Mod Pathol 37 (2024) 100419

MODERN PATHOLOGY

USCAP UNITED STATES AND CANADIAN ACADEMY OF PATHOLOGY
Creating a Better Pathologist

Journal homepage: https://modernpathology.org/

Research Article

Poor Diagnostic Reproducibility in the Identification of Nonconventional Dysplasia in Colitis Impacts the Application of Histologic Stratification Tools

Nadia Nasreddin[a], Marnix Jansen[b], Maurice B. Loughrey[c,d], Lai Mun Wang[e], Viktor H. Koelzer[f], Manuel Rodriguez-Justo[b], Marco Novelli[b], Jennifer Fisher[g], Matthew W. Brown[a], Ibrahim Al Bakir[h], Ailsa L. Hart[h], Philip Dunne[i], Trevor A. Graham[g], Simon J. Leedham[a,*]

[a] *Wellcome Centre Human Genetics, Roosevelt Drive, University of Oxford, Oxford, United Kingdom;* [b] *Department of Pathology, UCL Cancer Institute, University College London, London, United Kingdom;* [c] *Department of Cellular Pathology, Belfast Health and Social Care Trust, Belfast, Northern Ireland;* [d] *Centre for Public Health, Queen's University Belfast, Belfast, Northern Ireland;* [e] *Department of Laboratory Medicine, Changi General Hospital, Singapore, Singapore;* [f] *Department of Pathology and Molecular Pathology, University and University Hospital Zürich, Zürich, Switzerland;* [g] *Centre for Evolution and Cancer, Institute of Cancer Research, London, United Kingdom;* [h] *IBD Unit, St Mark's Hospital, Harrow, London, United Kingdom;* [i] *Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, United Kingdom*

ARTICLE INFO

Article history:
Received 6 July 2023
Revised 10 December 2023
Accepted 19 December 2023
Available online 27 December 2023

Keywords:
inflammatory bowel disease
dysplasia
colorectal cancer
classification
cancer risk

ABSTRACT

Due to their increased cancer risk, patients with longstanding inflammatory bowel disease are offered endoscopic surveillance with concomitant histopathologic assessments, aimed at identifying dysplasia as a precursor lesion of colitis-associated colorectal cancer. However, this strategy is beset with difficulties and limitations. Recently, a novel classification criterion for colitis-associated low-grade dysplasia has been proposed, and an association between nonconventional dysplasia and progression was reported, suggesting the possibility of histology-based stratification of patients with colitis-associated lesions. Here, a cohort of colitis-associated lesions was assessed by a panel of 6 experienced pathologists to test the applicability of the published classification criteria and try and validate the association between nonconventional dysplasia and progression. While confirming the presence of different morphologic patterns of colitis-associated dysplasia, the study demonstrated difficulties concerning diagnostic reproducibility between pathologists and was unable to validate the association of nonconventional dysplasia with cancer progression. Our study highlights the overall difficulty of using histologic assessment of precursor lesions for cancer risk prediction in inflammatory bowel disease patients and suggests the need for a different diagnostic strategy that can objectively identify high-risk phenotypes.

Introduction

Colitis-associated colorectal cancer (CRC) remains the most feared complication of inflammatory bowel disease (IBD). The first large meta-analysis estimated ulcerative colitis cancer risk to be 2% after 10 years, 8% after 20 years, and 18% after 30 years of disease duration.[1] Similarly, the overall cancer risk in patients with Crohn's disease was reported as being 2.3% for 10 years, 5.6% for 20 years, and 8.3% after 30 years of disease duration.[2] Since these publications, discrepant studies have reported highly variable colitis-associated CRC risk rates.[3-7] Despite uncertainty on the

* Corresponding author.
E-mail address: simon.leedham@well.ox.ac.uk (S.J. Leedham).

https://doi.org/10.1016/j.modpat.2023.100419

exact cancer risk in patients with IBD, several risk factors have been consistently associated with colitis-associated CRC, including younger age at IBD diagnosis, and extent, severity, and duration of inflammation.

To address increased cancer risk, IBD patients are currently offered endoscopic surveillance, often with concomitant biopsy and histologic assessment. This aims at detecting dysplasia as a precursor lesion of colitis-associated CRC.[8] However, this management strategy is an imperfect gold standard, being beset by both process and disease-intrinsic factors. Among the process-based issues are the difficulty in detecting dysplastic lesions at endoscopy, the poor interrater agreement among pathologists in grading dysplasia, and the difficulty in distinguishing colitis-associated dysplasia from sporadic lesions. Dysplastic polyps resembling sporadic adenomas are frequently detected in IBD patients, with an estimated prevalence of 6.2%, and it has previously been shown that they can be safely managed by endoscopic resections,[5,9,10] resulting in a cancer risk similar to that of sporadic lesions occurring in disease-free segments (0%-6%).[11] Additional difficulty is also contributed by disease-intrinsic factors, including the demonstration of field cancerization in some IBD patients. Field cancerization is the acquisition of widespread oncogenic mutations in extensive patches of epithelium, including the detection of mutations in areas without any histologic evidence of dysplasia.[12-14]

Together, these issues highlight the difficulties and inadequacies of current colitis-associated cancer risk prediction and contribute to postcolonoscopy (interval) CRC rates as high as 30%.[15] Furthermore, the chance of identifying occult cancer foci in surgical resections from patients undergoing prophylactic colectomy for colitis-associated dysplasia is as high as 25% and 50% in those with low-grade dysplasia (LGD) and high-grade dysplasia (HGD), respectively.[15] In contrast, other patients are subjected to frequent intrusive endoscopic surveillance regimes without ever developing CRC because most of the LGD do not progress. Consequently, there is an urgent need to identify applicable and effective risk stratification tools that can discriminate colitis-associated dysplasia patients into those with high and low risk of future cancer progression.

Regarding this, Choi et al[16] and Lee et al[17] investigated the histologic subtypes of colitis-associated colorectal dysplasia and their association with CRC risk.

Literature on colorectal dysplasia is mostly based on well-recognized intestinal-type adenomatous dysplasia, also referred to as conventional dysplasia. However, more recently, other histologic patterns, collectively referred to as nonconventional dysplasia, have been noted at high frequency in patients with IBD, particularly among those patients with concomitant CRC.[16] In a multicenter clinicopathologic study investigating 106 IBD patient-derived lesions, Choi et al[16] identified 6 morphologic patterns of nonconventional dysplasia and outlined morphologic criteria characterizing nonconventional dysplasia subtypes (Table 1). In a follow-up study investigating the potential association between nonconventional dysplasia and CRC in IBD patients, Lee et al[17] suggested nonconventional dysplasia, particularly crypt cell dysplasia, hypermucinous, goblet cell deficient, and sessile serrated lesion-like histologic subtypes, to be associated with a higher risk of colitis-associated CRC progression.

To test consensus agreement and practical application of the Choi et al[16] criteria in a real-world, multicenter setting, we asked 6 experienced gastrointestinal pathologists (M.J., M.B.L., L.M.W., V.H.K., M.R-J., M.N.) to histologically assess an independent cohort of IBD dysplasia using the Choi et al[16] criteria. This was to validate the published association of nonconventional dysplasia with increased cancer risk in our cohort and assess whether histologic classification could be used as a practical and replicable risk stratification tool in IBD dysplasia.

Material and Methods

Samples

Dysplastic lesions from IBD patients were identified and obtained from London North West Healthcare NHS Trust, Oxford University Hospitals NHS Foundation Trust, and Belfast Health and Social Care Trust, under the following ethical approval: London Stanmore committee 10/LO/1613, London, Fulham Research Ethics committee 18/LO/2051, Oxford gastrointestinal Illness ethics, 18/1L/JE/Early-detection, OCHRe:18/A11, London—Fulham Research Ethics committee 18/LO/2051, and 16/NI, 0030. All cases were formalin-fixed paraffin-embedded tissue blocks of lesions biopsied or resected at endoscopy procedures. The lesions were assessed for dysplasia before enrolling in the study by board-certified pathologists, including those contributing to the subsequent study assessment. However, all images were anonymized and shuffled at the study entry, and no individual author contributed more than 10% of the eventual cases used. Clinical annotation with patient outcome was known with a median follow-up time of 5.5 years across the cohort. Based on the clinical follow-up data, IBD patients were categorized as progressors, ie those progressing to cancer within 5 years of lesion detection, and non-progressors, those remaining cancer-free in the 5 years after detection.

Slide Staining, Scanning, and Visualization

Four-micron tissue sections were cut and stained for hematoxylin and eosin using the Leica ST500 Autostainer XL. Stained slides were scanned using an Aperio scanner at $\times$40 magnification. Slide scans were annotated using ImageScope. Each annotated area was a denominated region. Piecemeal resected lesions were divided into multiple separate areas, resulting in multiple regions, to improve the granularity of histologic assessment (70 lesions, 54.26% of the lesions, divided into a total of 173 regions). An illustrative example of a lesion that was divided into multiple regions can be found in Supplementary Figure S4. Biopsy lesions, which are by definition smaller, were annotated as a single region. Where lesions were divided into multiple regions, each region was annotated as a separate file. The annotated files were uploaded into the web image viewer SlideScore for assessors to access (www.slidescore.com). All regions were anonymized, renamed, and randomized, with each pathologist viewing the regions in a different order. The assessment and downstream analysis were performed on a per-region rather than per-lesion or per-patient basis.

Assessors

The study was undertaken with a panel of 6 experienced, tertiary referral center gastrointestinal pathologists. Each had been actively practicing gastrointestinal pathology for a minimum of 10 years. The panel was multicentered and international: 3 pathologists practicing in England, 1 in Northern Ireland, 1in Switzerland, and 1 in Singapore. In this study, the pathologists are referred to with Greek constellation names to maintain anonymity.

Table 1
Choi et al[16] morphologic criteria of colitis-associated nonconventional dysplasia

Nonconventional dysplasia subtypes	Hypermucinous type	Intestinal-type		Crypt cell type	Serrated type		
Dysplasia subtype	Hypermucinous	Dysplasia with increased Paneth cell differentiation	Goblet cell deficient	Crypt cell atypia/ dysplasia	Traditional serrated adenoma-like	Sessile serrated lesion-like	Serrated NOS
Architecture	Tubulovillous/villous	Tubular	Tubular	Flat	Tubulovillous/villous with serration	Tubular with serration	Tubular with serration
Defining features	Tall mucinous cells with typically mildly elongated, hyperchromatic nuclei	Intestinal-type cells with mostly elongated, hyperchromatic nuclei	Intestinal-type cells with mostly elongated, hyperchromatic nuclei	Mostly round-to-oval, nonstratified nuclei	Columnar cells with mostly elongated nuclei, intensely eosinophilic cytoplasm, and ectopic crypts	Prominent serration and dilation at the crypt base and surface, including dilated L- or inverted T-shaped crypts at the interface with muscularis mucosa	Often complex serration but without definite features of traditional serrated adenoma or sessile serrated lesion
	Hypermucinous component should represent >50% of the lesion	Increased Paneth cell differentiation involving at least 2 contiguous crypts in 2 different foci (beyond what is present in background mucosa)	Complete or near-complete absence of goblet cells	Atypia can be limited to the crypt base without obvious surface involvement	Traditional serrated adenoma-like component should represent >50% of the lesion	Sessile serrated lesion-like component should represent >50% of the lesion	Serrated lesion not otherwise specified component should represent >50% of the lesion
Other features	Degree of atypia tends to decrease from the crypts to the surface of the villi	Some loss of goblet cells allowed, but no complete or near-complete absence of goblet cells	Scattered Paneth cells allowed, but not in multiple clusters of dysplastic crypts as seen in dysplasia with increased Paneth cell differentiation	Some loss of goblet cells allowed, but no complete or near-complete absence of goblet cells		May show dysplasia, which may be confined to the lower portion or involve the entire thickness of the mucosa	May show dysplasia, which may be confined to the lower portion or involve the entire thickness of the mucosa
			Similar degree of Paneth cell differentiation is present in the lesion and in adjacent mucosa	Occasional Paneth cells may be seen, but similar degree of Paneth cell differentiation is present in the lesion and in adjacent mucosa			"Hyperplastic polyp-like" changes may be present in adjacent mucosa or represent the entire lesion

Adapted from Choi et al.[16]

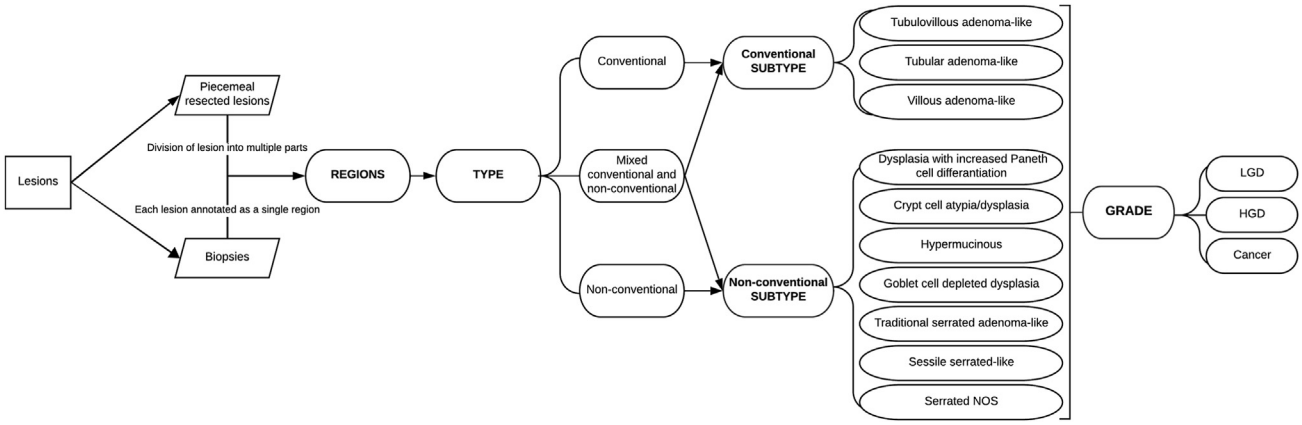


Figure 1.
Stepwise decision tree for the scoring of the regions. Colitis-associated dysplasia was annotated into regions for assessment. For biopsy samples, a region comprised the entirety of the sample. For piecemeal resected lesions, the samples were divided into multiple parts, which were annotated and assessed as separate regions. For each region, each pathologist was asked to determine a dysplasia type, subtype, and grade.

Assessment and Classification Criteria

Before the assessment, a consensus standard operating procedure for the scoring of the regions was set and jointly agreed upon by all the pathologists. Furthermore, exemplary cases from the literature and concrete diagnostic examples were discussed in light of the Choi et al criteria.[16] During the assessment period, each pathologist assessed all regions independently and scored the regions using the web questionnaire within the web viewer; no discussions were held between pathologists at this point. Scoring was performed on a per-region basis, following the criteria outlined by Choi et al[16] (reported in Table 1). The panel was asked to score the type, subtype, and grade for each region as follows (also displayed in Fig. 1). For dysplasia type, diagnostic possibilities of conventional (>80% of the tissue being conventional), nonconventional (>80% of the tissue being nonconventional), or mixed (for regions with mixed conventional and nonconventional histologic features, where no >80% majority tissue composition was found) could be chosen. For dysplasia subtype, diagnostic possibilities of tubular adenoma-like, tubulovillous adenoma-like, and villous adenoma-like (for conventional dysplasia) or hypermucinous, dysplasia with increased Paneth cell differentiation, crypt cell atypia/dysplasia, goblet cell deficient, traditional serrated adenoma-like, sessile serrated adenoma-like, and serrated lesion not otherwise specified (for nonconventional dysplasia) were provided. For mixed regions, ie, regions with 2 or more histologic components, a major component (comprising >50% of the tissue) and a minor component (comprising >50% of the tissue) could be scored with any conventional or nonconventional subtypes. Dysplasia grade was based on the worst grade identified within the region, with diagnostic possibilities of no dysplasia, LGD, HGD, or adenocarcinoma. The pathologists were not provided the diagnostic possibility of indefinite dysplasia for dysplasia grade because none of the dysplasia were originally signed out as indefinite for dysplasia and to avoid impacting statistical power by adding further diagnostic categories.

Outcome Measures

The outcome measures were interrater agreement in scoring a region's type, subtype, and grade; the percentage of progressor regions with a diagnosis of nonconventional dysplasia; and the percentage of progressor regions with high-risk histology diagnosis. In measuring the interrater agreement for each region, the type, subtype, and grade scoring of the pathologists was compared and the following were determined: (1) the agreement score of each pathologist, by calculating the mean of the percentage agreement of each pathologist across all assessed regions (referred to as agreement score); (2) study percentage agreement, determined by first calculating the percentage agreement across pathologists for each region and then taking the mean of such scores (referred to as study interrater agreement); (3) Cohen's kappa, and (4) Fleiss' kappa. A majority histologic diagnosis was reached when 4 or more pathologists were in agreement.

Assessment of Association Between Nonconventional Dysplasia and Progression

To assess for a correlation between nonconventional dysplasia and cancer progression, we correlated and statistically tested the association of histologic diagnosis with patient progressor/non-progressor status (Fisher exact test). Two levels of analysis were undertaken. First, majority diagnosis regions, defined above, were assessed. In the second analysis, some additional regions with mixed histologic diagnosis that had not reached consensus were included by deferring to the apparent worst morphologic grade reported (in accordance with standard histologic practice). Therefore, mixed conventional and nonconventional dysplasia scores were considered nonconventional (Schematic breakdown in Supplementary Fig. S1).

Assessment of Association Between Nonconventional Histologic Subtype and Progression

To assess for a correlation between nonconventional histologic subtypes and cancer progression,[17] we correlated and statistically tested the association of subtype diagnosis with patient progressor/non-progressor status (Fisher's exact tests). Two levels of analysis were undertaken. First, the assessment was carried out on all majority diagnosis regions, defined above. In the second assessment, some additional regions with mixed histologic subtype dysplasia that had not reached consensus were included by deferring to the apparent worst subtype morphologic grade

Table 2
Cohort clinical details

Patient group	Non-progressors	Progressors	SS	*P* value and test
No. of patients	56	33	–	–
Sex				
Female	12	12	NS	.14 (Fisher's exact test)
Male	44	21		
Age (y)				
Median	60	60	NS	.68 (Mann-Whitney *U* test)
Range	28-78	32-82		
PSC				
Yes	7	7	NS	.37 (Fisher's exact test)
No	49	26		
Disease duration				
Median	27	19	NS	.21 (Mann-Whitney *U* test)
Range	0-57 (±13.0)	0-50 (±12.7)		
Longstanding	–	4	NA	NA
Disease extent				
Extensive	54	25	NS	.59 (Fisher's exact test)
Left-sided	2	2		
Right-sided	–	–	NA	NA
NA	–	6	NA	NA
Time to progression				
Median	–	1.3 (±1.9)	NA	NA
Progression-free follow-up time				
Median	7.7 (±2.4)	–	NA	NA
No. of follow-up endoscopies/y follow-up				
Median	0.8 (±0.3)	1.4 (±1.5)	[a]	.009 (Mann-Whitney *U* test)
No. of biopsies/colonoscopies				
Median	10.8 (±7.2)	13.8 (±4.7)	[b]	.02 (Mann-Whitney *U* test)
Percentage of chromoendoscopies out of all colonoscopies				
Percentage	69 (±138.4)	50 (±37.5)	NS	.12 (Mann-Whitney *U* test)

NA, not applicable; NS, not significant; SS, statistical significance.
[a] $.001 \leq P$ value $< .01$.
[b] $.01 \leq P$ value $< .05$.

reported (in accordance with standard histologic practice). For example, for a region where hypermucinous and tubular adenoma-like histologies were reported, the region was denoted as hypermucinous, for the same rationale as above (Schematic breakdown in Supplementary Fig. S2).

Statistical Analyses

All statistical analyses were carried out on R (version 1.3.959). For the assessment of clinical and lesions' features, Fisher's exact tests (for categorical variables) and Mann-Whitney *U* tests (for continuous variables/medians) were used. Calculations of inter-rater agreeability were performed using the R package irr. The association between progression and histologic type/subtype was assessed using Fisher's exact test on R.

Results

Demographics and Lesion Details

The colitis-associated LGD specimens were collected from 89 IBD patients, with IBD duration ranging from 0 to 57 years. Of the 89 patients, 56 were denoted as progressors and 33 were non-progressors (Table 2). Progressor patients had a median follow-up period of 1.3 years from LGD diagnosis to progression, whereas the median follow-up period of non-progressors was 7.7 years, with all non-progressors being cancer-free for 5+ years after the LGD diagnosis. Progressor patients had a significantly greater number of endoscopies and biopsies taken within this shorter follow-up period, and this more intensive surveillance may have been prompted by an increased size of the index lesions in progressor patients (the majority of the progressor lesions were >10 mm; Table 3). The progressor LGD samples consisted of 64 different lesions, divided into 114 regions. The non-progressor LGD samples consisted of 65 lesions divided into 129 regions (Fig. 2). Most lesions were left-sided (58.1% non-progressor and 67.5% progressor). Furthermore, 58.9% of non-progressor lesions were polypoid, 34.1% were non-polypoid, 3.9% were invisible, and 3.1% had unspecified lesion shape. In contrast, of the progressor lesions, 46.5% were polypoid, 27.2% were non-polypoid, 9.6% were invisible, and 16.7% had unspecified lesion shape (Table 3).

Histology Examples

Examples of morphologic patterns of colitis-associated dysplasia are shown in Figure 3.

Diagnostic Agreement in the Assessment of Colitis Regions

First, the diagnostic agreement between pathologists in the assessment of colitis-associated dysplasia was examined. Poor interrater agreement was found in the typing and subtyping of colitis-associated dysplasia. The lowest diagnostic agreement

Table 3
Cohort lesions details

Patient group	Non-progressors	Progressors	SS	*P* value and test
No. of lesions	65	64	-	-
No. of regions	129	114	-	-
Lesion location				
Left	75	77	NS	.07 (Fisher's exact test)
Right	51	31		
Not specified	3	6	NA	NA
Lesion size				
<10 mm	57	29	NS	.06 (Fisher's exact test)
>10 mm	57	53		
Not specified	15	32	NA	NA
Lesion shape				
Invisible	5	11		
Polypoid	76	53	NS	.12 (Fisher's exact test)
Nonpolypoid	44	31		
Not specified	4	19		

NA, not applicable; NS, not significant; SS, statistical significance.

(percentage agreement of 57%) was observed for the dysplasia subtype, with all pathologists agreeing on the diagnosis of only 13 of 243 sample regions. Slightly better was the diagnostic agreement for dysplasia type, with an interrater agreement of 69%. In contrast, there was consensus in the grading of the dysplasia with an inter-rate agreement of 85%. These results confirm the known difficulty in assessing colitis-associated dysplasia (Fig. 4 and Supplementary Fig. S3) and raise concerns over the reproducibility and consistency of applying conventional versus nonconventional annotations.

Association Between Nonconventional Dysplasia and Progression

Next, we made an attempt to validate Choi et al findings that nonconventional histologic diagnosis is associated with progression risk.[16,17] From the initial 243 histologic regions, full consensus agreement was obtained for only 30 colitis regions, but majority diagnosis, where 4 or more pathologists agreed on a region's dysplasia type, was obtained for 166 regions. Of these, 73.5% were conventional, 21.1% were nonconventional, and 1.8% were mixed.

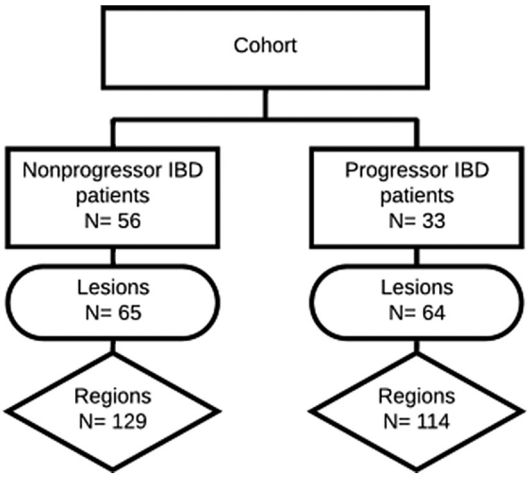


Figure 2.
Breakdown of the number of patients, lesions, and regions in the study.

Initially, we analyzed regions where we had reached a majority diagnosis of conventional, nonconventional, and mixed dysplasia ($n = 166$) and were unable to find a statistically significant correlation between nonconventional dysplasia and patient progression (*P* value =.318, Fig. 5A). We then extended our analysis, as outlined in the methods, which increased the number of regions included ($n = 198$). Despite these increased numbers, no association between nonconventional dysplasia and progression was found (*P* value =.368, Fig. 5B).

Interestingly, 6 regions that had been given a LGD diagnosis in their hospital of origin were scored as non-dysplastic by the pathologist panel, with a majority consensus being reached in all 6. Similarly, 14 other apparent low-grade regions were reclassified as HGD (11 of which with no consensus dysplasia type and 3 conventional/tubulovillous adenoma-like) and 5 regions as adenocarcinomas (4 with no consensus dysplasia type or subtype and 1 conventional/tubulovillous adenoma-like).

Altogether, we were unable to validate the published association between nonconventional dysplasia and patient progression in our cohort.

Association Between Progression and High-Risk Histologies

Having found no association between progression and nonconventional dysplasia in our data set, the next analysis focused on dysplasia subtypes. Consistent with published work, we were able to identify multiple histologic subtypes of colitis-associated dysplasia, with tubular adenoma-like as the most common histologic subtype, accounting for 52.8% of the regions. This was followed by tubulovillous adenoma-like regions (12.8%). The most common nonconventional subtype detected was hypermucinous dysplasia (16%), with smaller numbers of traditional serrated adenoma-like, and dysplasia, with increased Paneth cell differentiation regions. No cases of goblet cell deficient and crypt cell atypia/dysplasia were found in this cohort (Fig. 6).

Lee et al[17] had previously noted that 4 nonconventional histology subtypes (crypt cell atypia/dysplasia, hypermucinous, goblet cell deficient, and sessile serrated lesion-like) were more likely to be associated with HGD or CRC. Based on this, we categorized the histologic subtypes into high-risk histologies (comprising crypt cell atypia/dysplasia, hypermucinous, goblet cell deficient, and sessile serrated lesion-like) and low-risk histologies (ie, tubulovillous adenoma-like, villous adenoma-like, traditional serrated adenoma-like, tubular adenoma-like, and dysplasia with increased Paneth cell differentiation). No cancer risk prediction was given for serrated not otherwise specified dysplasia. We then applied this categorization to the dysplasia in our cohort and assessed the association of high- and low-risk subtypes with patient progression.

Initially, we analyzed regions where we had reached a majority single subtype diagnosis ($n = 113$) and were unable to find a statistically significant correlation between any high-risk dysplasia subtypes and patient progression (*P* value =.376). We then extended our analysis by annotating mixed regions according to the highest risk subtype visible ($n = 125$), as outlined in the methods (breakdown in Supplementary Fig. S2). Despite these increased numbers, we were unable to confirm the association between nonconventional dysplasia subtype and patient progression in our cohort (*P* value =.099, Fig. 6).

Collectively, data from the histologic assessment of colitis-associated dysplasia using the Choi et al[16] criteria confirmed the presence of the different morphologic subtypes of colitis-associated dysplasia yet was unable to validate the published

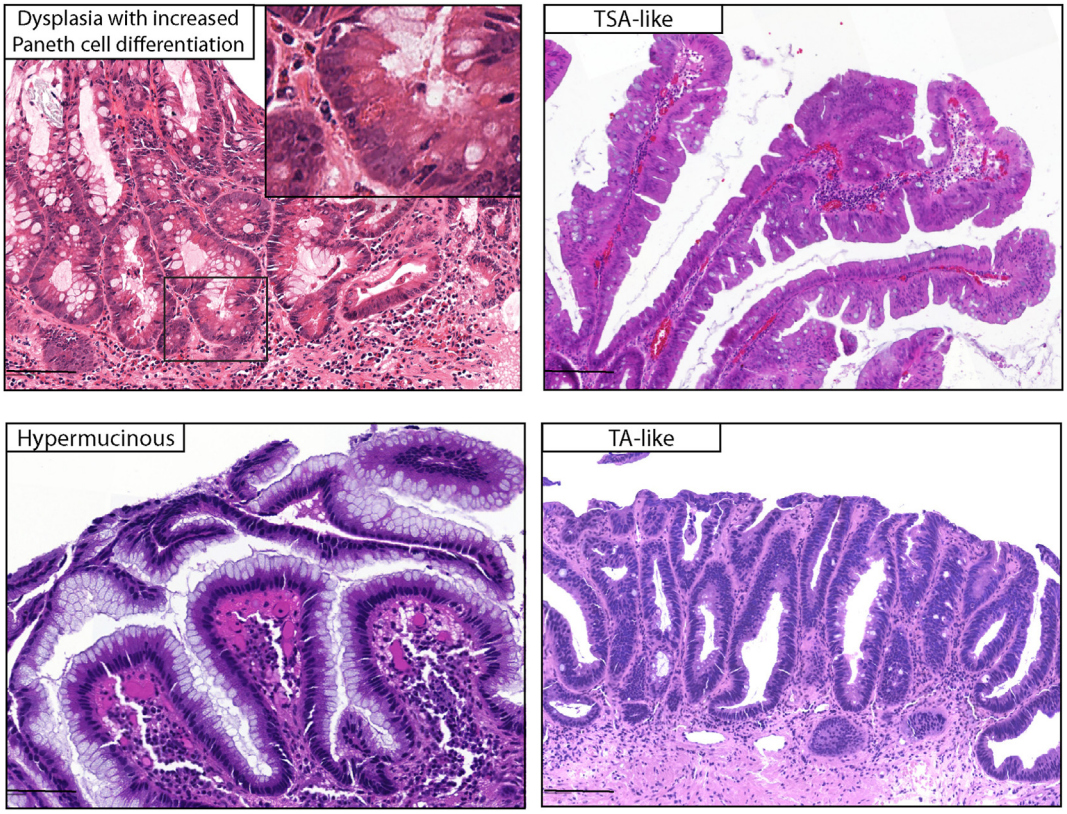


Figure 3.
Examples of morphologic patterns of colitis-associated dysplasia, including hypermucinous, dysplasia with increased Paneth cell differentiation, and traditional serrated adenoma-like dysplasia, as examples of nonconventional dysplasia, and tubulovillous adenoma-like dysplasia, as an example of conventional dysplasia.

finding of cancer risk prediction based on histologic subtype assessment in this cohort.

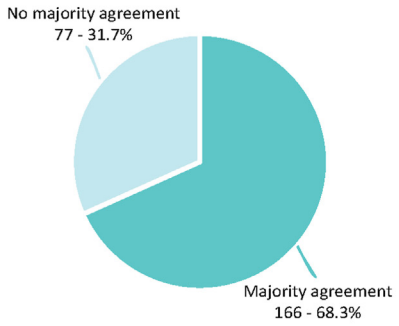
Discussion

Determination of cancer risk is pivotal in decision-making, particularly when considering management strategies. Currently, upon LGD detection, IBD patients are offered the possibility to undergo continued and frequent surveillance or preventive colectomy, without supportive individualized cancer risk estimates. On one hand, some patients undergo potentially unnecessary prophylactic colectomy due to fears of developing CRC, which inevitably has an impact on their quality of life. On the other hand, some patients opt out of surgery and risk high interval cancer rates.[18] Therefore, there is a clear clinical need for better evidence-based stratification tools in colitis-associated dysplasia management to aid in shared decision-making. Individualized CRC risk predictions would improve the effectiveness of IBD patients' management.[18] They would facilitate communication by aiding in the stratification of patients into low-risk, who could be reassured by undergoing surveillance, and high-risk, who would be more confident of their benefit from preventive colectomy.

The ineffectiveness of the current management strategy highlights the need for the identification of a risk predictor. Pathological classification currently relies on the grading of dysplasia into LGD and HGD as a measure of cancer risk. This has the benefit of being simple and reproducible, but it does not account for sporadic adenomas in colitis segments and is confounded by field cancerization, where there may be no detectable dysplasia. The histologic phenotype-based stratification tool proposed by Choi et al[16] was built on important work identifying notable histologic phenotype heterogeneity in colitis-associated dysplasia and attempted to stratify patient cancer risk on the basis of these specific histologic features. This was an important and laudable endeavor, as a histologic stratification tool benefits from applicability to routine clinical samples worldwide, without requiring novel sample processing techniques or additional costs. However, our assessment of the application of this technique by 6 experienced pathologists highlighted the difficulty in assessing and classifying colitis-associated dysplasia types and subtypes with robust consistency and reproducibility. The interrater agreement was negatively impacted by the complexity of the classification criteria, characterized by several categories, some of which were only minimally different and others allowing space for subjective interpretation. In support of this is the fact that interrater agreement was better in the grading of the dysplasia. The tool could potentially be improved by the application of digital pathology and artificial intelligence algorithms in the future. However, this might present its own difficulties, as it would be challenging to establish histologic ground truth in large training and discovery sets when experienced consensus agreement is so low.

Importantly, we were also unable to validate the association between nonconventional dysplasia and progression in this cohort. It is conceivable that sample size prevented us from detecting a genuine association with patient progression, although our cohort (243 regions from 129 lesions in 89 patients) was larger than that deployed in the original Choi et al[16] paper (106 lesions from 58 patients). It is likely that difficulties in

A Dysplasia type (conventional/non-conventional) scoring agreement among pathologists



Percentage agreement

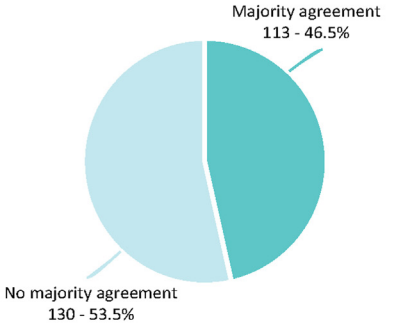
Assessors	Andromeda	Ara	Hydrus	Eridanus	Lyra	Tucana
Agreeability Score	63	56	64	51	62	56
Study inter-rater agreement			69			

Kappa values

Cohen's Kappa

Andromeda	Andromeda				
Ara	0.212	Ara			
Hydrus	0.384	0.257	Hydrus		
Eridanus	0.217	0.097	0.165	Eridanus	
Lyra	0.335	0.344	0.430	0.141	Lyra
Tucana	0.229	0.171	0.194	0.235	0.18
Fleiss' Kappa			0.219		

B Dysplasia subtype scoring agreement among pathologists



Percentage agreement

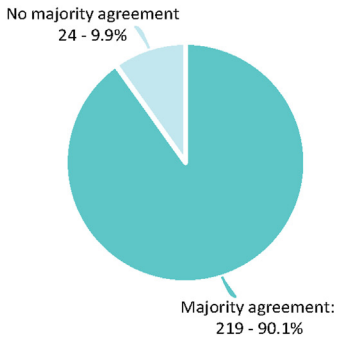
Assessors	Andromeda	Ara	Hydrus	Eridanus	Lyra	Tucana
Agreeability Score	48	45	49	40	50	43
Study inter-rater agreement			57			

Kappa values

Cohen's Kappa

Andromeda	Andromeda				
Ara	0.235	Ara			
Hydrus	0.353	0.256	Hydrus		
Eridanus	0.198	0.177	0.174	Eridanus	
Lyra	0.322	0.371	0.351	0.181	Lyra
Tucana	0.241	0.189	0.252	0.232	0.223
Fleiss' Kappa			0.244		

C Grading agreement among pathologists



Percentage agreement

Assessors	Andromeda	Ara	Hydrus	Eridanus	Lyra	Tucana
Agreeability Score	82	70	82	82	80	82
Study inter-rater agreement			85			

Kappa values

Cohen's Kappa

Andromeda	Andromeda				
Ara	0.261	Ara			
Hydrus	0.398	0.280	Hydrus		
Eridanus	0.429	0.232	0.356	Eridanus	
Lyra	0.439	0.407	0.368	0.298	Lyra
Tucana	0.390	0.231	0.450	0.382	0.222
Fleiss' Kappa			0.326		

Figure 4.
Measures of diagnostic agreement among pathologists in assessing colitis-associated low-grade dysplasia. Left: Breakdown of majority agreement in the assessment of the regions. Top right: percentage agreement measured in agreement score for each pathologist and the study's interrater agreement. Both reported as percentage values. Bottom right: Cohen's Kappa measuring agreement between 2 pathologists at a time and Fleiss' Kappa measuring agreement between all pathologists.

reaching consensus impacted sample size, and we note that this study is smaller than the follow-up study by Lee et al[17] (317 dysplastic lesions from 168 patients).

Given the overall poor correlation of nonconventional dysplasia with prospective cancer outcomes and the complexity of the classification of colitis-associated dysplasia, its practical use as a widespread, reproducible, clinically applicable tool is likely to be limited. Furthermore, even without the issues highlighted above, cancer risk stratification based on histologic classification of dysplasia is destined to be a suboptimal strategy, as the identification of dysplasia cannot necessarily account for field cancerization in non-dysplastic crypts. This is a major and unsolved issue in colitis-associated CRC surveillance, which is currently founded on endoscopic identification and histologic-based assessment of dysplasia.

The results of our study, together with the historical difficulties, suggest the need for a different diagnostic strategy that can objectively identify high-risk phenotypes, including patients with field cancerization. Recent studies[19-21] have used exome and targeted sequencing studies to find differences in single nucleotide alteration patterns between colitis-associated CRC and sporadic CRC, with colitis-associated CRC bearing single nucleotide alteration in a different set of genes to that of sporadic CRC. These differences could be used to differentiate true colitis-associated

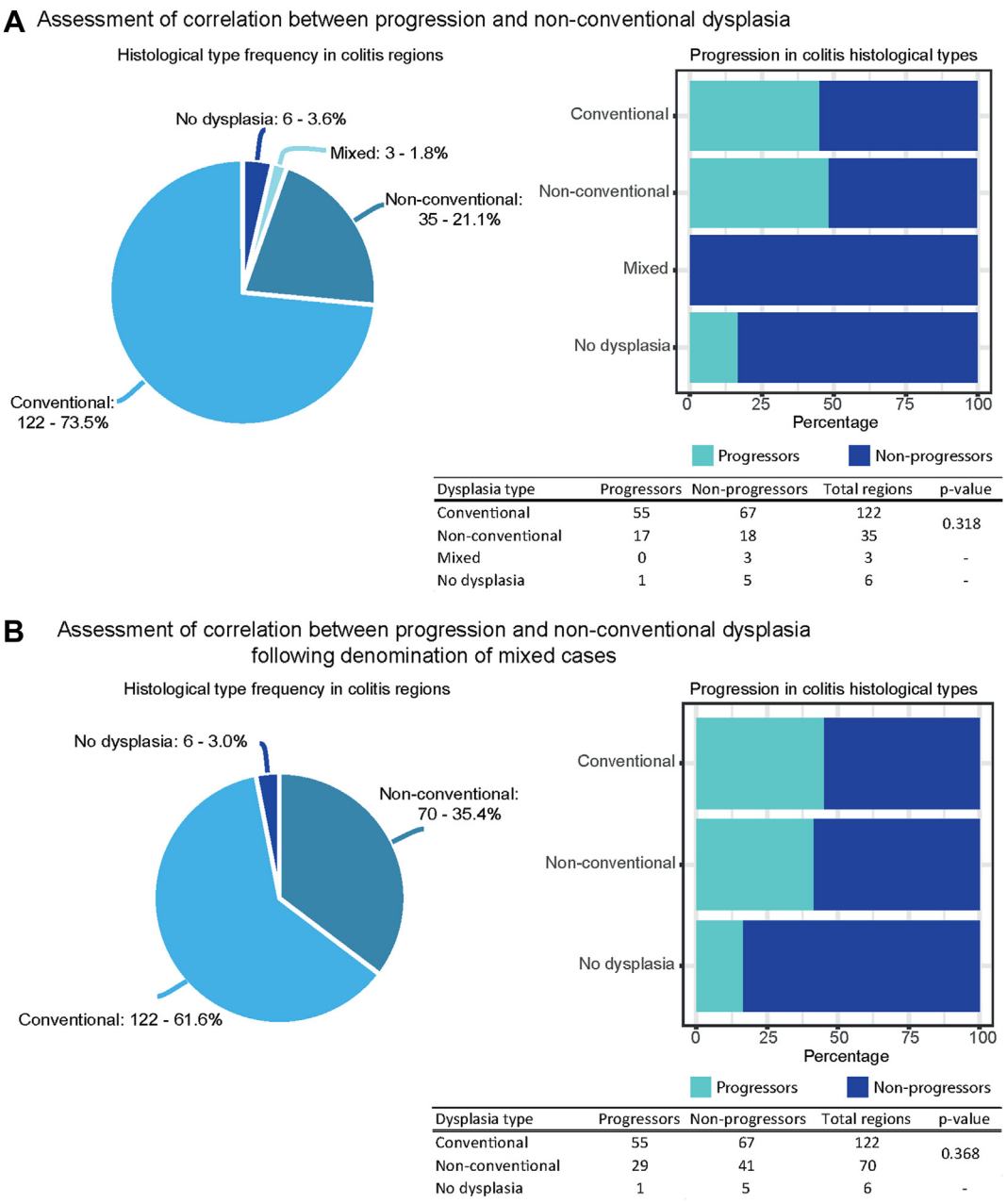
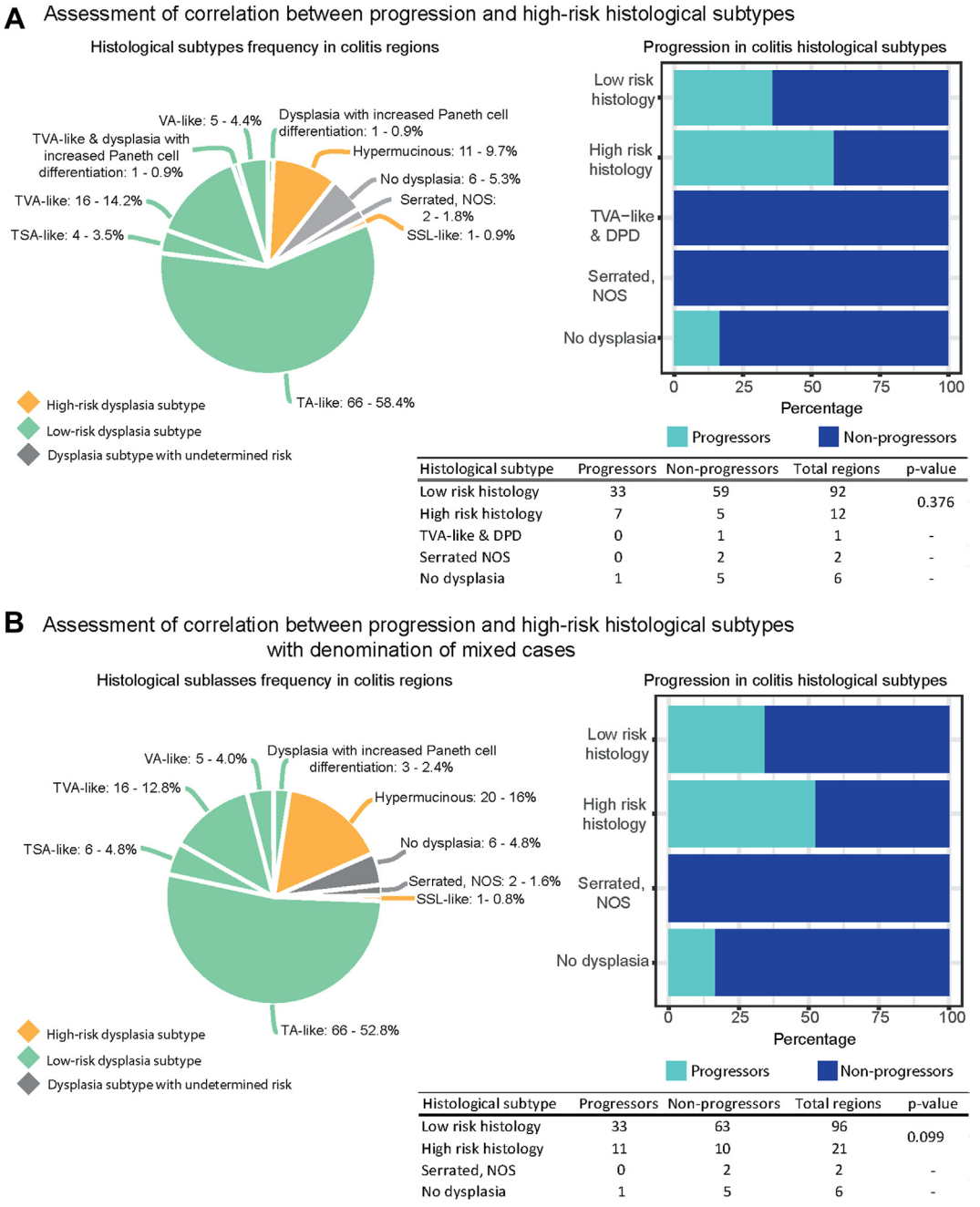


Figure 5.
Association of nonconventional dysplasia with progression. Left: a pie chart outlining the distribution of conventional, nonconventional and mixed dysplasia in the colitis cohort. Right: distribution of progressor and non-progressor low-grade dysplasia among the dysplasia regions. Bottom left: results of Fisher's exact test assessing whether nonconventional dysplasia is more likely to be progressors. (A) Results assessment without denotation of mixed scores. (B) Results assessment where scores of mixed low-grade dysplasia were denoted to the worse visible class.

lesions from sporadic lesions occurring in IBD patients. Other work has noted correlations between copy number alteration, histologic grade, and progression risk in colitis, with reports of an increase in copy number alteration in the progression from LGD to HGD.[19,22,23] Consequently, future work on molecular biomarkers may help provide early, objective identification of cancer risk and enable discrimination between colitis-associated and sporadic lesions, which can aid in the identification of patients with field cancerization. This would help inform prophylactic colectomy decisions and guide endoscopic surveillance regimens, including reducing endoscopic requirements in patients with identified low-risk lesions.

In conclusion, the Choi et al[16] article was the first to describe in detail the different morphologic patterns in colitis-associated dysplasia. The presence of different colitis-associated dysplasia subtypes is very important and warrants further studies to understand how and why these different morphologic features evolve. However, the complexity of the criteria, leading to poor reproducibility and interrater agreement is likely to limit the practical application of this histologic subclassification of dysplasia as a cancer risk prediction strategy. This could be improved in the future with the use of artificial intelligence algorithms, but in the meantime, other more promising tools, such as molecular testing, should also be explored.



Histological subtype	Progressors	Non-progressors	Total regions	p-value
Low risk histology	33	59	92	0.376
High risk histology	7	5	12	
TVA-like & DPD	0	1	1	-
Serrated NOS	0	2	2	-
No dysplasia	1	5	6	-

Histological subtype	Progressors	Non-progressors	Total regions	p-value
Low risk histology	33	63	96	0.099
High risk histology	11	10	21	
Serrated, NOS	0	2	2	-
No dysplasia	1	5	6	-

Figure 6.
Association of dysplasia with high-risk histologies with progression. Right: Pie chart outlining the distribution of dysplasia subtypes in the colitis cohort. Left: Distribution of progressor and non-progressor low-grade dysplasia among the high-risk and low-risk histologies. The histologic subtypes in the high-risk group are: hypermucinous and sessile serrated lesion-like (SSL-like). Those in the low-risk group are: tubulovillous adenoma-like (TVA-like), villous adenoma-like (VA-like), traditional serrated adenoma-like (TSA-like), tubular adenoma-like (TA-like), and dysplasia with increased Paneth cell differentiation. Bottom left: Results of Fisher's exact test assessing whether the high-risk histologies are more likely to be progressors. (A) Assessment without denotation of mixed scores. (B) Assessment where scores of mixed LGD were denoted to worse visible subtype.

Limitations

In this study, no crypt cell atypia/dysplasia and goblet cell-deficient nonconventional dysplasia subtypes were identified. Consequently, conclusions made on the association between the nonconventional dysplasia subtypes and progression might not necessarily hold true for these 2 subtypes. In this study, the median number of biopsies/scopes was in the range of 10.8 to 13.8 for progressors and non-progressors, respectively. This is considerably lower than the 33 biopsy/scope standard, but may be a consequence of the use of targeted biopsies in chromoendoscopy procedures. Chromoendoscopy has been recommended for IBD surveillance over the timescale that incorporates the follow-up period of many of the patients in this study. Chromoendoscopy was undertaken in a significant majority of non-progressor patients who underwent a larger proportion of more recent procedures, and thus, it is not believed that the lower median number of biopsies/scopes would have an effect on the ability to assess the

association between progression and nonconventional dysplasia histology. In the grading of dysplasia, no indefinite dysplasia category was provided. None of the dysplasia in this study was originally signed out as indefinite for dysplasia, and although reviewers were blinded to the original diagnosis, it was believed that adding further diagnostic categories would have impacted statistical power. Furthermore, interobserver reproducibility of indefinite for dysplasia is poor,[24] and the clinical management of such lesions remains a contentious issue.

Acknowledgments

The authors would like to thank Jan Hudecek (SlideScore) for his assistance with setting up the study on SlideScore; Myrtle van der Wel for her expert advice on statistical analyses; Andrew Barber and the rest of the Oxford Translational Gastroenterology Unit for their help in retrieving the cases of interest; the BCI histopathology Unit for sectioning and staining the samples; the Oxford Centre for Histopathology Research, for supplying and processing the samples, and St Mark's Hospital, for supplying the samples.

Author Contributions

N.N. and S.J.L. conceived and designed the project. Funding was obtained by S.J.L. and T.A.G. Experiments were conducted by N.N. and I.A.B. Clinical data collection was conducted by N.N. and J.F. Bioinformatic analysis was carried out by N.N. and M.W.B. Pathology support, image analysis, and intellectual input was provided by M.J., V.H.K., M.B.L., L.M.W., M.R-J., M.N., N.N., and S.J.L. Tissue and data provision were provided by P.D., T.A.G., and A.L.H. Conceptual input and data interpretation was provided by M.J., V.H.K., M.B.L., L.M.W., M.R-J., M.N., N.N., and S.J.L. The manuscript was written by N.N. and S.J.L.

Data Availability

Image data is available from the corresponding author upon reasonable request.

Funding

S.J.L. was supported by CRUK Program Grant (DRCNPG-Jun22\100002) and a Wellcome Trust (Senior Clinical Research Fellowship [206314/Z/17/Z]). N.N. was supported by National Institute for Health and Care Research (NIHR) Oxford Biomedical Research Centre. This research was supported by a CRUK Early Detection and Diagnosis award (25901). Core funding to the Wellcome Centre for Human Genetics was provided by the Wellcome Trust (090532/Z/09/Z). This research was funded, in part, by the Wellcome Trust. For the purpose of Open Access, the author has applied a CC BY public copyright license to any Author Accepted Manuscript version arising from this submission. The views expressed in this work are those of the author/s and not necessarily those of the NHS, the NIHR, or the Department of Health.

Declaration of Competing Interest

S.J. Leedham has received grant income from UCB Pharma. V.H. Koelzar has served as an invited speaker on behalf of Indica Labs. All other authors declare no competing interests.

Ethics Approval and Consent to Participate

Dysplastic lesions from IBD patients were obtained from London North West Healthcare NHS Trust, London, UK (London Stanmore committee 10/LO/1613, London – Fulham Research Ethics committee 18/LO/2051), Oxford University Hospitals NHS Foundation Trust, Oxford, UK (Oxford GI Illness ethics, 18/1L/JE/Early-detection, OCHRe:18/A11, London – Fulham Research Ethics committee 18/LO/2051) and Belfast Health and Social Care Trust (16/NI, 0030), Belfast, UK.

Supplementary Material

The online version contains supplementary material available at https://doi.org/10.1016/j.modpat.2023.100419

References

1. Eaden J, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut*. 2001;48(4):526–535. https://doi.org/10.1136/gut.48.4.526
2. Canavan C, Abrams KR, Mayberry JF. Meta-analysis: colorectal and small bowel cancer risk in patients with Crohn's disease. *Aliment Pharmacol Ther*. 2006;23(8):1097–1104. https://doi.org/10.1111/j.1365-2036.2006.02854.x
3. Castaño-Milla C, Chaparro M, Gisbert JP. Systematic review with meta-analysis: the declining risk of colorectal cancer in ulcerative colitis. *Aliment Pharmacol Ther*. 2014;39(7):645–659. https://doi.org/10.1111/apt.12651
4. Lutgens MWMD, Van Oijen MGH, Van Der Heijden GJMG, Vleggaar FP, Siersema PD, Oldenburg B. Declining risk of colorectal cancer in inflammatory bowel disease. *Inflamm Bowel Dis*. 2013;19(4):789–799. https://doi.org/10.1097/mib.0b013e31828029c0
5. Choi CHR, Ignjatovic-Wilson A, Askari A, et al. Low-Grade dysplasia in ulcerative colitis: Risk factors for developing High-Grade Dysplasia or colorectal cancer. *Am J Gastroenterol*. 2015;110(10):1461–1471. https://doi.org/10.1038/ajg.2015.248
6. Desai D, Shah S, Deshmukh A, et al. Colorectal cancers in ulcerative colitis from a low-prevalence area for colon cancer. *World J Gastroenterol*. 2015;21(12):3644. https://doi.org/10.3748/wjg.v21.i12.3644
7. Watanabe T, Konishi T, Kishimoto J, Kotake K, Muto T, Sugihara K. Ulcerative colitis-associated colorectal cancer shows a poorer survival than sporadic colorectal cancer: a nationwide Japanese study. *Inflamm Bowel Dis*. 2011;17(3):802–808. https://doi.org/10.1002/ibd.21365
8. Lamb CA, Kennedy NA, Raine T, et al. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut*. 2019;68(Suppl 3):s1–s106. https://doi.org/10.1136/gutjnl-2019-318484
9. Odze RD, Farraye FA, Hecht JL, Hornick JL. Long-term follow-up after polypectomy treatment for adenoma-like dysplastic lesions in ulcerative colitis. *Clin Gastroenterol Hepatol*. 2004;2(7):534–541. https://doi.org/10.1016/s1542-3565(04)00237-x
10. Kisiel JB, Loftus EV, Harmsen WS, Zinsmeister AR, Sandborn WJ. Outcome of sporadic adenomas and adenoma-like dysplasia in patients with ulcerative colitis undergoing polypectomy§†. *Inflamm Bowel Dis*. 2012;18(2):226–235. https://doi.org/10.1002/ibd.21687
11. Van Schaik FDM, Offerhaus GJA, Schipper MEI, Siersema PD, Vleggaar FP, Oldenburg B. Endoscopic and pathological aspects of colitis-associated dysplasia. *Nat Rev Gastroenterol Hepatol*. 2009;6(11):671–678. https://doi.org/10.1038/nrgastro.2009.162
12. Leedham SJ, Graham TA, Oukrif D, et al. Clonality, founder mutations, and field cancerization in human Ulcerative Colitis–Associated Neoplasia. *Gastroenterology*. 2009;136(2):542–550.e6. https://doi.org/10.1053/j.gastro.2008.10.086
13. Curtius K, Wright NA, Graham TA. An evolutionary perspective on field cancerization. *Nat Rev Can*. 2017;18(1):19–32. https://doi.org/10.1038/nrc.2017.102
14. Salk JJ, Bansal A, Lai LA, et al. Clonal expansions and short telomeres are associated with neoplasia in early-onset, but not late-onset, ulcerative colitis. *Inflamm Bowel Dis*. 2013;19(12):2593–2602. https://doi.org/10.1097/mib.0b013e3182a87640
15. Choi CHR, Rutter MD, Askari A, et al. Forty-year analysis of Colonoscopic Surveillance Program for neoplasia in ulcerative colitis: an updated overview. *Am J Gastroenterol*. 2015;110(7):1022–1034. https://doi.org/10.1038/ajg.2015.65
16. Choi W, Yozu M, Miller G, et al. Nonconventional dysplasia in patients with inflammatory bowel disease and colorectal carcinoma: a multicenter

clinicopathologic study. *Mod Pathol*. 2020;33(5):933–943. https://doi.org/10.1038/s41379-019-0419-1
17. Lee H, Rabinovitch PS, Mattis AN, Lauwers GY, Choi W. Non-conventional dysplasia in inflammatory bowel disease is more frequently associated with advanced neoplasia and aneuploidy than conventional dysplasia. *Histopathology*. 2020;78(6):814–830. https://doi.org/10.1111/his.14298
18. Curtius K, Kabir M, Bakir IA, et al. Multicentre derivation and validation of a colitis-associated colorectal cancer risk prediction web tool. *Gut*. 2021;71(4):705–715. https://doi.org/10.1136/gutjnl-2020-323546
19. Baker A, Cross W, Curtius K, et al. Evolutionary history of human colitis-associated colorectal cancer. *Gut*. 2018;68(6):985–995. https://doi.org/10.1136/gutjnl-2018-316191
20. Shivakumar BM, Rotti H, Vasudevan TG, et al. Copy number variations are progressively associated with the pathogenesis of colorectal cancer in ulcerative colitis. *World J Gastroenterol*. 2015;21(2):616. https://doi.org/10.3748/wjg.v21.i2.616
21. Curtius K, Bakir IA, Baker A, et al. 781—Quantifying evolution of early dysplastic lesions in ulcerative colitis predicts future colorectal cancer risk. *Gastroenterology*. 2019;156(6):S-163. https://doi.org/10.1016/s0016-5085(19)37198-7
22. Rubin CE, Haggitt RC, Burmer GC, et al. DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. *Gastroenterology*. 1992;103(5):1611–1620. https://doi.org/10.1016/0016-5085(92)91185-7
23. Hartmann DP, Montgomery EA, Carr N, Gupta PK, Azumi N. Flow cytometric DNA analysis of ulcerative colitis using paraffin-embedded biopsy specimens: comparison with morphology and DNA analysis of fresh samples. *PubMed*. 1995;90(4):590–596.
24. van der Wel MJ, Coleman HG, Bergman JJ, Jansen M, Meijer SL. Histopathologist features predictive of diagnostic concordance at expert level among a large international sample of pathologists diagnosing Barrett's dysplasia using Digital Pathology. *Gut*. 2019;69(5):811–822. https://doi.org/10.1136/gutjnl-2019-318985