Variability in the distribution of histological disease activity in the colon of patients with ulcerative colitis.

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Abbreviations

CI	confidence interval
GS	Geboes score
ICC	intraclass correlation
IQR	interquartile range
MES	Mayo endoscopic subscore
NHI	Nancy histological index
RHI	Robarts histological index

SD standard deviation

UC ulcerative colitis

UCEIS ulcerative colitis endoscopic index of severity

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Abstract:

Objective: Histological activity scores were developed and validated. Data on the distribution of the histological inflammation within one segment in patients with ulcerative colitis (UC) are lacking. This impacts on the reliability of the histological activity scores. The aim of this study was to assess the variability in histological activity within one endoscopic segment in patients with UC.

Design: Biopsies were taken in sequential patients with UC in 3 adjacent contiguous regions within a macroscopically homogeneous colonic segment. Biopsies were scored for Geboes score (GS), Robarts histological index (RHI) and Nancy histological index (NHI). Variability was assessed by Kappa statistics for categorical outcomes and intraclass correlation coefficient (ICC) for continuous outcomes.

Results: A total of 161 biopsy sets from 55 endoscopic segments of 21 patients were analysed. Endoscopic active disease was present in 45% of segments. The continuous histological scores showed excellent agreement between the different regions. The ICC for RHI in all segments was 0.974 (95% confidence interval (CI) 0,958 - 0,984; $p < 0.0001$) and 0.98 (95% CI: 0,968 - 0,988; $p < 0.0001$) for the numerically converted GS. The categorical NHI showed higher variability: $\kappa = 0.574$ (95% CI: 0.571-0.577; $p < 0.0001$). In all segments the highest variability was seen in samples with NHI=2. When dichotomizing based on histologic remission, substantial agreement was seen for all scores, with $\kappa > 0.734$ for all cut-offs. The homogeneity in the distribution of the histological disease activity was comparable between the colonic segments.

Conclusion: Distribution of histological disease activity in UC follows a homogeneous pattern in different locations of one segment.

Keywords: Histological scores, inflammatory bowel disease, clinical trial endpoints, advanced imaging, histological remission.

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Summary box:

What is already known about this subject?

- Validated histological scores have been developed to assess histological disease activity in ulcerative colitis.
- A disconnect exists between endoscopic and histological disease activity, especially in patients with low endoscopic disease activity.

What are the new findings?

- Histological disease activity in normal or macroscopically homogeneously inflamed colon is regularly distributed.
- The variability of the histological activity is dependent on the histological score that is used.
- Binary scoring of histological remission reduces the variability in the score.

How might this impact on clinical practice in the foreseeable future?

- Histological remission is reliable as a clinical trial endpoint as it is independent of the location where the biopsy is taken.
- A single biopsy is a reliable representation of the histological inflammation in a macroscopically homogeneously inflamed or normal colon.
- Histological scoring can be applied as a gold standard when developing advanced endoscopic techniques.

Introduction:

The pathogenesis of ulcerative colitis (UC) is a complex interaction between the immune system, the gut microbiome and environmental factors in genetically predisposed subjects. Inflammatory cells infiltrate the colonic mucosa. Typical histological features of active UC include diffuse architectural disturbance and increased mixed inflammatory infiltrate in the lamina propria, together with widespread epithelial damage and mucin depletion, and possibly Paneth cell metaplasia.^{1,2} In contrast to Crohn's disease the distribution of the macroscopic inflammation in UC is continuous starting from the anal margin and spreading to more proximal segments. Histological changes are assumed to follow the macroscopic pattern. Data that effectively support this assumption are however lacking. Moreover, some older studies suggest that, certainly after treatment, the distribution of the histological inflammation might become more patchy.^{3,4} A recent analysis of colectomy specimens showed significant microscopic heterogeneity of the inflammation in a single bowel segment. This study assessed the percentage of discrepancy in the histological scoring with the Robarts histological index (RHI)⁵ and Nancy histological index (NHI).^{6,7} The ongoing debate on how the histological inflammation is really distributed in UC has important implications for drug effectiveness testing and for clinical practice. Histological remission is currently proposed as a new endpoint in clinical trials.⁸ In that case, data on histological remission are only valuable if the location of the biopsy in an inflamed segment has no impact on the scoring of histological disease activity. Second, with advanced endoscopic techniques including magnifying endoscopy, computer-aided diagnosis and image analysis it is currently possible to assess in real-time the histological inflammation in UC.⁹⁻¹³ Measurements of histological inflammation with these emerging techniques are only

reliable if inflammation is indeed equally distributed within a colonic segment. Therefore, more insight in the distribution of histological inflammation in UC is needed before histological remission and real-time endoscopy-based histological assessments can be implemented as a target.

The aim of this study was to assess the variability in histological disease activity within one colonic segment in patients with UC.

Methods:

Data collection:

This was a prospective study in patients with UC undergoing endoscopic assessment during routine clinical care. All patients had a confirmed diagnosis of UC based on clinical, endoscopic and histological criteria.¹⁴ The indication for the endoscopy was the assessment of the severity and extent of disease activity or healing. Colonoscopy preparation was based on a water enema. Clinical symptoms (full Mayo score), UC treatments and disease duration were recorded. Biochemical data were analysed if available.

Endoscopic procedure:

All procedures were performed by the same endoscopist (PB) with a standard non-magnifying endoscope EC-760R-V/L type (Fujifilm, Tokyo, Japan) and processor VP-7000 (Fujifilm, Tokyo, Japan). A cap was mounted on the top of the endoscope to improve visualization of the region of interest. Prior to the assessment, the colonic wall was rinsed with a waterjet (water with diluted simethicone). The left colon was subdivided in three

segments based on the anatomical landmarks (rectum, sigmoid colon, descending colon).

The segments were evaluated from distal to proximal. All segments were scored with white light illumination using the Mayo endoscopic subscore (MES)¹⁵ and Ulcerative Colitis Endoscopic Index of Severity (UCEIS)¹⁶. The depth of intubation was dependent on the clinical indication and the tolerance of the patient. In all patients at least the rectum and sigmoid was evaluated. Then, per segment, three regions of interest were systematically evaluated with a standardized approach. These three regions of interest were adjacent and were located in an area with endoscopic continuous inflammation or continuous healed mucosa (i.e. not in the area surrounding the upper margin of the inflammatory extent). In case severe ulcers were present (i.e. MES= 3) the region of interest and corresponding biopsy location was located at the border of the ulcer. The middle region was the reference for the two other regions that were located 1-2 cm above (above region) and 1-2 cm below (below region) the middle region as illustrated in **figure 1**. After endoscopic evaluation and image capturing, biopsy (one per region) was taken with a biopsy forceps (Radial Jaw 4, Boston Scientific, Marlborough, Massachusetts, United states of America) in each region and fixed instantly in formaldehyde 4 %.

Histopathological assessment:

All biopsies were routinely processed. Five µm-thick serial sections at 2 different levels were mounted on glass slides and stained with hematoxyline-eosine. Biopsies were scored *blinded* by a histopathologist with >15 years of experience and expertise in scoring disease activity in UC (GDH). All biopsies were scored for the Geboes score (GS)¹⁷, RHI⁵ and NHI⁶. The Geboes score was converted in a numerical score to enable intraclass correlation

coefficient (ICC) statistics. Cut-offs for histological non-remission were defined as GS $\geq$ 2B.1, RHI >3 and >6 , NHI ≥ 2 .^{6,18,19}

Statistics:

Descriptive statistics for continuous variables are described using means with standard deviation (SD), categorical variables using medians and interquartile ranges (IQR). The variability of the histological scores between the different segments was calculated using Kappa statistics (Cohen and Fleiss kappa) for categorical scores and intraclass correlation coefficient (two way mixed model for consistency) for continuous or numeric scores. Following definitions for reliability were used: $\kappa < 0$ 'poor'; 0 to 0.20 'slight'; 0.21 to 0.4 'fair'; 0.41 to 0.60 'moderate'; 0.61 to 0.8 'substantial'; > 0.81 'almost perfect'.

Ethical aspects:

All patients provided written informed consent prior to the study procedures. The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki 2008 and in compliance with the principles of Good Clinical Practice, according to the International Conference on Harmonisation Harmonised Tripartite Guideline. The study was approved by the Ethics Committee of the University hospital Leuven (S59405 EC approval number).

Results:

Patient population

In total, 161 regions of interest were biopsied in 55 endoscopic segments originating from 21 patients with UC. The baseline characteristics are summarized in **table 1**. Five patients were in clinical remission based on the total Mayo score (≤ 2). In total 45% of the segments had active endoscopic lesions defined as UCEIS ≥ 2 . Fifty-five percent of the patients had active histological inflammation defined as NHI >1 in the mid segment.

Variability continuous scores:

The distribution of the inflammation in segments with an endoscopic homogeneous aspect was assessed by the variability of the histological scores from the 3 adjacent regions. The ICC values for all segments when using the RHI was 0.974 (95% confidence interval (CI) 0,958 - 0,984; $p < 0.0001$) and 0.98 (95% CI: 0,968 -0,988; $p < 0.0001$) when using the numerically transformed GS. Similar values were seen in a per segment analysis (**table 2**). These results reflect a very low variability and equal distribution of the microscopic inflammatory infiltrate measured by numerical scales.

Variability in ordinal score:

When comparing the results of the histological inflammation based on the ordinal NHI higher variability was seen. The comparison of all regions within one segment showed a κ -value of 0.574 (95% CI: 0.571-0.577; $p < 0.0001$). Similar findings of a moderate correlation were seen in a per segment evaluation and when comparing regions within one segment pair-wise. Details are summarized in **table 3**.

Variability in histological remission:

As thresholds for histological remission are used for treatment planning in the routine clinical care for patients with UC, we assessed the variability of the histological inflammation based on various established cut-offs for histological remission. Low variability was seen for the different cut-offs with κ -values all >0.73 . The lowest variability was seen for the cut-off $\text{RHI} > 6$, which is however a less strict threshold for histological remission. Similar findings were seen in a per segment evaluation and when comparing regions within one segment pair-wise. Details are summarized in **supplementary table 1**.

Impact endoscopic disease activity:

Since some patients had active histological inflammation in endoscopically normal appearing mucosa, we also compared the variability between segments with active endoscopic lesions and those without. We could find a higher overall variability in active segments based on RHI and NHI. For assessment based on the GS the variability was higher in the inactive segments. Details are summarized in **table 4**.

Discussion:

In this study, using a systematic endoscopic imaging and biopsy protocol, we demonstrated for the first time a low variability in histological inflammation in colonic segments with a uniform endoscopic appearance. The variability was influenced by the scoring system used for the assessment of the histological disease activity and by endoscopic activity. No clear difference in the distribution between different colonic segments was found although the variability was lower in the rectum compared to the sigmoid. Furthermore, we showed that

binary scoring of histological remission does not impact on the performance of the scoring system, moreover it decreases the variability of the NHI.

The distribution and mapping of the mucosal inflammatory infiltrate in patients with active UC has not been explored in vivo to date. The generally accepted opinion, stating that the histological disease activity is equally distributed, is extrapolated from the macroscopic appearance of the endoscopic activity of UC, but has not been formally proven. Additionally, there is a disconnect between endoscopic and histological disease activity, especially in the lower end of the disease spectrum.²⁰ Furthermore, several papers suggested that there might be a variance in the distribution of the inflammatory infiltrate in UC.^{3,4,7} Since histological remission is suggested to be a formal and new endpoint for UC therapy and may be important to tailor an exit strategy, we felt that the exact distribution of histologic disease features should be prospectively explored.

Our results have important implications for the conduct of clinical trials in UC, certainly in an era where the role of histological remission is gaining importance.²¹ First, as the variability in the distribution of the inflammation is low, the number of biopsies and their location within a segment are not so critical, as long as the biopsy is obtained from a macroscopically homogeneous inflamed (or normal) segment. Investigational protocols often state to take the biopsies between 20-25 cm from the anal margin. Our results now suggest this practice is not obligatory as the variability of the distribution of the inflammatory infiltrate is low. Furthermore, the variability is the lowest in the rectum compared to the sigmoid, which implies that histological data from patients with proctitis might be as reliable as patients with more extended disease. Second, our results underline the impact of the different histological scores on the results of histological outcomes in

clinical trials. The GS and the RHI are the preferred scoring systems when assessing histological disease activity as an absolute value, since these scores demonstrate a low variability. The NHI however has a higher variability, which is mainly driven by NHI grade 2 ($\kappa = 0.21$) and to a lesser extent grades 3 and 4. The inconsistency in the results of the NHI, can be overcome by using the histological score in a binary way. For all scores, the dichotomization between remission and non-remission results in a low variability that is comparable between the scores.

Our results also impact on daily clinical care for patients with UC. Since histological remission is the best predictor of a favourable disease course over time, efforts are made to harmonize the histological assessments in UC.^{22,23} Our results do further support the incorporation of histological sampling and scoring to guide clinical decision making in daily practice. Based on our study, it can be assumed that biopsies taken in an endoscopic homogeneously inflamed segment are a reliable representation of the histological disease activity of patient with UC. The performance of the histopathologist that provides the histological score is still a confounder. To maximally decrease the variability in the histological scoring, we recommend extensive training and the application of scoring conventions in clinical practice for those histopathologists that use the incorporate histological scoring for UC.^{5,24}

Finally, our study might have implications for the development of new endoscopic tools. Currently several magnifying endoscopy techniques have been proposed for real-time histological assessment either based on human evaluation^{11,25} or artificial intelligence-based computer-aided diagnosis^{9,10}. These techniques are dependent on the reproducibility of histological scoring at different sampled locations in a given colonic

segment. Moreover, other approaches use histological disease activity as the gold standard for the development of image analysis techniques of endoscopic images of a colonic segment^{26,27} Histological disease activity can only serve as a reference value if it represents the effective grade of histological inflammation of the visualised segment. By confirming the low variability in the distribution we can state that these newer techniques are effectively meaningful in assessing disease activity in an objective way when using histological scores as reference.

One strength of this study is the systematic sampling of different adjacent regions from one colonic segment based on standardized protocol. All procedures were furthermore performed by the same endoscopist and the evaluation of the biopsies by the same histopathologist to avoid interrater variability. Another strength is that patients were included from routine clinical care and in this way represent the overall UC population rather than a selected population as is seen in clinical trials. Our study also has some limitations. This was a monocentric study performed in a tertiary center and can for this be subjected to a selection bias. In this case external validation might be required. Although we aimed to evaluate the complete spectrum disease activity, patients with severe endoscopic lesions and patients with disconnect between endoscopic remission and persistent histological inflammation might be underrepresented. For this validation in a larger cohort is required. Finally, as mentioned above, we used a single observer evaluation for endoscopic and histological scoring to avoid interrater variability. This choice was made since no conclusive evidence exists that interrater variability in the scoring of histological lesions can be completely avoid by multiple reading and adjudication or averaging.²⁸

In conclusion, our study is the first to clearly map the distribution of the histological disease activity in patients with UC in a systematic way in vivo. Histological scores from mucosal biopsies taken in a homogeneous inflamed or normal segment are representative for the disease activity.

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Figure 1: Schematic representation of the different regions of interest that were assessed during the study.

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Table 1: Baseline characteristics of the patients include in the study (IQR: interquartile range; TNF: tumour necrosis factor; UCEIS: ulcerative colitis endoscopic index of severity)

Variable	Cohort (n=21)
Gender (male/female)	10/11
Median age (years; IQR)	48.4 (26.4-52.8)
Disease duration (months; IQR)	26 (5.6-90.7)
Maximal disease extent (Montreal classification E1/E2/E3)	1/16/4
Median C-reactive protein (mg/L; IQR) (n=19)	3.4 (1.1-9.8)
Current treatment	
5-Aminosalicylates	14
Steroids	5
Immunomodulator	2
Anti-TNF	3
Vedolizumab	2
Tofacitinib	2
Previous treatment	
5-Aminosalicylates	6
Steroids	10
Immunomodulator	3
Anti-TNF	4

Vedolizumab	4
Tofacitinib	1
Median UCEIS (IQR)	
Rectum	4 (0-5)
Sigmoid	0 (0-4)
Descending	1 (0-5)
Median Mayo endoscopic subscore (IQR)	
Rectum	2 (0-2)
Sigmoid	0 (0-2)
Descending	1 (0-2)
Median total Mayo score (IQR)	
Rectum	5 (3-8)
Sigmoid	3 (2-8)
Descending	4 (2-8)

Table 2: Result of the ICC comparing the histological scoring of the adjacent regions. (CI: confidence interval; ICC: intraclass correlation coefficient; RHI: Roberts histological index)

Histological score	Colonic segment	ICC	95% CI	p-value
RHI	All segments	0,974	0,958 - 0,984	<0.0001
	Rectum	0,965	0,928 - 0,985	<0.0001
	Sigmoid	0,978	0,952 - 0,991	<0.0001
	Descending	0,983	0,952 - 0,995	<0.0001
Geboes score	All segments	0,980	0,968 - 0,988	<0.0001
	Rectum	0,974	0,947 - 0,989	<0.0001
	Sigmoid	0,980	0,957 - 0,992	<0.0001
	Descending	0,986	0,962 - 0,996	<0.0001

Table 3: Result of the Cohen's and Fleiss kappa comparing the results of the Nancy histological index of the adjacent regions. (CI: confidence interval)

<i>Cohen's kappa</i>		mid/above	p-value	above/below	p-value	mid/below	p-value	
Nancy ordinal	All segments	0,59	<0.0001	0,518	<0.0001	0,605	<0.0001	
	Rectum	0,504	<0.0001	0,431	<0.0001	0,501	<0.0001	
	Sigmoid	0,558	<0.0001	0,618	<0.0001	0,682	0.003	
	Descending	0,747	<0.0001	0,5	0.005	0,644	<0.0001	
<i>Fleiss kappa</i>	All segments	95% CI	Rectum	95% CI	sigmoid	95% CI	descending	95% CI
Nancy ordinal *	0,574	0.571- 0.577	0,473	0.469- 0.478	0,636	0.631- 0.641	0,621	0.615- 0.628

*all p<0.0001

Table 4: comparison of the variability of the histological scoring of adjacent regions between endoscopic active and inactive segments. Intra class correlation coefficient for continues scores and Fleiss kappa for ordinal scores GS: Geboes score; RHI: Robarts histological index; Nancy histological index; CI: confidence interval)

	inactive segments	95% CI	active segments	95% CI
GS *	0.847	0.664-0.938	0.923	0.851-0.964
RHI *	0.963	0.928-0.982	0.901	0.809-0.953
NHI *	0.565	0.56-0.569	0.379	0.374-0.383

*all $p < 0.0001$

Figure 1