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Histopathological Features in Colonic Biopsies at Diagnosis Predict Long-term Disease Course in Patients with Crohn's Disease

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Abstract

Background and Aims: Crohn's disease [CD] is characterised by a heterogeneous disease course. Patient stratification at diagnosis using clinical, serological, or genetic markers does not predict disease course sufficiently to facilitate clinical decision making. The current study aimed to investigate the additive predictive value of histopathological features to discriminate between a long-term mild and severe disease course.

Methods: Diagnostic biopsies from treatment-naïve CD patients with mild or severe disease courses in the first 10 years after diagnosis were reviewed by two gastrointestinal pathologists after developing a standardised form comprising 15 histopathological features. Multivariable logistic regression models were built to identify predictive features and compute receiver operating characteristic [ROC] curves. Models were internally validated using bootstrapping to obtain optimism-corrected performance estimates.

Results: In total, 817 biopsies from 137 patients [64 mild, 73 severe cases] were included. Using clinical baseline characteristics, disease course could only moderately be predicted (area under receiver operating characteristic curve [AUROC]: 0.738 [optimism 0.018], 95% confidence interval [CI] 0.65–0.83, sensitivity 83.6%, specificity 53.1%). When adding histopathological features, in colonic biopsies a combination of [1] basal plasmacytosis, [2] severe lymphocyte infiltration in lamina propria, [3] Paneth cell metaplasia, and [4] absence of ulcers were identified and resulted in significantly better prediction of a severe course (AUROC: 0.883 [optimism 0.033], 95% CI 0.82–0.94, sensitivity 80.4%, specificity 84.2%).

Conclusions: In this first study investigating the additive predictive value of histopathological features in biopsies at CD diagnosis, we found that certain features of chronic inflammation in colonic biopsies contributed to prediction of a severe disease course, thereby presenting a novel approach to improving stratification and facilitating clinical decision making.

Key Words: Crohn's disease; pathology; disease course; biomarkers

1. Introduction

Crohn's disease [CD] is a chronic relapsing-remitting inflammatory disorder of the gastrointestinal tract, characterised by a heterogeneous presentation and highly variable disease course.[1] Current treatment strategies are directed towards chronic suppression of the immune system in order to promote endoscopic remission and prevent chronic inflammation. Ultimately, the goal is to achieve sustained corticosteroid-free remission.[2,3] Although step-up regimens are still being advocated in current guidelines, recent studies have shown that early intervention and tight control of mucosal inflammation using a top-down approach improve long-term outcomes in patients with an unfavourable phenotype.[4,5] However, balancing the benefits and risks [e.g., drug-related side effects, infections, and malignancies] associated with treatment remains challenging due to the heterogeneous nature of CD and the limitations of biomarkers currently used to accurately predict disease course.[6–9]

Despite major advances in inflammatory bowel disease [IBD] research, to date there are no validated markers in use in clinical practice for the prediction of disease course in CD at time of diagnosis. Current clinical predictors have several shortcomings. The Montreal Classification system, for example, has limited value for disease course prediction and clinical decision making, in part as a result of the inherent inability to account for changes in the clinical phenotype during the course of the disease.[10,11] Genetic markers such as the *NOD2* mutations, which have been associated with worse disease outcomes, also have limited predictive potential related to their low prevalence, and geographical dependence.[12] Furthermore, serological markers measured at diagnosis, such as antimicrobial antibodies, have proven inadequate in accurately predicting disease course.[13] The observations derived from these clinical, genetic, and serological markers warrant the identification of alternative and better predictors of disease course.[14,15]

In a recent study, we assessed disease activity patterns in patients with CD using long-term follow-up data from the population-based IBD South-Limburg [IBDSL] cohort.[16] Almost one-third of the population experienced a quiescent disease course during the first 10 years after diagnosis, underlining the importance of adequately stratifying patients to decrease the risk of overtreatment. Yet, another third of patients experienced a severe disease course and may benefit from early intervention and tight monitoring. At time of diagnosis, disease course could only moderately be predicted based on merely clinical parameters such as the Montreal classification.[16] These findings once more emphasise the major medical need for the identification of disease course predictors in order to improve patient stratification at diagnosis and distinguish between patients who are more likely to develop a mild or a severe disease course, to facilitate clinical decision making.

The European Crohn's and Colitis Organisation [ECCO] has published a consensus paper describing histopathological features characteristic of CD.[17,18] Although no findings are pathognomonic, histopathological features of endoscopic biopsies and resection material are routinely used alongside clinical findings and imaging, in order to contribute to the diagnosis and differentiate CD from ulcerative colitis [UC] and other diseases.[17] As histopathological features vary largely among patients, and tissue samples are available for the majority of patients, it could be useful to investigate their role in disease course prediction.

The relevance of histopathological features in biopsies from CD patients at diagnosis in relation to prediction of disease outcomes has been evaluated by only a limited number of studies.[19–21] These studies showed conflicting results and were limited by relatively short post-biopsy follow-up periods and assessments of biopsies taken after treatment initiation. Through retrospective analysis of biopsies from CD patients taken at time of diagnosis, the current study aimed to investigate the additive predictive value of histopathological features to discriminate between patients with a mild or severe disease course in the first 10 years after diagnosis.

2. Materials and Methods

2.1. Setting and patients

All patients in this multicentre retrospective cohort study originate from the population-based IBDSL cohort. In brief, this inception cohort was initiated in 1991 and contains all patients newly diagnosed with IBD, from the age of 18 years and older and living in the South Limburg region [NL]. As a result of the joint cooperation of all regional hospitals [Maastricht University Medical Center+ and Zuyderland Medical Centre Heerlen and Sittard], the cohort comprises at least 93% of all patients with IBD in the South Limburg region.[22] The IBDSL study design has been approved by the Ethics Committee of MUMC+ [NL31636.068.10], meets the ethical standard of the declaration of Helsinki, and is registered in ClinicalTrial.gov [NCT02130349].[23]

For the present study, CD patients diagnosed between January 1991 and January 2010, with a disease follow-up of at least 10 years after diagnosis, were identified. Diagnosis of CD was based on the combination of clinical presentation, imaging, endoscopy, and/or histological findings in biopsies.[14] For all patients, disease activity was assessed for each annual quarter since time of diagnosis, as has been described previously.[16] In short, active disease was defined by either: [1] activity on endoscopy or imaging [comprising all endoscopy and imaging reports in which the experts judged lesions to be due to active CD], [2] hospitalisation as a result of disease exacerbation [excluding, for example, elective admissions, drug administrations, admissions due to side effects], [3] surgery for active CD because of active luminal disease, active internal fistulas, active perianal disease, or stricturing complications [excluding diagnostic procedures], or [4] adjustment of treatment by the treating physician due to increased symptoms or biochemical changes.

Patients were eligible for inclusion in the present study if they had either a mild or a severe disease course in the first 10 years after diagnosis and when treatment-naïve endoscopic biopsies obtained at the time of CD diagnosis were available. Definitions for disease course were adapted from our previous work.[16] In our previous work, six disease course clusters were defined through expert consensus based on the number and distribution of active quarters in the first 10 years after diagnosis. In the current study, only CD patients from the mild or severe clusters with available diagnostic biopsies were included. Disease course was defined as 'mild' in case of two or more quarters of total activity in the first 10 years after diagnosis. Disease course was defined 'severe' in case of at least one quarter of disease activity per year in 8 or more years or six or more quarters of disease activity in total [with at least one quarter of activity every 2 years] in the first 10 years after diagnosis.

Relevant clinical data including age at diagnosis, disease phenotype at diagnosis according to the Montreal classification, gender, smoking status at diagnosis, medication use, and family history of all eligible patients were collected from patients' medical records using standardised registration forms. Histopathology reports of biopsies obtained during colonoscopy at time of diagnosis were reviewed for every patient, and total number and location of collected biopsies were noted.

2.2. Biopsy preparation

All diagnostic biopsies of eligible patients were retrieved from the histopathology archives of participating centres. Tissue sections of 3–5 µm were cut and stained with haematoxylin and eosin [H&E] using an automatic slide stainer [COT-20, Medite Medical GmbH, Burgdorf, Germany]. Subsequently, stained slides were scanned using a Pannoramic P1000 slide scanner [3DHISTECH Ltd., Budapest, Hungary], equipped with a Plan-Apochromat objective [magnification, 20x; numerical aperture, 0.8; Carl Zeiss AG, Jena, Germany] and a high-resolution Quartz colour camera [4096 x 3072 resolution, 5.5 x 5.5µm pixel size; Q-12A180, Adimec, The Netherlands], resulting in a dynamic whole-slide image resolution of 0.24 µm/pixel. Before histopathological evaluation, digitised slides were manually quality controlled by ARA and rescanned if necessary.

2.3. Histopathological evaluation

After a comprehensive literature review, a standardised scoring form was developed containing 15 histopathological features considering the most commonly described histological scoring indices in CD studies in literature and the ECCO consensus on histopathology, as well as input from two senior gastrointestinal pathologists [HIG and DG].[17,24–29]

Histopathological features with corresponding definitions and scoring ranges are presented in Table 1. A two-tier scoring system was used in which no option for 'moderate' was provided for features with a semi-quantitative scoring scale.[30] A 'not judgeable' option was included for all features. Representative examples of some key histopathological features are shown in Figure 1. All features selected in this study can be assessed based on H&E staining alone.

All biopsies were independently evaluated by two senior gastrointestinal pathologists [HIG, DG] using CaseViewer software [Version 2.3, 3DHISTECH, Budapest, Hungary]. Both pathologists were blinded to the corresponding disease course. For scoring of features, a per patient 'worst-case' approach was used in which the final score of a certain feature was based on the biopsy with presence of most severe features. Agreement between pathologists was assessed by measuring interobserver agreement for every feature and, in case of disagreements in scoring, a meeting was organised to discuss findings and obtain consent.

Scorings from ileal biopsies [i.e., biopsies from the ileum and ileocaecal valve] and colonic biopsies [including rectal biopsies] were analysed separately. After collection of all scores, a single maximum value was rendered for each histopathological feature for every patient for ileal biopsies and colonic biopsies. For example, if in three colonic biopsies of one patient one biopsy showed severe neutrophil infiltration in the lamina propria and two showed mild infiltration, the final score for colonic neutrophil infiltration would be 'severe'. This strategy was adapted from Naini *et al.*[25] Similar strategies have been used in histopathological evaluation studies on UC.[31,32]

2.4. Statistical analysis

Baseline characteristics are presented as means with corresponding standard deviations [SD] for numerical variables, where normality was checked using histograms and Q-Q plots, and as number of patients with corresponding percentage for categorical variables. For comparison of baseline characteristics between groups, the independent samples t test was used for numerical variables and the chi square test or Fisher's exact test, when applicable, for categorical variables. Interobserver agreement was measured using Cohen's kappa [κ].

In order to assess the additive predictive value of histopathological features for discriminating between a mild and a severe disease course, two multivariable logistic regression models were built. The first model assessed the predictive value of clinically relevant baseline characteristics alone. This model included the following variables [Model 1]: Montreal classification [including age at diagnosis, disease location, disease behaviour, presence of perianal disease], smoking status at diagnosis, and gender.[11,33–35] Upper gastrointestinal [GI] disease involvement at diagnosis, which is associated with worse disease outcomes, was not included in the model as a result of very low numbers in both disease course groups [i.e., in none of the patients with a mild and in four patients with a severe disease course].

For the second model [Model 2], two different model-building strategies were explored to identify predictive histopathological features. To adjust for clinically relevant baseline characteristics and determine additive predictive value, the first model was used as a starting point for the model containing the histopathological features. A logistic regression analysis with the forward stepwise likelihood ratio method was used where all histopathological features were considered for entry [if $p \leq 0.05$] and, if applicable, removal [if $p > 0.10$]. In addition, a more sophisticated, researcher-based approach for variable selection was applied. This model-building strategy, as proposed by Hosmer and Lemeshow, describes a purposeful selection of covariates algorithm.[36,37] As opposed to other strategies, this attempts to minimise overfitting while retaining important predictors and confounders, and assesses the importance of interaction variables. This method is particularly useful in situations where the number of predictor variables is relatively large in relation to the sample size. Subsequently, final models were compared using the likelihood ratio test, to check for improved model fit. All models were evaluated for multicollinearity using variance inflation factors [VIFs, >10 indicating a problem], and the impact of influential cases was evaluated by calculating Cook's distance [>1 indicating a problem]. The entire model-building processes were performed for both scorings from ileal biopsies and scorings from colonic biopsies.

Performance of prediction models was assessed by calculating predicted probabilities of logistic regression models to compute

Table 1. Overview of evaluated histopathological features, corresponding definitions and scoring range.

Histopathological feature	Definition	Scoring range
1. Architectural distortion	Ileum: widening, blunting, and/or shortening of villi[26,74] Colon: ranging from normal parallel crypts to irregularly arranged, branching crypts, crypt loss, crypt shortening, and surface villi formation[26,75]	0, absent; 1, mild; 2, severe
2. Patchiness	Change from continuous to discontinuous inflammation[17]	0, absent; 1, present
3. Neutrophil infiltration in lamina propria	Ranging from rare neutrophils in the lamina propria [mild], up to conspicuous infiltrates of neutrophils in the lamina propria [severe][26]	0, absent; 1, mild; 2, severe
4. Eosinophil infiltration in lamina propria	Ranging from rare eosinophils in the lamina propria [mild], up to conspicuous infiltrates in the lamina propria [severe][20,76]	0, absent; 1, mild; 2, severe
5. Lymphocyte and plasma cell infiltration in laminapropria	Ranging from rare lymphocytes and plasma cells at the bottom of the lamina propria [mild], up to conspicuous infiltration of the lamina propria [severe]. Infiltration can be sprinkling or band-like[26]	0, absent; 1, mild; 2, severe
6. Ulcers and/or erosion	Presence of granulation tissue in an area with loss of epithelial surface[26]	0, absent; 1, present
7. Granulomas	Collection of epithelioid cells, not related to crypt injury[17]	0, absent; 1, present
8. Cryptitis	Presence of neutrophils on top of a crypt epithelial cell[17]	0, absent; 1, present
9. Crypt abscess	Cluster of neutrophils inside the crypt lumen[17]	0, absent; 1, present
10. Paneth cell metaplasia	Evaluated only distal from hepatic flexure[26]	0, absent; 1, present
11. Pyloric gland metaplasia	Presence of gastric pyloric-type glands in either ileum or colon[74,76]	0, absent; 1, present
12. Mucin depletion	Ranging from rare examples of goblet cell reduction [mild], up to conspicuous reduction of goblet cells [severe][26]	0, absent; 1, mild; 2, severe
13. Lymphoid follicles with/without germinal centres	Focal agglomeration of lymphocytes with or without a germinal centre [GC][76]	0, absent; 1, present without GC; 2, present with GC
14. Basal plasmacytosis	Presence of a lymphoplasmacytic infiltrate between the base of the crypts and the muscularis mucosae[26,75]	0, absent; 1, present
15. Chronic inflammation of submucosa	Disruption of submucosa by features of chronic inflammation	0, absent; 1, present

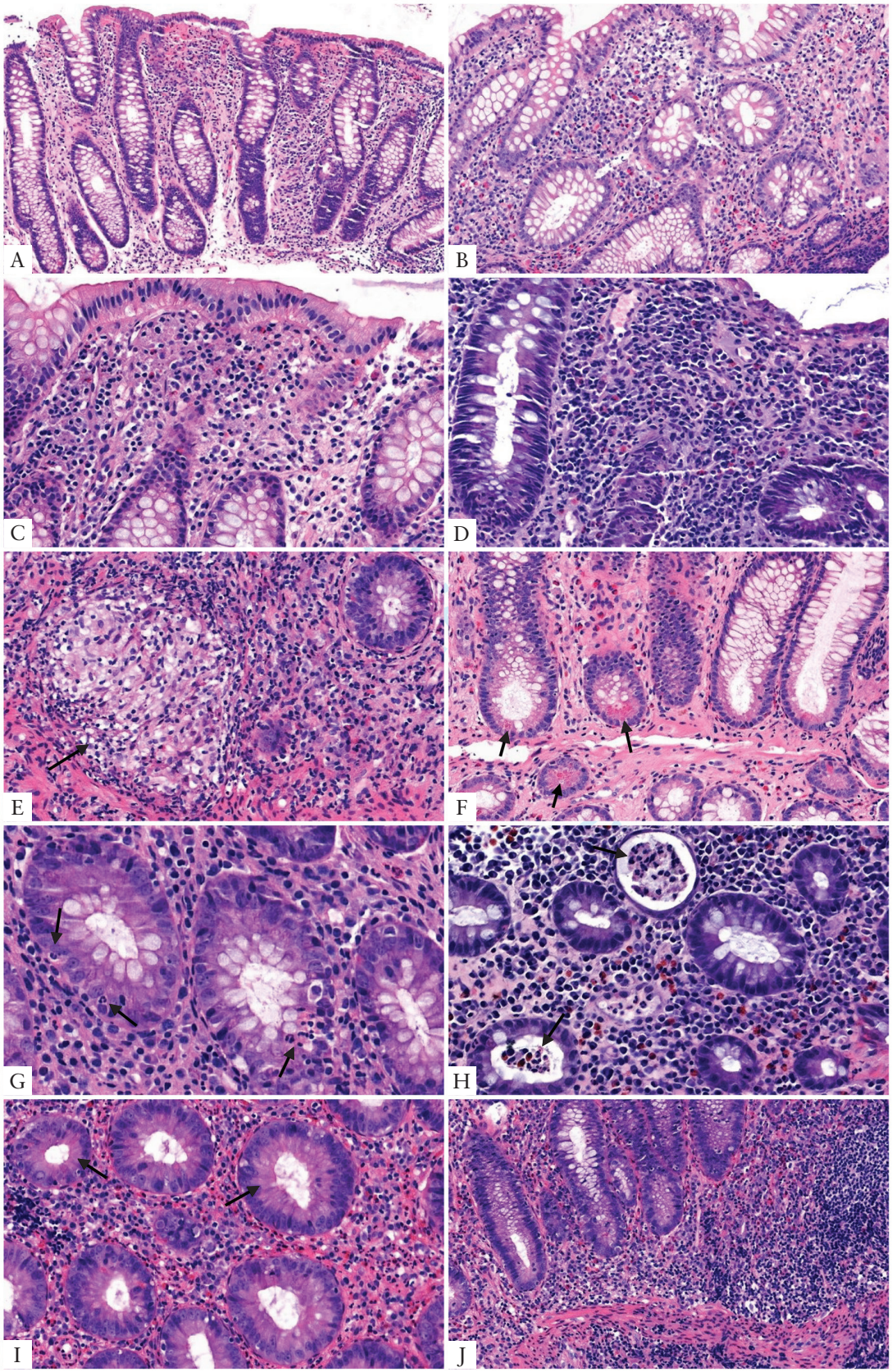

Figure 1. Representative examples of some key features from assessed biopsies. All materials were stained with haematoxylin and eosin, arrows indicate specific features. A: Mild architectural distortion [200x]. B: Severe architectural distortion [300x]. C: Mild lymphoplasmacytic infiltration in lamina propria [400x; 36 cells/mm^2]. D: Severe lymphocyte and plasma cell infiltration in lamina propria [400x; 66 cells/mm^2]. E: Granuloma [400x]. F: Paneth cell metaplasia [300x]. G: Cryptitis [600x]. H: Crypt abscess [400x]. I: Severe mucin depletion [400x]. J: Basal plasmacytosis [200x].

receiving operating characteristic [ROC] curves and obtain the subsequent area under ROC curves [AUROC] for each model. To determine if differences in predictive quality were statically significant, AUROCs of models were compared using the method of DeLong *et al.*[38] The Youden's J statistic was calculated to determine optimal cut-off points for sensitivity and specificity of each model. Internal validation was performed using bootstrapping, as proposed by Steyerberg *et al.*, to account for overfitting and to obtain a more accurate optimism-corrected estimate of model performance of both prediction models in similar new patients.[39] The modelling process was repeated by drawing 1000 bootstrap samples with replacement from the original sample. The shrinkage factor, obtained from the bootstrapping procedures, was used to calculate bias-corrected regression coefficients for the final prediction model formulas.

All statistical analyses were conducted using SPSS software [version 26.0, SPSS, Chicago, IL, USA], except for the internal validation procedure which was performed in R statistics [version 4.0.3] using the RMS and HMSIC packages. A two-sided p-value of ≤0.05 was considered statistically significant. The TRIPOD statement was followed as a guideline for analysis and reporting of prediction models.[40]

3. Results

3.1. Study population

In total, 146 and 176 patients from the IBDSL cohort with a CD diagnosis between 1991 and 2010 fulfilled the criteria for a mild or a severe disease course during the first 10 years after diagnosis, respectively. Of these, 89 patients in the mild and 127 patients in the severe disease course group underwent colonoscopy with biopsies at time of diagnosis. The remainder of patients were not eligible for inclusion for various reasons: 57 underwent resection at time of diagnosis [36 mild vs 21 severe course group], five patients had isolated upper GI disease at diagnosis [all severe disease course group], and in 44 patients [21 mild vs 23 severe course group] the initial diagnosis was based on a combination of clinical presentation and findings on imaging which was later confirmed by histopathology in all patients. To eliminate treatment-specific alterations at the histological level, patients who had received treatment before diagnostic biopsy collection were excluded. Eventually, biopsy material from 64 treatment-naïve patients with a mild and 73 treatment-naïve patients with a severe disease course were available for evaluation in the current study.

Baseline characteristics of the study population are presented in Table 2. At time of diagnosis, patients with a severe disease course in the first 10 years after diagnosis were on average significantly younger {28.2 years (standard deviation [SD] 10.6) vs 35.4 years (SD 14.4), $p = 0.001$} and a larger proportion of patients were active smokers [63.0% vs 42.2%, $p = 0.015$] compared with those with a mild course. Furthermore, patients with a severe disease course presented more frequently with complications [i.e., fistulas and/or strictures] [21.9% vs 6.3%, $p = 0.017$] and ileocolonic disease location [39.7% vs 17.2%, $p = 0.013$]. Gender, presence of IBD in family, and perianal disease at diagnosis were not significantly different between groups.

A total of 817 biopsies from 137 patients were analysed. The mean number of biopsies per patient was comparable between disease course groups (6.1 [SD 2.6] vs 5.9 [SD 2.7], $p = 0.682$) and no significant differences in location of biopsies taken were observed [Table 2].

3.2. Prevalence of histopathological features in patients with a mild and a severe disease course

Using univariable analysis, no significant differences were found in the prevalence of individual histopathological features between a mild and a severe disease course in the ileal biopsies [Supplementary Table 1, available as Supplementary data at *ECCO-JCC* online]. For colonic biopsies, basal plasmacytosis [$p = 0.001$], severe lymphocyte and plasma cell infiltration in the lamina propria [$p = 0.003$], and Paneth cell metaplasia [$p = 0.003$] all showed a higher prevalence in patients with a severe course as compared with those with a mild disease course [Table 3]. The overall prevalence of individual features in ileal and colonic biopsies, irrespective of disease course, is presented in Supplementary Table 2, available as Supplementary data at *ECCO-JCC* online. Interobserver agreement between pathologists was good (Cohen's kappa 0.7 [0.52–0.86], Supplementary Table 3, available as Supplementary data at *ECCO-JCC* online).

3.3. Predictive value of histopathological features at time of diagnosis for discrimination between mild and severe disease course

Results from the multivariable logistic regression models are presented in Table 4 [all model assumptions were met]. ROC analyses of Model 1, evaluating the predictive power of clinically relevant baseline characteristics alone [i.e., Montreal classification, smoking status, and gender], showed moderate discrimination between patients with a mild or a severe disease course, yielding an AUROC of 0.738 (95% confidence interval [CI] 0.65–0.83). At the optimal cut-off, a sensitivity of 83.6% and specificity of 53.1% were found for Model 1 [Figure 2].

Next, when histopathological features were added to Model 1, no additive predictive value was found for ileal biopsies using either model-building strategy. For colonic biopsies, both model-building strategies resulted in the identification of the same histopathological features [therefore, both strategies are listed under Model 2]. A combination of the presence of [1] basal plasmacytosis, [2] Paneth cell metaplasia, [3] severe lymphocyte and plasma cell infiltration in the lamina propria, and [4] absence of ulcers was identified as predictors for the development of a severe disease course. These features combined with those of Model 1 yielded an AUROC of 0.883 [95% CI 0.82–0.94] with a corresponding sensitivity of 80.4% and specificity of 84.2% at the optimal cut-off [Figure 2]. Compared with Model 1, addition of the combination of the four histopathological features [Model 2] resulted in a significantly better model fit to the data [$p < 0.001$]. Comparison of AUROCs also revealed a statistically significant increase in predictive quality [Model 2 vs Model 1 AUROC, $p = 0.001$].

Internal validation of both prediction models using bootstrapping revealed shrinkage factors of 0.915 [Model 1] and 0.799 [Model 2], and an optimism-corrected AUROC of 0.720 [optimism: 0.018] and 0.850 [optimism: 0.033] for Model 1 and Model 2, respectively, indicating minimal-to-low degree of overfitting in the original models [Figure 2]. Original and shrinkage factor-adjusted regression coefficients along with final prediction model formulas are presented in Table 4.

4. Discussion

In the present study, we evaluated the additive predictive value of histopathological features in biopsies from CD patients taken at time of diagnosis. Using multivariable prediction models, we found that in comparison with clinically relevant baseline characteristics alone,

Table 2. Baseline characteristics of CD patients with a mild or a severe disease course during the first 10 years after diagnosis.

	Mild disease course [*n* = 64]	Severe disease course [*n* = 73]	*p*-value
Patient characteristics			
Age at diagnosis in years, mean [SD]	35.4 [14.4]	28.2 [10.6]	**0.001***
Gender, *n* [%]			0.534
Male	27 [42.2%]	27 [37.0%]	
Female	37 [57.8%]	46 [63.0%]	
IBD in family,[a] *n* [%]	12 [28.6%]	13 [22.4%]	0.483
First degree relative with IBD, *n* [%]	3 [25.0%]	2 [15.4%]	0.691
Smoking at diagnosis, *n* [%]			**0.015***
Yes	27 [42.2%]	46 [63.0%]	
No	37 [57.8%]	27 [37.0%]	
Disease characteristics			
Age distribution at diagnosis,[b] *n* [%]			**0.027***
A1: <16 years	0 [0 %]	0 [0 %]	
A2: 17–40 years	42 [65.6%]	60 [82.2%]	
A3: >40 years	22 [34.4%]	13 [17.8%]	
Disease location at diagnosis,[b] *n* [%]			**0.013***
L1: ileal	21 [32.8%]	20 [27.4%]	
L2: colonic	32 [50.0%]	24 [32.9%]	
L3: ileocolonic	11 [17.2%]	29 [39.7%]	
L4: isolated upper GI disease	0 [0 %]	0 [0 %]	
Disease behaviour at diagnosis,[b] *n* [%]			**0.017***
B1: non-stricturing, non-penetrating	60 [93.8%]	57 [78.1%]	
B2: stricturing	4 [6.3%]	12 [16.4%]	
B3: penetrating	0 [0.0%]	4 [5.5%]	
Perianal disease at diagnosis, *n* [%]	6 [9.4%]	7 [9.6%]	0.966
Upper GI disease at diagnosis,[c] *n* [%]	0 [0.0%]	4 [5.5%]	0.123
Biopsy characteristics			
Biopsies per patient, mean [SD]	6.1 [2.6]	5.9 [2.7]	0.682
Total number of biopsies, *N*	388	429	0.682
Terminal ileum, *N* [%]	63 [16.2%]	104 [24.2%]	0.109
Ileocecal valve, *N* [%]	18 [4.6%]	22 [5.1%]	0.908
Colon, *N* [%]	307 [79.1%]	303 [70.6%]	0.178
Caecum	31 [10.1%]	36 [11.9%]	
Ascendens	18 [5.9%]	25 [8.3%]	
Transversum	73 [23.8%]	49 [16.2%]	
Descendens	30 [9.8%]	38 [12.5%]	
Sigmoid	88 [28.7%]	100 [33.0%]	
Rectum	67 [21.8%]	55 [18.2%]	

CD, Crohn's disease; IBD, inflammatory bowel disease; GI, gastrointestinal tract; *n*, number of patients; *N*, number of biopsies; SD, standard deviation.
[a]No data available on IBD in family in *n* = 22 and *n* = 15 in mild and severe courses, respectively.
[b]Phenotype at diagnosis according to Montreal Classification.
[c]Four patients with both upper and lower GI involvement.
*Significant *p* value <0.05, based on independent samples t test or chi square test.

addition of histopathological features from colon biopsies resulted in a significant increase in predictive value for discrimination between patients with a long-term mild or a severe disease course.

In line with current literature, younger age at diagnosis, smoking, and stricturing/penetrating disease were all associated with worse outcomes [i.e., severe course] in the logistic regression models.[41] Ileocolonic disease location at diagnosis was also found to be associated with a severe disease course in our cohort. Although ileocolonic disease has previously been linked to disease progression, isolated ileal disease has more frequently been associated with worse outcomes.[42–44] In contrast to findings by others, perianal disease at diagnosis was not significantly associated with a severe disease course in the current study.[45,46] These contradictory findings once again underline the heterogeneous behaviour of CD, as well as reflect the limitations of the Montreal classification in accurately stratifying patients at baseline. This is further supported by our and previous findings demonstrating that the Montreal classification in combination with other clinically relevant baseline characteristics such as smoking status, gender and need for surgery at diagnosis moderately predicted disease course [AUROC 0.738].[16]

A combination of presence of [1] basal plasmacytosis, [2] Paneth cell metaplasia, [3] severe lymphocyte and plasma cell infiltration in the lamina propria, and [4] absence of ulcers resulted in a significant increase in predictive power compared with the model containing only relevant baseline characteristics [AUROC Model 2: 0.883 vs Model 1: 0.738, $p = 0.001$]. Internal validation of prediction models revealed that both models suffered from a low degree of overfitting. In clinical practice, high sensitivity is important to capture patients who are at risk of developing a severe disease course and to ensure timely introduction of therapy, whereas a high specificity is important to prevent misclassification and potential overtreatment. The second model showed good sensitivity [80.4%], being comparable to the first model [83.6%], but

Table 3. Prevalence of evaluated histopathological features in colonic biopsies from CD patients at diagnosis.

	Mild disease course [*n* = 57]	Severe disease course [*n* = 56]	*p*-value
Architectural distortion, *n* [%]			0.288
Absent	15 [26.3%]	10 [17.9%]	
Mild	27 [47.4%]	24 [42.9%]	
Severe	15 [26.3%]	22 [39.3%]	
Patchiness, *n* [%]	38 [66.7%]	42 [75.0%]	0.330
Neutrophil infiltration in lamina propria, *n* [%]			0.507
Absent	18 [31.6%]	13 [23.2%]	
Mild	21 [36.8%]	26 [46.4%]	
Severe	18 [31.6%]	17 [30.4%]	
Eosinophil infiltration in lamina propria, *n* [%]			0.392
Absent	28 [49.1%]	22 [39.3%]	
Mild	24 [42.1%]	25 [44.6%]	
Severe	5 [8.8%]	9 [16.1%]	
Lymphocyte and plasma cell infiltration in lamina propria, *n* [%]			**0.003***
Absent	9 [15.8%]	3 [5.4%]	
Mild	27 [47.4%]	15 [26.8%]	
Severe	21 [36.8%]	38 [67.9%]	
Ulcers and/or erosions, *n* [%]	15 [26.3%]	14 [25.0%]	0.873
Granulomas, *n* [%]	7 [12.3%]	14 [25.0%]	0.082
Cryptitis, *n* [%]	41 [71.9%]	44 [78.6%]	0.414
Crypt abscess, *n* [%]	28 [50.9%]	24 [57.1%]	0.504
Paneth cell metaplasia, *n* [%]	13 [22.8%]	29 [51.8%]	**0.001***
Pyloric gland metaplasia, *n* [%]	1 [1.8%]	1 [1.8%]	1.000
Mucin depletion, *n* [%]			0.644
Absent	28 [49.1%]	23 [41.4%]	
Mild	18 [31.6%]	22 [39.3%]	
Severe	11 [19.3%]	11 [19.6%]	
Lymphoid follicles with/without germinal centres, *n* [%]			0.580
Absent	12 [21.1%]	15 [26.8%]	
Present without germinal centre	32 [56.1%]	26 [46.4%]	
Present with germinal centre	13 [22.8%]	15 [26.8%]	
Basal plasmacytosis, *n* [%]	32 [56.1%]	47 [83.9%]	**0.001***
Chronic inflammation of submucosa, *n* [%][a]	25 [54.3%]	29 [59.2%]	0.634

[a]Due to the nature of the biopsies, 'chronic inflammation of submucosa' could not be assessed in *n* = 11 and *n* = 7 in mild and severe course respectively
*Significant *p*-value <0.05 based on chi square test, or Fisher's exact test when applicable

showed remarkably better specificity [84.2% vs 53.1%], thereby being the preferred model to contribute to clinical decision making.

Basal plasmacytosis, lymphocyte and plasma cell infiltration in lamina propria, and Paneth cell metaplasia are all features of chronicity, whereas ulcers are features of an acute inflammatory process.[47,48] Basal plasmacytosis is recognised as a strong predictor for the presence of IBD with good reproducibility, and is commonly observed in both UC [63%] and CD biopsies [62%] at time of diagnosis.[49–51] These percentages are also in line with the total prevalence of basal plasmacytosis in our cohort [69.9% colonic, 62.5% ileal biopsies]. In UC, a combination of increased lamina propria cellularity and basal plasmacytosis is associated with an increased risk of relapse.[52,53] Data in CD are not available. Furthermore, the observed prevalence of infiltration of lymphocytes and plasma cells in the lamina propria and Paneth cell metaplasia was comparable to literature, although data are scarce.[48] Given the distribution in the univariable analyses, one would not expect ulcers to be identified in the multivariable analyses. However, the appearance of ulcers and its negative coefficient have to be interpreted in context of the other features in the model, as they were correlated. More specifically, all other three chronic features were more frequently observed in both disease course groups at diagnosis when ulcers were present in comparison with when ulcers were absent.

The presence of many of the features are likely to vary over time.[54] Schumacher *et al.* were the first to describe time-related changes of histopathological features in untreated patients with IBD.[55] Basal plasmacytosis, probably one of the earliest features, was observed in 38% of patients within the first 15 days after initiation of symptoms, progressing to 54% within 30 days, 81% within 120 days, and 89% within 300 days of symptom duration. In contrast, Paneth cell metaplasia appears to be a feature of more long-standing disease and its presence correlates with disease duration.[56–58] However, early, untreated IBD, may also show no or minimal histological changes.[48] Time-related changes for lamina propria infiltration and ulcers have not been described previously. Although elucidating the exact sequential changes on a histological level is challenging and requires a well-defined prospective approach, these data provide insight into a time-dependent effect on the presence of features. Based on the identified features of chronicity in our prediction models, Paneth cell metaplasia in particular, one may hypothesise that a longer period of histological disease activity preceded diagnosis of CD in patients with a subsequent severe course. Studies have shown that longer duration of untreated CD can induce irreversible damage, resulting in a reduced response to medical therapy and complicated disease course as a consequence.[59] To support this hypothesis, insight in time from onset of symptoms to the actual diagnosis is needed. However, it is

Table 4. Results from multivariable logistic regression models of clinically relevant baseline characteristics and upon addition of histopathological features from colonic biopsies.

Intercept and predictors	Model 1. Clinically relevant baseline characteristics				Model 2.[a] Clinically relevant baseline characteristics and combination of histopathological features			
	B	B[b]-corrected	*p*	Adjusted OR [95% CI]	B	B[b]-corrected	*p*	Adjusted OR [95% CI]
Age at diagnosis	-0.050	-0.046	**0.010***	0.95 [0.92–0.99]	-0.081	-0.065	**0.003***	0.92 [0.87–0.97]
Gender, female	-0.646	-0.591	0.17	0.52 [0.21–1.32]	-0.550	-0.440	0.32	0.58 [0.20–0.54]
Disease location			**0.025***				**0.018***	
L1	REF				REF			
L2 vs L1	0.690	0.631	0.28	1.99 [0.58–6.91]	-0.789	-0.630	0.37	0.45 [0.08–2.58]
L3 vs L1	1.735	1.588	**0.012***	5.67 [1.46–22.0]	1.011	0.807	0.25	2.75 [0.49–15.56]
Disease behaviour								
B1	REF				REF			
B2/B3 vs B1	1.180	1.080	0.17	3.25 [0.61–17.4]	2.643	2.111	**0.023***	14.06 [1.45–136.4]
Perianal disease	-0.026	-0.024	0.97	0.97 [0.25–3.77]	0.021	0.017	0.98	1.02 [0.18–5.73]
Smoking at diagnosis	0.568	0.520	0.18	1.76 [0.77–4.04]	0.817	0.652	0.13	2.26 [0.80–6.44]
Basal plasmacytosis	-				2.391	1.909	**0.006***	10.92 [2.01–59.30]
Paneth cell metaplasia	-				1.600	1.278	**0.004***	4.95 [1.65–14.86]
Lymphocyte infiltration [LP]								
Absent/mild	REF				REF			
Severe vs Absent/mild	-				1.445	1.154	**0.037***	4.24 [1.09–16.45]
Ulcer/erosion	-				-2.072	-1.655	**0.006***	0.13 [0.029–0.54]
Intercept	0.622				-0.337			
	AUROC [95% CI]: 0.738 [0.647–0.828] Bootstrap optimism-corrected AUROC: 0.720				AUROC [95% CI]: 0.883 [0.821–0.944] Bootstrap optimism-corrected AUROC: 0.850			

The predicted probability for a severe course can be calculated by using the following formulas. Coefficients in the final prediction model formulas are bias-corrected using the shrinkage factor obtained from the internal validation procedure. Predictor value is 1 when present and 0 when absent, except for AgeAtDiagnosis which is continuous.

Model 1 formula: *P*[*severe disease course*] = 1/[1 + exp [-[0.622 + AgeAtDiagnosis*-0.046 + GenderFemale*-0.591 + DiseaseLocationL2*0.631 + DiseaseLocationL3*1.588 + PerianalDisease*-0.024 + SmokingAtDiagnosis*0.568]]].

Model 2 formula: *P*[*severe disease course*] = 1/[1 + exp [-[-0.337 + AgeAtDiagnosis*-0.065 + GenderFemale*-0.440 + DiseaseLocationL2*-0.630 + DiseaseLocationL3*0.807 + PerianalDisease*0.017 + SmokingAtDiagnosis*0.652 + BasalPlasmacytosis*1.909 + PanethCellMetaplasia*1.278 + LymphocyteInfiltrationLPSevere*1.154 + Ulcer/Erosion*-1.655]]].

All model assumptions were met with VIFs <1.5 and Cook's distance <1 across both models.

L1, ileal; L2, colonic; L3, ileocolonic; B1, non-stricturing/non-penetrating disease; B2/B3, stricturing/penetrating disease; OR, odds ratio; CI, confidence interval; REF, reference category; *p*, *p*-value; B, regression coefficient; AUROC, area under receiver operating characteristiccurve; VIFs, variance inflation factors.

[a]Same final model obtained after forward stepwise and Hosmer & Lemeshow methods.

[b]Regression coefficient [B] from Model 1 and 2 multiplied by the shrinkage factor obtained from the bootstrapping procedure [Model 1: 0.9152; Model 2: 0.7986].

*Significant *p*-value <0.05.

Prediction Models	AUROC [95% CI]	Bootstrap-corrected AUROC [optimism]	Sensitivity; Specificity‡	PPV; NPV
1: Baseline characteristics[a]	0.738 [0.65-0.83]	0.720 [0.018]	83.6%; 53.1%	67.0%; 73.9%
2: Baseline characteristics and combination of features[b]	0.883 [0.82-0.94]	0.850 [0.033]	80.4%; 84.2%	83.3%; 81.4%

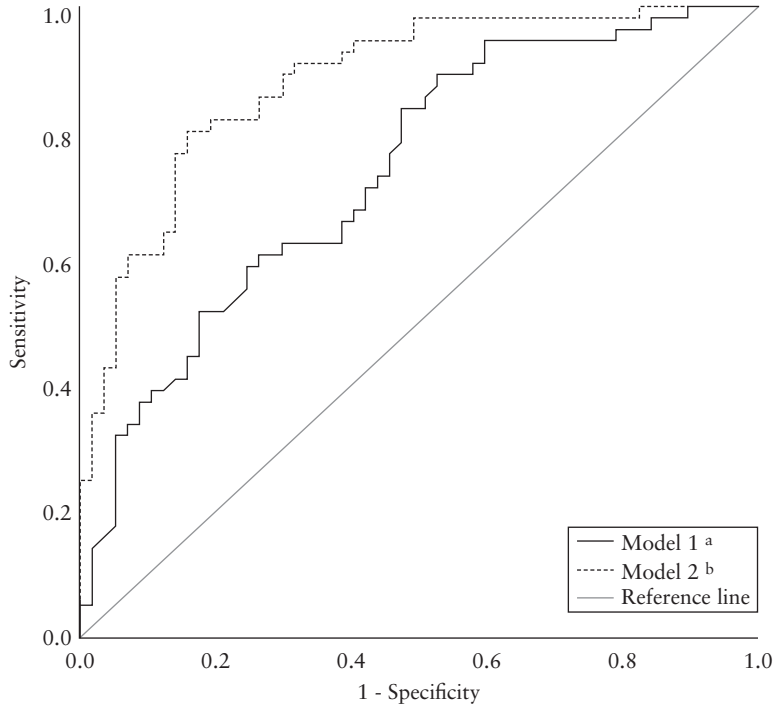

Figure 2. Results from ROC analyses for discrimination between development of a mild and a severe disease course [state variable] and corresponding diagnostic measures. [a]Model 1: baseline characteristics [i.e., Montreal classification, smoking status at diagnosis, and gender]; [b]Model 2: includes all variables from Model 1 and [1] basal plasmacytosis, [2] severe lymphocyte and plasma cell infiltration in lamina propria, [3] Paneth cell metaplasia, and [4] absence of ulcers. ‡Sensitivity and specificity at the optimal cut-off based on Youden's J statistic. AUROC, area under the receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

also known that CD can have a subclinical course before onset of symptoms, which makes it challenging to attest this hypothesis.[60,61]

In contrast to colonic biopsies, we did not observe any statistically significant differences in biopsies from the ileal region between patients with mild and severe disease course, and no features could be identified being of additive predictive value. This may in part be due to the relatively small sample size in this group. The majority of patients [82.5%] had colonic biopsies taken at time of diagnosis, whereas in only 58.3% of patients ileal biopsies were collected. Our findings on these particular biopsies should therefore be interpreted with care.

To our best knowledge, this is the first study to assess the additive predictive value of multiple histopathological features in diagnostic biopsies from CD patients to discriminate between disease courses. Klein *et al.* also investigated biopsies from the first documented colonoscopy. However, they did not evaluate characteristic features of IBD. Instead they made use of additional stainings to perform morphometric analysis on colonic biopsies from CD patients and were able to differentiate between inflammatory and stricturing/penetrating phenotypes [B1 vs B2 AUROC: 0.74; B1 vs B3: 0.78] based on crypt areas and optical densities of collagen.[19] Other studies have primarily focused on predicting relapse and complications during the course of the disease or on the predictive value of single features [i.e., granulomas] with conflicting results. Hofstädter *et al.* retrospectively examined intestinal biopsies from 63 CD patients with a mean disease follow-up of 3.4 years before onset of fistulas and strictures, by analysing 34 histopathological features, and found that a combination of lymphocyte infiltration in lamina propria, crypt atrophy, and absence of lymphocytes in the epithelium were best to predict an uncomplicated course [sensitivity 67%; specificity 83%].[20] Brennan *et al.* correlated histological abnormalities found during elective colonoscopies in CD patients in clinical remission with subsequent flares, and observed that the presence of neutrophils and eosinophils in lamina propria was associated with an increased risk of flares within 24 months.[21] No influence on outcome was observed in more than half of the studies evaluating the role of granulomas, whereas in certain studies the risks of complications or recurrence were either positively or negatively correlated with granuloma presence.[62–68] One explanation for the contradictory findings of the previous studies is the effect of treatment on histological findings. Several studies have shown that treatment can alter histological findings with high treatment-specific variability.[48,54] Combination treatment with prednisolone and sulphasalazine, for example, can result in a high degree of histological normalisation,

whereas anti-tumour necrosis factor [TNFα] exposure can impede granuloma formation and induce reduction of chronic features after a single dose.[69–72] Another possible explanation for the conflicting findings arises from the methodological approaches. None of the studies adjusted their findings for all well-established relevant baseline characteristics. Finally, even though granulomas have proven reliable for distinguishing between CD and UC, they are not always present. The overall prevalence of granulomas in biopsies is relatively low and granulomas can develop in several other infectious colitides, such as tuberculosis, which creates differences across observations and limits the potential predictive value of granulomas as a single feature.[50,73] In the present study, granulomas were more frequently observed in diagnostic colonic and ileal biopsies from patients with a severe disease course. However, neither observation was statistically significant and overall prevalence, in line with literature, was low [ileal 11.3%, colonic 18.6%].[48]

The main strength of the present study is the inclusion of subjects, originating from the IBDSL cohort, with well-defined disease courses and follow-up during the first 10 years after diagnosis, enabling long-term predictive value. Of note, baseline characteristics of the included study population did not significantly differ from the original cohort for both disease course groups [data not shown].[16] Furthermore, all patients were treatment-naïve, thereby eliminating treatment-dependent effects on histological findings. Other important strengths of our study are: the use of a scoring form developed through a comprehensive literature search and input from two senior GI pathologists to ensure incorporation of all possible relevant features, and the adjustment for clinically relevant baseline characteristics. In addition, we performed internal validation of our predictions models using bootstrapping to account for overfitting and to obtain bias-corrected performance estimates to enhance generalisability of results. Nevertheless, we also recognise this study's limitations. First, the retrospective character resulted in dependence on biopsies available, which were taken according to routine clinical practice at the time and may have introduced sampling bias. However, by taking a 'worst case' approach we tried to minimise the effect of random sampling. Second, our findings are limited to patients with a mild or severe disease course and a potential role of histopathological features as disease course predictors for the remaining population, with moderately active [i.e. intermediate] disease courses, remains to be determined. This is an important next step since these patients also highly benefit from adequate stratification at diagnosis. Third we used a 'worst case' approach, based on the segment of most severe histological inflammation, to identify relevant features, instead of only assessing the macroscopic disease location at time of diagnosis. Though, by taking this approach we were able to identify and account for possible histological inflammation in the absence of macroscopic inflammation. Last, we did not quantify number of cells to differentiate between severity of certain features, instead used semi-quantitative scoring scales as generally used in histopathological evaluation studies and clinical practice.

In conclusion, the results of this study suggest that presence of certain features of chronicity in colonic biopsies from CD patients, taken at time of diagnosis, contribute to the prediction of patients who are prone to have a severe, instead of a mild, disease course in the first 10 years after diagnosis. Considering that histological sampling and assessment of the selected histopathological features in this study are part of the workup in diagnosis of CD, this presents a potential novel approach to improve patient stratification at diagnosis and may aid in optimising choice of adequate treatment regimens, thereby finding a balance between under- and overtreatment and improving long-term outcomes. Nonetheless, before clinical implementation, the findings of this study should be validated in independent cohorts and prospective follow-up studies.

Data Availability Statement

A data management plan for IBDSL is made in collaboration with DataHub MUMC+. DataHub works according to the FAIR principles. Metadata of IBDSL are available for interested researchers and data are available after approval of research proposals by the IBDSL committee [contact: m.pierik@mumc.nl].

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Conflict of Interest

DJ reports grants from Top Knowledge Institute [Well on Wheat], grants from Horizon 2020 DISCOvERIE, grants from NWO-CCC Partnership programme [Carbokinetics], all outside the submitted work. MP reports grants and non-financial support from Falk Pharma, grants from European Commission, grants from ZONMW [Dutch national research fund], grants and non-financial support from Takeda, grants and non-financial support from Johnson and Johnson, grants and non-financial support from Abbvie, non-financial support from Ferring, non-financial support from Immunodiagnostics, non-financial support from MSD, all outside the submitted work. The other authors have nothing to disclose relevant to this publication.

Author Contributions

ARA, MP, and DJ conceptualised the study. ARA retrieved all biopsy materials, was involved in preparation of biopsy materials, and digitised all biopsies for scoring purposes. DG and HIG provided input during development of the scoring form and critically revised and scored all biopsy materials. ARA, DW, and MR collected the data. ARA, DJ, and BW analysed and interpreted the data. ARA and DJ drafted the manuscript. All authors critically reviewed and revised the manuscript for intellectual content. All authors approved the final manuscript.

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Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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