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Serum Calprotectin - A novel diagnostic and prognostic marker in Inflammatory Bowel Diseases

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- 23 ulcerative colitis; biomarkers; diagnosis; management
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25 Abstract

26 Introduction

27 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

29 investigate the diagnostic and prognostic utility of serum calprotectin (SC) in IBD.

30 Methods

A total of 171 patients (n=96 IBD, n=75 non-IBD) were prospectively recruited. A multi-

32 biomarker model was derived using multivariable logistic regression analysis. Cox

33 proportional hazards model was derived to assess the contribution of each variable to disease

34 outcomes.

35 **Results**

- 36 SC correlated strongly with current biomarkers including faecal calprotectin (FC) (n=50, rho
- 37 = 0.50, p=1.6x10⁻⁴). SC was the strongest individual predictor of IBD diagnosis (odds ratio
- 38 (OR): 9.37(95%CI: 2.82-34.68), p= 4.00×10^{-4}) compared with other markers (CRP: OR
- 39 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
- 40 a subset of 50 patients with paired SC and FC, the area under receiver operating characteristic
- 41 discriminating IBD from controls was better for FC than SC (0.99, (95% CI 0.87-1.00) and
- 42 0.87 (95% CI:0.78-0.97) respectively; p=0.01).
- 43 At follow up (median 342 days; IQR: 88-563), SC predicted treatment escalation and/or
- 44 surgery in IBD (HR 2.7, 95% CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3).

45	A model incorporating SC and either CRP or albumin has a positive likelihood ratio of 24.14
46	for IBD. At 1 year, our prognostic model can predict treatment escalation in IBD in 65% of
47	cases (95% CI: 43-79%) and 80% (95% CI: 31-94%) in CD if 2 or more blood marker
48	criteria are met.
49	Conclusions
50	A diagnostic and prognostic model that combines SC and other blood-based biomarkers
51	accurately predicts the inflammatory burden in IBD and has the potential to predict disease
52	and its outcomes. Our data warrants further detailed exploration and validation in large multi-
53	centre cohorts.
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71 Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis 72 (UC), are chronic, debilitating inflammatory disorders of the gastrointestinal tract affecting 73 74 adults and children (1,2). A recent systematic review showed rising trends in the incidence 75 and prevalence of IBD worldwide(3) associated with significant healthcare costs amounting 76 to around £470 million in the UK, up to \notin 5.6 billion annually in Europe and over \$6 billion 77 annually in the USA (3-8). With an ever expanding therapeutic repertoire, it is important to 78 select patients who may benefit from early use of immunosuppressants' and/or biological 79 therapies in order to minimise irreversible luminal damage and prevent long-term complications. 80 Blood-based biomarkers provide a non-invasive estimation of the inflammatory burden in 81 82 newly diagnosed IBD. However relatively few blood-based biomarkers have been 83 extensively validated in IBD, and fewer still are in routine use in the clinic (9). There is an 84 emerging interest in discovering novel markers using multi-omic platforms that may be 85 valuable in a variety of clinical settings including IBD diagnostics, disease activity assessments, predicting disease outcomes and response to therapy (9–11). 86 The S100 family of proteins including S100A8/A9 (calprotectin) and S100A12 (calgranulin) 87 have been implicated in disease pathogenesis and investigated as potential markers of 88 89 inflammation (12, 13). In IBD, faecal calprotectin (FC) has emerged as a particularly 90 informative tool(14). A recent meta-analysis of 13 studies and 1041 patients found that FC 91 had a pooled sensitivity and specificity of 0.93 (0.85-0.97) and 0.96 (0.79-0.99) respectively 92 for IBD and identified those individuals requiring endoscopy for suspected IBD(13). There 93 are also data on the role of FC in other clinical settings, such as predicting post-operative CD 94 recurrence and predicting outcomes in acute severe colitis(15,16). However, there are 95 limitations to FC testing in clinical practice. Faecal collection can be a hurdle for patients(17)

96	and sample delivery and processing delays can hinder its clinical utility. In active UC, FC
97	shows high within-day variability and the optimal timing for sampling is not clear(18,19). A
98	blood based biomarker such as serum calprotectin (SC) may be more convenient in routine
99	practice and more acceptable to patients. SC has been studied in diseases such as
100	inflammatory arthropathies and cystic fibrosis (20-24). In Rheumatoid arthritis, SC was
101	independently predictive of a 10 year radiographic disease progression (25), while in cystic
102	fibrosis, SC predicted exacerbation and lung function decline(20,21).
103	More recently, SC has been investigated in IBD to predict response to- and relapse following
104	anti-tumour necrosis factor (anti-TNF) therapy(17,26). In CD patients, SC has a similar
105	profile to high sensitivity C-reactive protein (hsCRP) and compliments FC and hsCRP for
106	prediction of relapse after anti-TNF withdrawal (p=0.0173, 0.0024 and 0.0002; HR: 3.191,
107	3.561 and 4.120 respectively) (17). In murine models, TNBS induced colitis is associated
108	with higher SC levels that correlate closely to macroscopic and microscopic disease
109	scores(27). We are yet to understand fully the relationship between SC and the other
110	currently available biomarkers in IBD and the diagnostic and prognostic value of SC in IBD.
111	Our study aims to investigate the role of SC in this clinical setting.

112 **Methods**

113 Study Design

114 A prospective, single centre case control study was performed in patients with suspected or

- 115 confirmed IBD at their first presentation to a tertiary gastrointestinal clinic. Data were
- 116 collected for patient demographics including age, sex, age at diagnosis and date of diagnosis
- 117 (Table 1). Details of drug therapy and concomitant medications were recorded. Laboratory
- 118 markers including C-reactive protein (CRP) and albumin were measured as part of the
- research protocol while other routine markers including haemoglobin, white cell count,
- 120 platelets and faecal calprotectin were recorded within 30 days from recruitment.

121 Inclusion criteria

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

127 Sample collection and processing

- 128 For SC analysis, blood samples were collected prospectively and serum was processed within
- 129 2 hours of sampling (using centrifugation at 2500G for 15 min) and subsequently stored at
- -80° C until further use. Samples were analysed in duplicate using the CalproTM AS
- 131 calprotectin ELISA (Calpro AS, Norway) according to manufacturer's instructions. Samples
- 132 with a calprotectin result of >2500 ng/ml were diluted and retested. Coefficients of variation
- 133 of <10% were included in the analysis.

134 Ethics Statement

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

137 Clinical Course in IBD

- 138 Case note review was performed for all IBD cases. Treatment escalation was defined as the
- need for escalation and establishment of 2 or more 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
- 142 a new diagnosis, requiring emergency colectomy during their index admission.

143 Statistical analysis

- 144 Data were analysed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) and R
- 145 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Continuous data are

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146	presented as medians and interquartile ranges and were analysed using a Mann-Whitney U-
147	test. Categorical data are presented as numbers and percentages, and were analysed using
148	Fisher's exact tests. Spearman's rank-order correlation test was applied for correlations
149	between variables. To determine the accuracy of blood parameter measurements as a
150	prognostic test capable of diagnosing IBD, receiver operating characteristic (ROC) analyses
151	were performed by plotting sensitivity against specificity using the 'pROC' package in R(32).
152 153	<i>Building diagnostic and prognostic models</i> After univariable analyses, the most significant laboratory parameters (CRP, albumin and
154	serum calprotectin) were included in multivariable models for IBD diagnosis and prognosis.
155	CRP and serum calprotectin were log transformed to more closely approximate a normal
156	distribution for further multivariable analysis. ROC analyses were used to define the optimal
157	cut-points (highest sum of sensitivity + specificity) for both models. The optimal models
158	were then selected by performing backward stepwise regression using the smallest Akaike
159	information criterion (AIC) values and adjusted for treatment exposure.
160	For the diagnostic model, an integer score was attributed to each variable according to its
161	relative contribution in the model (as determined by the coefficients) and positive and
162	negative predictive values were then calculated for each total score.
163	For the prognostic model, a Cox proportional hazards model was derived to assess the
164	contribution of each variable to disease outcomes. Thresholds were then identified using
165	ROC analyses to allow stratification of patients to either a benign or an aggressive disease
166	course (requiring treatment escalation and/or surgery), and to allow creation of survival
167	curves.

168 **Results**

- 169 Comparison of Serum Calprotectin with conventional biomarkers
- 170 Overall, serum calprotectin was analysed in 171 patient serum samples from August 2013 to
- April 2015. Serum calprotectin correlated positively with CRP (rho=0.61, p= 6.9×10^{-19}) and
- negatively with albumin (rho= -0.54, p= 3.3×10^{-14}). Paired full blood count was available
- 173 within 30 days (median 0 days; range: -26 to 16 days) of recruitment in 147 patients. Serum
- 174 calprotectin correlated positively with Neutrophil count (rho=0.65, p= 1.9×10^{-20}) and
- negatively with Haemoglobin (rho= 0.42, p=6.1 x10⁻⁸). 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).
- 177 Serum calprotectin correlated significantly with faecal calprotectin (Spearman's rho = 0.50,
- 178 $p=1.6x10^{-4}$). Table 1 summarises the correlation of blood and faecal parameters. SC
- demonstrated a stronger correlation with white cell count (William's test, p=0.02) and
- neutrophils (p=0.03) in controls compared to IBD cases.

181 Diagnostic utility of Serum Calprotectin in IBD

In a subset of 156 patients (83 IBD and 73 non-IBD), blood sampling was performed within

- 183 90 days from diagnosis (median 0 days; IQR 0-6). There were 35 patients with a diagnosis of
- 184 CD, 45 patients with UC and 3 patients with IBDU in the IBD group. In CD, 44% had L3 +/-
- L4 disease and 62% had B1 behaviour according to the Montreal classification (33). In UC,
- 186 33% had pancolitis (E4) and 11% had limited proctitis (E1) as per the Paris classification
- 187 (30). Table 2 and Table 3 summarise the demographics and blood and faecal parameters for
- the IBD and control cohorts. SC was significantly increased in IBD compared with controls
- 189 (1010 ng/ml [IQR 796-1426ng/ml] vs 506ng/ml [IQR 362-725ng/ml], p=3.7 x 10⁻¹⁵) (Figure
- 190 1). CRP and albumin were also significantly different between IBD and controls (CRP
- 191 $p=8.9 \times 10^{-15}$; albumin $p=4.9 \times 10^{-14}$). There was no difference in SC between CD and UC
- 192 (1015 ng/ml [IQR 740-1518 ng/ml] vs 911µg/g [IQR 809-1413ng/ml], p=0.79) and within
- controls (HC: 432 ng/ml [IQR 359-586] vs symptomatic controls: 563ng/ml [IQR 382-787];

- 194 p=0.12). SC was not significantly associated with sex (p=0.14), age (rho -0.06, p=0.43) or
- smoking status (p=0.49). Serum calprotectin and CRP were able to discriminate IBD from
- 196 controls with similar areas under the receiver operator characteristics curve (AUROC) of 0.87
- 197 (95% confidence interval [CI] 0.82-0.93) and 0.86 (95% CI 0.80-0.91) respectively (Figure 2)
- 198 (p=0.64 DeLong's test for comparison of ROC curves). In those with paired SC and FC
- 199 within 30 days, the AUROC for discriminating cases and controls was superior for FC (0.99,
- 200 95% CI 0.98-1.00) than SC (0.87, 95% CI 0.78-0.97) (p=0.01 De Long's test), as shown in
- 201 Figure 2.

202 Multivariable analysis

- 203 Multivariable logistic regression analysis of predictors of IBD was performed on 155 cases
- 204 (83 IBD, 72 non-IBD) where the data for the predictors were complete. Albumin, male
- 205 gender, log transformed CRP and log transformed SC were significant predictors of IBD.
- **Table 4** summarises the statistical significance of each covariate.

207 Building an IBD Diagnosis Score

- 208 Using the multivariable model, continuous variables were categorised using integer cut-
- 209 points guided by the ROC curves and observed relationship with diagnosis. The final scoring
- system for the diagnosis of IBD included SC>852ng/ml, Albumin<38g/L, CRP≥3.5mg/L and
- 211 male gender. To formulate a numerical risk score, each variable was given a score based on
- the odds ratio generated from the linear model. **Table 5** summarises the positive and negative
- 213 predictive values for each score. Using this model, a SC> 852ng/ml and either a
- 214 CRP≥3.5mg/L or albumin <38g/L has a sensitivity of 67%, specificity of 97% and a positive
- 215 likelihood ratio (LR) of 24.14 for IBD.

216 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

219	with disease beyond the rectum (> $E1$) compared to proctitis alone ($E1$) (median SC
220	1078ng/ml IQR 820-1418 vs 812ng/ml IQR 698-821, p=0.03). Albumin also predicted
221	disease extent in UC (p=0.01) but not CRP (p=0.05). In CD however, there was no significant
222	difference in SC, CRP or albumin by disease location (p=0.47, 0.55 and 0.20 respectively).
223 224	Predicting Disease outcomes in IBD Kaplan-Meier analyses were performed on a total of 83 patients with IBD. There were 35
225	patients with a diagnosis of CD, 45 patients with UC and 3 patients with IBDU. The median
226	age was 31 years (IQR: 26-41) and 69% were male (n=58). A total of 1(33%), 16 (46%),
227	23(51%) patients required treatment escalation in the IBDU, CD and UC group respectively.
228	Using backwards stepwise selection, albumin<37 g/L and SC \geq 1046 ng/ml remained
229	significant predictors of treatment escalation in IBD (logrank test $p=5.1 \times 10^{-5}$). Both
230	biomarkers had similar hazards ratio (HR) as shown in Table 6a. A score was generated using
231	both biomarkers at these thresholds. At a year, the estimated chance of treatment escalation
232	was 21% (95% CI: 1-37%) if none of the criteria were met, 40% (95% CI: 17-56%) for
233	patients meeting one criterion and 65% (95% CI: 43-78%) for those meeting both criteria
234	(Figure 3a).
235	In order to assess whether the time lag between diagnosis and blood sampling had an impact
236	on the final model, stepwise regression analyses was performed for samples within 60 days
237	(n=74) and 30 days (n=60) from diagnosis. SC remained a significant predictor of disease
238	outcomes at 60 days and 30 days (p=0.003 and p=0.004 respectively).
239	In 28 patients, paired FC was available within 30 days from diagnosis. Using a multivariate
240	model which included age, gender, CRP, albumin, FC and SC, backward stepwise regression
241	analysis was performed and only SC remained as a significant predictor (p=0.0004). FC did

243	Further regression analyses were performed within the subgroups UC (Table 6b) and CD
244	(Table 6c). In CD, CRP>24mg/L and SC>991ng/ml and albumin<26g/L predicted treatment
245	escalation (logrank test p=0.003). At 1 year, the estimated chance of treatment escalation was
246	11% (95%CI: 0-29%) for patients meeting none of the criteria, 30% (95% CI: 0-51%) for
247	patients meeting one criterion and 80% (95% CI: 31-94%) for patients meeting two or more
248	criteria (Figure 3b).
249	In UC, albumin< 37g/L and CRP>2.5g/L predicted a more aggressive disease course
250	(logrank test p=0.001). At 1 year, the estimated chance of treatment escalation was 0 for
251	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).

253 **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 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
- 260 IBD (HR 2.7, 95%CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3).
- 261 Calprotectin, a member of the S100 proteins, represents 45% of all cytosolic proteins in
- neutrophils compared to 1% in monocytes (34,35). Given the short half-life of SC (5 hours),
- it may provide a more dynamic test of the current inflammatory status compared with
- conventional inflammatory markers (half-life of CRP 18 hours, albumin 19 days) (36). SC
- correlates better with neutrophil count in controls compared to IBD patients; in IBD, SC
- levels may reflect calprotectin release from activated neutrophils and other immune cells such

267	as monocytes, macrophages and epithelial cells (Figure 4). SC shows a strong correlation
268	with other markers such as CRP (r= 0.61 , p= 6.9×10^{-19}) similar to published studies (r= 0.33 -
269	(0.59)(17,25,37,38) and a moderate correlation between SC and FC (0.50, p=1.6x10 ⁻⁴).
270	As a diagnostic blood based marker, SC is the strongest predictor of IBD 9.37 (95%CI: 2.82-
271	34.68). In a clinical setting, blood markers such as CRP are often available, therefore
272	investigating the utility of a combined marker may be more relevant as this allows for greater
273	specificity in diagnostics (39,40). We generated an IBD scoring system that would allow
274	clinicians to predict IBD in patients at their index clinical visit. If 2 blood marker criteria are
275	met (score of 8 or above), there is a high likelihood of IBD (positive LR 24.14).
276	FC has a high NPV but a low PPV for IBD vs functional disease (cut off 50 μ g/g, NPV 93%
277	PPV 37%)(41). In practice, a blood biomarker model can complement the existing FC
278	screening of patients with gastrointestinal symptoms in primary care. In the current climate of
279	optimal tertiary care resource management, this model can be utilised for patients being
280	referred for suspected IBD and help select and prioritise investigations for individuals with a
281	high IBD score and a high likelihood of disease. The AUC for FC is superior to SC in our
282	study (0.87 and 0.99 respectively, p=0.01). FC has an established role in IBD diagnostics,
283	however in clinical practice faecal sampling and testing can be challenging. One
284	consideration in interpreting these data is the lag between SC and FC testing. The median
285	time lag between SC and FC testing was 0 days (IQR -5 to 5 days), but there were individuals
286	with upto 30 days between SC and FC testing. Nonetheless, any time lag represents real life
287	experience with faecal testing as often FC is not available until a few weeks after the clinic
288	visit. There is a large variability in the concentration of FC in stool within a single day and
289	storage conditions can impact on FC levels(18). Sampling faeces can be a hurdle for patients
290	and individuals can either decline FC testing, fail to provide a sample or provide insufficient
291	sample for analysis. These factors impact on the practical utility of FC. SC testing has the

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292	potential to provide a more timely assessment of inflammation on the day of the visit. The
293	cost per sample for performing SC testing are comparable to FC (\pounds 5; \$7.3 equivalent). In
294	addition, other costs related to sample handling and processing are likely to be lower as
295	serum testing is often automated.
296	Beyond diagnostics, studies have investigated the utility of non-invasive markers in
297	predicting endoscopic activity. A recent meta-analyses evaluated the diagnostic accuracy of
298	CRP, FC and stool lactoferrin (SL) for the assessment of endoscopically defined activity in
299	IBD. The pooled AUC for CRP, FC and SL were 0.49(95% CI: 0.34-0.64), 0.88(CI: 0.84-
300	0.90) and 0.73(CI: 0.66-0.79)(42). There was however heterogeneity in the endoscopic index
301	used. Other factors such as inclusion criteria, in particular time lag between blood/faecal
302	sampling and endoscopy (0-7 days) differed(43–46). There is a need for future prospective
303	studies investigating the performance of non-invasive endoscopic activity markers such as
304	SC.
305	In our study, SC predicts treatment escalation and/or surgery in IBD (HR 2.7, 95%CI: 1.1-
306	4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3). We also generate blood-based prognostic
307	models incorporating CRP, albumin and SC. At 1 year, our model can predict treatment
308	escalation in IBD in 65% of cases (95% CI: 43-79%) and 80% (95% CI: 31-94%) in CD if 2
309	or more criteria are met. Predicting the disease course early in individuals is becoming
310	increasingly important in order to identify patients who would benefit from more aggressive
311	therapy. In clinical practice, there is an unmet need for early indicators of persistent activity,
312	either in a continuous or a relapsing-remitting manner despite initial induction therapy(31).
313	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* identified age, the

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317	presence of perianal disease and requirement for steroids at diagnosis as independent
318	predictive factors for a disabling course(47). However, biological markers were not analysed
319	in that study. Since then, the role of biomarkers in predicting the disease course has been the
320	focus of many studies, although their effectiveness in predicting outcomes vary(31,48–51).
321	Most studies suggest that CRP predicts relapse in IBD(48-50), although one study found it
322	had no predictive value (52). There are several reasons for this observed variation and
323	includes differences in defining an aggressive disease course, disease heterogeneity and
324	disease duration prior to analyses. It is also possible that variations in CRP genotype may
325	explain variations in its performance in adult cohort studies. This has been described in the
326	paediatric population(53), but yet to be explored in adults. The role of FC in predicting
327	colectomy in acute severe colitis (ASUC) has been investigated previously (AUC 0.65,
328	p=0.04) and more recently, SC has been shown to predict colectomy in ASUC with an AUC
329	of 0.69 (95%CI 0.53-0.81) compared to FC (AUC 0.58; 95%CI 0.35-0.81) and CRP (AUC
330	0.71; 95%CI: 0.56-0.86) (16,38). SC has also been studied as a prognostic marker in
331	predicting relapse after anti-TNF withdrawal and complements FC (>250 μ g/g) and
332	hsCRP(>5mg/L) (p=0.0173, 0.0024 and 0.0002; HR: 3.191, 3.561 and 4.120 respectively)
333	(17). Our study however is the first to explore the prognostic utility of SC at diagnosis. Future
334	studies incorporating periodic SC testing to predict disease course in IBD may be useful.
335	Our study does have certain limitations. The results are from a single tertiary centre and
336	based on a select cohort of newly diagnosed IBD patients. The relatively small numbers
337	within the sub-type of IBD limits the power to dissect factors predicting phenotype and our
338	diagnostic and prognostic models require further validation. There were more females with
339	functional bowel disease in the control cohort and this alone may underly the observation that
340	male gender is a risk factor for IBD in our study. The major strengths of this study include a
341	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 342
- potential clinical use in IBD. 343

Conclusion 344

- 345 SC shows promise as a blood based biomarker in diagnosing and predicting disease course in
- 346 IBD. A diagnostic and prognostic model that combines SC and other blood-based biomarkers
- 347 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 348
- multicentre cohorts. 349

349	multicentre cohorts.
350	
351	 What is Current Knowledge Serum calprotectin (SC) has been studied as a prognostic marker in predicting
352	relapse after anti-TNF withdrawal and complements FC and hsCRP for the
353	prediction of relapse.SC can predict colectomy in acute severe colitis with an AUC of 0.69,
354	comparable to CRP.
355	• SC correlates with other inflammatory blood markers such as CRP
356	• SC has been studied in diseases such as inflammatory arthropathies and cystic
357	fibrosis and can predict disease progression.
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366	What is New Here
367	• Serum Calprotectin (SC) is a strong individual predictor of a diagnosis of IBD
368	• SC correlates with faecal calprotectin (FC) and is useful in diagnosis
369	• SC can predict treatment escalation and/or surgery in IBD, in particular CD
370	• Blood based diagnostic and prognostic models can provide an accurate reflection
371	of the inflammatory burden and have the potential to predict disease and its
372	outcomes.
373	
374	Competing interests
375	The study was supported by Calpro AS TM , Norway who provided the ELISA kits for serum
376	calprotectin testing.
377 378	Author contributions Study design RK and JS. Patient recruitment and sample processing NTV, RK, NAK, RKB.
379	Experimental work RK and MV. Data Analysis RK, NAK, ATA and NTV. RK wrote the
380	manuscript. All authors were involved in critical review, editing, revision and approval of the
381	final manuscript.
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529	Figure Legends
530	Figure 1: Serum Calprotectin levels in patients with Crohn's disease (CD), Ulcerative colitis
531	(UC), Inflammatory bowel disease unclassified (IBDU), symptomatic controls (non-IBD) and
532	healthy controls (HC)
533 534	Footnote : Boxplots represent median and inter-quartile ranges for serum calprotectin within each subcohort
535	Figure 2: Receiver operating curve analysis (ROC) of serum calprotectin (SC) and other
536	blood based markers in differentiating Inflammatory bowel diseases (IBD) from non-IBD and
537	ROC analysis of SC and faecal calprotectin (FC) (within 30 days) in discriminating
538	Inflammatory bowel diseases (IBD) from non-IBD.
539	Footnote: WCC:white cell count; CRP:C-reactive protein; Hb:Haemoglobin
540	Figure 3a: Kaplan Meier survival curves of disease course using blood biomarkers to predict
541	outcomes in newly diagnosed Inflammatory Bowel Diseases (IBD). Single marker represents
542	either or albumin<37 g/L or serum calprotectin \geq 1046ng/ml. Dual markers represents a
543	combination of both variables.
544	Figure 3b: Kaplan Meier survival curves of disease course using blood biomarkers to predict
545	outcomes in newly diagnosed Crohn's disease (CD)
546 547 548	Footnote : '1 marker' represents either CRP>24mg/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.

- 549 Figure 3c: Kaplan Meier survival curves of disease course using blood biomarkers to predict
- outcomes in newly diagnosed Ulcerative Colitis (UC) 550
- 551 Footnote: Single marker represents either albumin<37 g/L or CRP >2.5mg/L. Dual markers
- 552 represents all the categorical variables as a combined biomarker.
- Figure 4: Correlation between log transformed serum calprotectin and neutrophil count in 553
- Inflammatory Bowel Diseases (IBD) versus controls (Non-IBD). 554

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- 2 Inflammatory Bowel Diseases
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- 22 <u>Keywords:</u> Crohn's disease; inflammatory bowel diseases; Ulcerative colitis; acute severe
- 23 ulcerative colitis; biomarkers; diagnosis; management
- 24 Word count: 3473

25 Abstract

26 Introduction

- 27 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
- 29 investigate the diagnostic and prognostic utility of serum calprotectin (SC) in IBD.

30 Methods

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

35 **Results**

- 36 <u>SC correlated strongly with current biomarkers including faecal calprotectin (FC)</u>
- 37 (n=50, rho = 0.50, p=1.6x10⁻⁴). SC was the strongest individual predictor of IBD
- diagnosis (odds ratio (OR): 9.37(2.82-34.68), p=4.00×10⁻⁴) compared with other markers
- 39 (CRP: OR 8.52(95%CI: 2.75-28.63), p=2.80×10⁻⁴); albumin: OR 6.12(95%CI: 1.82-
- 40 <u>22.16</u>), p=0.004). In a subset of 50 patients with paired SC and FC, the area under
- 41 receiver operating characteristic discriminating IBD from controls was better for FC
- 42 than SC (0.99, (95% CI 0.87-1.00) and 0.87 (95% CI: 0.78-0.97) respectively; p=0.01).
- 43 At follow up (median 342 days; IQR: 88-563), <u>SC predicted treatment escalation and/or</u>
- 44 surgery in IBD (HR 2.7, 95% CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3).

45	A model incorporating SC and either CRP or albumin has a positive likelihood ratio of
46	24.14 for IBD. At 1 year, our prognostic model can predict treatment escalation in IBD in
47	65% of cases (95% CI: 43-79%) and 80% (95% CI: 31-94%) in CD if 2 or more blood
48	marker criteria are met.
49	Conclusions
50	A diagnostic and prognostic model that combines SC and other blood-based biomarkers
51	accurately predicts the inflammatory burden in IBD and has the potential to predict
52	disease and its outcomes. Our data warrants further detailed exploration and validation in
53	large multi-centre cohorts.
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71 Introduction

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis 72 (UC), are chronic, debilitating inflammatory disorders of the gastrointestinal tract affecting 73 74 adults and children (1,2). A recent systematic review showed rising trends in the incidence 75 and prevalence of IBD worldwide(3) associated with significant healthcare costs amounting 76 to around £470 million in the UK, up to \notin 5.6 billion annually in Europe and over \$6 billion 77 annually in the USA (3-8). With an ever expanding therapeutic repertoire, it is important to 78 select patients who may benefit from early use of immunosuppressants' and/or biological 79 therapies in order to minimise irreversible luminal damage and prevent long-term complications. 80 Blood-based biomarkers provide a non-invasive estimation of the inflammatory burden in 81 82 newly diagnosed IBD. However relatively few blood-based biomarkers have been 83 extensively validated in IBD, and fewer still are in routine use in the clinic (9). There is an 84 emerging interest in discovering novel markers using multi-omic platforms that may be 85 valuable in a variety of clinical settings including IBD diagnostics, disease activity assessments, predicting disease outcomes and response to therapy (9–11). 86 The S100 family of proteins including S100A8/A9 (calprotectin) and S100A12 (calgranulin) 87 have been implicated in disease pathogenesis and investigated as potential markers of 88 89 inflammation (12, 13). In IBD, faecal calprotectin (FC) has emerged as a particularly 90 informative tool(14). A recent meta-analysis of 13 studies and 1041 patients found that FC 91 had a pooled sensitivity and specificity of 0.93 (0.85-0.97) and 0.96 (0.79-0.99) respectively 92 for IBD and identified those individuals requiring endoscopy for suspected IBD(13). There 93 are also data on the role of FC in other clinical settings, such as predicting post-operative CD recurrence and predicting outcomes in acute severe colitis(15,16). However, there are 94 95 limitations to FC testing in clinical practice. Faecal collection can be a hurdle for patients(17)

96	and sample delivery and processing delays can hinder its clinical utility. In active UC, FC
97	shows high within-day variability and the optimal timing for sampling is not clear(18,19). A
98	blood based biomarker such as serum calprotectin (SC) may be more convenient in routine
99	practice and more acceptable to patients. SC has been studied in diseases such as
100	inflammatory arthropathies and cystic fibrosis (20-24). In Rheumatoid arthritis, SC was
101	independently predictive of a 10 year radiographic disease progression (25), while in cystic
102	fibrosis, SC predicted exacerbation and lung function decline(20,21).
103	More recently, SC has been investigated in IBD to predict response to- and relapse following
104	anti-tumour necrosis factor (anti-TNF) therapy(17,26). In CD patients, SC has a similar
105	profile to high sensitivity C-reactive protein (hsCRP) and compliments FC and hsCRP for
106	prediction of relapse after anti-TNF withdrawal (p=0.0173, 0.0024 and 0.0002; HR: 3.191,
107	3.561 and 4.120 respectively) (17). In murine models, TNBS induced colitis is associated
108	with higher SC levels that correlate closely to macroscopic and microscopic disease
109	scores(27). We are yet to understand fully the relationship between SC and the other
110	currently available biomarkers in IBD and the diagnostic and prognostic value of SC in IBD.
111	Our study aims to investigate the role of SC in this clinical setting.

112 **Methods**

113 Study Design

- 114 A prospective, single centre case control study was performed in patients with suspected or
- 115 confirmed IBD at their first presentation to a tertiary gastrointestinal clinic. Data were
- 116 collected for patient demographics including age, sex, age at diagnosis and date of diagnosis
- 117 (Table 1). Details of drug therapy and concomitant medications were recorded. Laboratory

118 markers including C-reactive protein (CRP) and albumin were measured as part of the

119 research protocol while other routine markers including haemoglobin, white cell count,

120 platelets and faecal calprotectin were recorded within 30 days from recruitment.

121 Inclusion criteria

- 122 Patients with a new diagnosis of IBD were included in the study. The Lennard-Jones,
- 123 Montreal and Paris criteria were used for diagnosis and classification of clinical
- 124 phenotypes(28–30). The control cohort consisted of healthy lab volunteers (HC) and
- 125 patients with gastrointestinal symptoms (symptomatic controls) who had no discernible
- 126 inflammatory disease, and a diagnosis of functional bowel disease at follow up.
- 127 Sample collection and processing
- 128 For SC analysis, blood samples were collected prospectively and serum was processed within
- 129 2 hours of sampling (using centrifugation at 2500G for 15 min) and subsequently stored at
- -80° C until further use. Samples were analysed in duplicate using the CalproTM AS
- 131 calprotectin ELISA (Calpro AS, Norway) according to manufacturer's instructions. Samples
- 132 with a calprotectin result of >2500 ng/ml were diluted and retested. Coefficients of variation
- 133 of <10% were included in the analysis.

134 Ethics Statement

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

137 Clinical Course in IBD

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

143 Statistical analysis

- 144 Data were analysed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) and R
- 145 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Continuous data are

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146	presented as medians and interquartile ranges and were analysed using a Mann-Whitney U-
147	test. Categorical data are presented as numbers and percentages, and were analysed using
148	Fisher's exact tests. Spearman's rank-order correlation test was applied for correlations
149	between variables. To determine the accuracy of blood parameter measurements as a
150	prognostic test capable of diagnosing IBD, receiver operating characteristic (ROC) analyses
151	were performed by plotting sensitivity against specificity using the 'pROC' package in R(32).
152 153	Building diagnostic and prognostic models After univariable analyses, the most significant laboratory parameters (CRP, albumin and
154	serum calprotectin) were included in multivariable models for IBD diagnosis and prognosis.
155	CRP and serum calprotectin were log transformed to more closely approximate a normal
156	distribution for further multivariable analysis. ROC analyses were used to define the optimal
157	cut-points (highest sum of sensitivity + specificity) for both models. The optimal models
158	were then selected by performing backward stepwise regression using the smallest Akaike
159	information criterion (AIC) values and adjusted for treatment exposure.
160	For the diagnostic model, an integer score was attributed to each variable according to its
161	relative contribution in the model (as determined by the coefficients) and positive and
162	negative predictive values were then calculated for each total score.
163	For the prognostic model, a Cox proportional hazards model was derived to assess the
164	contribution of each variable to disease outcomes. Thresholds were then identified using
165	ROC analyses to allow stratification of patients to either a benign or an aggressive disease
166	course (requiring treatment escalation and/or surgery), and to allow creation of survival
167	curves.

168 **Results**

- 169 Comparison of Serum Calprotectin with conventional biomarkers
- 170 Overall, serum calprotectin was analysed in 171 patient serum samples from August 2013 to
- 171 April 2015. Serum calprotectin correlated positively with CRP (rho=0.61, p=6.9x10⁻¹⁹)
- 172 <u>and negatively with albumin (rho= 0.54, p=3.3x10⁻¹⁴). Paired full blood count was</u>
- 173 available within 30 days (median 0 days; range: -26 to 16 days) of recruitment in 147
- 174 patients. Serum calprotectin correlated positively with Neutrophil count (rho=0.65,
- 175 $p=1.9x10^{-20}$ and negatively with Haemoglobin (rho= 0.42, p=6.1 x10⁻⁸). Paired FC was
- 176 available within 30 days (median 0 days, IQR: -5 to 5 days) of SC in 50 patients (IBD
- 177 <u>n=31, non-IBD n=19). Serum calprotectin correlated significantly with faecal</u>
- 178 <u>calprotectin (Spearman's rho = 0.50, p= 1.6×10^{-4})</u>. Table 1 summarises the correlation of
- blood and faecal parameters. SC demonstrated a stronger correlation with white cell count
- (William's test, p=0.02) and neutrophils (p=0.03) in controls compared to IBD cases.
- 181 Diagnostic utility of Serum Calprotectin in IBD
- 182 In a subset of 156 patients (83 IBD and 73 non-IBD), blood sampling was performed
- 183 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 IBDU in the IBD group. In CD,
- 185 44% had L3 +/- L4 disease and 62% had B1 behaviour 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 summarise the demographics and blood and
- 188 faecal parameters for the IBD and control cohorts. SC was significantly increased in IBD
- 189 compared with controls (1010 ng/ml [IQR 796-1426ng/ml] vs 506ng/ml [IQR 362-
- 190 <u>725ng/mll, p=3.7 x 10⁻¹⁵</u> (Figure 1). CRP and albumin were also significantly different
- between IBD and controls (CRP $p=8.9x10^{-15}$; albumin $p=4.9x10^{-14}$). There was no
- 192 difference in SC between CD and UC (1015 ng/ml [IQR 740-1518 ng/ml] vs 911µg/g [IQR
- 193 <u>809-1413ng/ml</u>], p=0.79) and within controls (HC: 432 ng/ml [IQR 359-586] vs

194 **symptomatic controls: 563ng/ml [IQR 382-787]; p=0.12)**. SC was not significantly

- associated with sex (p=0.14), age (rho -0.06, p=0.43) or smoking status (p=0.49). Serum
- 196 calprotectin and CRP were able to discriminate IBD from controls with similar areas under

197 the receiver operator characteristics curve (AUROC) of 0.87 (95% confidence interval

- 198 [CI] 0.82-0.93) and 0.86 (95% CI 0.80-0.91) respectively (Figure 2) (p=0.64 DeLong's
- 199 test for comparison of ROC curves). In those with paired SC and FC within 30 days, the

200 AUROC for discriminating cases and controls was superior for FC (0.99, 95% CI 0.98-

201 <u>1.00) than SC (0.87, 95% CI 0.78-0.97) (p=0.01 De Long's test), as shown in Figure 2.</u>

202 Multivariable analysis

- 203 Multivariable logistic regression analysis of predictors of IBD was performed on 155 cases
- 204 (83 IBD, 72 non-IBD) where the data for the predictors were complete. Albumin, male
- 205 gender, log transformed CRP and log transformed SC were significant predictors of IBD.
- **Table 4** summarises the statistical significance of each covariate.

207 Building an IBD Diagnosis Score

- 208 Using the multivariable model, continuous variables were categorised using integer cut-
- 209 points guided by the ROC curves and observed relationship with diagnosis. The final scoring
- system for the diagnosis of IBD included SC>852ng/ml, Albumin<38g/L, CRP≥3.5mg/L and
- 211 male gender. To formulate a numerical risk score, each variable was given a score based on
- the odds ratio generated from the linear model. **Table 5** summarises the positive and negative
- 213 predictive values for each score. Using this model, a SC> 852ng/ml and either a

214 <u>CRP≥3.5mg/L or albumin <38g/L has a sensitivity of 67%, specificity of 97% and a</u>

215 **positive likelihood ratio (LR) of 24.14 for IBD.**

216 **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

219	with disease beyond the rectum (> E1) compared to proctitis alone (E1) (median SC
220	1078ng/ml IQR 820-1418 vs 812ng/ml IQR 698-821, p=0.03). Albumin also predicted
221	disease extent in UC (p=0.01) but not CRP (p=0.05). In CD however, there was no significant
222	difference in SC, CRP or albumin by disease location (p=0.47, 0.55 and 0.20 respectively).
223 224	Predicting Disease outcomes in IBD Kaplan-Meier analyses were performed on a total of 83 patients with IBD. There were 35
225	patients with a diagnosis of CD, 45 patients with UC and 3 patients with IBDU. The median
226	age was 31 years (IQR: 26-41) and 69% were male (n=58). A total of 1(33%), 16 (46%),
227	23(51%) patients required treatment escalation in the IBDU, CD and UC group respectively.
228	<u>Using backwards stepwise selection, albumin<37 g/L and SC ≥1046 ng/ml remained</u>
229	significant predictors of treatment escalation in IBD (logrank test p=5.1×10 ⁻⁵). Both
230	biomarkers had similar hazards ratio (HR) as shown in Table 6a. A score was generated
231	using both biomarkers at these thresholds. At a year, the estimated chance of treatment
232	escalation was 21% (95% CI: 1-37%) if none of the criteria were met, 40% (95% CI:
233	17-56%) for patients meeting one criterion and 65% (95% CI: 43-78%) for those
234	meeting both criteria (Figure 3a).
235	In order to assess whether the time lag between diagnosis and blood sampling had an impact
236	on the final model, stepwise regression analyses was performed for samples within 60 days
237	(n=74) and 30 days (n=60) from diagnosis. SC remained a significant predictor of disease
238	outcomes at 60 days and 30 days (p=0.003 and p=0.004 respectively).
239	In 28 patients, paired FC was available within 30 days from diagnosis. Using a multivariate
240	model which included age, gender, CRP, albumin, FC and SC, backward stepwise regression
241	analysis 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).
243	Further regression ana	lyses were per	rformed within the	e subgroups UC	(Table 6b) and CD
					· · · · · · · · · · · · · · · · · · ·

- 244 (Table 6c). In CD, CRP>24mg/L and SC>991ng/ml and albumin<26g/L predicted treatment
- escalation (logrank test p=0.003). At 1 year, the estimated chance of treatment escalation was
- 246 11% (95%CI: 0-29%) for patients meeting none of the criteria, 30% (95% CI: 0-51%) for
- 247 patients meeting one criterion and 80% (95% CI: 31-94%) for patients meeting two or more
- criteria (Figure 3b).
- In UC, albumin< 37g/L and CRP>2.5g/L predicted a more aggressive disease course
- 250 (logrank test p=0.001). At 1 year, the estimated chance of treatment escalation was 0 for
- 251 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).

253 **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 the role of SC in patients with a new diagnosis of IBD. <u>SC independently</u>
- 257 predicts a diagnosis of IBD with an OR of 9.37 (95%CI: 2.82-34.68). A combined blood-

258 based biomarker diagnostic model including SC and either CRP or albumin has a high

- 259 positive LR for IBD (positive LR 24.14). Similarly, SC can predict treatment escalation
- 260 and/or surgery in IBD (HR 2.7, 95%CI: 1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-
- 261 <u>15.3).</u>
- 262 Calprotectin, a member of the S100 proteins, represents 45% of all cytosolic proteins in
- neutrophils compared to 1% in monocytes (34,35). Given the short half-life of SC (5 hours),
- it may provide a more dynamic test of the current inflammatory status compared with
- conventional inflammatory markers (half-life of CRP 18 hours, albumin 19 days) (36). SC
- correlates better with neutrophil count in controls compared to IBD patients; in IBD, SC

267	levels may reflect calprotectin release from activated neutrophils and other immune cells such
268	as monocytes, macrophages and epithelial cells (Figure 4). SC shows a strong correlation
269	with other markers such as CRP (r=0.61, p= 6.9×10^{-19}) similar to published studies (r=0.33-
270	0.59)(17,25,37,38) and <u>a moderate correlation between SC and FC (0.50, p=1.6x10⁻⁴).</u>
271	As a diagnostic blood based marker, SC is the strongest predictor of IBD 9.37 (95%CI:
272	<u>2.82-34.68</u>). In a clinical setting, blood markers such as CRP are often available, therefore
273	investigating the utility of a combined marker may be more relevant as this allows for greater
274	specificity in diagnostics (39,40). We generated an IBD scoring system that would allow
275	clinicians to predict IBD in patients at their index clinical visit. If 2 blood marker criteria
276	are met (score of 8 or above), there is a high likelihood of IBD (positive LR 24.14).
277	FC has a high NPV but a low PPV for IBD vs functional disease (cut off 50 μ g/g, NPV 93%
278	PPV 37%)(41). In practice, a blood biomarker model can complement the existing FC
279	screening of patients with gastrointestinal symptoms in primary care. In the current climate of
280	optimal tertiary care resource management, this model can be utilised for patients being
281	referred for suspected IBD and help select and prioritise investigations for individuals with a
282	high IBD score and a high likelihood of disease. The AUC for FC is superior to SC in our
283	study (0.87 and 0.99 respectively, p=0.01). FC has an established role in IBD diagnostics,
284	however in clinical practice faecal sampling and testing can be challenging. One
285	consideration in interpreting these data is the lag between SC and FC testing. The median
286	time lag between SC and FC testing was 0 days (IQR -5 to 5 days), but there were individuals
287	with upto 30 days between SC and FC testing. Nonetheless, any time lag represents real life
288	experience with faecal testing as often FC is not available until a few weeks after the clinic
289	visit. There is a large variability in the concentration of FC in stool within a single day and
290	storage conditions can impact on FC levels(18). Sampling faeces can be a hurdle for
291	patients and individuals can either decline FC testing, fail to provide a sample or

- 292 **provide insufficient sample for analysis**. These factors impact on the practical utility of FC.
- 293 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:
- 295 <u>\$7.3 equivalent</u>). In addition, other costs related to sample handling and processing are
- 296 <u>likely to be lower as serum testing is often automated.</u>
- 297 Beyond diagnostics, studies have investigated the utility of non-invasive markers in
- 298 predicting endoscopic activity. A recent meta-analyses evaluated the diagnostic
- 299 <u>accuracy of CRP, FC and stool lactoferrin (SL) for the assessment of endoscopically</u>
- 300 defined activity in IBD. The pooled AUC for CRP, FC and SL were 0.49(95% CI: 0.34-
- 301 <u>0.64), 0.88(CI: 0.84-0.90) and 0.73(CI: 0.66-0.79)(42). There was however heterogeneity</u>
- 302 in the endoscopic index used. Other factors such as inclusion criteria, in particular time
- 303 lag between blood/faecal sampling and endoscopy (0-7 days) differed(43–46). There is a
- 304 <u>need for future prospective studies investigating the performance of non-invasive</u>
- 305 endoscopic activity markers such as SC.
- 306 <u>In our study, SC predicts treatment escalation and/or surgery in IBD (HR 2.7, 95%CI:</u>
- 307 <u>1.1-4.9), in particular CD (HR 4.2, 95% CI 1.2-15.3).</u> 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 or more 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
- 313 persistent activity, either in a continuous or 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

317	disease course (31). Clinical predictors have been studied previously. In CD, Beaugerie et al
318	identified age, the presence of perianal disease and requirement for steroids at diagnosis as
319	independent predictive factors for a disabling course(47). However, biological markers were
320	not analysed in that study. Since then, the role of biomarkers in predicting the disease course
321	has been the focus of many studies, although their effectiveness in predicting outcomes
322	vary(31,48-51). Most studies suggest that CRP predicts relapse in IBD(48-50), although one
323	study found it had no predictive value (52). There are several reasons for this observed
324	variation and includes differences in defining an aggressive disease course, disease
325	heterogeneity and disease duration prior to analyses. It is also possible that variations in CRP
326	genotype may explain variations in its performance in adult cohort studies. This has been
327	described in the paediatric population(53), but yet to be explored in adults. The role of FC in
328	predicting colectomy in acute severe colitis (ASUC) has been investigated previously (AUC
329	0.65, p=0.04) and more recently, SC has been shown to predict colectomy in ASUC with an
330	AUC of 0.69 (95%CI 0.53-0.81) compared to FC (AUC 0.58; 95%CI 0.35-0.81) and CRP
331	(AUC 0.71; 95%CI: 0.56-0.86) (16,38). SC has also been studied as a prognostic marker in
332	predicting relapse after anti-TNF withdrawal and complements FC (>250µg/g) and
333	hsCRP(>5mg/L) (p=0.0173, 0.0024 and 0.0002; HR: 3.191, 3.561 and 4.120 respectively)
334	(17). Our study however is the first to explore the prognostic utility of SC at diagnosis.
335	Future studies incorporating periodic SC testing to predict disease course in IBD may
336	<u>be useful.</u>
337	Our study does have certain limitations. The results are from a single tertiary centre and
338	based on a select cohort of newly diagnosed IBD patients. The relatively small numbers
339	within the sub-type of IBD limits the power to dissect factors predicting phenotype and our
340	diagnostic and prognostic models require further validation. There were more females with
341	functional bowel disease in the control cohort and this alone may underly the

342 observation that male gender is a risk factor for IBD in our study. The major strengths
343 of this study include a prospective design and a cohort of newly diagnosed IBD aiming for
344 the first time to explore the correlation of SC with current biomarkers and build diagnostic
345 and prognostic models for potential clinical use in IBD.

346 **Conclusion**

- 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
- 351 multicentre cohorts.

352	
353	What is Current Knowledge
	• Serum calprotectin (SC) has been studied as a prognostic marker in predicting
354	relapse after anti-TNF withdrawal and complements FC and hsCRP for the
255	prediction of relapse.
355	• SC can predict colectomy in acute severe colitis with an AUC of 0.69,
356	comparable to CRP
357	• SC correlates with other inflammatory blood markers such as CRP
358	• SC has been studied in diseases such as inflammatory arthropathies and cystic
359	fibrosis and can predict disease progression.
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366	What is New Here
367	• Serum Calprotectin (SC) is a strong individual predictor of a diagnosis of IBD
368	• SC correlates with faecal calprotectin (FC) and is useful in diagnosis
369	• SC can predict treatment escalation and/or surgery in IBD, in particular CD
370	• Blood based diagnostic and prognostic models can provide an accurate reflection
371	of the inflammatory burden and have the potential to predict disease and its
372	outcomes.
373	
374	Competing interests
375	The study was supported by Calpro AS TM , Norway who provided the ELISA kits for serum
376	calprotectin testing.
377 378	Author contributions Study design RK and JS. Patient recruitment and sample processing NTV, RK, NAK, RKB.
379	Experimental work RK and MV. Data Analysis RK, NAK, ATA and NTV. RK wrote the
380	manuscript. All authors were involved in critical review, editing, revision and approval of the
381	final manuscript.
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528	
529	Figure Legends
530	Figure 1: <u>Serum Calprotectin levels in patients with Crohn's disease (CD), Ulcerative</u>
531	colitis (UC), Inflammatory bowel disease unclassified (IBDU), symptomatic controls
532	(non-IBD) and healthy controls (HC)
533	Footnote: Boxplots represent median and inter-quartile ranges for serum calprotectin
534	within each subcohort
535	Figure 2: Receiver operating curve analysis (ROC) of serum calprotectin (SC) and other
536	blood based markers in differentiating Inflammatory bowel diseases (IBD) from non-IBD and
537	ROC analysis of SC and faecal calprotectin (FC) (within 30 days) in discriminating
538	Inflammatory bowel diseases (IBD) from non-IBD.
539	Footnote: WCC:white cell count; CRP:C-reactive protein; Hb:Haemoglobin
540	Figure 3a: Kaplan Meier survival curves of disease course using blood biomarkers to predict
541	outcomes in newly diagnosed Inflammatory Bowel Diseases (IBD). Single marker represents
542	either or albumin<37 g/L or serum calprotectin \geq 1046ng/ml. Dual markers represents a
543	combination of both variables.
544	Figure 3b: Kaplan Meier survival curves of disease course using blood biomarkers to predict
545	outcomes in newly diagnosed Crohn's disease (CD)
546	Footnote: '1 marker' represents either CRP>24mg/L or albumin<26 g/L or serum
547	calprotectin >991 ng/ml. '2 or 3 marker' represents a combination of any 2 or 3 of the above
548	mentioned variables.

- 549 Figure 3c: Kaplan Meier survival curves of disease course using blood biomarkers to predict
- outcomes in newly diagnosed Ulcerative Colitis (UC) 550
- 551 Footnote: Single marker represents either albumin<37 g/L or CRP >2.5mg/L. Dual markers
- 552 represents all the categorical variables as a combined biomarker.
- Figure 4: Correlation between log transformed serum calprotectin and neutrophil count in 553
- Inflammatory Bowel Diseases (IBD) versus controls (Non-IBD). 554

scases (i

Parameters	Number of patients	rho(spearman r	ho) P va	lue
SC – CRP	171	0.61	6.9x	10 ⁻¹⁹
SC – WCC	147	0.61	3.8 x	10 ⁻¹⁷
SC – Neut	147	0.65	1.9x	10 ⁻²⁰
SC – Lymp	147	-0.03	0.68	
SC – Alb	171	-0.54	3.3x	10 ⁻¹⁴
SC-Hb	147	-0.42	6.1 x	10 ⁻⁸
SC-Plts	147	0.49	1.3×	10-10
SC-FC	50	0.50	1.6x	10 ⁻⁴
Parameters	IBD(n)	rho(p-value)	Non- IBD(n)	rho(p-value)
SC – CRP	96	$0.41(3.9 \times 10^{-5})$	75	0.30(0.01)
SC – WCC	92	$0.37(2.3 \times 10^{-4})$	55	$0.66(4.3 \times 10^{-8})$
SC - Neut	92	$0.43(2.2 \times 10^{-5})$	55	$0.68(1.0 \times 10^{-8})$
SC – Lymp	92	-0.18(0.08)	55	$0.48(2.0 \times 10^{-4})$
SC – Alb	95	-0.39(6.9x10 ⁻⁵)	75	-0.09(0.46)
SC-Hb	92	$-0.41(4.7 \times 10^{-5})$	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)

 Table 1: Correlation coefficient (spearman rho) between Serum Calprotectin (SC) and blood

 and faecal parameters

Abbreviations: SC: serum calprotectin, WCC: white cell count; Neut: neutrophil count; Lymp: lymphocyte count; Alb: albumin; Hb: haemoglobin; FC: faecal calprotectin; Plts: Platelet count



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

 Table 2: Study demographics, Montreal classification, disease behaviour for newly

 diagnosed Inflammatory Bowel Diseases (IBD) and control cohorts

Footnote: CD: Crohn's disease; UC: ulcerative colitis; IBD-U: Inflammatory bowel disease unclassified; HC: healthy controls; SC: symptomatic controls.

Smoking status was available in 81 patients with IBD and 63 patients with non-IBD.

 Table 3: Blood and faecal parameters for Inflammatory Bowel Disease (IBD) and control cohorts

		IBD	Controls		
Test	Number of patients	Median (IQR)	Number of patients	Median (IQR)	
Haemoglobin (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)	
Faecal 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	1015(811-1442)	73	506(362-725)	
			°L2		

 Table 4: Multiple logistic regression of predictors of Inflammatory Bowel Diseases versus 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 (9.55-18512.49)	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>3.5mg/L	8.52(2.75-28.63)	2.80×10 ⁻⁴			
Serum Calprotectin >852ng/ml	9.37(2.82-34.68)	4.00×10 ⁻⁴			
Albumin<38g/L	6.12 (1.82-22.16)	0.004			
Male gender	2.87(0.97-9.24)	0.06			

Table 5a: Sensitivity, specificity, positive and negative likelihood ratios (LR) of the Inflammatory Bowel Diseases (IBD) scoring parameters. Each variable score is based on the odds ratio generated from the linear model

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

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

Table 5b: Sensitivity, specificity, 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>3.5mg/L	0.70	0.86	5.03	0.35
Albumin<38 g/L	0.66	0.88	5.30	0.39
Serum Calprotectin>852ng/ml	0.69	0.90	7.06	0.35

	Categorical variable analysis						
Categorical variableCategorical thresholdHazards ratio (95%CI)AICP-value f threshold							
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			

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

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	Hazards 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)	>2.5mg/L	2.6(0.7-9.6)	147.0	0.15		

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	P-value for thresholds						
C-reactive protein(CRP)	>24mg/L	2.7(1.0-7.4)	93.3	0.06			
Albumin	<26 g/L 2.6(0.8-9.1)	2.6(0.8-9.1)	91.9	0.13			
Serum Calprotectin	>991ng/ml	4.2(1.2-15.3)	95.1	0.03			



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)

Footnote: Boxplots represent median and inter-quartile ranges for serum calprotectin within each subcohort



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 faecal calprotectin (FC) (within 30 days) in discriminating Inflammatory bowel diseases (IBD) from non-IBD. Footnote: WCC:white cell count; CRP:C-reactive protein; Hb:Haemoglobin





Figure 3a: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Inflammatory Bowel Diseases (IBD). Single marker represents either or albumin<37 g/L or serum calprotectin \geq 1046ng/ml. Dual markers represents a combination of both variables.





Figure 3b: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Crohn's disease (CD)

Footnote: '1 marker' represents either CRP>24mg/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.



Figure 3c: Kaplan Meier survival curves of disease course using blood biomarkers to predict outcomes in newly diagnosed Ulcerative Colitis (UC)

Footnote: Single marker represents either albumin<37 g/L or CRP >2.5mg/L. Dual markers represents all the categorical variables as a combined biomarker.



Figure 4: Correlation between log transformed serum calprotectin and neutrophil count in Inflammatory Bowel Diseases (IBD) versus controls (Non-IBD).

Supplementary Table 1: Disease outcomes in patients with Ulcerative colitis (UC) and Crohn's disease (CD).

Diagnosi s	Treatment Escalation?	Age	Sex	Total follow up time (days)	Diagnosis to SC sampling (days)	SC sampling to Rx escalation(days)	Rx immediately prior escalation	Treatment escalation
UC	Yes	46	М	126	1	30	Prednisolone	Azathioprine and 2nd course of Prednisolone
UC	Yes	54	М	374	73	27	5ASA	Azathioprine and Prednisolone
UC	Yes	35	F	547	-14	316	5ASA	Azathioprine and Prednisolone
UC	Yes	33	F	609	18	67	5ASA	Azathioprine and Prednisolone
UC	Yes	53	М	871	2	47	5ASA	Azathioprine and Prednisolone
UC	Yes	20	М	893	-14	269	5ASA	Azathioprine and Prednisolone
UC	Yes	29	М	201	0	44	Prednisolone	Ciclosporin and Azathioprine during 2nd admission
UC	Yes	19	М	470	2	43	5ASA	Ciclosporin, Prednisolone and Azathioprine
UC	Yes	31	М	781	-1	408	5ASA	Ciclosporin, Prednisolone and Azathioprine
UC	Yes	34	М	498	37	225	Azathioprine	Golilumab
UC	Yes	33	F	69	41	26	Azathioprine	Infliximab
UC	Yes	26	F	549	8	44	Azathioprine	Infliximab
UC	No	35	М	118	0	NA	5ASA	No change in therapy
UC	No	38	F	132	43	NA	5ASA	No change in therapy
UC	No	32	М	145	0	NA	5ASA	No change in therapy
UC	No	32	М	145	0	NA	5ASA	No change in therapy
UC	No	53	М	345	12	NA	5ASA	No change in therapy
UC	No	40	М	367	24	NA	5ASA	No change in therapy
UC	No	24	F	410	0	NA	5ASA	No change in therapy
UC	No	27	F	456	57	NA	5ASA	No change in therapy
UC	No	37	F	472	78	NA	5ASA	No change in therapy
UC	No	48	М	484	68	NA	5ASA	No change in therapy
UC	No	41	М	499	0	NA	Azathioprine	No change in therapy
UC	No	57	М	518	23	NA	5ASA	No change in therapy
UC	No	27	М	575	-39	NA	5ASA	No change in therapy
UC	No	24	F	578	0	NA	5ASA	No change in therapy

UC	No	58	М	604	75	NA	5ASA	No change in therapy
UC	No	50	М	637	61	NA	5ASA	No change in therapy
UC	No	62	М	657	32	NA	5ASA	No change in therapy
UC	No	26	М	670	30	NA	No medications	No change in therapy
UC	No	57	F	788	45	NA	5ASA	No change in therapy
UC	No	38	М	795	27	NA	5ASA	No change in therapy
UC	No	52	М	801	78	NA	5ASA	No change in therapy
UC	No	34	М	837	-1	NA	Azathioprine	No change in therapy
UC	Yes	29	М	366	0	7	IVMP and Ciclosporin	Panproctocolectomy
UC	Yes	44	М	451	2	6	MP and Prednisolone	Panproctocolectomy
UC	Yes	21	М	126	13	35	Prednisolone and Ciclosporin	Subtotal colectomy
UC	Yes	59	М	433	5	1	5ASA	Subtotal colectomy
UC	Yes	43	F	602	25	323	MP	Subtotal colectomy
UC	Yes	22	М	643	22	303	5ASA	Subtotal colectomy
UC	Yes	27	F	672	4	153	5ASA	Subtotal colectomy
UC	Yes	22	М	712	0	23	5ASA	Subtotal colectomy
UC	Yes	23	F	871	1	496	5ASA and Azathioprine	Subtotal colectomy
UC	Yes	29	М	385	-12	12	Prednisolone and Ciclosporin	Subtotal colectomy
UC	Yes	39	М	342	0	161	MP	Vedolizumab
CD	Yes	42	М	602	1	413	Azathioprine	Adalimumab
CD	Yes	27	М	852	0	311	Azathioprine	Adalimumab
CD	Yes	27	М	870	0	614	MP	Adalimumab
CD	Yes	18	М	358	44	275	Infliximab	Azathioprine
CD	Yes	24	F	510	0	47	Infliximab	Azathioprine
CD	Yes	25	М	454	0	91	Prednisolone	Azathioprine and 2 nd course Prednisolone
CD	Yes	19	М	525	35	27	Prednisolone	Azathioprine and Infliximab
CD	Yes	23	М	504	34	69	5ASA	Azathioprine and Prednisolone
CD	Yes	27	F	644	11	566	5ASA	Azathioprine and Prednisolone
CD	Yes	27	F	762	50	84	MP and Infliximab	Ileocaecal resection
CD	No	29	М	768	-2	6	IVMP	Ileocaecal resection at index admission

CD	Yes	32	М	618	30	161	MP	Infliximab
CD	Yes	19	F	664	20	45	MP	Infliximab
CD	Yes	33	F	718	1	42	Azathioprine	Infliximab
CD	Yes	30	М	803	-27	64	Azathioprine	Infliximab
CD	Yes	24	М	837	0	140	Azathioprine	Infliximab
CD	No	41	М	150	-4	NA	Azathioprine	No change in therapy
CD	No	59	F	160	-2	NA	Polymeric diet	No change in therapy
CD	No	31	М	177	-113	NA	No medications	No change in therapy
CD	No	72	М	347	46	NA	No medications	No change in therapy
CD	No	29	М	408	52	NA	No medications	No change in therapy
CD	No	38	М	417	87	NA	No medications	No change in therapy
CD	No	29	М	506	-18	NA	Azathioprine	No change in therapy
CD	No	52	М	513	57	NA	5ASA	No change in therapy
CD	No	31	М	555	0	NA	Azathioprine	No change in therapy
CD	No	29	F	582	-36	NA	Azathioprine	No change in therapy
CD	No	18	М	637	28	NA	No medications	No change in therapy
CD	No	57	F	719	33	NA	5ASA	No change in therapy
CD	No	40	F	721	18	NA	Azathioprine	No change in therapy
CD	No	23	М	726	22	NA	No medications	No change in therapy
CD	No	19	М	781	-6	NA	Azathioprine	No change in therapy
CD	No	38	F	821	64	NA	No medications	No change in therapy
CD	No	36	F	914	76	NA	5ASA	No change in therapy
CD	Yes	21	М	504	4	99	MP	Subtotal colectomy
CD	Yes	22	F	319	-206	NA	Azathioprine	No change in therapy

Footnote: F: Female; M: Male; NA: Not applicable; 5ASA: 5-aminosalicylates; MP: mercaptopurine; IVMP: Intravenous methylprednisolone

Supplementary Table 2a: Clinical symptoms (Simple Clinical Colitis Activity Index) at recruitment in patients with Ulcerative Colitis (UC) and Inflammatory Bowel Disease-Unclassified (IBDU)

		General		SCCAI Day	SCCAI Night	SCCAI	SCCAI Blood	
Diagnosis	Rx naive	Well Being	Complications	BO	BO	Urgency	PR	SCCAI Score
UC	Y	2	0	0	0	1	3	6
UC	Ν	1	1	0	0	1	2	5
UC	Ν	1	0	1	1	NA	3	NA
UC	Y	1	0	1	0	1	0	3
UC	Y	1	0	0	0	1	2	4
UC	Ν	0	1	0	0	0	0	1
UC	Y	1	0	0	0	0	2	3
UC	Y	1	0	2	1	1	3	8
UC	Y	1	0	2	1	0	2	6
UC	Y	2	0	3	1	1	3	10
UC	Y	3	1	1	1	1	2	9
UC	Ν	2	0	2	1	1	3	9
UC	Ν	0	0	0	0	1	3	4
UC	Ν	0	0	0	0	0	0	0
UC	Ν	0	0	1	0	1	2	4
UC	Ν	1	0	2	1	1	2	7
UC	Ν	1	0	0	0	0	2	3
UC	Ν	0	0	0	1	2	0	3
UC	Y	2	0	3	2	1	3	11
UC	Y	1	0	3	0	3	3	10
UC	Ν	1	0	1	0	1	3	6
UC	Ν	1	0	1	2	1	3	8
UC	Y	2	0	0	2	1	2	7
UC	Y	1	0	2	0	1	3	7
UC	N	NA	NA	NA	NA	NA	NA	NA
UC	N	1	0	3	1	1	3	9
UC	Y	1	0	0	0	1	0	2
UC	N	1	0	3	2	1	3	10
UC	Y	1	0	0	0	1	2	4

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UC	Ν	2	0	0	1	1	2	6
UC	Ν	4	0	3	2	3	3	15
UC	Y	2	0	3	2	3	3	13
UC	Y	1	0	2	1	1	2	7
UC	Y	1	1	0	0	0	3	5
UC	Ν	0	0	0	0	1	3	4
UC	Y	2	0	NA	NA	1	3	NA
UC	Ν	1	0	0	0	0	1	2
UC	Y	1	1	0	0	1	2	5
UC	Y	1	0	2	0	1	3	7
UC	Y	1	1	1	0	1	2	6
UC	Ν	1	1	0	0	1	2	5
UC	Ν	1	1	0	1	1	2	6
UC	Ν	1	0	0	0	2	3	6
UC	Y	3	0	1	1	1	2	8
UC	Ν	2	0	1	2	2	3	10
IBDU	Y	0	0	0	0	0	2	2
IBDU	Ν	1	0	1	0	1	0	3
IBDU	Y	2	0	1	1	2	3	9

			General	Abdominal		Abdominal		
Diagnosis	Rx naive	Weight loss	Well Being	Pain	BO per day	mass	Complications	HBI Score
CD	Y	Ν	0	0	2	NA	0	NA
CD	Ν	Ν	NA	1	11	0	1	NA
CD	Y	Y	1	0	1	0	1	3
CD	Y	Y	1	0	1	0	0	2
CD	Ν	Y	2	1	10	0	1	14
CD	Y	Ν	1	3	9	0	0	13
CD	Y	Y	2	2	5	1	0	10
CD	Y	Ν	1	1	1	2	0	5
CD	Y	Y	2	2	0	1	0	5
CD	Y	Y	0	0	1	0	0	1
CD	Y	Y	2	2	1	0	1	6
CD	Y	Y	0	0	1	0	0	1
CD	Ν	Ν	1	0	11	0	0	12
CD	Y	Y	NA	3	10	0	1	NA
CD	Y	Y	1	1	2	0	0	4
CD	Y	Y	1	0	1	0	1	3
CD	Y	Y	NA	NA	NA	NA	NA	NA
CD	Y	Ν	0	2	1	3	0	6
CD	Ν	Y	1	1	2	0	1	5
CD	Y	Ν	1	1	2	0	2	6
CD	Y	Y	1	2	4	0	0	7
CD	Ν	Y	1	3	2	0	2	8
CD	Ν	Y	1	0	5	0	1	7
CD	Y	Y	2	2	10	0	2	16
CD	Y	Y	1	2	7	0	1	11
CD	Y	Y	1	1	10	0	0	12
CD	Y	Y	2	2	8	0	0	12
CD	Y	Ν	2	0	5	0	1	8
CD	Y	Ν	2	2	7	NA	2	NA

Supplementary Table 2b: Clinical symptoms (Harvey Bradshaw Index) at recruitment in patients with Crohn's Disease (CD)

CD	Y	Ν	0	0	0	0	2	2
CD	Y	NA	NA	0	1	0	0	NA
CD	Y	NA	1	3	3	0	2	9
CD	Y	Ν	1	1	2	0	0	4
CD	Y	N	0	0	4	0	0	4
CD	Y	Y	1	3	2	0	0	6

Footnote: Rx naïve: Treatment naïve status at sampling; SC: serum calprotectin; BO: Bowel habits; SCCAI: Simple clinical colitis activity index; HBI: Harvey Bradshaw Index; NA: Not available; Y: yes; N: No

, SC: seil

Supplementary Table 3: Summary of investigations undertaken in the control cohort

			Faecal Calprotectin		
Diagnosis	Age	Sex	(µg/g)	Colonoscopy	Radiology
HC	47	Μ	NA	no	no
HC	42	М	NA	no	no
HC	32	М	NA	no	no
HC	30	М	NA	no	no
HC	31	F	NA	no	no
HC	24	F	NA	no	no
HC	33	М	NA	no	no
HC	34	F	NA	no	no
HC	32	F	NA	no	no
HC	30	М	NA	no	no
HC	32	F	NA	no	no
HC	54	F	NA	no	no
HC	39	М	NA	no	no
HC	43	М	NA	no	no
HC	59	F	NA	no	no
HC	35	F	NA	no	no
HC	25	F	NA	no	no
HC	20	F	NA	no	no
HC	31	М	NA	no	no
HC	19	F	NA	no	no

HC	64	F	NA	no	no
HC	49	F	NA	no	no
HC	24	М	NA	no	no
HC	30	М	NA	no	no
HC	30	Μ	NA	no	no
НС	33	Μ	NA	no	no
HC	34	Μ	NA	no	no
Non-IBD	31	F	19	no	no
Non-IBD	22	М	19	yes	no
Non-IBD	33	F	19	no	yes
Non-IBD	21	М	19	yes	yes
Non-IBD	31	М	19	yes	no
Non-IBD	21	М	19	no	no
Non-IBD	29	F	19	yes	no
Non-IBD	37	F	19	yes	no
Non-IBD	28	Μ	NA	yes	no
Non-IBD	28	F	NA	yes	no
Non-IBD	33	F	NA	yes	no
Non-IBD	27	F	19	no	no
Non-IBD	23	F	30	yes	yes
Non-IBD	33	F	19	no	no
Non-IBD	29	F	NA	yes	no
Non-IBD	23	F	19	yes	no
Non-IBD	44	F	NA	yes	no
Non-IBD	41	Μ	NA	no	yes
Non-IBD	28	F	120	yes	yes
Non-IBD	26	F	19	yes	yes
Non-IBD	33	Μ	19	yes	no
Non-IBD	41	F	19	no	no
Non-IBD	19	F	19	yes	yes

Non-IBD	22	F	19	yes	yes
Non-IBD	29	F	30	yes	no
Non-IBD	39	F	19	yes	yes
Non-IBD	34	F	210	yes	yes
Non-IBD	28	F	80	yes	no
Non-IBD	23	Μ	19	yes	yes
Non-IBD	29	F	NA	yes	no
Non-IBD	21	Μ	19	yes	no
Non-IBD	22	Μ	19	no	no
Non-IBD	44	Μ	470	yes	yes
Non-IBD	39	Μ	19	yes	no
Non-IBD	32	М	NA	yes	no
Non-IBD	30	F	160	yes	no
Non-IBD	37	F	190	yes	no
Non-IBD	36	Μ	NA	yes	no
Non-IBD	34	F	110	yes	yes
Non-IBD	43	М	NA	yes	no
Non-IBD	45	Μ	NA	yes	no
Non-IBD	23	Μ	19	no	no
Non-IBD	41	Μ	NA	yes	yes
Non-IBD	21	Μ	19	no	no
Non-IBD	20	F	19	yes	no
Non-IBD	20	F	19	yes	no

Footnote: HC: Healthy lab volunteers; Non-IBD: Symptomatic controls; M: Male; F: Female; NA: Not available
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Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT		Blood-based Diagnostic and Prognostic Models in IBD: The Utility of Serum Calprotectin	1
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy	6 and 7
		(such as sensitivity, specificity, predictive values, or AUC)	
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions	2 and 3
		(for specific guidance, see STARD for Abstracts)	
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	4 and 5
	4	Study objectives and hypotheses	4 and 5
METHODS			~
Study design	5	Whether data collection was planned before the index test and reference standard	6
Dauticia ante	~	The sector for the sector study or after (retrospective study)	C
Participants	0 7	Eligipility criteria	D C
	/	(such as symptoms, results from previous tests, inclusion in registry)	б
	Q	(Such as symptoms, results from previous tests, inclusion in registry)	6
	0 Q	Whether participants formed a consecutive, random or convenience series	6
Test methods	9 10a	Index test in sufficient detail to allow replication	6
rest methods	10a 10h	Reference standard in sufficient detail to allow replication	6
	100	Rationale for choosing the reference standard (if alternatives evict)	6
	17a	Definition of and rationale for test positivity cut-offs or result categories	6
	120	of the index test, distinguishing pre-specified from exploratory	0
	12b	Definition of and rationale for test positivity cut-offs or result categories	6
		of the reference standard, distinguishing pre-specified from exploratory	·
	13a	Whether clinical information and reference standard results were available	7
		to the performers/readers of the index test	
	13b	Whether clinical information and index test results were available	7
		to the assessors of the reference standard	
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy	7
	15	How indeterminate index test or reference standard results were handled	7
	16	How missing data on the index test and reference standard were handled	7
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	7
	18	Intended sample size and how it was determined	7
RESULTS			
Participants	19	Flow of participants, using a diagram	NA
	20	Baseline demographic and clinical characteristics of participants	8
	21 a	Distribution of severity of disease in those with the target condition	8
	21b	Distribution of alternative diagnoses in those without the target condition	8
	22	Time interval and any clinical interventions between index test and reference standard	10
Test results	23	Cross tabulation of the index test results (or their distribution)	8
		by the results of the reference standard	~
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	8
	25	Any adverse events from performing the index test or the reference standard	NA
DISCUSSION	~~		12
	26 27	Study initiations, including sources of potential blas, statistical uncertainty, and generalisability	13
ОТНЕР	21	implications for practice, including the intended use and clinical role of the index test	15
	70	Registration number and name of registry	6
	20 20	Where the full trudy protocol can be accessed	υ
	27 20	Sources of funding and other support, role of funders	11/A 15
	50	ScholarOne, 375 Greenbrier Drive, Charlottesville, VA, 22901	1.5





STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition**. This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on <u>http://www.equator-network.org/reporting-guidelines/stard.</u>

