Journal of the Canadian Association of Gastroenterology, 2021, 4(5), e92–e100
doi: 10.1093/jcag/gwaa033
Original Article
Advance Access publication 15 October 2020

OXFORD

Original Article

Clinical and Laboratory Characteristics Are Associated With Biologic Therapy Use in Pediatric Inflammatory Bowel Disease: A Retrospective Cohort Study

Mary E. Sherlock, MD PhD[1], Mary Zachos, MD[1], Robert M. Issenman, MD[1], Daniel J. Mulder, MD PhD[2]

[1]Department of Pediatrics, Division of Pediatric Gastroenterology, McMaster Children's Hospital, Hamilton, Ontario, Canada; [2]Division of Gastroenterology, Hepatology and Nutrition, Hospital for Sick Children, Toronto, Ontario, Canada

Correspondence: Daniel J. Mulder, MD, PhD, Division of Gastroenterology, Hepatology and Nutrition, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada, e-mail: daniel.mulder@sickkids.ca

Abstract

Background: Biologic agents are a highly useful class of medications for treating inflammatory bowel disease (IBD). Limited evidence exists to guide initiation of biologic therapy, especially in pediatric patients. It is unclear if disease severity is connected to biologic response. We hypothesized that the clinical, biochemical and radiographic characteristics of pediatric IBD at diagnosis were associated with subsequent initiation of biologic therapy.

Methods: We performed a retrospective analysis of the charts of all pediatric patients diagnosed with IBD at our centre over 14 years. Kaplan–Meier curves evaluated patient characteristics at diagnosis with time to initiation of biologic therapy. A Cox proportional hazards model was used for multivariate characteristic analysis.

Results: A total of 198 patients were included, 57.6% had Crohn's disease, 27.8% had ulcerative colitis and 14.6% had IBD type unclassified. Mean follow-up time was 47.8 months. About 55.5% of the patients received a biologic medication, the mean time to biologic initiation was 21.5 months. Earlier initiation of biologic therapy was frequently associated with older age, higher disease activity index and lower serum albumin.

Conclusions: Older pediatric patients with more severely active disease and lower serum albumin levels at the time of IBD diagnosis were more likely to initiate biologic therapy when considering biologic initiation, even many years after diagnosis. Identification of these characteristics may help inform decisions to initiate biologic therapy earlier in the IBD disease course.

Keywords: *Biologic therapy; IBD natural history; IBD outcomes; IBD therapy*

Introduction

Inflammatory bowel disease (IBD) is a heterogeneous group of inflammatory conditions that primarily affect the gastrointestinal tract. The etiology of IBD is currently unknown. The natural history of IBD spans a wide spectrum of severity. This creates a wide variety of manifestations along the IBD disease spectrum, ranging from mild disease that may not require ongoing treatment to severe disease that is relapsing and remitting in spite of aggressive therapy (1).

Treatment of IBD is aimed at inducing and then maintaining remission. Therapeutic strategies are currently in evolution. Traditionally, IBD therapy has been approached in a stepwise fashion, where increasingly potent immunosuppressive medications are used until the disease is clinically and biochemically controlled. In recent years, biologic therapy has become more readily available (2). These biologically derived molecules target specific immunologic molecules known to be integral to

Received: May 22, 2020; Accepted: September 15, 2020.

the IBD inflammatory cascade, such as tumour necrosis factor alpha (TNF-α).

Although multiple trials have demonstrated the efficacy of biologic therapy, there is limited objective evidence regarding the indications for initiating biologic therapy in children. For example, biologic therapy has been shown to aid in linear growth impairment recovery (prepuberty) (3) and lead to longer sustained healing in fistulizing disease (4). It has also been demonstrated that starting this treatment earlier in the IBD disease course leads to improved outcomes (5,6).

Despite strong evidence of clinical effect, biologic therapy is not for all patients. Limited evidence exists to guide the decision to start biologic therapy, especially in pediatric patients (7,8). Commonly, features suggestive of severe disease course are used as justification for starting biologic therapy. It is likely that some patients that are not at high risk for severe disease would still experience improved outcomes with biologic therapy. Thus, identifying specific patients who would benefit from biologic therapy is imperative. Additionally, it will be important to establish which patients will not respond to biologic therapy, so as not to expose them to potential side effects. Ideally, patient characteristics identified at the time of diagnosis could be used to guide the decision to start biologic therapy.

We hypothesized that in our patients there were specific clinical, biochemical, endoscopic and histologic characteristics of IBD at diagnosis that were correlated with the eventual initiation of biologic therapy. In this study, we demonstrated the characteristics of IBD at diagnosis that were statistically associated with subsequent biologic therapy.

PATIENTS AND METHODS

Study Population

This study was approved by the McMaster University Health Sciences Research Ethics Board and conforms with the principles outlined in the Declaration of Helsinki. We identified all patients diagnosed from April 2001 to August 2015 with IBD followed at McMaster Children's Hospital, Hamilton, Canada through our patient database, which was assembled for the purpose of this study. Patients were sub-classified by phenotype as Crohn's disease (CD), ulcerative colitis (UC) or IBD-unclassified (IBD-U). We excluded patients with <9 months of follow-up and patients referred to our centre with an already established diagnosis of IBD.

Data Collection

We collected the clinical, biochemical, endoscopic, radiologic and histologic features of disease present at diagnosis. Details of the data collected are summarized in Supplementary Tables 1–3 and additionally included time to biologic initiation (or time to censoring) and a narrative description of the course of therapy for each patient up to this time. Date of diagnosis was defined as the date of the diagnostic endoscopy. We considered any data to be collected from 3 months prior to the diagnosis until 3 months after the date of diagnosis, with priority given to using data collected as close to the initial diagnostic investigation as was available. The assay and normal range for C-reactive protein at our centre was changed mid-way through the study period, so this parameter was not included in our analysis. For patients whose IBD subtype diagnosis eventually changed (for example, IBD-U to CD), the date of diagnosis was not altered, but the most recent subtype classification was considered to be the patient's definitive IBD subtype. Patients diagnosed with IBD-U were assessed by both validated scores of disease activity: the Pediatric Crohn's Disease Activity Index (PCDAI) (9) and Pediatric Ulcerative Colitis Activity Index (PUCAI) (10). In terms of endoscopic evaluation, 21 patients (10.6%) did not undergo upper endoscopy at the time of colonoscopy and 2 patients (1.0%) did not have a colonoscopy at the time of diagnosis (due to a diagnostic upper endoscopy and then delayed lower endoscopy). Overall, the proportion of missing data for all fields was 4.8%, 11.2% and 7.8% for CD, UC and IBD-U, respectively.

To define specific cut offs to facilitate time series analysis of our data, we began with stepwise evaluation of increasing thresholds examining for significance. If multiple thresholds reached significance, we chose the strongest one. Comparisons between similar threshold values with significance levels are included in Supplementary Tables 1–3.

We also documented the medications used to establish and maintain disease control over time for each patient, especially with respect to biologic initiation and months from diagnosis to most recent follow-up appointment. To be classified as having initiated a biologic, the patient was required to have received at least three doses. There were no institutional criteria used for when to start a patient on biologic therapy at our centre, thus the initiation was dependent on physician impression and did not require endoscopic or detailed imaging prior to initiation. Biologic use increased over the study period (Supplementary Figure 1). Seven pediatric gastroenterologists were involved in diagnosis and initiation of biologic therapy in this population. Overall, potential bias was minimized by standardized data collection, quantitative predefined outcomes and a priori standards for excluding samples with missing data.

Data Analysis

We compared the presenting features of those patients who received biologic therapy at any time (cases) with those who did not receive biologic therapy (controls). The value of each characteristic was compared with the time to biologic initiation (or right censoring) on a Kaplan–Meier curve. A log-rank (Mantel–Cox) test was used to determine statistical

Table 1. Baseline cohort characteristics at diagnosis of IBD

Characteristic	Mean (SD) (unless otherwise stated)
Age at diagnosis, years	10.5 (3.9)
Male sex (%)	59.1
Weight *z*-score	−0.6 (1.3)
Height *z*-score	−1.1 (1.3)
Albumin (g/L)	32.3 (7.5)
Erythrocyte sedimentation rate (mm/h)	33.0 (20.6)
Disease subtype, % (*n*)	
Crohn's disease	57.6 (*n* = 114)
Ulcerative colitis	27.8 (*n* = 56)
IBD-unclassified	14.6 (*n* = 28)
PCDAI	23.9 (17.8)
PUCAI	39.5 (22.4)
Paris classification* (%)	
Crohn's disease	
A1a	40.4
A1b	57.9
A2	1.8
L1	2.6
L2	23.5
L3	72.2
L4a†	48.7
L4b	18.3
B1	66.1
B2	13.9
B3	18.3
B2B3	0.8
p (perianal disease)	23.5
Ulcerative colitis	
E1	8.9
E2	25.0
E3	8.9
E4	57.1
S0	83.9
S1	16.1
PGAS (%)	
Mild	40.4
Moderate	47.5
Severe	11.6
Follow-up, months	47.8 (35.2)

IBD, Inflammatory bowel disease; PCDAI, Pediatric Crohn's Disease Activity Index; PGAS, Physician Global Assessment Score; PUCAI, Pediatric Ulcerative Colitis Activity Index.

*Paris classification for pediatric IBD (11).

†As per the Paris classification, L4a and L4b can co-exist with L1-3.

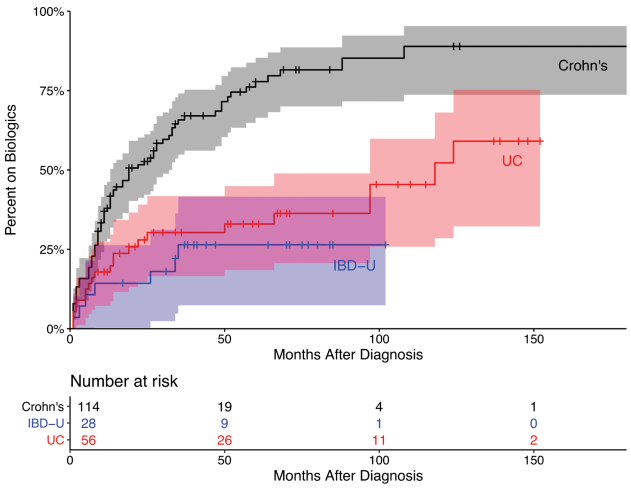

Figure 1. Kaplan–Meier curve of time to initiation of biologic treatment stratified by disease subtype. Shaded regions indicate the 95% confidence interval over time.

significance for categorical variables. Continuous parameters were correlated by calculating the nonparametric Spearman's rank correlation coefficient and performing univariate linear regression. Continuous parameters were dichotomized using cut-point values nearest those that were estimated by the maximally selected rank statistic (as calculated using the R package 'maxstat', version 0.7.25), and were also outside of the normal range of the test, and simplified to a rounded integer value (for ease of interpretation in a clinical setting).

A multivariate Cox proportional hazards model was calculated by initializing the models with the variables that were statistically significant in the univariate analysis. Subsequent models were evaluated sequentially by addition of individual variables that did not reach univariate statistical significance and optimized to the highest concordance while maintaining statistical significance and limiting variable number to five or less. In all cases, statistical significance was defined as $P < 0.05$. A minimum of 10 patients in each category was required for comparison for each variable in both the univariate and multivariate models. Statistical analysis and graphical representation were performed using GraphPad Prism version 7.0 (GraphPad, La Jolla, CA) and R (version 3.6) with software packages 'survminer' (version 0.4.8) and 'survival' (version 3.2.3).

RESULTS

Two hundred twenty-four patients were diagnosed with IBD at our centre during the study dates. Out of this cohort, eight patients were excluded from analysis because the diagnosis was made before contact with our team or the diagnosis

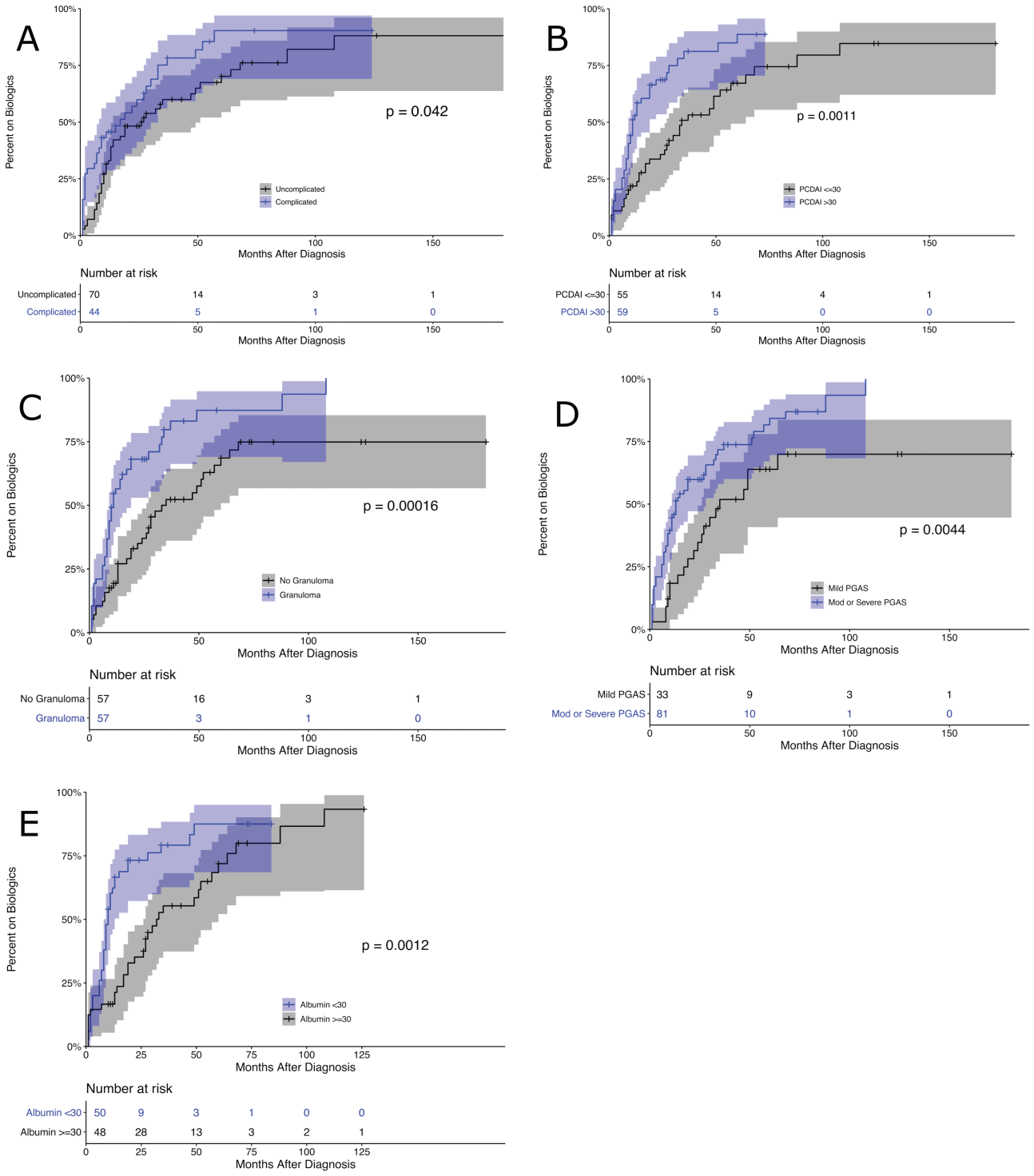

Figure 2. Kaplan–Meier curves comparing individual characteristics at diagnosis of Crohn's disease that were significantly associated with earlier initiation of biologics: (**A**) complicated disease (defined as penetrating or stricturing disease), (**B**) higher Pediatric Crohn's Disease Activity Index (PCDAI), (**C**) presence of well-formed granuloma, (**D**) moderate or severe Physician Global Assessment Score (PGAS) and (**E**) serum albumin <30. Shaded regions indicate the 95% confidence interval over time.

was not clearly IBD. A further 18 patients were excluded because less than 9 months of follow-up data were available. Baseline characteristics for the 198 patients included in the analysis are summarized in Table 1. The mean follow-up time was 47.8 months (SD = 35.1 months) and ranged from 9 to 181 months (Supplementary Figure 2). Patients ranged from 1 year 7 months old to 17 years 11 months old. Average age at diagnosis was 10.5 years old (SD = 3.9 years),

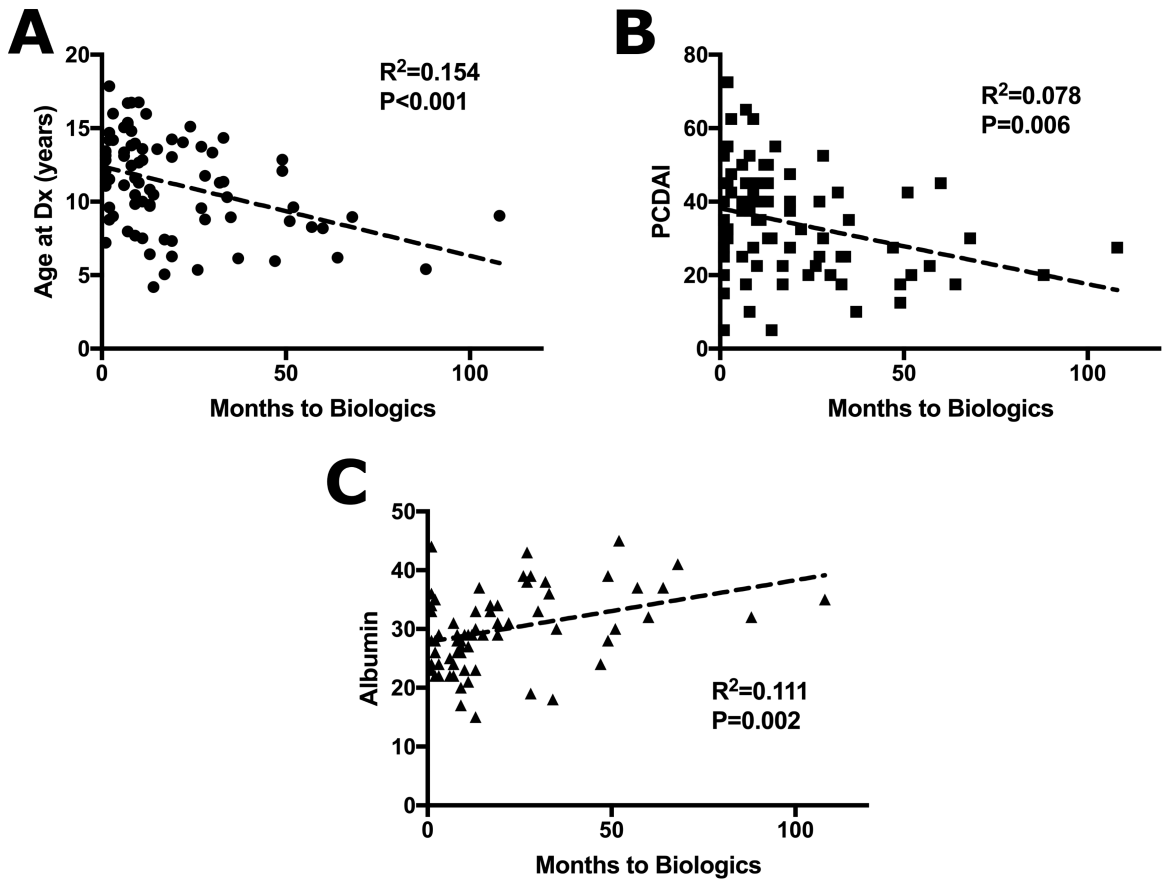

Figure 3. Scatterplots showing univariate linear regression of individual characteristics at diagnosis of Crohn's disease that were significantly correlated with time to initiation of biologics, including: (**A**) age, (**B**) Pediatric Crohn's Disease Activity Index (PCDAI) and (**C**) serum albumin level.

median age at diagnosis was 11.1 years old (interquartile range [IQR] = 6.1 years). In terms of disease subtype, 57.6% (n = 114) of patients had CD, 27.8% (n = 56) had UC and 14.6% (n = 28) had IBD-U (Figure 1).

Biologic therapy was used in 71% (n = 81) of patients with CD, 39% (n = 22) with UC and 25% (n = 7) with IBD-U. Overall, 55.5% (n = 110) of the patients received biologic therapy, the mean time to initiation was 21.5 months (SD = 26 months), the median time was 11 months (IQR = 22.5 months) and ranged from 0 to 124 months after diagnosis. Biologics were initiated within 1 month of diagnosis in 6.6% (n = 13) of all patients (7.9% with CD, 5.4% with UC and 3.4% with IBD-U, respectively; Supplementary Table 4). Biologics were initiated within 12 months of diagnosis in 28.8% (n = 57) of all patients (37.7% with CD, 17.9% with UC and 14.3% with IBD-U, respectively).

Kaplan–Meier curves were used to compare time to initiation of biologic therapy for those patients with and without each clinical characteristic in a univariate fashion. For patients with CD, individual characteristics at diagnosis that were individually associated with earlier initiation of biologic therapy included complicated disease behaviour (defined as penetrating or stricturing disease), higher PCDAI score (>30), presence of well-formed granuloma, moderate or severe Physician Global Assessment Score (PGAS) and lower serum albumin level (<30; Figure 2).

We analyzed all continuous variables by correlation and univariate linear regression analysis for those patients who received biologic therapy. For patients with CD who received biologic therapy, individual characteristics at diagnosis that were significantly correlated with earlier initiation of biologic therapy included older age, higher PCDAI and lower serum albumin level (Figure 3).

For patients with UC, Kaplan–Meier analysis of individual characteristics at diagnosis that were significantly associated with earlier initiation of biologic therapy curves included age over 10 years, male sex, pancolitis, PUCAI score ≥35 and serum albumin <35 g/L (Figure 4). For continuous variables, individual characteristics at diagnosis of UC that were significantly correlated with time to initiation of biologic therapy included older age at diagnosis, higher PUCAI score and lower serum albumin level (Figure 5).

Similar analysis for the 28 patients with IBD-U including Kaplan–Meier and regression analysis for continuous variables yielded no statistically significant disease characteristics associated with IBD-U and time to biologic initiation. This analysis included

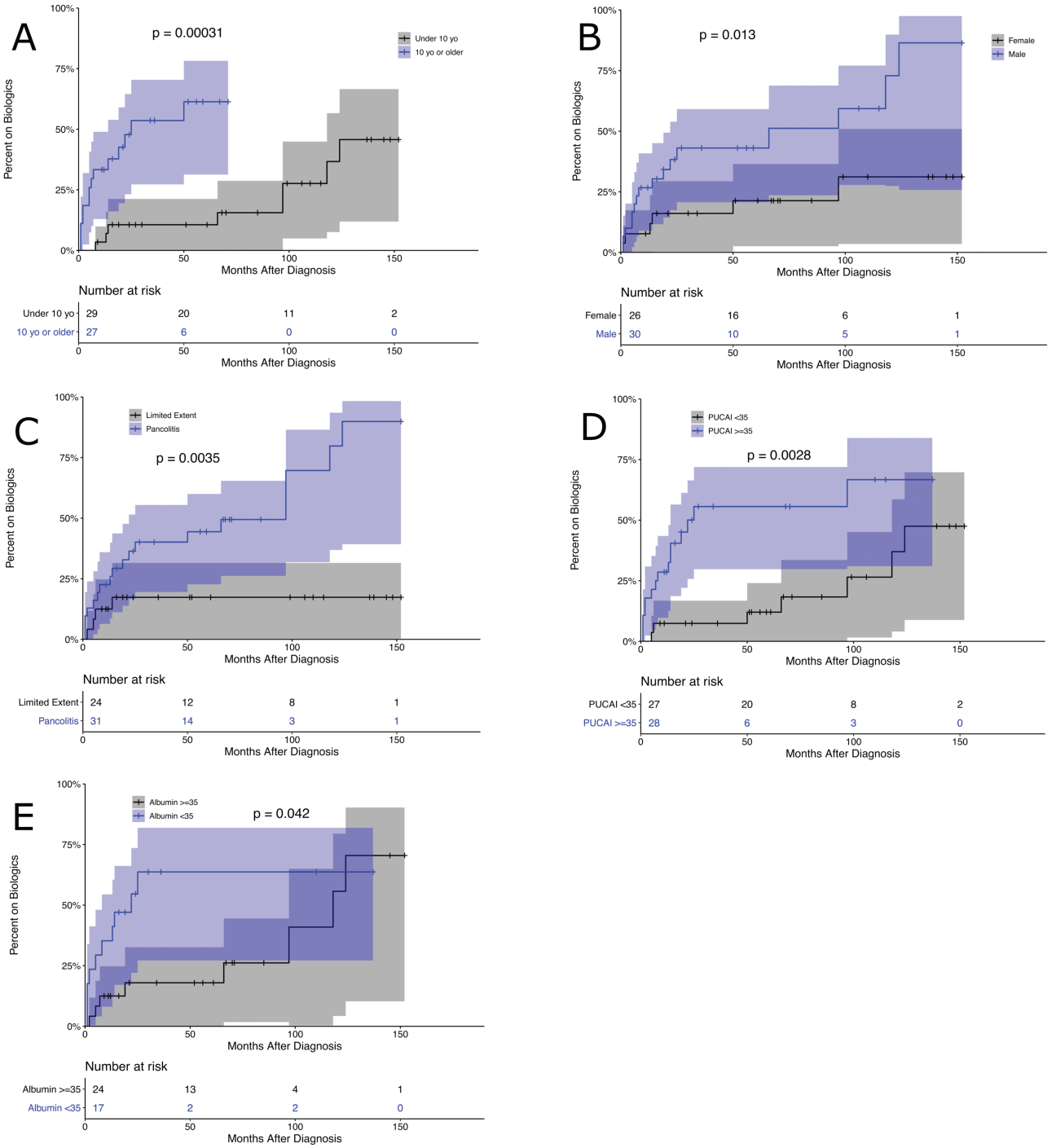

Figure 4. Kaplan–Meier curves comparing individual characteristics at diagnosis of ulcerative colitis that were significantly associated with earlier initiation of biologics: (**A**) age of 10 years or older, (**B**) male sex, (**C**) pancolitis, (**D**) Pediatric Ulcerative Colitis Activity Index (PUCAI) ≥35 and (**E**) serum albumin <35. Shaded regions indicate the 95% confidence interval over time.

both disease activity indices. Full details of statistical significance for each parameter evaluated are described in Supplementary Tables 1–3 (for CD, UC and IBD-U, respectively).

An optimized multivariate Cox proportional hazards model for CD included age, presence of granuloma, albumin and BMI *z*-score (Figure 6A). This model showed an overall concordance of 0.721. For UC, an optimized multivariate Cox proportional hazards model included age, sex, PUCAI, hemoglobin and erythrocyte sedimentation rate (Figure 6B). This model had an overall concordance of 0.863. Summary statistics for each model are found in Supplementary Table 5. Both final models were statistically significant by likelihood ratio test, Wald test

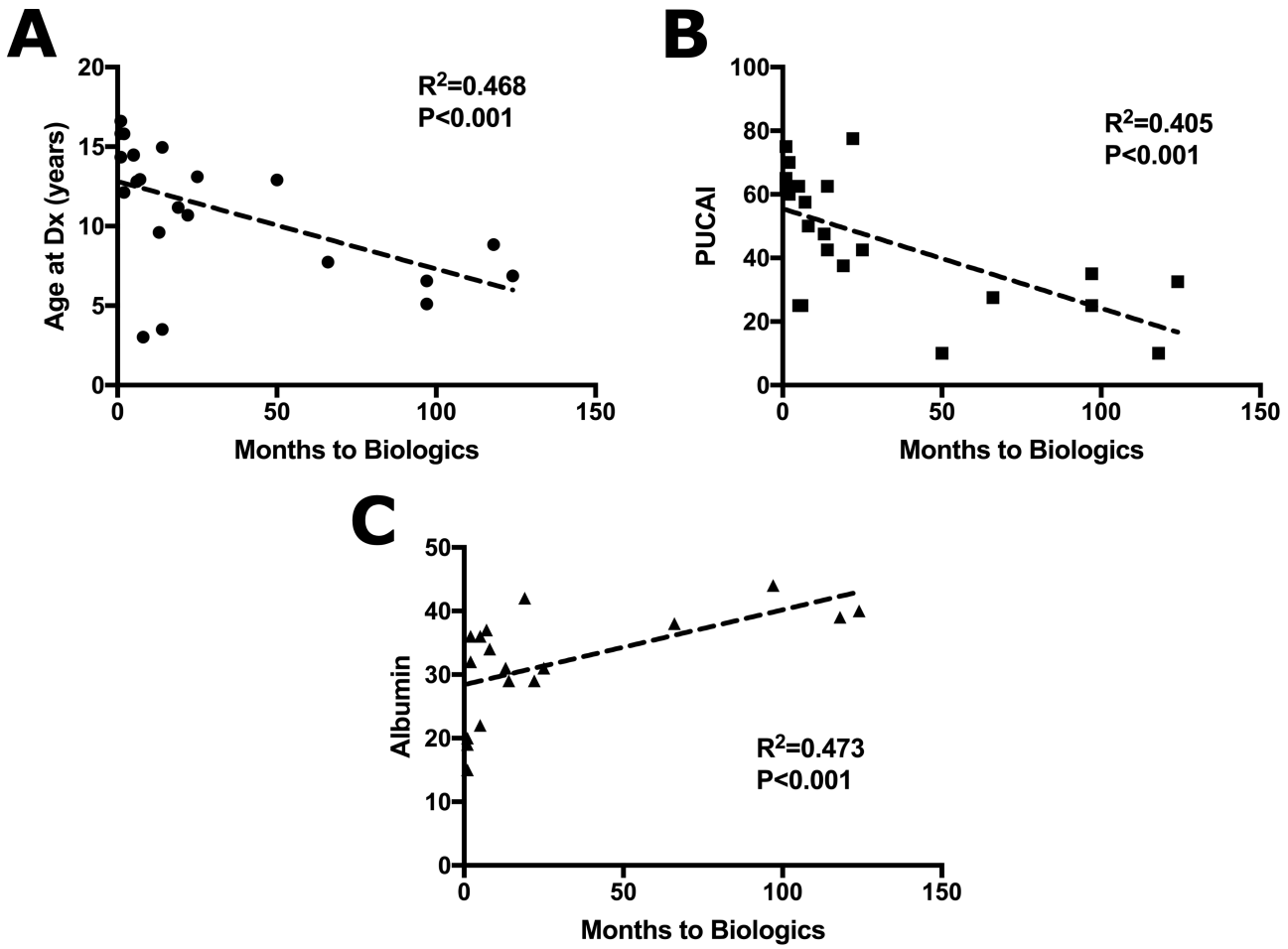

Figure 5. Scatterplots showing univariate linear regression of individual characteristics by at diagnosis of ulcerative colitis that were significantly correlated with time to initiation of biologics, including (**A**) age at diagnosis, (**B**) Pediatric Ulcerative Colitis Disease Activity Index (PUCAI) and (**C**) serum albumin level.

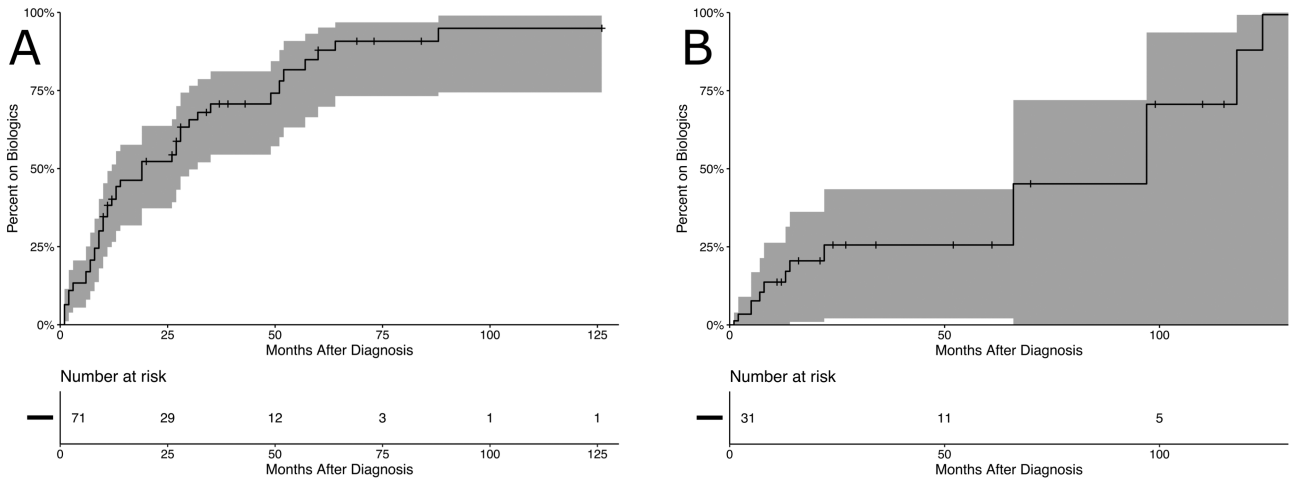

Figure 6. Survival curves of the optimal multivariate models created using Cox proportional hazard models. (**A**) Crohn's disease model, which included age, presence of granuloma, albumin and body mass index (BMI) *z*-score (concordance = 0.721). (**B**) Ulcerative colitis model, which included age, sex, Pediatric Ulcerative Colitis Disease Activity Index (PUCAI), hemoglobin and erythrocyte sedimentation rate (ESR) (concordance = 0.863). Shaded regions indicate the 95% confidence interval over time. Summary statistics for these models can be found in Supplementary Table 5.

and log-rank test. No multivariate model for the IBD-U cohort reached statistical significance.

Discussion

In the present study, we identified patient characteristics at the time of IBD diagnosis associated with subsequent biologic therapy in a large pediatric cohort with lengthy follow-up. Parameters associated with disease severity, such as age and higher disease activity index scores, were higher at presentation in patients who eventually received biologic therapy. This finding suggests that patients presenting with more severe disease were more likely to be started on biologic therapy. Thus, patients who have more severe disease at presentation may benefit from earlier initiation of biologic therapy than those presenting with milder disease. It has been assumed that biologic therapy is more appropriate for those with more severe disease, but data supporting which features of IBD are associated with need for biologic therapy in children were previously lacking.

The precise indications for starting biologic therapy for a child with IBD are not clear. Until 2019, there were no guidelines for biologic therapy initiation for pediatric IBD in North America (8). In the European guidelines, recommendations for CD management are based on expert opinion and data surrounding potential predictors of severe disease (7). Not all patients respond to biologic therapy (12), nor does relatively earlier biologic therapy appear to prevent stricturing disease (13). Our study demonstrates that there were features present at diagnosis that differentiated patients who were prescribed biologic therapy from those who were not prescribed biologic therapy. These features could be useful in future studies as potential indicators for biologic initiation.

When considering disease severity and poor outcomes as starting points for identifying IBD patients who would benefit from biologics, there is extensive adult literature to support this strategy (14). Factors that are known to correlate with poor outcomes in pediatric IBD are often disease-related changes (as opposed to demographics) and include acute phase reactant levels (15), growth failure (13) and disease activity (16). Our study approached biologic use *at any time* as potentially a function of such markers at the time of diagnosis, providing a novel perspective on biologic initiation. Our analysis supports the previous findings that severity markers (such as growth failure, disease activity and acute phase reactants) play a strong role in patients eventually starting biologics. Demographics such as age and sex have also previously been seen to influence disease severity (13) and indeed in our study these factors were similarly associated with biologic initiation.

Statistically, the characteristics at diagnosis that we identified were weakly correlated when examined individually, but the correlation increased substantially when these characteristics were combined in a multivariate model.

Prediction of outcomes in IBD has been challenging (17). Kugathasan et al. performed a prospective cohort study that used age, race, disease location and antimicrobial serologies as predictors of IBD complications in a pediatric cohort (13). Our study aimed to identify unique features of IBD at presentation that were associated with biologic use. We did not identify perianal disease as predictors of biologic initiation, although this feature is considered indicative of poor prognosis (7). This difference from previous studies may be due the fact that we limited our data collection to the immediate diagnostic period, whereas these disease features would be more likely to appear over time.

Data from previous studies suggest that hypoalbuminemia is a marker for overall disease severity in patients with IBD (18,19). Our study supports this finding, in that hypoalbuminemia at diagnosis was also associated with earlier initiation of biologic therapy when compared to patients with normal serum albumin levels, and the severity of the hypoalbuminemia was linearly correlated with earlier initiation of biologic therapy. Those patients presenting with normal serum albumin levels who eventually received biologic therapy may have subsequently developed hypoalbuminemia. It seems plausible that severely lowered serum albumin levels may be considered a possible harbinger of future need for biologic therapy.

This study was limited by its single centre retrospective design in that the initiation of biologic therapy in this patient cohort did not necessarily imply improved outcomes. Practice with respect to biologic initiation changed over the course of our study. The process of disease classification also changed over the course of the study (11). However, given the lack of pre-existing data to guide which patients should be started on biologic therapy, this study provides a foundation for further prospective evaluation that will help to determine if initiation based on these patient characteristics at the time of IBD diagnosis does indeed lead to improved clinical outcomes.

In summary, for both CD and UC, our patient population possessed unique clinical characteristics present at diagnosis that were strongly associated with earlier initiation of biologic therapy, notably demographics (such as age and sex), disease severity and altered acute phase reacts. These findings imply that it may be possible to differentiate patients at the time of IBD diagnosis who would and would not benefit from biologic therapy. Eventually, this information could be used to guide treatment decisions in IBD, thus establishing better disease control and minimizing complications.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of the Canadian Association of Gastroenterology* online.

Conflicts of Interest: The authors declare that there is no conflict of interests in this study.

Author Contributions: DJM, MZ, RMI and MES designed the study and contributed to the data analysis, drafting and editing of the manuscript. MES and DJM collected the data. All authors read and approved the final manuscript.

References

1. Solberg IC, Lygren I, Jahnsen J, et al.; IBSEN Study Group. Clinical course during the first 10 years of ulcerative colitis: Results from a population-based inception cohort (IBSEN Study). Scand J Gastroenterol 2009;44(4):431–40.
2. Devlin SM, Panaccione R. Evolving inflammatory bowel disease treatment paradigms: Top-down versus step-up. Gastroenterol Clin North Am 2009;38(4):577–94.
3. Walters TD, Gilman AR, Griffiths AM. Linear growth improves during infliximab therapy in children with chronically active severe Crohn's disease. Inflamm Bowel Dis 2007;13(4):424–30.
4. Sands BE, Anderson FH, Bernstein CN, et al. Infliximab maintenance therapy for fistulizing Crohn's disease. N Engl J Med 2004;350(9):876–85.
5. D'Haens G, Baert F, van Assche G, et al.; Belgian Inflammatory Bowel Disease Research Group; North-Holland Gut Club. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: An open randomised trial. Lancet 2008;371(9613):660–7.
6. Walters TD, Kim MO, Denson LA, et al.; PRO-KIIDS Research Group. Increased effectiveness of early therapy with anti-tumor necrosis factor-α vs an immunomodulator in children with Crohn's disease. Gastroenterology 2014;146(2):383–91.
7. Ruemmele FM, Veres G, Kolho KL, et al.; European Crohn's and Colitis Organisation; European Society of Pediatric Gastroenterology, Hepatology and Nutrition. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. J Crohns Colitis 2014;8(10):1179–207.
8. Mack DR, Benchimol EI, Critch J, et al. Canadian Association of Gastroenterology Clinical Practice Guideline for the Medical Management of Pediatric Luminal Crohn's Disease. J Can Assoc Gastroenterol 2019;2(3):e35–63.
9. Hyams JS, Ferry GD, Mandel FS, et al. Development and validation of a pediatric Crohn's Disease Activity Index. J Pediatr Gastroenterol Nutr 1991;12(4):439–47.
10. Turner D, Otley AR, Mack D, et al. Development, validation, and evaluation of a Pediatric Ulcerative Colitis Activity Index: A prospective multicenter study. Gastroenterology 2007;133(2):423–32.
11. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: The Paris classification. Inflamm Bowel Dis 2011;17(6):1314–21.
12. D'Haens GR. Top-down therapy for IBD: Rationale and requisite evidence. Nat Rev Gastroenterol Hepatol 2010;7(2):86–92.
13. Kugathasan S, Denson LA, Walters TD, et al. Prediction of complicated disease course for children newly diagnosed with Crohn's disease: A multicentre inception cohort study. Lancet 2017;389(10080):1710–8.
14. Danese S, Vuitton L, Peyrin-Biroulet L. Biologic agents for IBD: Practical insights. Nat Rev Gastroenterol Hepatol 2015;12(9):537–45.
15. Alper A, Zhang L, Pashankar DS. Correlation of erythrocyte sedimentation rate and C-reactive protein with pediatric inflammatory bowel disease activity. J Pediatr Gastroenterol Nutr 2017;65(2):e25–7.
16. Dotson JL, Hyams JS, Markowitz J, et al. Extraintestinal manifestations of pediatric inflammatory bowel disease and their relation to disease type and severity. J Pediatr Gastroenterol Nutr 2010;51(2):140–5.
17. Sands BE. Biomarkers of inflammation in inflammatory bowel disease. Gastroenterology 2015;149(5):1275–1285.e2.
18. Tromm A, Tromm CD, Hüppe D, et al. Evaluation of different laboratory tests and activity indices reflecting the inflammatory activity of Crohn's disease. Scand J Gastroenterol 1992;27(9):774–8.
19. Suzuki Y, Matsui T, Ito H, et al. Circulating interleukin 6 and albumin, and infliximab levels are good predictors of recovering efficacy after dose escalation infliximab therapy in patients with loss of response to treatment for Crohn's disease: A prospective clinical trial. Inflamm Bowel Dis 2015;21(9):2114–22.