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The Number of Colon Crypts in Digital Mucosal Samples: A New Independent Parameter for Diagnosing Ulcerative Colitis

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Abstract. *Background/Aim: It has been demonstrated that most routine biopsies from the colon and rectum display cross-cut crypts (CCC). The aim was to assess the number of CCC in microscopic isometric digital samples (0.500 mm^2) from routine colon biopsies. Patients and Methods: Colon biopsies from 224 patients were investigated: 99 in patients with ulcerative colitis (UC), 31 UC in remission (UCR), 28 infectious colitis (IC), 7 resolved IC (RIC), 19 diverticular sigmoiditis (DS), and 40 normal colon mucosa (NCM). Results: A total of 8,024 CCC were registered: 2,860 (35.6%) in UC, 1,319 UCR (16.4%), 849 (10.6%) in IC, 340 (4.2%) in RIC, 795 (9.9%) in DS, and 1,861 (23.2%) in NCM. The CCC frequencies in UC and IC were significantly lower ($p<0.05$) than those in UCR, RIC, DS, and NCM. Conclusion: By the simple algorithm of counting CCC in standardized isometric microscopic digital circles measuring 0.500 mm^2, it was possible to differentiate between UC (long-lasting inflammation) and IC (short-lasting inflammation) on the one hand, and UCR, RIC, DS (persistent inflammation), and NCM, on the other. The counting of CCC in the algorithm by five pathologists working in three disparate European Countries, was found to be reproducible.*

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The histopathological evaluation of colon biopsies together with clinical, endoscopic, and radiological findings are important cornerstones of the final diagnosis of ulcerative colitis (UC) (1). There is international agreement regarding the parameters required for a histological diagnosis of UC (2-9). 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 xvi) Abnormal regeneration (2-9).

For many years, our subjective impression was that most of the routine biopsies in patients with UC were cross-cut during the technical laboratory work-up. In the early 1980's, we investigated the potential significance of the cutting mode by assessing the frequency of cross-cut crypts in isometric microscopic areas of rectal biopsies in 51 patients with UC, 61 patients with UC in remission (UCR), and in 124 controls with no inflammation (10-12). The diameter of the crypts, the distance between cross-cut crypts, and the number of chronic inflammatory cells within the lamina propria found within the limits of an ocular provided with a graded frame

measuring 30×30 mm, were recorded. The ocular frame standardized the field of view (FOV) at 995.4 mm^2. The results of that quantitative method permitted to differentiate between normal rectal mucosa, rectal mucosa with UC, and rectal mucosa with UCR. The possibility of semi-automatically quantitate these variables by the aid of Digital Image Analysis, was then further discussed (11).

We recently tested our subjective impression that most of the colon biopsies were also being crosscut at the laboratory (13). For that purpose, the percentage of routine biopsies with cross-cut crypts were assessed in 447 colon biopsies (376 with UC and 71 controls with mucosal inflammation: 50 with infectious colitis and 21 with sigmoid diverticulitis) (13). It was found that out of 376 colon biopsies with UC, 73% exhibited ≥60% CCC. Importantly, out of the 237 biopsies showing ≥80% CCC, as many as 71% exhibited 100% CCC in individual biopsies. Similar percentages were found in control biopsies (13). Thus, most of the colon biopsies found in a routine setting often display cross-cut crypts. This knowledge is crucial, considering that several UC histological parameters, such as surface epithelial damage (flattening, metaplastic changes focal cell loss, mucosal breaks such as erosions, or ulcers), villous irregular mucosal surface due to finger-like outgrowths around wide crypt orifices, basal plasmacytosis (≥3 plasma cells between the base of the crypts and the *muscularis mucosae*), chronic inflammation beneath the bottom of the crypts, and the shortening of the crypts (crypt atrophy) are recorded in well oriented (vertical) sections and not in cross-cut crypts. In contrast, parameters such as increased number of chronic inflammatory cells, crypt destruction, epithelial regeneration, goblet cell depletion, crypts differing in size and in diameter are detected both in cross-cut sections and in well oriented (vertical) sections (*i.e.*, regardless of the cutting orientation of the mucosal fragments during the laboratory work up). From these elaborations, it became apparent that the cutting mode of the biopsies at the laboratory may influence the perception of some of the histological parameters in routine biopsies in UC. Since the crypts in UC are irregular in shape and may be far apart from each other due to the interstitial ongoing inflammation, the aim of the present study was to assess the frequency of CCC in isometric areas of colon biopsies in UC. The results were compared to those recorded in UC in remission (UCR), infectious colitis (IC), resolved infectious colitis (RIC), diverticular sigmoiditis (DS), and normal colon mucosa (NCM, *i.e.*, with no pathological changes).

Patients and Methods

Study design. The material was retrieved from the electronic archive, Institute of Pathology, Klinikum Bayreuth; DC Systeme, Heiligenhaus, Germany. Patients 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).

Table I. *The frequency of isometric mucosal aliquots in colon mucosa biopsies and the age of patients with ulcerative colitis (UC), with ulcerative colitis in remission (UCR), with infectious colitis (IC), with resolving infectious colitis (RIC), with sigmoid diverticulitis (SD) and with normal colon mucosa (NMC).*

Clinical diagnosis	No. cases	No. mucosal rings	Age (years)
UC	99	2,860 28.9 (11-59)	49.6 (12-82)
UCR	31	1,319 42.5 (21-60)	48.1 (20-81)
IC	28	849 30.3 (6-50)	55.1 (21-77)
RIC	7	340 45.6 (36-86)	61.0 (28-90)
DS	19	795 41.8 (14-70)	67.8 (54-79)
NCM	40	1,861 46.5 (32-114)	48.8 (22-85)
All	224	8,024 1,337.3 (6-114)	330.4 55.0 (12-90)

The mean and (range) are given for each subgroup.

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 sampled mucosal clones were standardized by drawing a digital circle of 500 mm^2 (see below) on whole slide scans from colon biopsies in 224 patients (Table I): 99 with UC, 31 with UC in remission, 28 with infectious colitis (IC), 7 with resolved IC, 19 with diverticular sigmoiditis, and 40 having normal colon mucosa.

The indications for a colonoscopy with biopsies in patients with NCM were: i) screening, ii) microscopic colitis, iii) irritable bowel syndrome, iv) colon polyp, v) <14 days diarrhea, vi) diarrhea/vomiting/sickness, vii) hematochezia/lower abdominal pain, viii) hematochezia/NSAID intake, ix) celiac disease, normal duodenum, and x) weight loss (known gastric cancer).

Rejected from the study were 52 additional biopsies (12 with UC, 11 with IC, 6 with diverticular sigmoiditis, and 23 with normal colon mucosa), as the width of the mucosal fragments were not encompassed by the digital circle.

The isometric digital circle. Searching for the adequate standard digital circles that could include a relatively large number of biopsies with cross-cut colon crypts, various digital ring dimensions were tested. After multiple trials, a digital circle measuring 0.500 mm^2 was finally adopted as standard to assess in a relatively large cohort of biopsies, the number of cross-cut crypts per mucosal aliquot.

Counting cross-cut crypts in isometric digital mucosal samples. Each cloned-figure depicting a 0.500 mm^2 ring containing cross-cut crypts, was stored in an electronic library. By ticking at individual images stored in the library, the figures could be retrieved in a Preview display. By ticking the sketch function of the Preview display, a doodle

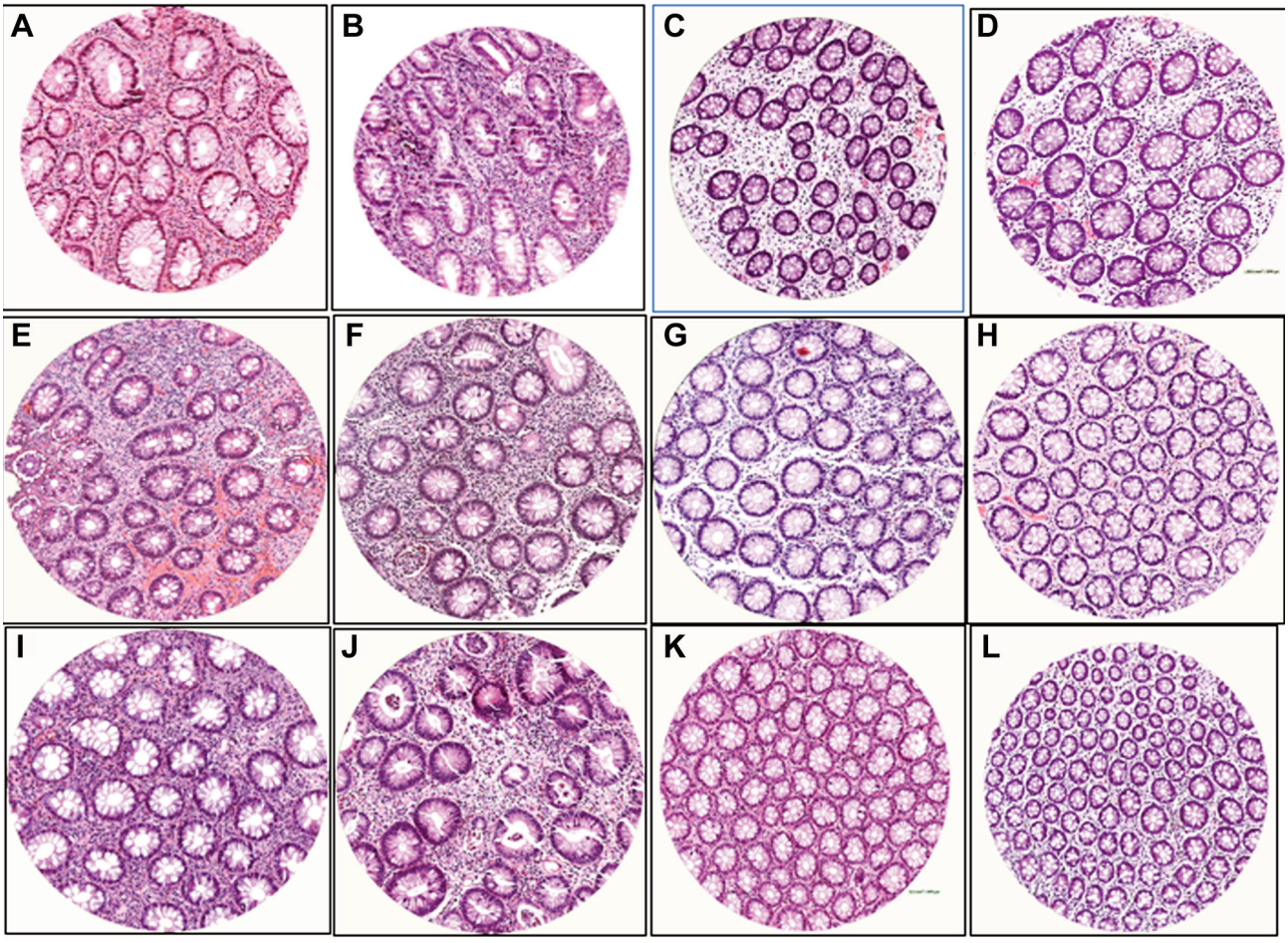

Figure 1. *Examples of digital mucosal samples from cross-cut colon biopsies measuring 0.500 mm^2. A, B) Ulcerative colitis (H&E, original ×10). C, D) Ulcerative colitis in remission (H&E, original ×10). E, F) Infectious colitis (H&E, original ×10). G, H) Resolving infectious colitis (H&E, original ×10). I, J) Sigmoid diverticulitis (H&E, original ×10). K, L) Normal colon mucosa (H&E, original ×10).*

line was activated, which permitted to mark and count successively cross-cut crypts. In addition, this procedure ensured that all cross-cut crypts present in the isometric cloned samples were counted.

Counting procedure. Counting of cross-cut colon crypts was done as previously described for cross-cut rectal crypts: entire cross-cut colon crypts found within the ring as well as those cut by the right half side of the ring (clockwise 12 to 6 h) were counted, but not those cut by the left side of the ring (clockwise 6 to 12 h). A crypt branching (14) was counted as one crypt. Individual countings were registered in a Microsoft Excel spreadsheet.

Statistical analysis. The non-parametric Mann–Whitney *U* two-tail test was applied to compare differences between two groups, the Pearson's correlation coefficient to measure the statistical relationship between two continuous variables, and one-way ANOVA including Tukey HSD, to assess possible statistical differences between the mean of the independent groups. Statistical significance was defined as $p<0.05$ (SPSS software, Stockholm, Sweden).

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

Results

Numbers of cross-cut crypts registered in digital mucosal samples. The results condensed in Table I show that a total of 8,024 cross-cut crypts were registered in digital mucosal samples: 2,860 (35.6%) in 99 cases with UC, 1,319 in 31 cases with UCR (16.4%), 849 (10.6%) in 28 cases with IC, 340 (4.2%) in seven cases with resolving IC, 795 (9.9%) in 19 cases with DS, and 1,861 (23.2%) in 40 cases with NCM. The statistical analysis showed that the frequency of cross-cut crypts in standard digital circles was significantly lower in UC than in the following ailments: UCR (significant at $p<0.05$, *p*-

Table II. *The frequency of cross-cut crypts found in isometric mucosal aliquots of colon mucosa in 110 males and in 114 females.*

Clinical diagnosis	Males	No. of crypts	Females	No. of crypts	Total sex	No. of crypts
UC	48	1,387 28.9 (11-59)	51	1,473 28.8 (11-51)	99	2,860
UCR	16	724 45.2 (21-94)	15	595 39.6 (26-60)	31	1,319
IC	11	283 25.7 (7-50)	17	566 33.3 (6-50)	28	849
RIC	4	168 42.0 (39-45)	3	172 57.3 (36-86)	7	340
SD	9	358 39.7 (16-70)	10	437 43.7 (14-67)	19	795
NCM	22	1,024 46.5 (32-86)	18	837 46.5 (33-114)	40	1,861
All	110		114		224	8,024

The mean and (range) are given for each subgroup. UC: Ulcerative colitis; UCR: ulcerative colitis in remission; IC: infectious colitis, RIC: resolving infectious colitis; SD: sigmoid diverticulitis; NMC: normal colon mucosa.

Table III. *Counting´s reliability by five pathologists working in three disparate European Countries.*

	Pathologist No. 1	Pathologist No. 2	Pathologist No. 3	Pathologist No. 4	Pathologist No. 5
No. of samples	26	28	31	40	27
No. crypts	1,384	1,151	1,108	1,127	1,180
Mean (range)	53.2 (28-109)	44.6 (24-97)	42.6 (20-96)	43.4 (32-72)	43.7 (25-97)

value≤0.00001), RIC (significant at $p<0.05$, p-value=0.00052), DS (significant at $p<0.05$, p-value=0.0018), and NCM (significant at $p<0.05$, p-value≤0.00001). On the other hand, no statistical difference was found in the frequency of cross-cut crypts in standard circles, between UC and IC [not significant at $p<0.05$, p-value=0.4777 (Mann–Whitney two-tailed test)].

Examples of isometric mucosal samples for UC, UCR, IC, RIC, DS, and NCM are illustrated in Figure 1.

Possible confounders. Age. The frequency of cross-cut rings found in cloned isometric rings from colon mucosa in males and females is shown in Table I. The difference between various ailments was not significant at $p<0.05$ for UC, $p=0.585881$; for UCR, $p=0.25014$; for IC, $p=0.218045$, for SD, $p=0.648034$; and for NCM, $p=0.328834$ (Pearson correlation coefficient). The numbers of cases with RIC were too few for analysis.

Sex. The frequency of cross-cut rings found in cloned isometric rings from colon mucosa in males and females is shown in Table II. The difference between males and females was not significant at $p<0.05$: for UC, $p=0.67448$; for UCR, $p=0.25014$; for IC, $p=0.1096$; for SD, $p=0.6818$; and for NCM, $p=0.9681$ (Mann–Whitney, two tailed). The numbers of cases with RIC were too few for analysis.

Testing counting reliability. All countings were primarily carried out by one of us (CAR). To test the reliability of the counting method, ≥25 unselected isometric ring samples including ≥one of the six groups in Table I were e-mailed to the four co-authors, together with instructions of how to assess the number of cross-cut crypts in isometric rings (Methods, vide supra). The 152 countings, together with the countings of the senior author (n=40) were tested with One Way ANOVA, including Tukey HSD. The results in Table III show that the difference between countings by the five pathologists was not significant ($p=0.131087$).

Discussion

In this analysis, cross-cut digital microscopic mucosal fragments of colon biopsies displaying CCC were analyzed. The frequency of CCC in isometric digital cloned mucosal samples of colon biopsies was significantly lower in UC than in i) UCR, ii) RIC, iii) DS, and iv) NCM. However, no statistical difference was found between UC and IC. The similar frequency of CCC in isometric mucosal samples in UC and IC, strongly suggest that the number of CCC per isometric digital sample, decreased during ongoing mucosal inflammation. Notably, the frequency of CCC both in UC and

IC were significantly lower than that in DS, suggesting that inflammation per se might not be the undisputable cause for the low number of crypts in UC and IC. The possible cause(s) for the similarity in the low frequency of CCC in cloned isometric mucosal samples in UC and IC, remains elusive.

The frequency CCC in isometric colon mucosal samples with various colon ailments including NCM listed in Table I, was not influenced by confounders such as the age and sex of the patients. This fact reinforced the conviction that the low frequency of CCC in isometric mucosal samples with UC and IC were genuine findings, inherent to those two inflammatory conditions. It should be stressed that the low frequency of CCC both in UC and IC might not result in a clinical difficulty, since UC is a long-lasting disease, and IC a short-lived disease, lasting approximately 1-3 days in children and about 7 days in adults (15).

In conclusion, this survey showed that by the simple digital algorithm of counting CCC in standardized microscopic mucosal samples from cross-cut colon biopsies, it was possible to differentiate between patients with UC (long-lasting mucosal inflammation) and IC (short-lasting inflammation) on the one hand, and UCR, RIC, DS (persistent inflammation) and NCM, on the other. These findings are remarkable, considering that only 0.500 mm^2 of a calculated large bowel mucosal surface of 2 m^2 (16), were analyzed. The counting of CCC in the algorithm by five pathologists working in three disparate European Countries was found to be reproducible.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

CAR was responsible for the conceptualization, conducting the project, visualization, writing the original draft, and data curation, formal analysis, investigation, and methodology. CL-S and MV scanned the sections. MV, CL-S, CM, and KK participated in the counting reliability quiz and reviewed and edited the original draft. The final draft was approved by all Authors.

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