Serum Calprotectin: A Novel Diagnostic and Prognostic Marker in Inflammatory Bowel Diseases

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OBJECTIVES: **There is an unmet need for novel blood-based biomarkers that offer timely and accurate diagnostic and prognostic testing in inflammatory bowel diseases (IBD). We aimed to investigate the diagnostic and prognostic utility of serum calprotectin (SC) in IBD.**

METHODS: **A total of 171 patients (*n*=96 IBD, *n*=75 non-IBD) were prospectively recruited. A multi-biomarker model was derived using multivariable logistic regression analysis. Cox proportional hazards model was derived to assess the contribution of each variable to disease outcomes.**

RESULTS: **SC correlated strongly with current biomarkers, including fecal calprotectin (FC) (*n*=50, ρ=0.50, *P*=1.6×10^{-4}). SC was the strongest individual predictor of IBD diagnosis (odds ratio (OR): 9.37 (95% confidence interval (CI): 2.82–34.68), *P*=4.00×10^{-4}) compared with other markers (C-reactive protein (CRP): OR 8.52 (95% CI: 2.75–28.63), *P*=2.80×10^{-4}); albumin: OR 6.12 (95% CI: 1.82–22.16), *P*=0.004). In a subset of 50 patients with paired SC and FC, the area under receiver operating characteristic discriminating IBD from controls was better for FC than for SC (0.99, (95% CI 0.87–1.00) and 0.87 (95% CI:0.78–0.97), respectively; *P*=0.01). At follow-up (median 342 days; interquartile range: 88–563), SC predicted treatment escalation and/or surgery in IBD (hazard ratio (HR) 2.7, 95% CI: 1.1–4.9), in particular Crohn's disease (CD) (HR 4.2, 95% CI 1.2–15.3). A model incorporating SC and either CRP or albumin has a positive likelihood ratio of 24.14 for IBD. At 1 year, our prognostic model can predict treatment escalation in IBD in 65% of cases (95% CI: 43–79%) and 80% (95% CI: 31–94%) in CD if ≥2 blood marker criteria are met.**

CONCLUSIONS: **A diagnostic and prognostic model that combines SC and other blood-based biomarkers accurately predicts the inflammatory burden in IBD and has the potential to predict disease and its outcomes. Our data warrant further detailed exploration and validation in large multicenter cohorts.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

Am J Gastroenterol 2016; 111:1796–1805; doi:10.1038/ajg.2016.342; published online 6 September 2016

INTRODUCTION

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic, debilitating inflammatory disorders of the gastrointestinal tract affecting adults and children (1,2). A recent systematic review showed rising trends in the incidence and prevalence of IBD worldwide (3) associated with significant health-care costs amounting to around £470 million in the United Kingdom, up to €5.6 billion annually in Europe, and >$6 billion annually in the United States of America (3–8). With an ever expanding therapeutic repertoire, it is important to select patients who may benefit from early use of immunosuppressants' and/or biological therapies in order to minimize irreversible luminal damage and prevent long-term complications.

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Received 15 February 2016; accepted 21 July 2016

Blood-based biomarkers provide a non-invasive estimation of the inflammatory burden in newly diagnosed IBD. However, relatively few blood-based biomarkers have been extensively validated in IBD, and fewer still are in routine use in the clinic (9). There is an emerging interest in discovering novel markers using multiomic platforms that may be valuable in a variety of clinical settings, including IBD diagnostics, disease activity assessments, predicting disease outcomes, and response to therapy (9–11).

The S100 family of proteins, including S100A8/A9 (calprotectin) and S100A12 (calgranulin), have been implicated in disease pathogenesis and investigated as potential markers of inflammation (12,13). In IBD, fecal calprotectin (FC) has emerged as a particularly informative tool (14). A recent meta-analysis of 13 studies and 1,041 patients found that FC had a pooled sensitivity and specificity of 0.93 (0.85–0.97) and 0.96 (0.79–0.99), respectively, for IBD and identified those individuals requiring endoscopy for suspected IBD (13). There are also data on the role of FC in other clinical settings, such as predicting postoperative CD recurrence and predicting outcomes in acute severe colitis (15,16). However, there are limitations to FC testing in clinical practice. Fecal collection can be a hurdle for patients (17) and sample delivery and processing delays can hinder its clinical utility. In active UC, FC shows high within-day variability and the optimal timing for sampling is not clear (18,19). A blood-based biomarker such as serum calprotectin (SC) may be more convenient in routine practice and more acceptable to patients. SC has been studied in diseases such as inflammatory arthropathies and cystic fibrosis (20–24). In rheumatoid arthritis, SC was independently predictive of a 10-year radiographic disease progression (25), while in cystic fibrosis, SC predicted exacerbation and lung function decline (20,21).

More recently, SC has been investigated in IBD to predict response to and relapse following anti-tumor necrosis factor (anti-TNF) therapy (17,26). In CD patients, SC has a similar profile to high-sensitivity C-reactive protein (CRP) and compliments FC and high-sensitivity CRP for prediction of relapse after anti-TNF withdrawal (P=0.0173, 0.0024, and 0.0002; hazard ratio (HR): 3.191, 3.561, and 4.120, respectively) (17). In murine models, trinitrobenzene sulfonic acid–induced colitis is associated with higher SC levels that correlate closely to macroscopic and microscopic disease scores (27). We are yet to understand fully the relationship between SC and the other currently available biomarkers in IBD and the diagnostic and prognostic value of SC in IBD. Our study aims to investigate the role of SC in this clinical setting.

METHODS

Study design

A prospective, single-center case control study was performed in patients with suspected or confirmed IBD at their first presentation to a tertiary gastrointestinal clinic. Data were collected for patient demographics, including age, sex, age at diagnosis, and date of diagnosis (**Table 1** and **Supplementary Table 2** online). Details of drug therapy and concomitant medications were recorded. Laboratory markers, including CRP and albumin, were measured as part of the research protocol while other routine markers, including hemoglobin, white cell count, platelets, and FC, were recorded within 30 days of recruitment.

Table 1. Correlation coefficient (Spearman's ρ) between serum calprotectin (SC) and blood and fecal parameters

Parameters	Number of patients	ρ (Spearman's ρ)	*P* value
SC–CRP	171	0.61	6.9×10^{-19}
SC–WCC	147	0.61	3.8×10^{-17}
SC–Neut	147	0.65	1.9×10^{-20}
SC–Lymp	147	–0.03	0.68
SC–Alb	171	–0.54	3.3×10^{-14}
SC–Hb	147	–0.42	6.1×10^{-8}
SC–Plts	147	0.49	1.3×10^{-10}
SC–FC	50	0.50	1.6×10^{-4}

Parameters	IBD (*n*)	ρ (*P* value)	Non-IBD (*n*)	ρ (*P* value)
SC–CRP	96	0.41 (3.9×10^{-5})	75	0.30 (0.01)
SC–WCC	92	0.37 (2.3×10^{-4})	55	0.66 (4.3×10^{-8})
SC–Neut	92	0.43 (2.2×10^{-5})	55	0.68 (1.0×10^{-8})
SC–Lymp	92	–0.18 (0.08)	55	0.48 (2.0×10^{-4})
SC–Alb	95	–0.39 (6.9×10^{-5})	75	–0.09 (0.46)
SC–Hb	92	–0.41 (4.7×10^{-5})	55	–0.05 (0.75)
SC–Plts	92	0.31 (0.002)	55	0.27 (0.04)
SC–FC	31	–0.07 (0.72)	19	0.13 (0.59)

Alb, albumin; CRP, C-reactive protein; FC, fecal calprotectin; Hb, hemoglobin; Lymp, lymphocyte count; Neut, neutrophil count; Plts, Platelet count; WCC, white cell count.

Inclusion criteria

Patients with a new diagnosis of IBD were included in the study. The Lennard–Jones, Montreal, and Paris criteria were used for diagnosis and classification of clinical phenotypes (28–30). The control cohort consisted of healthy laboratory volunteers (HC) and patients with gastrointestinal symptoms (symptomatic controls) who had no discernible inflammatory disease and a diagnosis of functional bowel disease at follow-up (**Supplementary Table 3**).

Sample collection and processing

For SC analysis, blood samples were collected prospectively and serum was processed within 2 h of sampling (using centrifugation at 2500 *g* for 15 min) and subsequently stored at −80 °C until further use. Samples were analyzed in duplicate using the Calpro AS calprotectin ELISA (Calpro AS, Lysaker, Norway) according to the manufacturer's instructions. Samples with a calprotectin result of >2500 ng/ml were diluted and retested. Coefficients of variation of <10% were included in the analysis.

Ethics statement

The NHS Lothian SAHSC Bioresource granted approval for this study (reference number SR558) with all patients giving written and informed consent (15/ES/0094).

Clinical course in IBD

Case note review was performed for all IBD cases. Treatment escalation was defined as the need for escalation and establishment of ≥2 immunomodulatory therapies and/or surgery for disease flare after initial induction of disease remission (criteria previously used by Lee *et al.* (31)). In UC, the definition of treatment escalation also included any patient with a new diagnosis, requiring emergency colectomy during their index admission (**Supplementary Table 1**).

Statistical analysis

Data were analyzed using Microsoft Excel 2010 (Microsoft, Redmond, WA) and R 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Continuous data are presented as medians and interquartile ranges (IQRs) and were analyzed using a Mann–Whitney *U*-test. Categorical data are presented as numbers and percentages and were analyzed using Fisher's exact tests. Spearman's rank-order correlation test was applied for correlations between variables. To determine the accuracy of blood parameter measurements as a prognostic test capable of diagnosing IBD, receiver operating characteristic (ROC) analyses were performed by plotting sensitivity against specificity using the 'pROC' package in R (32).

Building diagnostic and prognostic models

After univariable analyses, the most significant laboratory parameters (CRP, albumin, and SC) were included in multivariable models for IBD diagnosis and prognosis. CRP and SC were log transformed to more closely approximate a normal distribution for further multivariable analysis. ROC analyses were used to define the optimal cut points (highest sum of sensitivity+specificity) for both models. The optimal models were then selected by performing backward stepwise regression using the smallest Akaike information criterion values and adjusted for treatment exposure.

For the diagnostic model, an integer score was attributed to each variable according to its relative contribution in the model (as determined by the coefficients) and positive and negative predictive values (PPV and NPV, respectively) were then calculated for each total score.

For the prognostic model, a Cox proportional hazards model was derived to assess the contribution of each variable to disease outcomes. Thresholds were then identified using ROC analyses to allow stratification of patients to either a benign or an aggressive disease course (requiring treatment escalation and/or surgery) and to allow creation of survival curves.

RESULTS

Comparison of SC with conventional biomarkers

Overall, SC was analyzed in 171 patient serum samples from August 2013 to April 2015. SC correlated positively with CRP (ρ=0.61, P=6.9×10^{-19}) and negatively with albumin (ρ=−0.54, P=3.3×10^{-14}). Paired full blood count was available within 30 days (median 0 days; range: −26 to 16 days) of recruitment in 147 patients. SC correlated positively with neutrophil count (ρ=0.65, P=1.9×10^{-20}) and negatively with hemoglobin (ρ=−0.42, P=6.1×10^{-8}). Paired FC was available within 30 days (median 0 days, IQR: −5 to 5 days) of SC in 50 patients (IBD n=31, non-IBD n=19). SC correlated significantly with FC (Spearman's ρ=0.50, P=1.6×10^{-4}). **Table 1** summarizes the correlation of blood and fecal parameters. SC demonstrated a stronger correlation with white cell count (William's test, P=0.02) and neutrophils (P=0.03) in controls compared with IBD cases.

Diagnostic utility of SC in IBD

In a subset of 156 patients (83 IBD and 73 non-IBD), blood sampling was performed within 90 days from diagnosis (median 0 days; IQR 0–6). There were 35 patients with a diagnosis of CD, 45 patients with UC, and 3 patients with IBD unclassified (IBDU) in the IBD group. In CD, 44% had L3 +/− L4 disease and 62% had B1 behavior according to the Montreal classification (33). In UC, 33% had pancolitis (E4) and 11% had limited proctitis (E1) as per the Paris classification (30). **Table 2** and **Table 3** summarize the demographics and blood and fecal parameters for the IBD and control cohorts. SC was significantly increased in IBD compared with controls (1,010 ng/ml (IQR 796–1,426 ng/ml) vs. 506 ng/ml (IQR 362–725 ng/ml), P=3.7×10^{-15}; **Figure 1**). CRP and albumin were also significantly different between IBD and controls (CRP P=8.9×10^{-15}; albumin P=4.9×10^{-14}). There was no difference in SC between CD and UC (1,015 ng/ml (IQR 740–1,518 ng/ml) vs. 911 µg/g (IQR 809–1,413 ng/ml), P=0.79) and within controls (HC: 432 ng/ml (IQR 359–586) vs. symptomatic controls: 563 ng/ml (IQR 382–787); P=0.12). SC was not significantly associated with sex (P=0.14), age (ρ −0.06, P=0.43), or smoking status (P=0.49). SC and CRP were able to discriminate IBD from controls with similar areas under the receiver operator characteristics curve (AUC) of 0.87 (95% confidence interval (CI) 0.82–0.93) and 0.86 (95% CI 0.80–0.91), respectively (**Figure 2**; P=0.64 DeLong's test for comparison of ROC curves). In those with paired SC and FC within 30 days, the AUC for discriminating cases and controls was superior for FC (0.99, 95% CI 0.98–1.00) than for SC (0.87, 95% CI 0.78–0.97; P=0.01 De Long's test), as shown in **Figure 2**.

Multivariable analysis

Multivariable logistic regression analysis of predictors of IBD was performed on 155 cases (83 IBD, 72 non-IBD) where the data for the predictors were complete. Albumin, male gender, log-transformed CRP, and log-transformed SC were significant predictors of IBD. **Table 4** summarizes the statistical significance of each covariate.

Building an IBD diagnosis score

Using the multivariable model, continuous variables were categorized using integer cut points guided by the ROC curves and observed relationship with diagnosis. The final scoring system for the diagnosis of IBD included SC>852 ng/ml, Albumin <38 g/l, CRP≥3.5 mg/l, and male gender. To formulate a numerical risk score, each variable was given a score based on the odds ratio (OR) generated from the linear model. **Tables 5a and 5b** summarize the PPV and NPV for each score. Using this model, a SC> 852 ng/ml and either a CRP≥3.5 mg/l or albumin <38 g/l has

Table 2. Study demographics, Montreal classification, and disease behavior for newly diagnosed inflammatory bowel diseases (IBD) and control cohorts

Variables	Inflammatory bowel diseases (*n*=83)	Controls (*n*=73)
Subtype IBD (CD:UC:IBDU)	35:45:3	
Subtype control group (HC:IBS)		27:46
Males (%)	58 (69)	33 (45)
Smoking status (current: never: ex)	14:41:26	12:36:15
Median age (range)	31 (18–73)	31 (19–64)
Montreal classification for CD		
L1+/–L4	10	
L2+/–L4	10	
L3+/–L4	15	
Montreal behavior for CD		
B1	21	
B2	2	
B3	6	
Not available	6	
Paris extent for UC		
E1	5	
E2	14	
E3	10	
E4	15	
Not available	1	

CD, Crohn's disease; HC: healthy controls; IBDU, IBD unclassified; IBS, irritable bowel syndrome; UC, ulcerative colitis.
Smoking status was available for 81 patients with IBD and for 63 patients with non-IBD.

a sensitivity of 67%, specificity of 97%, and a positive likelihood ratio (LR) of 24.14 for IBD.

Predicting disease extent in IBD

SC, CRP, and albumin were not able to differentiate between IBD subtypes (CRP P=0.45; albumin P=0.67; SC P=0.49). Within the UC cohort, SC was significantly higher in those with disease beyond the rectum (>E1) compared with proctitis alone (E1) (median SC 1,078 ng/ml IQR 820–1,418 vs. 812 ng/ml IQR 698–821, P=0.03). Albumin also predicted disease extent in UC

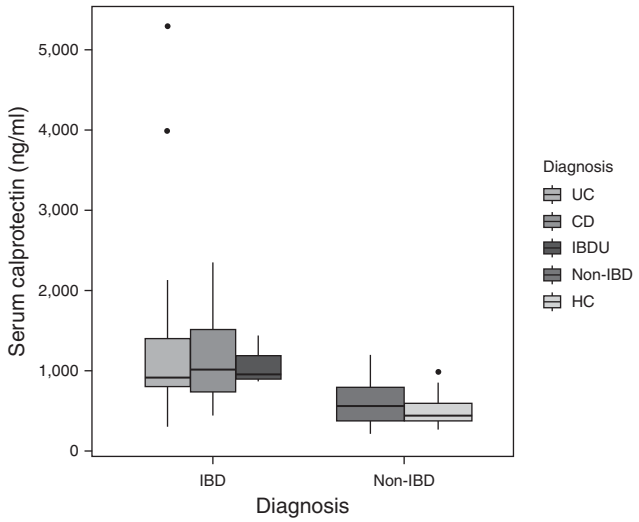

Figure 1. Serum calprotectin levels in patients with Crohn's disease (CD), ulcerative colitis (UC), inflammatory bowel disease unclassified (IBDU), symptomatic controls (Non-IBD), and healthy controls (HC). Boxplots represent median and interquartile ranges for serum calprotectin within each subcohort. For color figure, please see full text version.

Table 3. Blood and fecal parameters for inflammatory bowel disease (IBD) and control cohorts

Test	IBD		Controls	
	Number of patients	Median (IQR)	Number of patients	Median (IQR)
Hemoglobin (g/l)	79	128 (119–139)	53	140 (135–150)
Neutrophil (10^9/l)	79	5.8 (3.9–7.5)	53	3.2 (2.5–4.1)
Lymphocyte (mg/l)	79	1.7 (1.2–2.1)	53	1.8 (1.5–2.2)
White cell count (10^9/l)	79	8.3 (6.5–10.7)	53	5.8 (4.7–6.6)
Platelet count (10^9/l)	79	335 (269–432)	53	244 (209–277)
C-reactive protein (mg/l)	83	11 (3.0–35.5)	73	1.0 (0.5–2.0)
Fecal calprotectin within 30 days (μg/g)	31	770 (660–880)	19	19 (19–25)
Albumin (g/l)	83	35 (29.0–39.0)	73	42 (39–46)
Serum calprotectin (ng/ml)	83	1,015 (811–1,442)	73	506 (362–725)

IQR, interquartile range.

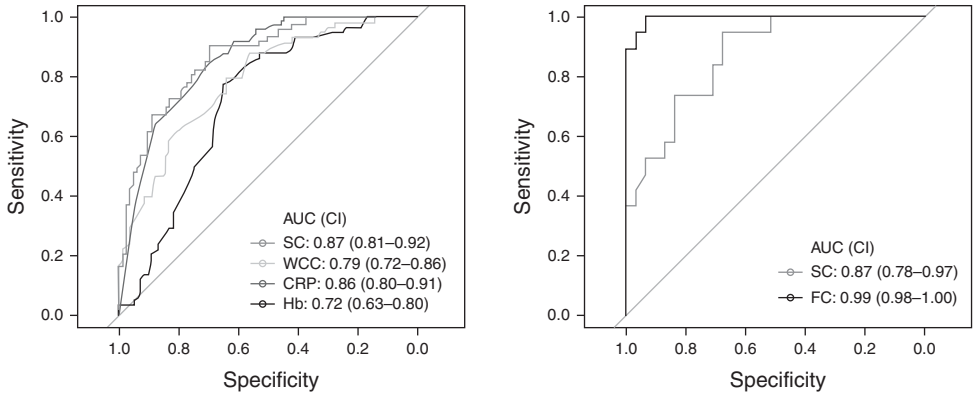

Figure 2. Receiver operating curve analysis (ROC) of serum calprotectin (SC) and other blood based markers in differentiating inflammatory bowel diseases (IBD) from non-IBD, and ROC analysis of SC and fecal calprotectin (FC) (within 30 days) in discriminating IBD from non-IBD. For color figure, please see full text version. CI, confidence interval; CRP, C-reactive protein; Hb, hemoglobin; WCC, white cell count.

Table 4. Multiple logistic regression of predictors of inflammatory bowel diseases vs. controls (*n*=155)

Continuous variable analysis		
Variable	**Odds ratio (95% CI)**	***P* value**
Log (C-reactive protein)	6.60 (2.18–23.67)	0.002
Log (serum calprotectin)	296.85 (9.55–18,512.49)	0.003
Albumin	0.85 (0.75–0.94)	0.003
Gender	4.00(1.22–14.68)	0.03
Categorical variable analysis		
Categorical threshold	**Odds ratio (95% CI)**	***P* value for thresholds**
C-reactive protein >3.5 mg/l	8.52 (2.75–28.63)	2.80×10^{-4}
Serum calprotectin >852 ng/ml	9.37 (2.82–34.68)	4.00×10^{-4}
Albumin <38 g/l	6.12 (1.82–22.16)	0.004
Male gender	2.87 (0.97–9.24)	0.06

CI, confidence interval.

Table 5a. Sensitivity, specificity, and positive and negative likelihood ratios (LR) of the inflammatory bowel diseases (IBD) scoring parameters

Variable	Score
Serum calprotectin >852 ng/ml	5
Albumin <38 g/l	3
CRP ≥3.5 mg/l	4
Male gender	1

IBD Score	Sensitivity	Specificity	Positive LR	Negative LR
≥1	0.96	0.31	1.39	0.12
≥3	0.89	0.68	2.79	0.16
≥4	0.85	0.75	3.41	0.20
≥5	0.84	0.89	7.57	0.18
≥6	0.74	0.93	10.71	0.28
≥7	0.68	0.96	16.39	0.33
≥8	0.67	0.97	24.14	0.34

Each variable score is based on the odds ratio generated from the linear model.

(*P*=0.01) but not CRP (*P*=0.05). In CD, however, there was no significant difference in SC, CRP, or albumin by disease location (*P*=0.47, 0.55, and 0.20, respectively).

Predicting disease outcomes in IBD

Kaplan–Meier analyses were performed on a total of 83 patients with IBD. There were 35 patients with a diagnosis of CD, 45 patients with UC, and 3 patients with IBDU. The median age was 31 years (IQR: 26–41) and 69% were male (*n*=58). A total of 1 (33%), 16 (46%), and 23 (51%) patients required treatment escalation in the IBDU, CD, and UC groups, respectively. Using backwards stepwise selection, albumin <37 g/l and SC ≥1,046 ng/ml remained significant predictors of treatment escalation in IBD (log-rank test $P=5.1\times10^{-5}$). Both biomarkers had similar HR as shown in **Table 6a**. A score was generated using both biomarkers at these thresholds. At a year, the estimated chance of treatment escalation was 21% (95% CI: 1–37%) if none of the criteria

Table 5b. Sensitivity, specificity, and positive and negative likelihood ratios (LR) of the inflammatory bowel diseases (IBD) of the individual markers

Test	Sensitivity	Specificity	Positive LR	Negative LR
C-reactive protein >3.5 mg/l	0.70	0.86	5.03	0.35
Albumin <38 g/l	0.66	0.88	5.30	0.39
Serum calprotectin >852 ng/ml	0.69	0.90	7.06	0.35

Table 6a. Multivariable analysis for predictive factors for an aggressive disease course in patients with inflammatory bowel diseases (*n*=83): final Cox proportional hazards model

Categorical variable analysis				
Categorical variable	**Categorical threshold**	**Hazard ratio (95% CI)**	**AIC**	***P* value for thresholds**
Serum calprotectin (SC)	≥1046 ng/ml	2.7 (1.3–5.6)	309.2	0.007
Albumin	<37 g/l	2.5 (1.1–5.6)	306.5	0.03

AIC, Akaike information criterion; CI, confidence interval.

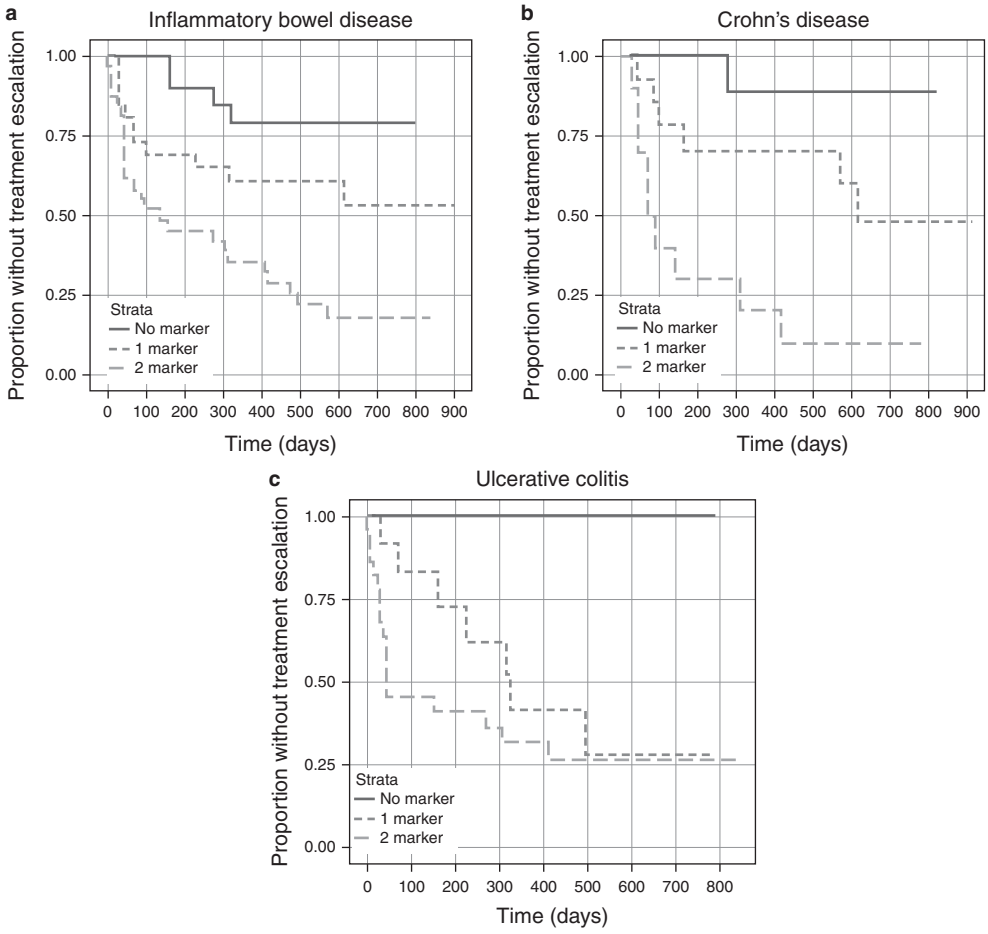

Figure 3. (**a**) Kaplan–Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed inflammatory bowel diseases. Single marker represents either albumin <37 g/l or serum calprotectin ≥1046 ng/ml. Dual markers represent a combination of both variables. (**b**) Kaplan–Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Crohn's disease. '1 marker' represents either C-reactive protein (CRP) >24 mg/l or albumin<26 g/l or serum calprotectin >991 ng/ml. '2 or 3 marker' represents a combination of any 2 or 3 of the above-mentioned variables. (**c**) Kaplan–Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed ulcerative colitis. Single marker represents either albumin <37 g/l or CRP >2.5 mg/l. Dual markers represents all the categorical variables as a combined biomarker. For color figure, please see full text version.

were met, 40% (95% CI: 17–56%) for patients meeting one criterion, and 65% (95% CI: 43–78%) for those meeting both criteria (**Figure 3a**).

In order to assess whether the time lag between diagnosis and blood sampling had an impact on the final model, stepwise regression analyses was performed for samples within 60 days (*n*=74) and 30 days (*n*=60) from diagnosis. SC remained a significant predictor of disease outcomes at 60 and 30 days (*P*=0.003 and *P*=0.004, respectively).

In 28 patients, paired FC was available within 30 days from diagnosis. Using a multivariate model that included age, gender, CRP, albumin, FC, and SC, backward stepwise regression analy-

Table 6b. Multivariable analysis for predictive factors for an aggressive disease course in patients with ulcerative colitis (*n*=45): final Cox proportional hazards model

Categorical variable analysis				
Categorical variable	**Categorical threshold**	**Hazard ratio (95% CI)**	**AIC**	***P* value for thresholds**
Albumin	<37 g/l	3.8 (1.2–11.9)	148.0	0.02
C-reactive protein (CRP)	>2.5 mg/l	2.6 (0.7–9.6)	147.0	0.15

AIC, Akaike information criterion; CI, confidence interval.

Table 6c. Multivariable analysis for predictive factors for an aggressive disease course in patients with Crohn's disease (*n*=35): final Cox proportional hazards model

Categorical variable analysis				
Categorical variable	**Categorical threshold**	**Hazard ratio (95% CI)**	**AIC**	***P* value for thresholds**
C-reactive protein (CRP)	>24 mg/l	2.7 (1.0–7.4)	93.3	0.06
Albumin	<26 g/l	2.6 (0.8–9.1)	91.9	0.13
Serum calprotectin	>991 ng/ml	4.2 (1.2–15.3)	95.1	0.03

AIC, Akaike information criterion; CI, confidence interval.

sis was performed and only SC remained as a significant predictor (P=0.0004). FC did not predict disease outcomes in this cohort (P=0.85, HR=1.0).

Further regression analyses were performed within the subgroups UC (**Table 6b**) and CD (**Table 6c**). In CD, CRP>24 mg/l and SC>991 ng/ml and albumin <26 g/l predicted treatment escalation (log-rank test P=0.003). At 1 year, the estimated chance of treatment escalation was 11% (95% CI: 0–29%) for patients meeting none of the criteria, 30% (95% CI: 0–51%) for patients meeting one criterion, and 80% (95% CI: 31–94%) for patients meeting two or more criteria (**Figure 3b**).

In UC, albumin <37 g/l and CRP>2.5 g/l predicted a more aggressive disease course (log-rank test P=0.001). At 1 year, the estimated chance of treatment escalation was 0 for patients meeting none of the criteria, 38% (95% CI: 0–61%) for patients meeting one criterion, and 68% (95% CI: 41–83%) for patients meeting two criteria (**Figure 3c**).

DISCUSSION

There is an unmet need for accurate diagnostic and prognostic biomarkers in IBD as currently available blood biomarkers lack sensitivity and/or specificity. Our study is the first to investigate

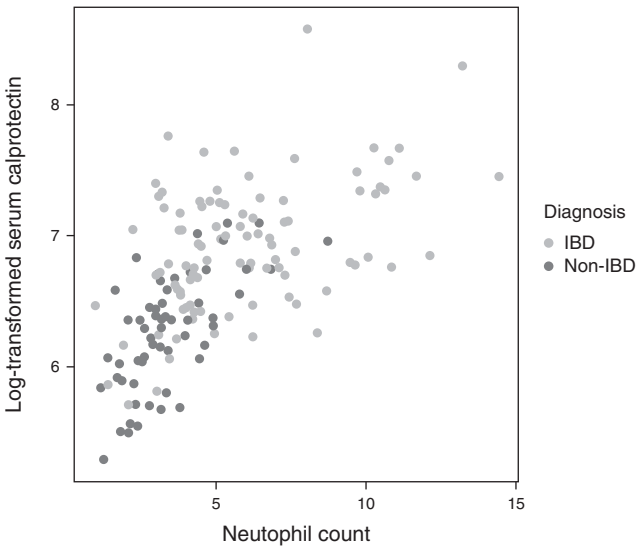

Figure 4. Correlation between log-transformed serum calprotectin and neutrophil count in inflammatory bowel diseases (IBD) vs. controls (Non-IBD). For color figure, please see full text version.

the role of SC in patients with a new diagnosis of IBD. SC independently predicts a diagnosis of IBD with an OR of 9.37 (95% CI: 2.82–34.68). A combined blood-based biomarker diagnostic model, including SC and either CRP or albumin, has a high positive LR for IBD (positive LR 24.14). Similarly, SC can predict treatment escalation and/or surgery in IBD (HR 2.7, 95% CI: 1.1–4.9), in particular CD (HR 4.2, 95% CI 1.2–15.3).

Calprotectin, a member of the S100 proteins, represents 45% of all cytosolic proteins in neutrophils compared with 1% in monocytes (34,35). Given the short half-life of SC (5 h), it may provide a more dynamic test of the current inflammatory status compared with conventional inflammatory markers (half-life of CRP 18 h, albumin 19 days) (36). SC correlates better with neutrophil count in controls compared with IBD patients; in IBD, SC levels may reflect calprotectin release from activated neutrophils and other immune cells such as monocytes, macrophages, and epithelial cells (**Figure 4**). SC shows a strong correlation with other markers such as CRP (r=0.61, P=6.9×10^{-19}) similar to published studies (r=0.33–0.59) (17,25,37,38) and a moderate correlation between SC and FC (0.50, P=1.6×10^{-4}).

As a diagnostic blood-based marker, SC is the strongest predictor of IBD 9.37 (95% CI: 2.82–34.68). In a clinical setting, blood markers such as CRP are often available, therefore investigating the utility of a combined marker may be more relevant as this allows for greater specificity in diagnostics (39,40). We generated an IBD scoring system that would allow clinicians to predict IBD in patients at their index clinical visit. If 2 blood marker criteria are met (score of ≥8), there is a high likelihood of IBD (positive LR 24.14).

FC has a high NPV but a low PPV for IBD vs. functional disease (cutoff 50 µg/g, NPV 93%, PPV 37%) (41). In practice, a blood biomarker model can complement the existing FC screening of

patients with gastrointestinal symptoms in primary care. In the current climate of optimal tertiary care resource management, this model can be utilized for patients being referred for suspected IBD and help select and prioritize investigations for individuals with a high IBD score and a high likelihood of disease. The AUC for FC is superior to SC in our study (0.87 and 0.99, respectively, P=0.01). FC has an established role in IBD diagnostics; however, in clinical practice fecal sampling and testing can be challenging. One consideration in interpreting these data is the lag between SC and FC testing. The median time lag between SC and FC testing was 0 days (IQR −5 to 5 days), but there were individuals with upto 30 days between SC and FC testing. Nonetheless, any time lag represents real-life experience with fecal testing as often FC is not available until a few weeks after the clinic visit. There is a large variability in the concentration of FC in stool within a single day and storage conditions can impact on FC levels (18). Sampling feces can be a hurdle for patients and individuals can decline FC testing, fail to provide a sample, or provide insufficient sample for analysis. These factors impact on the practical utility of FC. SC testing has the potential to provide a more timely assessment of inflammation on the day of the visit. The cost per sample for performing SC testing are comparable to FC (£5; $7.3 equivalent). In addition, other costs related to sample handling and processing are likely to be lower as serum testing is often automated.

Beyond diagnostics, studies have investigated the utility of non-invasive markers in predicting endoscopic activity. A recent meta-analyses evaluated the diagnostic accuracy of CRP, FC, and stool lactoferrin for the assessment of endoscopically defined activity in IBD. The pooled AUC for CRP, FC, and stool lactoferrin were 0.49 (95% CI: 0.34–0.64), 0.88 (CI: 0.84–0.90), and 0.73 (CI: 0.66–0.79) (42). There was, however, heterogeneity in the endoscopic index used. Other factors such as inclusion criteria, in particular time lag between blood/fecal sampling and endoscopy (0–7 days), differed (43–46). There is a need for future prospective studies investigating the performance of non-invasive endoscopic activity markers, such as SC.

In our study, SC predicts treatment escalation and/or surgery in IBD (HR 2.7, 95% CI: 1.1–4.9), in particular CD (HR 4.2, 95% CI 1.2–15.3). We also generate blood-based prognostic models incorporating CRP, albumin, and SC. At 1 year, our model can predict treatment escalation in IBD in 65% of cases (95% CI: 43–79%) and 80% (95% CI: 31–94%) in CD if ≥2 criteria are met. Predicting the disease course early in individuals is becoming increasingly important in order to identify patients who would benefit from more aggressive therapy. In clinical practice, there is an unmet need for early indicators of persistent activity, either in a continuous or in a relapsing–remitting manner despite initial induction therapy (31). These patients will often go on to require further immunomodulators, biological therapies, and/or surgery. As quiescent IBD do not require such treatment escalations, we used the requirement of such treatment escalations to define an aggressive disease course (31). Clinical predictors have been studied previously. In CD, Beaugerie *et al.* (47) identified age, the presence of perianal disease, and requirement for steroids at diagnosis as independent predictive factors for a disabling course. However, biological markers were not analyzed in that study. Since then, the role of biomarkers in predicting the disease course has been the focus of many studies, although their effectiveness in predicting outcomes vary (31,48–51). Most studies suggest that CRP predicts relapse in IBD (48–50), although one study found that it had no predictive value (52). There are several reasons for this observed variation and includes differences in defining an aggressive disease course, disease heterogeneity, and disease duration prior to analyses. It is also possible that variations in CRP genotype may explain variations in its performance in adult cohort studies. This has been described in the pediatric population (53), but yet to be explored in adults. The role of FC in predicting colectomy in acute severe colitis has been investigated previously (AUC 0.65, P=0.04), and more recently, SC has been shown to predict colectomy in acute severe colitis with an AUC of 0.69 (95% CI 0.53–0.81) compared with FC (AUC 0.58; 95% CI 0.35–0.81) and CRP (AUC 0.71; 95% CI: 0.56–0.86) (16,38). SC has also been studied as a prognostic marker in predicting relapse after anti-TNF withdrawal and complements FC (>250 μg/g) and high-sensitivity CRP (>5 mg/l) (P=0.0173, 0.0024, and 0.0002; HR: 3.191, 3.561, and 4.120, respectively) (17). Our study, however, is the first to explore the prognostic utility of SC at diagnosis. Future studies incorporating periodic SC testing to predict disease course in IBD may be useful.

Our study does have certain limitations. The results are from a single tertiary center and based on a select cohort of newly diagnosed IBD patients. The relatively small numbers within the subtype of IBD limits the power to dissect factors predicting phenotype and our diagnostic and prognostic models require further validation. There were more females with functional bowel disease in the control cohort and this alone may underly the observation that male gender is a risk factor for IBD in our study. The major strengths of this study include a prospective design and a cohort of newly diagnosed IBD aiming for the first time to explore the correlation of SC with current biomarkers and build diagnostic and prognostic models for potential clinical use in IBD.

SC shows promise as a blood-based biomarker in diagnosing and predicting disease course in IBD. A diagnostic and prognostic model that combines SC and other blood-based biomarkers accurately predicts the inflammatory burden in IBD and has the potential to predict disease and its outcomes. Our findings warrant further exploration and validation within large multicenter cohorts.

CONFLICT OF INTEREST

Guarantor of the article: J. Satsangi, DPhil, FRCP.

Specific author contributions: Study design: R.K. and J.S. Patient recruitment and sample processing: N.T.V., R.K., N.A.K., and R.K.B. Experimental work: R.K. and M.R.V. Data analysis: R.K., N.A.K., A.T.A., and N.T.V. R.K. wrote the manuscript. All authors were involved in critical review, editing, revision, and approval of the final manuscript.

Financial support: The study was supported by Calpro AS, Norway who provided the ELISA kits for serum calprotectin testing.

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Serum calprotectin (SC) has been studied as a prognostic marker in predicting relapse after anti-TNF withdrawal and complements fecal calprotectin (FC) and high-sensitivity C-reactive protein (CRP) for the prediction of relapse.
- ✓ SC can predict colectomy in acute severe colitis with an AUC of 0.69, comparable to CRP.
- ✓ SC correlates with other inflammatory blood markers, such as CRP.
- ✓ SC has been studied in diseases such as inflammatory arthropathies and cystic fibrosis and can predict disease progression.

WHAT IS NEW HERE

- ✓ SC is a strong individual predictor of a diagnosis of IBD.
- ✓ SC correlates with FC and is useful in diagnosis.
- ✓ SC can predict treatment escalation and/or surgery in inflammatory bowel diseases (IBD), in particular CD.
- ✓ Blood-based diagnostic and prognostic models can provide an accurate reflection of the inflammatory burden and have the potential to predict disease and its outcomes.

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