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. 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. 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. 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.

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. 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. Furthermore, serological markers measured at diagnosis, such as antimicrobial antibodies, have proven inadequate in accurately predicting disease course. The observations derived from these clinical, genetic, and serological markers warrant the identification of alternative and better predictors of disease course.

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. 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. 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. 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. 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. 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. 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].

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. For all patients, disease activity was assessed for each annual quarter since time of diagnosis, as has been described previously. 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 strictureing 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 if treatment-naive endoscopic biopsies obtained at the time of CD diagnosis were available. Definitions for disease course were adapted from our previous work. 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 as ‘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 eligible patients were collected from patients’ medical records using smoking status at diagnosis, medication use, and family history of all type at diagnosis according to the Montreal classification, gender, 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].

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. 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.11,32

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 ≤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.16,37 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
| Histopathological feature | Definition                                                                                                                                                                                                                                                                                                                                 | Scoring range   |
|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|
| 1. Architectural distortion | Ileum: widening, blunting, and/or shortening of villi\cite{26,74} Colon: ranging from normal parallel crypts to irregularly arranged, branching crypts, crypt loss, crypt shortening, and surface villi formation\cite{26,74} | 0, absent; 1, mild; 2, severe |
| 2. Patchiness              | Change from continuous to discontinuous inflammation\cite{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\cite{20}                                                                                                                                                      | 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\cite{20,76}                                                                                                                                                       | 0, absent; 1, mild; 2, severe |
| 5. Lymphocyte and plasma cell infiltration in lamina propria | 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\cite{26}                                                                                     | 0, absent; 1, mild; 2, severe |
| 6. Ulcers and/or erosion   | Presence of granulation tissue in an area with loss of epithelial surface\cite{26}                                                                                                                                                                                                 | 0, absent; 1, present |
| 7. Granulomas              | Collection of epithelioid cells, not related to crypt injury\cite{7}                                                                                                                                                                                                                                                                      | 0, absent; 1, present |
| 8. Cryptitis               | Presence of neutrophils on top of a crypt epithelial cell\cite{17}                                                                                                                                                                                                                | 0, absent; 1, present |
| 9. Crypt abscess          | Cluster of neutrophils inside the crypt lumen\cite{17}                                                                                                                                                                                                                           | 0, absent; 1, present |
| 10. Paneth cell metaplasia | Evaluated only distal from hepatic flexure\cite{26}                                                                                                                                                                                                                               | 0, absent; 1, present |
| 11. Pyloric gland metaplasia | Presence of gastric pyloric-type glands in either ileum or colon\cite{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\cite{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]\cite{16}                                                                                                                                                                                              | 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\cite{26,73}                                                                                                                                                                | 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/mm²]. D: Severe lymphocyte and plasma cell infiltration in lamina propria [400x; 66 cells/mm²]. E: Granuloma [400x]. F: Paneth cell metaplasia [300x]. G: Cryptitis [600x]. H: Crypt abscess [400x]. I: Severe mucin depletion [400x]. J: Basal plasmacytosis [200x].
to obtain a more accurate optimism-corrected estimate of model as proposed by Steyerberg. 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. 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 HMSCI 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.

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-naive patients with a mild and 73 treatment-naive 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,
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.41,42 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.43,44 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 2. Baseline characteristics of CD patients with a mild or a severe disease course during the first 10 years after diagnosis. |
|-------------------------------------------------|-------------------------------------------------|-----------------|
| Patient characteristics                         | Mild disease course \( n = 64 \) | Severe disease course \( n = 73 \) |
| Age at diagnosis in years, mean [SD]            | 35.4 [14.4] | 28.2 [10.6] | 0.001* |
| Gender, \( n \) [%]                              | Male: 27 [42.2%] | 27 [37.0%] | 0.534 |
|                                                 | Female: 37 [57.8%] | 46 [63.0%] |               |
| IBD in family, \( n \) [%]                       | 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, \( n \) [%]                | Yes: 27 [42.2%] | 46 [63.0%] | 0.015* |
|                                                 | No: 37 [57.8%] | 27 [37.0%] |               |
| Disease characteristics                         | Age distribution at diagnosis, \( n \) [%]        | 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, \( n \) [%]       | 0.013* |
|                                                 | L1: ileal | 21 [32.8%] | 20 [27.4%] |
|                                                 | L2: colonic | 32 [50.0%] | 24 [32.9%] |
|                                                 | L3: ileocolic | 11 [17.2%] | 29 [39.7%] |
|                                                 | L4: isolated upper GI disease | 0 [0 %] | 0 [0 %] |
| Disease behaviour at diagnosis, \( n \) [%]      | 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, \( n \) [%]       | 0.966 |
|                                                 | 0 [0.0%] | 4 [5.5%] | 0.123 |
| Upper GI disease at diagnosis, \( n \) [%]       | 0.178 |
|                                                 | 0 [0.0%] | 303 [70.6%] | 0.178 |
| Biopsy characteristics                          | 6.1 [2.6] | 5.9 [2.7] | 0.682 |
| Biopsies per patient, mean [SD]                  | 388 | 429 | 0.682 |
| Total number of biopsies, \( N \)                | 63 [16.2%] | 104 [24.2%] | 0.109 |
| Terminal ileum, \( N \) [%]                     | 18 [4.6%] | 22 [5.1%] | 0.908 |
| Ileocecal valve, \( N \) [%]                     | 307 [79.1%] | 303 [70.6%] |               |
| Colon, \( N \) [%]                               | 31 [10.1%] | 36 [11.9%] |               |
| Caecum                                          | 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.

*No data available on IBD in family in \( n = 22 \) and \( n = 15 \) in mild and severe courses, respectively.

4Phenotype at diagnosis according to Montreal Classification.

4Four patients with both upper and lower GI involvement.

*Significant \( p \) value <0.05, based on independent samples t test or chi square test.
The presence of many of the features are likely to vary over time. Schumacher et al. were the first to describe time-related changes of histopathological features in untreated patients with IBD. 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. However, early, untreated IBD, may also show no or minimal histological changes. 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. To support this hypothesis, insight in time from onset of symptoms to the actual diagnosis is needed. However, it is

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

| Feature                                              | 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 [45.1%]                     | 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 [%]                               | 28 [49.1%]                  | 23 [41.4%]                     | 0.644   |
| Absent                                              | 18 [31.6%]                  | 22 [39.3%]                     |         |
| Mild                                                 | 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 [%]*            | 25 [54.3%]                  | 29 [59.2%]                     | 0.634   |

*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. 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. 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. 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. 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.
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. Clinically relevant baseline characteristics and combination of histopathological features |
|-------------------------|-----------------------------------------------------|--------------------------------------------------------------------------------------------------|
|                         | Model 1                                             | Model 2.                                                                                         |
|                         | B         | B\textsuperscript{b}-corrected | \textit{p} | Adjusted OR [95% CI] | B         | B\textsuperscript{b}-corrected | \textit{p} | Adjusted OR [95% CI] |
| Age at diagnosis        | -0.050   | -0.046                      | 0.010\textsuperscript{*} | 0.95 [0.92–0.99]    | -0.081 | -0.065                      | 0.003\textsuperscript{*} | 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        |          |                             |       |                    |          |                             |       |                    |
| 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\textsuperscript{*} | 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\textsuperscript{*} | 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\textsuperscript{*} | 10.92 [2.01–59.30]  |
| Paneth cell metaplasia  | -        |                             |       |                    | 1.600   | 1.278                      | 0.004\textsuperscript{*} | 4.93 [1.63–14.86]   |
| Lymphocyte infiltration \[LP\] |          |                             |       |                    |          |                             |       |                    |
| Absent/mild             |          | REF                         |       |                    |          | REF                         |       |                    |
| Severe vs Absent/mild   | -        |                             |       |                    |          | 1.445 | 1.154                      | 0.037\textsuperscript{*} | 4.24 [1.09–16.45]   |
| Ulcer/erosion           | -        |                             |       |                    | -2.072  | -1.655                      | 0.006\textsuperscript{*} | 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: \( P[\text{severe disease course}] = \frac{1}{1 + e^{-[0.622 + \text{AgeAtDiagnosis} - 0.046 + \text{GenderFemale} - 0.591 + \text{DiseaseLocationL2} - 0.631 + \text{DiseaseLocationL3} - 1.588 + \text{PerianalDisease} - 0.024 + \text{SmokingAtDiagnosis} - 0.568]}}\).

Model 2 formula: \( P[\text{severe disease course}] = \frac{1}{1 + e^{-[-0.337 + \text{AgeAtDiagnosis} + 0.065 + \text{GenderFemale} - 0.440 + \text{DiseaseLocationL2} + 0.630 + \text{DiseaseLocationL3} - 0.807 + \text{PerianalDisease} - 0.017 + \text{SmokingAtDiagnosis} - 0.625 + \text{BasalPlasmacytosis} - 1.909 + \text{PanethCellMetaplasia} + 1.278 + \text{LymphocyteInfiltrationLPSevere} + 1.154 + \text{Ulcer/Erosion} + 1.655]}}\).

All model assumptions were met with VIFs < 1.5 and Cook’s distance < 1 across both models. L1, jej; 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 characteristic curve; 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].

\(^{c}\)Significant \( p \)-value < 0.05.
also known that CD can have a subclinical course before onset of symptoms, which makes it challenging to attest this hypothesis. 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.

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. 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%].

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. 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. 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.

Combination treatment with prednisolone and sulphasalazine, for example, can result in a high degree of histological normalisation.

### Table: Prediction Models AUROC [95% CI] Bootstrap-corrected AUROC [optimism] Sensitivity; Specificity PPV; NPV

| Prediction Models                        | AUROC [95% CI] | Bootstrap-corrected AUROC [optimism] | Sensitivity | Specificity | PPV | NPV |
|-----------------------------------------|----------------|--------------------------------------|-------------|-------------|-----|-----|
| 1: Baseline characteristics             | 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 | 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.  
†Model 1: baseline characteristics [i.e., Montreal classification, smoking status at diagnosis, and gender]; ‡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.
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 [Carboskinetics], 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 digested 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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