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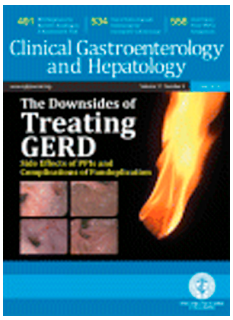

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Abbreviations: FC – fecal calprotectin

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Abstract

Background & Aims

Mucosal healing is associated with improved outcomes in patients with Crohn's disease (CD), but assessment typically requires ileocolonoscopy. Calprotectin can be measured in fecal samples to determine luminal disease activity in place of endoscopy—this measurement is an important component of the treat to target strategy. We investigated whether levels of fecal calprotectin associate with subsequent CD progression.

Methods

We performed a retrospective study of 918 patients with CD (4218 patient-years of follow-up; median, 50.6 months; interquartile range [IQR], 32.8–76.0 months) managed at a tertiary medical center in Edinburgh, United Kingdom, from 2003 through 2015. Patients were included if they had 1 or more fecal calprotectin measurement made 3 months or more following their diagnosis. We collected clinical data and fecal calprotectin measurements and analyzed these data to identify factors associated with a composite outcome of progression in Montreal behavior, hospitalization, and resection.

Results

Increased level of fecal calprotectin at index visit was associated with subsequent progression of CD, independent of symptoms or disease location. The median level of fecal calprotectin at the index visit was 432 μg/g (IQR, 1365–998 μg/g) in patients who reached the composite endpoint vs 180 μg/g (IQR, 50–665 μg/g) in patients who did not. In multivariable analysis, a cutoff of 115 μg/g calprotectin identified patients who met the endpoint with a hazard ratio on of 2.4 (95% CI, 1.8–3.1; P<.0001).

Conclusion

In a retrospective analysis of patients with CD, we found that measurements of fecal calprotectin made during routine monitoring can identify patients at risk for disease progression, independent of symptoms or disease location. It is therefore important to screen asymptomatic patients for mucosal inflammation and pursue complete resolution of inflammation.

Keywords

IBD; biomarker; prognostic factor; non-invasive

Introduction

Crohn's disease (CD), a form of inflammatory bowel disease (IBD), is characterized by relapsing episodes of intestinal inflammation and the accumulation of irreversible digestive damage. Prognosis is highly variable between individuals,[1] such that the identification of patients at greatest risk of poor outcomes is an urgent research priority. Some clinical phenotypes, such as disease location and environmental factors such as smoking, have been clearly associated with poorer outcomes.[2,3] However, accurate prediction remains difficult. Over the past decade, there has been a paradigm shift away from treating until symptom resolution and towards mucosal healing as persistent subclinical bowel inflammation leads to poorer outcomes.[4–8] However this has typically required ileocolonoscopy, which is invasive, expensive and carries risk for patients.[9]

Fecal calprotectin (FC) has become well-established as a biomarker of intestinal inflammation. Calprotectin is a 36.5 kDa protein that constitutes 60% of the contents of granules in neutrophils.[10] Its use as a screening test to distinguish IBD from irritable bowel syndrome is well-supported by multiple studies, with an AUROC of 0.95 in meta-analysis.[11] Several groups have demonstrated that FC correlates well with endoscopic measures of disease activity.[12–16] There has been greater uncertainty of its role in small bowel CD, but more recently FC has been shown to correlate well with both MRI[17] and capsule endoscopy findings.[18,19]

The use of FC as a prognostic marker has been demonstrated in the context of medically- and surgically-induced remission.[20–22] In both contexts, baseline FC predicts disease flare over a follow-up period of two years, though there is also a rise notable in FC 3-4 months prior to clinical disease flare. The recent CALM study has demonstrated the effectiveness of a treat to target strategy incorporating FC in Crohn's disease.[23] However, it has still not yet been demonstrated whether elevations in FC, irrespective of clinical symptoms, are associated with disease progression. This information would provide further support to the principle of treating beyond symptoms.

We aimed to use a large, extensively-phenotyped cohort of CD patients followed over time to determine the value of FC to predict progression of disease. We focused on endpoints associated with digestive damage: progression of Montreal behaviour[24], surgical resection or hospitalization for severe flare.

Methods

This was a retrospective cohort study of CD patients managed at the Western General Hospital, Edinburgh, UK, a teaching hospital that cares for secondary- and tertiary-referred patients with IBD. The primary inclusion criteria were a diagnosis of CD and at least one FC more than three months post-diagnosis. The *a priori* primary endpoint was a composite of progression in Montreal luminal disease behavior (B1 to B2/B3 or B2 to B3), hospitalization for flare and resectional surgery. These individual components were also defined as separate secondary endpoints. In order to reduce the possibility of merely measuring the FC at the time of the disease flare that caused the endpoint, any events that happened within 90 days after the index FC were regarded as having already happened and were not included in the endpoint analysis.

We obtained FC data from the Edinburgh FC Registry (EFCR), a record of every FC done in Edinburgh since its introduction in 2003. Patients in this initial cohort had their first FC between 2003 and 2014 and were followed up until 2015. Fecal calprotectins were requested as part of routine monitoring and also directed by patients' symptoms. These data represent a convenience sample, and include all patients tested during that period who met our inclusion criteria.

We matched these data to existing research and clinical databases to identify patients with a known diagnosis of CD. We then interrogated the electronic and paper medical records to obtain information on demographics, symptoms, disease location and behavior over time, hospitalizations, surgical procedures, investigations and drug therapy. Disease location and behavior were classified according to the Montreal classification.[24] Changes in disease behavior were defined as occurring

when the first investigation that demonstrated the change was performed, for example an MRI scan showing stricturing small bowel disease.

Patients were regarded as symptomatic either by Harvey Bradshaw Index (HBI) > 4 and/or by physician global assessment of active symptomatic luminal disease[25]. Each of the previous medical therapies was categorized as having ever taken versus never, with immunomodulators defined as azathioprine, mercaptopurine and methotrexate. Data were stored in a Microsoft Access 2003 database (Microsoft, Redmond, WA, USA).)

FC collection kits were given to patients and samples returned to the hospital biochemistry laboratories either directly or via their GP practice (samples forwarded the same day). Upon arrival at the laboratories samples are stored at -20 °C. FC was measured using a standard enzyme-linked immunosorbent assay (ELISA) technique (Calpro AS, Norway). All assays were performed utilizing the same protocol in the Department of Clinical Biochemistry at the Western General Hospital, Edinburgh. The manufacturer's reference range for distinguishing inflammatory bowel disease from functional gut disorders is >50 µg/g.

Statistical analysis was done using R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). The Mann Whitney U test was performed for continuous non-parametric data, while Fisher's exact tests were done for categorical data. Survival analysis was performed using Kaplan Meier and Cox proportional hazards models.[26] For the survival models, we have reported the outcome as the proportion with maintained digestive health, i.e. the inverse of our primary endpoint. Patients were excluded from the specific analysis of progression in Montreal behavior if they were already B3 at baseline.

FC was analyzed using log-transformed data and using a predefined threshold of 250 µg/g. The optimum threshold for FC on survival analysis was then explored by examining the p values of the likelihood ratio test and the Akaike Information Criteria for Cox proportional hazard models. Variable

selection for multivariable models was done using a stepwise backwards method based on Akaike Information Criterion. We performed Cox proportional hazards analyses of the effect of drug therapy up to 3 months pre or 6 months post fecal calprotectin on the primary outcome; for this analysis, patients who had disease progression within the first six months or who were censored in that period were excluded from analysis. The multistate transition data for disease progression in the overall cohort was done using the empirical transition matrix method.[27]

The principal analysis was done using the first FC for each patient where there was more than one. Owing to the retrospective nature of this dataset, these were not taken at uniform intervals. Exploratory analysis of multiple FCs was performed using the median for each rolling six-month period centered on each month following diagnosis and stratified by progression in Montreal behavior. FCs were excluded from this analysis where the patient was symptomatic at the time of sampling.

This study was conducted as a service evaluation using data collected routinely as part of clinical care, and therefore following guidance from the UK Health Research Authority did not require specific ethical approval or consent.

Results

We identified 918 CD patients meeting our inclusion criteria (Figure 1). 61.1% were female, and median age at the index FC measurement was 40.7 years (interquartile range [IQR] 28.5-54.8) (Table 1). Median follow-up time was 50.6 months (IQR 32.8-76.0), with a total of 4218 patient-years of follow-up across the cohort. At diagnosis, 81% had an inflammatory (B1) phenotype, 12% stricturing (B2) and 8% penetrating (B3). By 30 years post-diagnosis, the proportions of B1, B2 and B3 were estimated as 29%, 36% and 36% respectively (Figure 2). FC was significantly higher in patients with L3 (median 315[IQR 90 – 866] µg/g) and L2 disease (median 289 [IQR 69 – 909] µg/g) than in those with L1 disease (median 180 [IQR 65 – 445 µg/g]; p<0.0001).

Demographic and biomarker data on the cohort stratified by whether the patients reached the composite endpoint or not are shown in table 2. On univariable cox proportional hazards analysis, FC was strongly associated with an elevated risk of reaching the primary endpoint (Table 3), with a hazard ratio (HR) of 1.79 (95% CI 1.50 – 2.14, $p = 1.9\times10^{-10}$) for $\log_{10}$(FC). The only other blood tests nominally associated with FC on univariable analysis were CRP (p=0.016), hemoglobin (p=0.011) and platelets (p=0.003). There were also associations with younger age at diagnosis (p=0.010), female sex (p=0.021), prior immunomodulator use (p=0.012), symptoms at index visit ($p=1.2\times10^{-7}$). Smoking status, previous intestinal resection, previous anti-TNF and time period of FC measurement (pre/post 2008) use were not associated with the primary endpoint, nor was there a significant difference in the time since diagnosis at the index FC.

On multivariable Cox proportional hazards analysis, disease progression was independently associated with elevated FC, female sex, younger age, ileal/ileocolonic disease, previous immunomodulator use and symptoms (Table 3).

A further analysis was performed to explore the effect of changes in treatment before and after measurement of calprotectin (Supplementary Table 1). This was restricted to patients who did not have disease progression and were not censored within the first six months. There were no significant associations with changes in medication in the three months leading up to the measurement of fecal calprotectin. Use of steroids in the six months following calprotectin was significantly associated with disease progression (HR 1.5 [95% CI 1.16 - 2.03], p=0.003). However, this was no longer significant in a multivariable analysis that also included the FC result (Supplementary Table 2).

Above a threshold FC of 250 µg/g, the hazard ratio for reaching the primary endpoint was 1.9 (95% CI 1.5 – 2.3, $p = 5.5\times10^{-8}$, figure 3A). Using analysis of different thresholds of FC (Supplementary Figure 1), the most significant difference in progression to the primary composite endpoint with a

cut-point of 115 µg/g (figure 3B) yielding a hazard ratio on multivariable analysis of 2.4 (95% CI 1.8 – 3.1, $p = 7.2\times10^{-10}$). Differences in progression were seen in all three principal Montreal locations (L1, L2 and L3; Supplementary Figure 2), in all three secondary endpoints (Supplementary Figure 3) and independent of symptom status at the index visit (Supplementary Figure 4).

Using the Kaplan-Meier estimates, the positive predictive value of an index FC >115 µg/g was 28%, 43%, 52% and 59% at 2, 4, 6 and 8 years respectively. The negative predictive value of an index FC ≤115 µg/g was 88%, 80%, 74% and 65% at 2, 4, 6, and 8 years respectively.

In a sensitivity analysis by quartiles of time from diagnosis to first fecal calprotectin, the association between calprotectin and disease progression was seen for quartiles 2 to 4, but not for the patients in the first quartile; these patients had 3 to 15.5 months between diagnosis and first fecal calprotectin (Supplementary Figure 5).

We performed an exploratory analysis using all of the available CD FC data and excluding FC taken when patients had symptoms. This analysis included 1456 FCs from 396 patients. The rolling median FC can clearly be seen to differ between those 35/396 patients with a subsequent progression in Montreal behavior and those that did not (Supplementary Figure 6).

Discussion

This study demonstrates that elevated FC is associated with increased disease progression, both as defined by a composite primary endpoint of advance in Montreal luminal behavior, surgical resection and hospitalization and by each of these endpoints when considered individually.

Mucosal healing is recognized as a target for therapy in Crohn's disease, with poorer prognosis and a higher risk of surgery associated with increased endoscopic disease activity[4–8] There is a strong correlation between FC, endoscopic disease activity and ulcer depth.[12,28] Our data show more directly that elevated FC can be used as a marker of increased risk of progression.

Although absolute index FC levels were lower in L1 patients, FC better predicted poorer outcomes in patients with L1/L3 rather than L2 disease distribution. Patients with active colonic disease may be more likely to exhibit symptoms, and thus have earlier intervention. In contrast, patients with active ileal disease may tolerate a higher level of subclinical inflammation, resulting in delay of treatment with a greater risk of progression and complications.

Other variables associated with an adverse outcome in our analysis included younger age, which has previously been identified as an adverse prognostic factor[1], and previous immunomodulator use which is likely to be a marker for a more aggressive prior disease course. Symptomatically active disease was associated with an increased rate of disease progression, independently of elevated FC. This validates a treat-to-target approach aiming for a combination of resolution of symptoms as well as mucosal healing, with FC a marker of the latter.

Thresholds for prediction of disease relapse have varied across the literature, influenced by the disease cohort being studied and the assay used. Several studies have identified a cut-off of 250 µg/g as being useful to distinguish active from inactive disease.[20,22,29] In the present study, the optimal separation between survival curves for progression of disease was seen using a lower threshold of 115 µg/g, suggesting that lower levels of inflammatory activity may still be associated with an adverse outcome. However, any such threshold needs to be interpreted in the context of the methods of FC extraction and measurement. For example, others have shown significant variability in FC measurement between weight-based and other methods of FC extraction and similarly when comparing ELISA kits from different manufacturers'.[30,31]

We have shown that elevated FC at any point in disease course beyond the first year correlates with poorer outcome. Previous studies have demonstrated an increase in symptomatic relapse in patients with elevation of FC;[20–22] our study further indicates that this is associated with an increase in disease progression. The CALM study has recently demonstrated better outcomes at 52 weeks when

a strategy incorporating symptoms, CRP and FC was compared with clinical disease activity alone.[32] Together, these data now clearly support a treat-to-target strategy combining a patient-reported symptom score with FC as a marker of mucosal inflammation.

Strengths of the present study include the large number of patients and duration of follow-up, with a median follow-up time following index FC of greater than four years. A clinically relevant definition of disease progression was selected *a priori*, and rich phenotype information was available. Restricting measurement of endpoints to at least 90 days after the index FC should reduce bias from measuring disease activity associated with an exacerbation that went on to cause hospital admission or surgical resection. It can also be observed that the survival curves in figures 3–6 continue to separate for many months after the index FC. This suggests that identification of mucosal inflammation at any point in patient follow up, even at relatively modest levels previously considered acceptable (i.e. FC 115-250ug/g), should warrant careful monitoring and low threshold for treatment escalation decisions.

Limitations of this study relate to its retrospective nature. FCs were not collected at fixed intervals, but as determined by the treating clinician. However, routine monitoring of FC including in asymptomatic patients was established quite early on in Edinburgh after the full roll-out of the test in 2005. The study was also performed at a single centre, which may reduce heterogeneity but at the expense of generalizability. Nonetheless, although the Western General Hospital is a referral centre, it also has a large secondary care population from the local catchment. Finally, medication data were completed as accurately as was possible, but it is possible some courses of steroids, particularly those in primary care, may have been missed. This is unlikely to have introduced any systematic bias.

In conclusion, we have shown in this study that elevated fecal calprotectin is associated with an increased risk of disease progression over time in Crohn's disease. Further studies should continue

to explore the utility of repeated FC measurements, and to assess whether intervention based on FC can alter disease outcome.

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Table 1 – Baseline demographics of the cohort (n=918)

Variable		Median (IQR) / Number (%)
Sex	Female	561 (61.1%)
Age at diagnosis/years		27.4 (20.1 - 42.8)
Age at calprotectin/years		40.7 (28.5 - 54.8)
Months to first calprotectin		75.5 (15.5 - 183.8)
Year of first calprotectin		2010 (2008 - 2011) (Range 2003 – 2014)
Smoking at diagnosis	Current	229 (32.5%)
	Ex	101 (14.3%)
	Never	375 (53.2%)
Montreal location	L1±L4	289 (31.7%)
	L2±L4	328 (36.0%)
	L3±L4	288 (31.6%)
	Isolated L4	6 (0.7%)
Montreal behavior at diagnosis	B1	741 (80.7%)
	B2	106 (11.5%)
	B3	71 (7.7%)
Montreal behavior at index calprotectin	B1	564 (61.4%)
	B2	200 (21.8%)
	B3	154 (16.8%)
New medication in 3 months prior to fecal calprotectin		
Steroids		91 (9.91%)
Immunomodulator		58 (6.32%)
Anti-TNF		16 (1.74%)
Any of these		146 (15.90%)
New medication in 6 months following fecal calprotectin		
Steroids		170 (18.52%)
Immunomodulator		105 (11.44%)
Anti-TNF		47 (5.12%)
Any of these		239 (26.03%)

Table 2 Demographics and investigations at index visit stratified by whether individuals reached the composite primary endpoint of progression in Montreal behavior, surgical operation or hospitalization

Variable			Primary endpoint		P
			Not reached	Reached	
Sex	M		235 (42.4%)	105 (32.5%)	0.005
	F		320 (57.7%)	217 (67.4%)	
Age at diagnosis/years			28.2 (20.9 - 45.0)	24.7 (17.9 - 38.1)	2.3×10^{-4}
Age at calprotectin/years			41.9 (30.0 - 56.3)	38.0 (26.7 - 49.8)	2.7×10^{-4}
Months to first calprotectin			69.3 (13.4 - 183.8)	85.1 (20.0 - 189.5)	0.234
Montreal location	L1		167 (30.3%)	110 (34.5%)	1.7×10^{-4}
	L2		224 (40.6%)	88 (27.6%)	
	L3		159 (28.8%)	117 (36.7%)	
Smoker at visit	No		263 (75.1%)	142 (68.9%)	0.115
	Yes		87 (24.9%)	64 (31.1%)	
Previous resection			231 (41.6%)	146 (45.3%)	0.289
Previous immunomodulator			255 (45.9%)	166 (51.6%)	0.123
Previous anti-TNF			110 (19.8%)	68 (21.1%)	0.664
Symptomatic at index visit			195 (53.4%)	162 (78.3%)	2.4×10^{-9}
Investigation		**n**			
Fecal calprotectin (ug/g)		877	180 (50 - 665)	432 (136 - 998)	6.9×10^{-12}
CRP (mg/L)		375	7 (3 - 19)	10 (4 - 27)	0.023
ESR (mm/hr)		202	21 (11 - 36)	26 (14 - 41)	0.045
Albumin (g/L)		350	40 (36 - 43)	38 (32 - 43)	0.097
Hemoglobin (g/L) (scaled to male range)		500	148 (139 - 155)	145 (133 - 154)	0.009
WCC ($\times10^9$/L)		507	7.5 (5.9 - 9.4)	7.3 (5.8 - 9.5)	0.785
Platelets ($\times10^9$/L)		489	277 (225 - 342)	305 (249 - 377)	4.9×10^{-4}

Values shown are medians (interquartile ranges) and numbers (percentages) as appropriate.

P values calculated using Mann Whitney U and Fisher's exact tests for continuous and categorical data respectively.

Table 3 Univariable and multivariable analyses using Cox proportional hazards models for time to reaching primary endpoint

Variable	Univariable		Multivariable	
	HR (95% CI)	p	HR (95% CI)	p
Sex (female)	1.31 (1.04 - 1.65)	0.021	1.66 (1.23 - 2.24)	0.001
Age at diagnosis/years	0.99 (0.98 - 1.00)	0.010		
Age at calprotectin/years	0.99 (0.98 - 1.00)	0.001	0.99 (0.98 - 1.00)	0.010
No ileal involvement (Montreal L2)	0.66 (0.51 - 0.84)	7.9×10^{-4}	0.60 (0.44 - 0.82)	0.001
Previous immunomodulator	1.32 (1.06 - 1.64)	0.012	1.39 (1.04 – 1.84)	0.024
Previous anti-TNF	1.12 (0.86 - 1.46)	0.411		
Symptomatic at index visit	2.45 (1.76 - 3.42)	1.2×10^{-7}	2.07 (1.46 – 2.93)	4.1×10^{-5}
Fecal calprotectin (ug/g)*	1.79 (1.50 - 2.14)	1.9×10^{-10}	1.49 (1.17 – 1.89)	0.001
CRP (mg/L)*	1.44 (1.07 - 1.93)	0.016		
Hemoglobin (g/L) (scaled to male range)	0.99 (0.98 - 1.00)	0.011		
Platelets (x10^9/L	1.00 (1.00 - 1.00)	0.003		

* Variable $\log_{10}$ transformed prior to use in the model. Hazard ratio is for each 10-fold increase in the variable.

HR: Hazard Ratio; CI: Confidence Interval

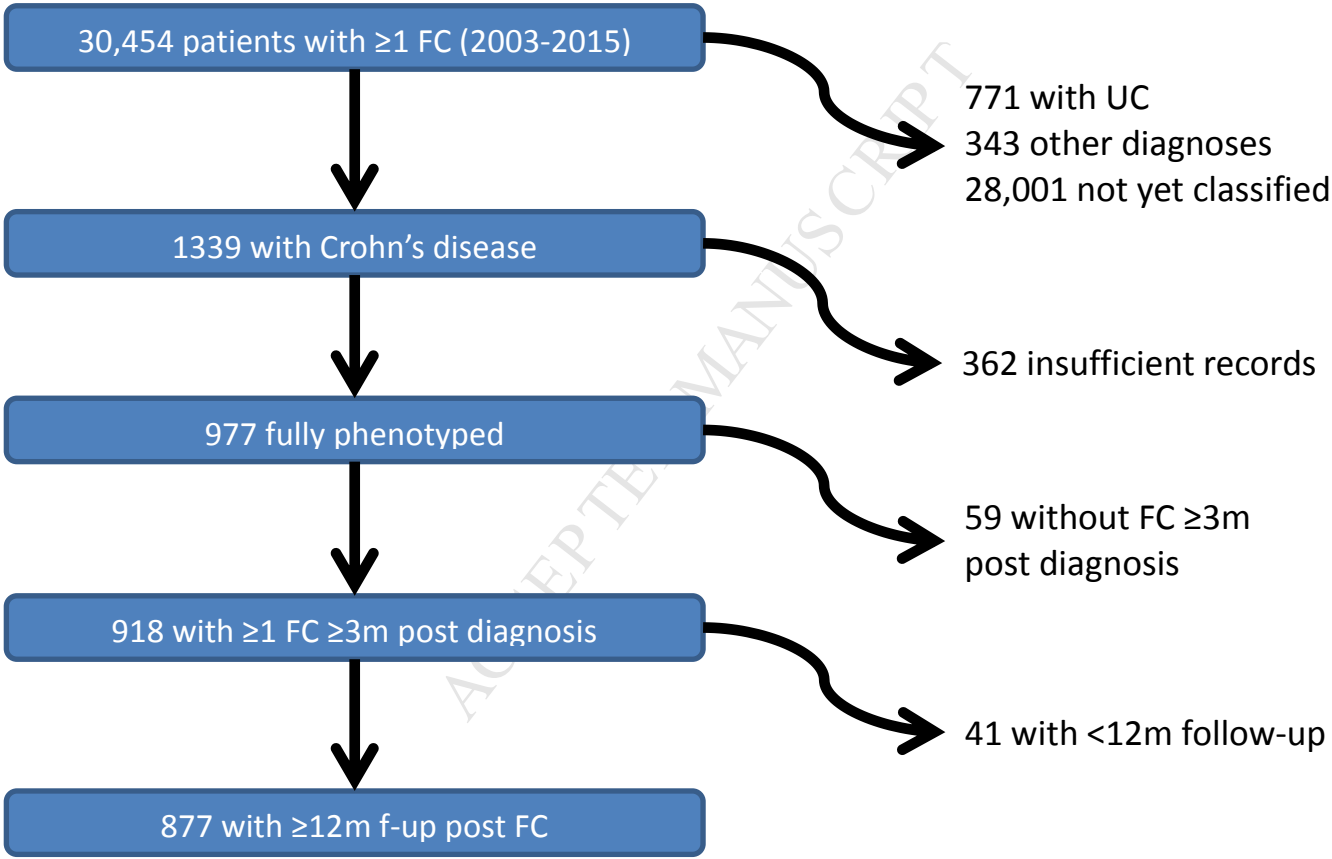
Figure legends

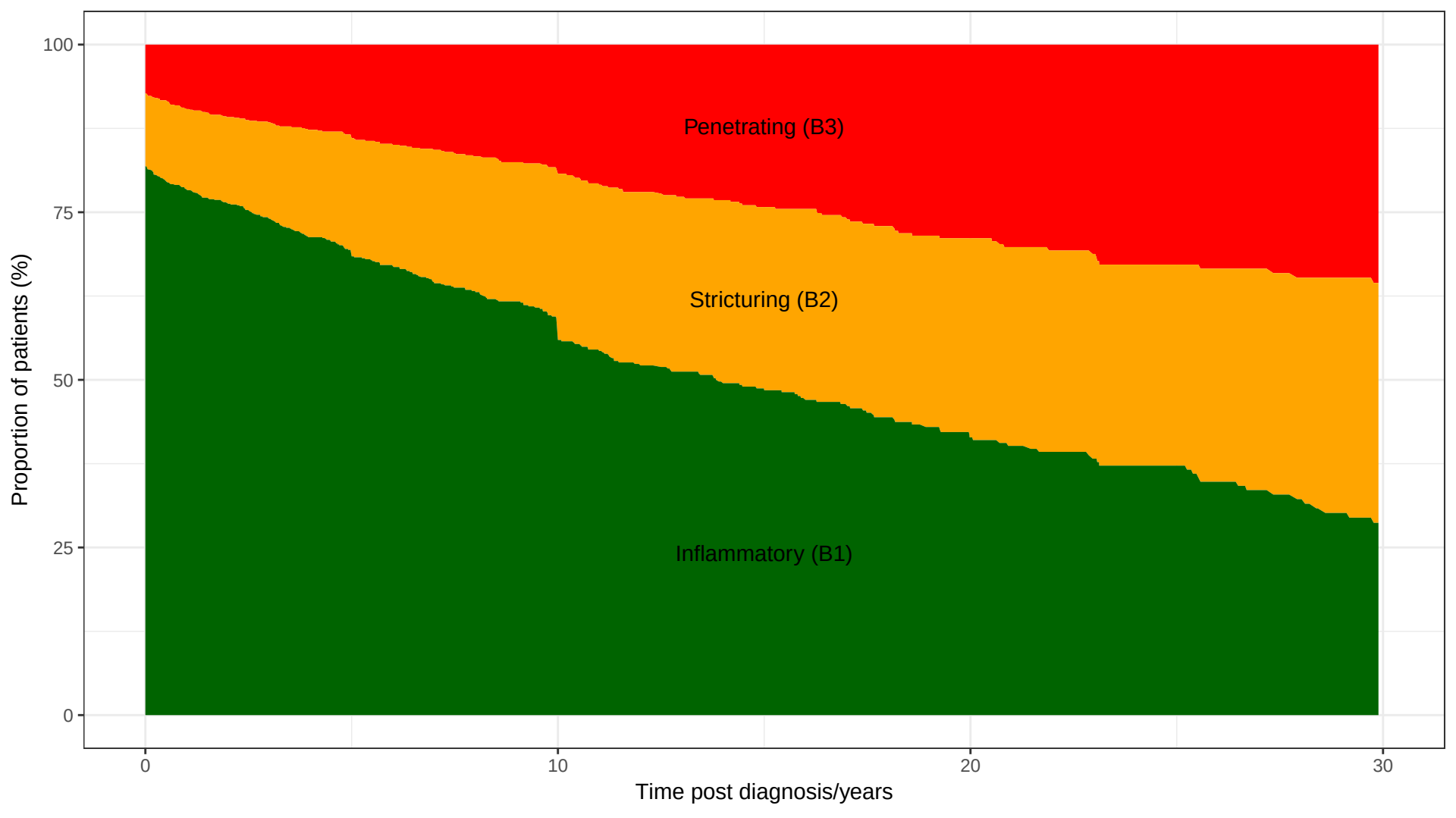
Figure 1 – Derivation of the cohort of patients with Crohn's disease, fecal calprotectin (FC) and follow-up data

Figure 2 – Disease progression over time in the whole cohort as estimated by the empirical transition matrix method

Figure 3 – Kaplan-Meier plot of time to reaching primary endpoint stratified by fecal calprotectin > 250 µg/g (A) and > 115 µg/g (B) at index visit

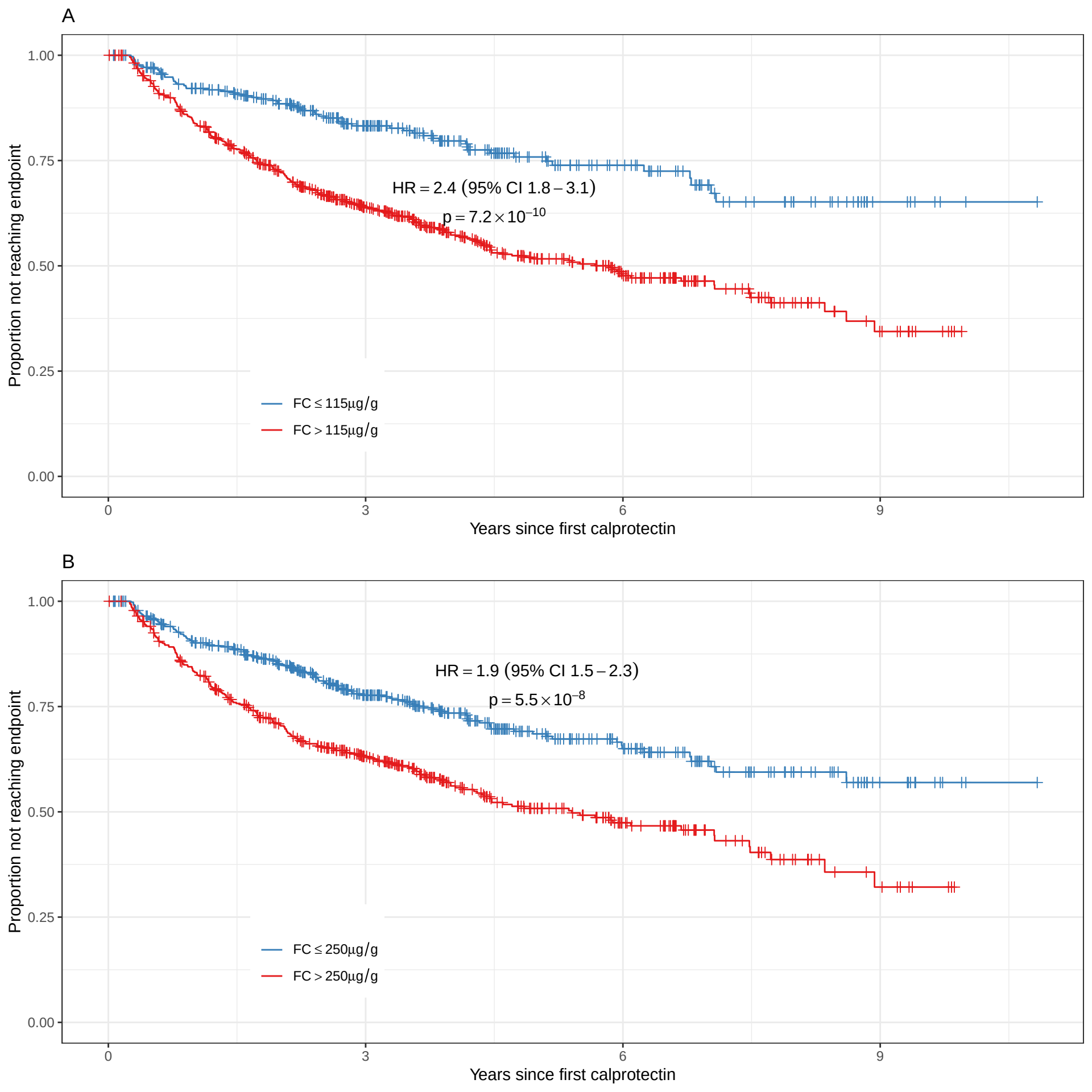
The outcome of maintained digestive health is defined here as the inverse of the primary study endpoint (a composite of progression in Montreal behavior, hospitalization or surgery)





Number at risk

0	10	20	30
918	722	514	361	245	157	101


A
1.00
0.75
0.50
0.25
0.00
Proportion not reaching endpoint
HR = 2.4 (95% CI 1.8 – 3.1)
p = 7.2 × 10−10
FC ≤ 115µg/g
FC > 115µg/g
0
3
6
9
Years since first calprotectin
B
1.00
0.75
0.50
0.25
0.00
Proportion not reaching endpoint
HR = 1.9 (95% CI 1.5 – 2.3)
p = 5.5 × 10−8
FC ≤ 250µg/g
FC > 250µg/g
0
3
6
9
Years since first calprotectin

What you need to know

Background and Context

Fecal calprotectin is a marker of luminal Crohn's disease (CD) activity. We investigated whether fecal calprotectin associates with subsequent CD progression.

Findings

We have now shown that an increased fecal calprotectin is associated with a long-term increase in disease progression, including hospitalisation, surgery and advance in Montreal behaviour.

Implications for patient care

It is important to screen asymptomatic patients for mucosal inflammation and pursue complete resolution of inflammation.