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Prediction of complicated disease course for children newly diagnosed with Crohn's disease: a multicentre inception cohort study

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SK, LAD, TDW, M-OK, MCS, RNB, JFM, BJA, JSH, and MCD conceived and designed the study, analysed data, and drafted the Article. UMM, MS, KM, CL, and GG analysed data, and drafted the Article. AG, JDN, WVC, SSn, SR, JRR, JMS, SG, DRM, RK, MDK, SSt, DEM, DK, SC, MO-H, MBH, ARO, SSB, JSE, BSK, ASP, and DZ recruited patients, gathered data, and revised the Article. BCT collected data and revised the Article. FAS and JC designed the study and revised the Article. RJX and CH collected and analysed data, and revised the Article.

Declaration of interests

We declare no competing interests.

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Summary

Background—Stricturing and penetrating complications account for substantial morbidity and health-care costs in paediatric and adult onset Crohn's disease. Validated models to predict risk for complications are not available, and the effect of treatment on risk is unknown.

Methods—We did a prospective inception cohort study of paediatric patients with newly diagnosed Crohn's disease at 28 sites in the USA and Canada. Genotypes, antimicrobial serologies, ileal gene expression, and ileal, rectal, and faecal microbiota were assessed. A competing-risk model for disease complications was derived and validated in independent groups. Propensity-score matching tested the effect of anti-tumour necrosis factor α (TNFα) therapy exposure within 90 days of diagnosis on complication risk.

Findings—Between Nov 1, 2008, and June 30, 2012, we enrolled 913 patients, 78 (9%) of whom experienced Crohn's disease complications. The validated competing-risk model included age, race, disease location, and antimicrobial serologies and provided a sensitivity of 66% (95% CI 51–82) and specificity of 63% (55–71), with a negative predictive value of 95% (94–97). Patients who received early anti-TNFα therapy were less likely to have penetrating complications (hazard ratio

[HR] 0·30, 95% CI 0·10–0·89; p=0·0296) but not stricturing complication (1·13, 0·51–2·51; 0·76) than were those who did not receive early anti-TNFα therapy. *Ruminococcus* was implicated in stricturing complications and *Veillonella* in penetrating complications. Ileal genes controlling extracellular matrix production were upregulated at diagnosis, and this gene signature was associated with stricturing in the risk model (HR 1·70, 95% CI 1·12–2·57; p=0·0120). When this gene signature was included, the model's specificity improved to 71%.

Interpretation—Our findings support the usefulness of risk stratification of paediatric patients with Crohn's disease at diagnosis, and selection of anti-TNFα therapy.

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Introduction

Crohn's disease is a chronic inflammatory disorder of the gastrointestinal tract characterised by a relapsing and remitting course. Evidence suggests that host genetics and microbial dysbiosis have a fundamental role in pathogenesis[1] of Crohn's disease, and childhood is the fastest-growing incident age group.[2] Most children present with an inflammatory (non-penetrating, non-stricturing) phenotype. A subgroup rapidly progress to complicated disease behaviours, with stricturing and possible bowel obstruction or internal penetrating fistulas, or both, often resulting in intra-abdominal sepsis.[3] Previous reports about the natural history of Crohn's disease have shown rates of complicated disease ranging from 48% to 52% 5 years after diagnosis.[4] Factors associated with complicated disease behaviours include age at diagnosis, ileal disease location, serological responses to various microbial antigens, and possibly cumulative genetic risk.[4]

Despite substantial progress in understanding of the immune pathogenesis of Crohn's disease, little is known about the precise mechanisms responsible for disease complications.[5] Wound healing triggered by inflammation might lead to tissue repair or fibrosis depending on the balance between production and degradation of extracellular matrix proteins.[4] In Crohn's disease, stricturing occurs when regeneration and repair fail to restore normal tissue architecture, and bowel wall thickening leads to luminal narrowing.[6] Internal penetration develops as a result of active transmural inflammation of the bowel wall with or without distal luminal narrowing. The substantial heterogeneity in disease course suggests a strong host biological component, with conditioning affected by environmental and intestinal microbial factors.

The impact of available therapies, specifically anti-tumour necrosis factor α (TNFα) agents, on the natural history of Crohn's disease remains unclear.[7] Discovery of factors contributing to disease complications and prediction of the risk of such complications in children presenting with non-stricturing and non-penetrating Crohn's disease is crucial to guide therapeutic decisions. In 2008, the Risk Stratification and Identification of Immunogenetic and Microbial Markers of Rapid Disease Progression in Children with Crohn's Disease (RISK) study was initiated to characterise prospectively the natural history of newly diagnosed Crohn's disease in children presenting with an uncomplicated disease state. We

aimed to derive a risk-stratification model based on clinical, host biology, and microbial factors defined at diagnosis, and treatments including anti-TNFα therapy.

Methods

Study population and outcome classification

We did an inception cohort study at 28 sites in the USA and Canada. Patients younger than 18 years old with suspected inflammatory bowel disease were enrolled between Nov 1, 2008, and June 30, 2012 (ClinicalTrials.gov identifier NCT00790543; appendix). Patients who did not have gut inflammation on endoscopy served as controls for these studies. Patients with diagnoses of inflammatory bowel disease other than Crohn's disease were excluded, as were those for whom incomplete information on disease location was available, those who experienced complications either at or within 90 days of diagnosis, and those who did not attend at least one follow-up visit.

The institutional review board at each site reviewed and approved the protocol and informed written consent or assent was obtained in all cases from parents or guardians. All patients provided appropriate assent. This study was approved by national regulatory authorities and by local ethics committees or institutional review boards.

Procedures

Patients were managed according to the dictates of their physicians, not by standardised protocols. Samples for microbial and gene expression studies were obtained before patients began therapy.

Early anti-TNFα exposure was defined as complication-free initiation of therapy within 90 days of diagnosis and successful completion of both induction doses (three doses infliximab and two doses adalimumab) and at least one maintenance dose. We defined disease behaviour and location on the basis of the Montreal classification system.[8] Stricturing disease (B2) was defined as persistent luminal narrowing with pre-stenotic dilatation as shown by small bowel contrast imaging. Internal penetrating disease (B3) was defined as intra-abdominal fistulising disease resulting in intra-abdominal or pelvic abscesses or fistulas to an adjacent organ (excluding the vagina or perianal region). B1 refers to an uncomplicated disease state.

At enrolment, blood sample for DNA and plasma were taken from all patients. Stool samples and mucosal biopsies from colonoscopic examinations were collected when available. Serological determination of perinuclear anti-neutrophil cytoplasmic antibodies (pANCA), anti-*Saccharomyces cerevisiae* antibodies (ASCA) IgG, ASCA IgA, anti-CBir1, and anti-outer membrane protein C precursor (OmpC) was done at Cedars-Sinai Hospital (Los Angeles, CA, USA).[9] Granulocyte–macrophage colony-stimulating factor (GM-CSF) autoantibodies were measured at Cincinnati Children's Hospital Medical Center (OH, USA).[10] *NOD2* genotypes (rs2066844, rs2066845, and rs2066847) were extracted from previously published data[11] or determined with TaqMan (Thermo Fisher Scientific, Waltham, MA, USA) single nucleotide polymorphism genotyping assays. We used the score routine available in PLINK to generate a weighted genetic risk score on the basis of

Immunochip data[11] for 137 single nucleotide polymorphisms associated with Crohn's disease and inflammatory bowel disease.[1]

DNA was isolated from ileal and rectal biopsies and stool samples and subjected to 16S rRNA amplicon sequencing as previously reported.[12] The data were subsequently reanalysed with advanced software tools and analysis strategies (appendix). For RNA sequencing, biopsies were taken from the ileum, rectum, and other colonic locations. However, we present results only for ileal biopsies because both B2 and B3 complications mainly occur in the terminal ileum. RNA was isolated from ileal biopsies and global patterns of gene expression were determined with RNA sequencing as previously reported (appendix).

Statistical analysis

We planned to enrol 1100 patients, anticipating a 9% dropout rate and a 15% complication rate during 3 years' follow-up on the basis of historical cohort data (n=583). This sample size was expected to sufficiently power identification of ten risk factors for B2 and B3 outcomes. The planned cohort was also anticipated to allow prediction of complication risk with sufficient precision estimated through split sample validation (projected SD of 3% for positive predictive value and 1% for negative predictive value based on 1000 simulated data).

Potential risk factor data were missing either by study design or in small fractions of the cohort. Study design genotyping, microbial community profiling, and RNA sequencing were done in different subsets of the cohort. The subsets with complete data were analysed. Serology samples were not analysable in 28 patients, and missing cases were imputed as seronegative to be conservative.

We deemed time to complication as the response and analysed the data with a competing-risk model. Whereas standard Cox proportional hazard regression is concerned with only one type of event (event *vs* no event), in this case the response time was defined by two complication outcomes, and three mutually exclusively defined disease behaviour states—stricturing (B2), penetrating (B3), or no event (B1)—were considered. Therefore, we used a competing-risk model to extend the proportional hazards model and estimate complication-specific hazards.[13]

Clinical variables tested included age at diagnosis, sex, race, disease location,[8] disease severity measured by the Paediatric Crohn's Disease Activity Index (PCDAI),[14] perianal disease, height Z-score, weight Z-score, and body-mass index Z-score. Laboratory variables measured included *NOD2* genotype, the Crohn's disease genetic risk score, albumin, haemoglobin, ESR, C-reactive protein (CRP), GM-CSF autoantibody, antimicrobial serologies, pANCA, and ileal gene expression. Ileal gene expression data for each participant were reduced to principal component 1 (PC1) for the extracellular matrix production biological pathway before testing in the model. Variables selected to remain in the model included those with p values less than 0·1 for either the stricturing or penetrating outcome, or, in the case of isolated ileal location, before evidence linking this factor to disease complications.[15]

Patients who received anti-TNFα within 90 days of diagnosis and met the criteria for medication exposure were included in a propensity score analysis. Early anti-TNFα exposure was defined in a per-protocol, rather than intention-to-treat, manner, to assess the biological effect in the matched groups. However, only six participants who received anti-TNFα therapy within 90 days did not meet the per-protocol exposure definition. We modelled the propensity of early anti-TNFα exposure by regressing the effects of baseline factors affecting anti-TNFα use (age, race, sex, disease location, perianal disease, height Z-score, weight Z-score, PCDAI score at diagnosis, any deep ulcer on colonoscopy, and interaction between age and PCDAI) on early anti-TNFα use. Scores representing the propensity of early anti-TNFα exposure were computed and used to match each of the patients who received early anti-TNFα with a patient who did not. We used the greedy-matching algorithm[16] with caliper of 0·1 SDs.

The study was reported as per the STROBE statement for observational cohort studies. R package cmprsk (version 2.2–2.7) was used in R 3.2.2 for competing-risk modelling. Descriptive statistics were computed in SAS (version 9.3).

Role of the funding source

The funders of the study had no role in study design; data collection, analysis, or interpretation; or writing of the Article. All the authors had access to all the study data and were responsible for the decision to submit the Article for publication.

Results

1813 patients were enrolled in our study (table 1). 402 did not have gut inflammation on endoscopy and were controls for microbial and gene expression studies. 204 patients were diagnosed with ulcerative colitis, and 111 with unspecified inflammatory bowel disease. Of the 1096 patients diagnosed with Crohn's disease, we excluded 18 for whom incomplete information on disease location was available, 42 who experienced complications at diagnosis, 17 who experienced complications within 90 days, and 106 who did not attend at least one follow-up visit. Thus, 913 patients who were free of complications 90 days after diagnosis were included in the final analysis.

Median age at diagnosis was 12·4 years (IQR 10·0–14·7). 565 (62%) participants were male, 681 (75%) were white, and 121 (13%) were African American or mixed race. Stricturing disease was associated with ASCA, CBir1, and GM-CSF seropositivity, and penetrating disease was associated with older age, African American race, and ASCA and CBir1 seropositivity in univariate analyses (data not shown). Patients with perianal involvement were more likely to be male, to receive anti-TNFα therapy within 90 days of diagnosis, and to be positive for both antimicrobial and GM-CSF autoantibody serologies than where those without perianal involvement (appendix).

Survival curves showing time to complicating disease behaviours are shown in figure 1A. 54 patients had stricturing complications and 24 had penetrating complications during follow-up (p=0·0007 for the comparison). Within 90 days of diagnosis, 191 patients (21%) received anti-TNFα therapy (180 infliximab and 11 adalimumab) and 413 (45%) were treated with an

immunomodulator (thiopurine or methotrexate). We used propensity-score matching to obtain a sample of 191 pairs of patients, one of whom received early anti-TNFα and one who did not (appendix). Survival curve analysis of this matched cohort showed similar progression to stricturing behaviour in patients, irrespective of early anti-TNFα exposure (figure 1B). By comparison, progression to penetrating behaviour was three-times reduced in those who received early anti-TNFα compared with patients who did not receive early anti-TNFα, although this reduction did not reach significance in the unadjusted analysis (p=0·0675; data not shown). 32 patients received both anti-TNFα therapy and an immunomodulator. The small size of this sample prevented further analysis of this important subgroup. Response to early anti-TNFα, which was defined as achieving corticosteroid-free remission 6 months after diagnosis, was noted in 124 (71%) of the 175 participants with available data for this outcome. We did not note a difference in frequency of B2 or B3 complications after 6 months in anti-TNFα responders compared with non-responders, although the small sample size of these subgroups precluded drawing firm conclusions (data not shown).

The competing-risk model based on clinical and serological variables is shown in table 2. Neither *NOD2* genotype (p=0·14 for stricturing disease and 0·39 for penetrating disease) nor a polygenic Crohn's disease risk score (p=0·46 for stricturing disease and p=0·27 for penetrating disease) reached significance (data not shown). Only CBir1 seropositivity was significantly associated with stricturing behaviour. Older age, African American race, and ASCA IgA and CBir1 seropositivity were associated with penetrating behaviour (table 2). We tested the discriminant power of the model by randomly splitting the cohort into equal test and validation groups and averaging the performance over 1000 iterations. These test characteristics included a sensitivity of 66% (95% CI 51–82), specificity of 63% (95% CI 55–71), positive predictive value of 14% (95% CI 12–17), and negative predictive value of 95% (95% CI 94–97). The comparative effectiveness of early anti-TNFα was assessed after adjustment for differences in factors in the competing-risk model. Early anti-TNFα was not associated with a reduction in stricturing behaviour (hazard ratio [HR] 1·13, 95% CI 0·51–2·51; p=0·76) but was associated with a reduction in penetrating behaviour (0·30, 0·10–0·89; 0·0296; table 2).

We identified 14 genera associated with paediatric Crohn's disease (appendix). In addition to organisms previously implicated including *Clostridiales, Pasteurellaceae, Veillonellaceae, Erysipelotrichaceae*, and *Bacteroidales*,[12] we identified new organisms, such as *Campylobacter, Akkermansia, Collinsella*, and *Desulfovibrio* species. The largest increase was detected for *Aggregatibacter*, and the greatest decrease was noted for *Roseburia*. Several organisms were associated with disease complications (appendix). *Rothia* and *Ruminococcus* were implicated in the development of strictures. *Collinsella* numbers were increased in patients who developed penetrating disease (B3), and *Veillonella* frequency was increased specifically in the ileum. However, the subcohort for whom microbial data were available differed from the whole cohort in key baseline clinical features, and so the individual taxa were not incorporated in the risk-prediction model for further assessment.

Although there was considerable heterogeneity in the ileal global pattern of gene expression (appendix), comparisons between groups showed significant differences in gene expression.

Genes regulating extracellular matrix accumulation were induced at diagnosis in patients who developed strictures (appendix), whereas genes regulating the acute inflammatory response to microbes were induced in those developing penetrating disease (appendix). The balance between antimicrobial acute inflammatory and extracellular matrix accumulation pathways within patients who developed penetrating (B3) and stricturing (B2) complications is shown in figure 2A. The extracellular matrix structural constituent molecular function was induced to a greater degree in patients who developed stricturing complications relative to both those who remained complication-free (B1), and those progressing to penetrating disease (figure 2B).

We then tested for differences in ileal gene expression between patients who were predicted to be at low risk for a stricturing complication but whose disease progressed, and those who were predicted to be at high risk of stricturing disease, but remained free of complications at 36 months. This analysis identified enrichment for a mitochondrial function gene signature in patients in the at-risk group remaining complication free (figure 2C; appendix). Conversely, we also noted enhancement of the extracellular matrix gene signature in patients who were predicted to be low risk but nonetheless progressed to a stricture (figure 2C; appendix).

The first principal component of the extracellular matrix structural constituent molecular function gene signature was associated with stricturing behaviour in the risk model (HR 1·70, 95% CI 1·12–2·57; p=0·0120; table 3). Leave-one-out cross validation showed that the model performance increased as this gene signature was added, with an area under the curve of 0·72, sensitivity of 69%, and specificity of 71% (table 3). These improved statistics were evident despite being based on only a quarter of the sample size of the full cohort available for gene expression, in large part because the transcriptome and serology risk factors were almost orthogonal (appendix). In fact, eight additional patients who had a B2 complication were classified as high risk on the basis of the extracellular matrix gene signature (appendix), who otherwise would have been classified as low risk in the model on the basis only of clinical factors including ileal location and serology.

Discussion

Because no validated models for prediction of risk of stricturing or penetrating complications are available in Crohn's disease, we did a prospective, inception cohort study in children with newly diagnosed Crohn's disease. We constructed a risk-prediction model based on clinical, serological, and host biology. Additionally, we showed that early anti-TNFα therapy reduced penetrating but not stricturing complications.

Patients with Crohn's disease typically show inflammatory disease behaviour at diagnosis, with some patients' disease then progressing to stricturing or internal penetrating complications.[15] Despite increased use of anti-TNFα therapy over the past decade, a population-level decline in rates of obstructive complications and surgeries has not been recorded.[7,17] Although a Danish cohort study showed a reduction in Crohn's disease surgeries between 1979 and 2011, an association with specific drugs was not established.[18] Disease complications, particularly penetrating complications, substantially increase health-

care costs. Additionally, anti-TNFα therapy is costly. Therefore, models to predict which patients with Crohn's disease are at the highest risk of complications, and estimates of the benefit of early anti-TNFα exposure, are urgently needed.[19]

We found that older age, African-American race, and ASCA and CBir1 seropositivity were associated with disease complications. The strongest effect was noted for CBir1 seropositivity to bacterial flagellin, which is induced at an early age, and could be involved in pathogenesis of Crohn's disease.[20] Flagellin is a dominant antigen driving mucosal T-cell responses in Crohn's disease, and both flagellin-specific T cells and antiflagellin antibodies exacerbate inflammation in murine colitis.[21] We therefore tested for an association between ileal microbiota and complications. We identified 14 genera associated with paediatric Crohn's disease. The largest increase was detected for *Aggregatibacter*, a bacterial taxon associated with a decrease in mucosal pattern-recognition receptors.[22] The greatest decrease was noted for *Roseburia,* including butyrate-producing bacteria that promote the epithelial barrier.[23] Distinct taxa were in turn associated with progression to complications, suggesting a role in modulation of host biology. These findings will require validation to determine their usefulness in a predictive model.

Largely because of the low prevalence of complications in the cohort, the positive predictive value of the clinical and serological model was low. We postulated that the ileal global pattern of gene expression, as an integrated readout for local host biology, might improve the specificity and positive predictive value of the model. The mucosa overlying established Crohn's disease strictures has a pro-fibrotic pattern of gene expression.[24] Whether ileal gene signatures would reveal these processes at an uncomplicated stage of disease was unknown. We detected a pronounced extracellular matrix gene signature in patients who ultimately progressed to strictures.[25] This gene signature was associated with stricturing complications, and improved the discriminant power of the model with respect to specificity and positive predictive value. If validated in a future study, the signature detected in the stricturing subgroup could help to inform more efficient enrolment into trials of antifibrotic therapies.

This study was ideally suited to test the effectiveness of early anti-TNFα in preventing complications. We first did propensity-score matching to account for clinical factors associated with early anti-TNFα use, and then adjusted for risk factors for complications. Early anti-TNFα in the propensity-matched cohort was associated with a substantial reduction in penetrating but not fibrostenotic complications. This finding is consistent with previous reports,[26,27] although only for penetrating complications. Therefore, earlier introduction of anti-TNFα therapy could reduce progression to internal abscesses and fistulas, with attendant high morbidity and costs.

Our study has two main limitations. We did not do a randomised controlled trial, which would be the gold standard to test the noted reduction in penetrating complications with early anti-TNFα use, and therapeutic drug monitoring was not mandated, which could have contributed to variation in treatment responses.[28] However, this variation probably would not have led to a bias between the three outcome groups. In terms of patient phenotyping, we did not use central reading protocols for small bowel imaging with MRI or CT enterography, which could have led to variation in classification of disease complications. In particular, the

possible underestimation of children with early fibrosis at diagnosis could explain why anti-TNFα therapy did not affect progression to stricturing.[24,29] Most participants had endoscopic ileal inflammation and so biopsies were taken from inflamed tissue. However, we were not able to obtain ileal biopsies from inflamed and non-inflamed segments in the same patients, and so cannot directly address the degree to which acute inflammation affected the gene signatures.

Substantial morbidity is associated with Crohn's disease complications and thus tools that help to communicate the risk of the disease allow for more informed risk–benefit discussions about treatment. We have done a large multicentre inception cohort study and derived and validated a prognostic model for disease complications, which is suitable for use in clinical practice. Older age at diagnosis, African American race, ileal disease location, and ASCA and CBir1 seropositivity were associated with increased risk for disease complications. These patients could be prioritised for early anti-TNFα therapy, with could reduce internal penetrating complications that account for high morbidity and health-care costs in Crohn's disease. Conversely, the high negative predictive value of the model could be used to classify patients at low risk for complications with a high degree of confidence. On the basis of the validated competing-risk model including clinical and serological factors, 108 (57%) patients who received early anti-TNFα therapy were projected to be at low risk and would have been deprioritised for this approach if the treatment decision had been based on the risk model. For these patients, treatment decisions might be guided by other considerations including corticosteroid-dependent or refractory inflammatory disease behaviour, or growth failure. In this regard, our previous report in the RISK cohort showed that early anti-TNFα therapy was associated with higher rates of corticosteroid-free remission and improved growth 1 year after diagnosis compared with patients who did not receive early anti-TNFα therapy.[30] We now extend those findings by showing a reduction in rates of penetrating complications with early anti-TNFα therapy. The clinical usefulness and positive predictive value of our risk model was improved by the addition of ileal gene signatures, which merit further exploration and validation. Collectively these results advance understanding of the pathogenesis of disease complications, and inform more personalised approaches for children newly diagnosed with Crohn's disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Research in context

Evidence before this study

Evidence-based models for rapidly progressive Crohn's disease to guide the use of expensive medical treatments such as anti-tumour necrosis factor α (TNFα) therapy are urgently needed. We searched PubMed and Embase with the terms "Crohn's disease", "TNF alpha therapy", "children", "complication", "stricture", "fistula", "penetration", "biologic", "natural history", and "predictive model" for population-based evidence and prospective and retrospective cohort studies of the natural history of disease complications in paediatric Crohn's disease, and associated clinical, serological, and genetic predictors. The studies identified by our search had small sample sizes, which precluded validation of predictors, and most did not follow up patients prospectively from the time of diagnosis. Although in several studies investigators developed models that included clinical, demographic, genetic, and serological factors, none included data for the global pattern of gene expression in the affected gut at diagnosis, or the associated microbiota. The available predictive models were therefore limited by lack of validation and lack of insight into the biology of refractory disease.

Added value of this study

In our study of 913 children with Crohn's disease at 28 sites in Canada and the USA, we derived and validated a risk-stratification model based on clinical and serological factors defined at diagnosis. Our model has very high negative predictive value. We showed that early anti-TNFα therapy was associated with reduced rates of internal penetrating, but not stricturing, disease complications. We detected a novel ileal extracellular matrix gene signature, which, if present at diagnosis, is associated with future stricturing complications. Inclusion of this gene signature improved the discriminant power of the model with respect to specificity and positive predictive value, and showed the importance of fibrogenesis in this subgroup who did not benefit from early anti-TNFα therapy.

Implications of all the available evidence

Our study adds to data for the natural history of paediatric Crohn's disease in the era of anti-TNFα therapy and provides definitive validation of previously reported clinical and serological predictors of disease complications. The diverging rates of stricturing versus penetrating behaviour in our cohort with time suggested potential differences in disease pathogenesis, and we showed that early anti-TNFα therapy will prevent internal penetrating but not stricturing complications. We provide a mechanistic basis for this observation by defining a novel ileal pro-fibrotic signature detectable at diagnosis. Collectively, these data could facilitate both appropriate treatment choices (early anti-TNFα to prevent penetrating complications) and rationale testing of novel antifibrotic approaches in patients at high risk for stricturing disease in future clinical trials.

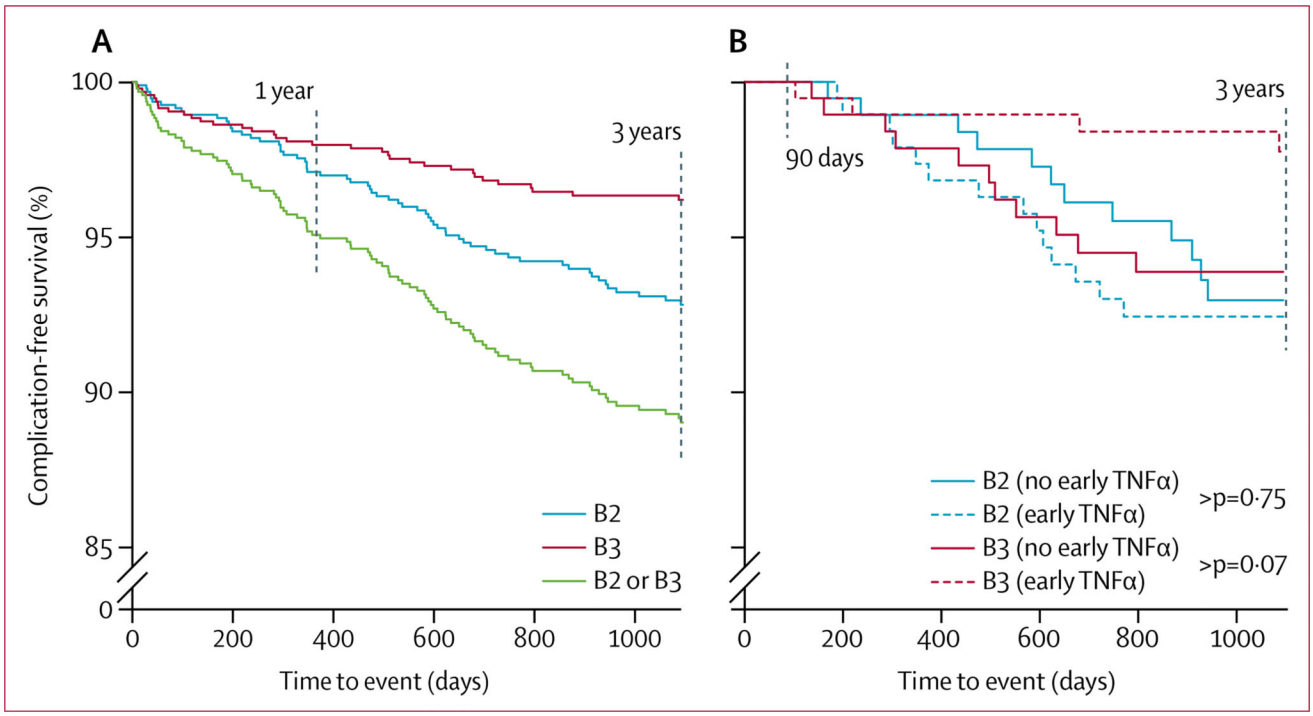

Figure 1. Development of stricturing or penetrating complications during follow-up in the competing-risk analysis in the whole cohort (A) and in the propensity-matched cohort (B)

B2 refers to stricturing behaviour, and B3 to penetrating behaviour. (A) includes 35 patients with Crohn's disease who either developed complications during the first 90 days after diagnosis, or did not have complete information for disease location at diagnosis, and were therefore excluded from the primary analysis. 97 patients developed complications, 63 with stricturing behaviour and 34 with penetrating behaviour. In (B), early anti-TNFα therapy was defined as exposure within 90 days of diagnosis, and the survival probabilities were computed among those staying complication free by 90 days. Among those treated with early anti-TNFα therapy, 18 patients developed complications, 14 with stricturing behaviour and four with penetrating behaviour. 23 patients developed complications, 12 with stricturing behaviour and 11 with penetrating behaviour, among the matched group not treated with early anti-TNFα therapy. TNFα=tumour necrosis factor α.

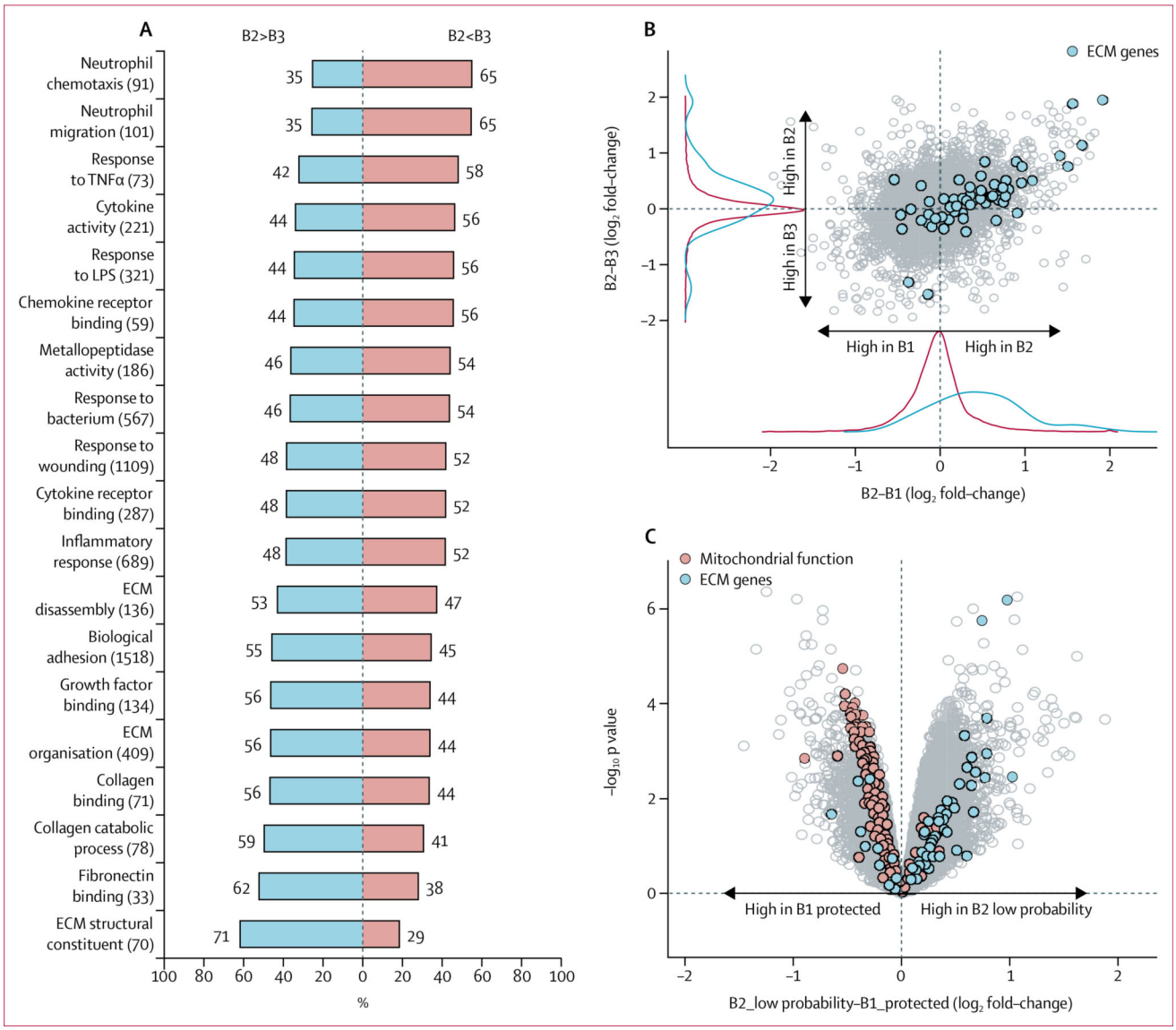

Figure 2. Ileal gene signatures associated with development of disease complications

(A) shows the proportion of ileal genes upregulated in patients who developed stricturing (B2, blue bars) versus penetrating (B3, red bars) complications for 19 gene ontology pathways significantly enriched for genes upregulated in pairwise comparisons between the subgroups (appendix). Names of pathways are shown, along with the total number of genes included in each pathway in parentheses.* (B) is a combined scatterplot and density plot of $\log_2$ fold changes in ileal gene expression for patients who exhibited stricturing (B2) versus complication-free (B1, x-axis) and stricturing (B2) versus penetrating (B3, y-axis) behaviour, with higher mean expression in B2 either to the right or to the top (and correspondingly, higher expression in B1 to the left, or B3 to the bottom). Genes involved in ECM remodelling (n=70, gene ontology pathway 0005201) are depicted with blue filled dots and blue lines. Background values for all genes (n=17 081) are shown with grey dots and black lines. (C) shows a volcano plot of significance (negative logarithm of the p value on the y-axis) against difference in average ileal gene expression ($\log_2$ scale, x-axis) between

patients with Crohn's disease with low risk for complications who nonetheless developed stricturing behaviour (B2 low probability†) and patients with Crohn's disease with high risk for complications who nonetheless remained complication free (B1 protected‡). Genes involved in the mitochondrial respiratory chain (n=179, gene ontology pathways 0022900 and 0045333, in dark red) are almost all upregulated in B1 protected, whereas genes involved in remodelling of the ECM (n=68, gene ontology pathway 0005201, in light blue) are upregulated in B2 low probability. TNFα=tumour necrosis factor α. ECM=extracellular matrix. LPS=lipopolysaccharide. *Sample sizes were n=18 (B2), n=11 (B3), and n=214 (B1). The pathways were selected as highly significant in the enrichment analyses of B2 *vs* B1 or B3 *vs* B1 (appendix). †Refers to nine patients who developed B2 despite a 3-year probability of complication from the competing-risk model below the median of all B2 (mean 0·048, range 0·029–0·072). ‡Refers to 22 patients who did not develop any complications despite being among the top 10% of 3-year probability of complication according to the competing-risk model (mean 0·152, range 0·121–0·241). The small sample size results in slight significance values, but the coherence of the two pathways is strongly indicated by the polarity of the genes in the two signatures on either side of zero.

Table 1

Clinical and demographic characteristics of the study cohort stratified by disease behaviour during follow-up

	Inflammatory (B1) (n=835)	Stricturing (B2) (n=54)	Penetrating (B3) (n=24)
Demographics and follow-up			
Age at diagnosis (years)	12·3 (9·9 to 14·5)	12·9 (10·6 to 15·1)	15·6 (12·9 to 16·4) *
Female sex (%)	316 (38%)	19 (35%)	13 (54%)
African American or mixed race	103 (12%)	9 (17%)	9 (38%) †
Duration of follow-up (months)	47 (36 to 55)	41 (36 to 50) ‡	40 (34 to 49) ‡
Time to behaviour change (days)	..	520 (301 to 722)	497 (260 to 680)
Disease activity and treatment exposures			
Moderate-to-severe disease activity	388 (46%)	23 (43%)	12 (50%)
Height Z-score	−0·25 (−0·94 to 0·45)	−0·6 (−1·34 to 0·09)	−0·35 (−1·23 to 0·33)
BMI Z-score	−0·66 (−1·63 to 0·15)	−0·85 (−1·88 to 0·26)	−0·76 (−1·84 to 0·18)
Anti-TNFα within 90 days of diagnosis	173 (21%)	14 (26%)	4 (17%)
Immunomodulator within 90 days	378 (45%)	21 (39%)	14 (58%)
Small bowel imaging within 6 months	608 (73%)	47 (87%) ‡	19 (79%)
Disease location at diagnosis			
Isolated terminal ileum with or without caecum involvement	166 (20%)	16 (30%)	7 (29%)
Isolated colonic	210 (25%)	10 (19%)	3 (13%)
Ileo-colonic	459 (55%)	28 (52%)	14 (58%)
Perianal	115 (14%)	7 (13%)	4 (17%)
Serological reactivity status at diagnosis			
ASCA IgA	182 (22%)	22 (41%) †	14 (58%) *
ASCA IgG	182 (22%)	19 (35%) ‡	9 (38%)
CBir1	293 (35%)	32 (59%) *	16 (67%) †
Granulocyte–macrophage colony-stimulating factor autoantibody	381 (46%)	35 (65%) †	14 (58%)
Outer membrane protein C precursor	54 (6%)	4 (7%)	2 (8%)
Perinuclear anti-neutrophil cytoplasmic antibodies	129 (15%)	5 (9%)	3 (13%)

In the Montreal classification of disease behaviour, B1 corresponds to inflammatory behaviour with no stricturing or luminal penetrating complications, B2 to stricturing behaviour with no luminal penetrating complications, and B3 to luminal penetrating behaviour with or without concurrent stricturing complications.

BMI=body-mass index. TNFα=tumour necrosis factor α. ASCA=anti-*Saccharomyces cerevisiae* antibodies.

* p value <0·001 for comparisons of B2 vs B1 and B3 *vs* B1.

† p value <0·01 for comparisons of B2 *vs* B1 and B3 *vs* B1.

‡ p value <0·05 for comparisons of B2 *vs* B1 and B3 *vs* B1. A version of this table with exact p values is included in the appendix.

Table 2

Competing-risk model for disease complications and early anti-TNFα comparative effectiveness analysis

	Stricturing behaviour (B2)		Penetrating behaviour (B3)	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Competing-risk model (n=913)				
Age at diagnosis	1·07 (0·97–1·17)	0·16	1·45 (1·17–1·80)	0·0008
African American race	1·08 (0·52–2·22)	0·84	3·19 (1·39–7·31)	0·0061
Isolated ileal location (L1)	1·60 (0·88–2·91)	0·12	1·23 (0·51–2·95)	0·64
ASCA IgA positive	1·69 (0·94–3·07)	0·0816	2·68 (1·19–6·04)	0·0171
CBir1 positive	2·30 (1·26–4·20)	0·0070	3·01 (1·31–6·93)	0·0097
Early anti-TNFα comparative effectiveness analysis of propensity-score matched cohort (n=382)				
Age at diagnosis	1·13 (0·97–1·31)	0·11	1·37 (1·03–1·81)	0·0278
African American race	1·25 (0·43–3·63)	0·68	3·02 (0·97–9·39)	0·0555
Isolated ileal location (L1)	1·66 (0·65–4·26)	0·29	1·26 (0·36–4·43)	0·72
ASCA IgA positive	2·87 (1·21–6·82)	0·0165	2·09 (0·71–6·12)	0·18
CBir1 positive	1·52 (0·63–3·70)	0·35	4·82 (1·53–15·2)	0·0072
Early anti-TNFα	1·13 (0·51–2·51)	0·76	0·30 (0·10–0·89)	0·0296

In the competing-risk model in the overall cohort (n=913), AUROC was 0·7 (95% CI 0·64–0·76), sensitivity 66% (95% CI 51–82), specificity 63% (95% CI 55–71), positive predictive value 14% (95% CI 12–17), negative predictive value 95% (95% CI 94–97), and prevalence of complications 8·5%. These characteristics were calculated from 1000 random split samples; for each, one half was used to validate predictions based on the other half. The cutoff set to balance sensitivity with specificity was 0·077. By contrast, for the model based on clinical factors alone, AUROC was 0·6 (95% CI 0·56–0·69), sensitivity 56% (95% CI 38–67), specificity 63% (95% CI 56–71), positive predictive value 12% (95% CI 10–15), and negative predictive value 94% (95% CI 92–95). In the early anti-TNFα comparative effectiveness analysis, the propensity of early anti-TNFα therapy use within 3 months of diagnosis in individual patients was analysed, and estimated propensity scores were used to obtain 191 matched pairs, each consisting of one patient who received early anti-TNFα therapy and one patient with similar baseline characteristics who did not. Prevalence of complications was 10·7% (6·8% for B2 and 3·9% for B3).

TNFα=tumour necrosis factor α. HR=hazard ratio. ASCA=anti-*Saccharomyces cerevisiae* antibodies. AUROC=area under the receiver operator characteristic curve.

Table 3

Competing-risk model including extracellular matrix gene signature

	Stricturing behaviour (B2)		Penetrating behaviour (B3)	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Age at diagnosis	1·07 (0·91–1·27)	0·42	1·45 (0·98–2·14)	0·0606
African American race	0·30 (0·04–2·47)	0·27	2·31 (0·4–13·27)	0·35
Isolated ileal location (L1)	1·09 (0·39–2·99)	0·87	1·36 (0·37–4·93)	0·64
ASCA IgA positive	1·48 (0·58–3·75)	0·41	2·92 (0·81–10·48)	0·10
CBir1 positive	2·14 (0·84–5·44)	0·11	7·99 (1·89–33·77)	0·0047
Extracellular matrix gene signature	1·70 (1·12–2·57)	0·0120	1·21 (0·53–2·73)	0·65

This table includes data for a sub-cohort of 243 patients for whom ileal gene expression data were available. When the extracellular matrix gene signature was excluded, AUROC was 0·66, sensitivity 69%, specificity 66%, positive predictive value 22%, negative predictive value 94%, and prevalence of complications 11·9% (7·4% for B2 and 4·5% for B3). With the addition of the extracellular matrix gene signature, AUROC was 0·72, sensitivity 69%, specificity 71%, positive predictive value 24%, and negative predictive value 94%. The discriminant power was computed via leave-one-out cross-validation.

HR=hazard ratio. ASCA=anti-*Saccharomyces cerevisiae* antibodies. AUROC=area under the receiver operator characteristic curve.