating inflammation in the rectal mucosa. III. Chronic ulcerative colitis. *Scand J Gastroenterol* 17(8): 1083-1087, 1982. PMID: 7167739.
- Rubio CA and Schmidt PT: Morphological classification of corrupted colonic crypts in ulcerative colitis. *Anticancer Res* 38(4): 2253-2259, 2018. PMID: 29599347. DOI: 10.21873/anticancer.12469

Received March 22, 2023

Revised April 3, 2023

Accepted April 4, 2023