# Chronic abdominal pain, fatigue and inflammatory bowel disease in children

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# CHAPTER 4

## TEST STRATEGIES TO PREDICT INFLAMMATORY BOWEL DISEASE AMONG CHILDREN WITH NON-BLOODY DIARRHOEA

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**ABSTRACT**

**OBJECTIVE:** We evaluated four diagnostic strategies to predict the presence of inflammatory bowel disease (IBD) in children who present with chronic non-bloody diarrhoea and abdominal pain.

**METHODS:** We conducted a prospective cohort study, including 193 patients aged 6–18 years, who underwent a standardised diagnostic work-up in secondary or tertiary care hospitals. Each patient was assessed for symptoms, c-reactive protein (>10 mg/L), haemoglobin (<-2 SD for age and gender) and faecal calprotectin (≥250 μg/g). Patients with rectal bleeding or perianal disease were excluded because the presence of these findings prompted endoscopy regardless of their biomarkers. Primary outcome was IBD confirmed by endoscopy, or IBD ruled out by endoscopy or uneventful clinical follow-up for 6 months. We measured the predictive performance of each strategy with AUC and decision curves.

**RESULTS:** 22 of 193 (11%) children had IBD. The basic prediction model was based on symptoms only. Adding blood or stool markers increased the AUC from 0.718 [95%CI: 0.604-0.832] to 0.930 [95%CI: 0.884-0.977] and 0.967 [95%CI: 0.945-0.990]. Combining symptoms with blood and stool markers outperformed all other strategies (AUC 0.997 [95%CI: 0.993-1.000]). Triaging with a strategy that involves symptoms, blood markers and calprotectin will result in 14 of 100 patients being exposed to endoscopy. Three of them will not have IBD, and no IBD-affected child will be missed.

**CONCLUSION:** Evaluating symptoms plus blood and stool markers in patients with non-bloody diarrhoea is the optimal test strategy that allows paediatricians to reserve a diagnostic endoscopy for children at high-risk for IBD.

**INTRODUCTION**

Persistent rectal bleeding or perianal disease in children and teenagers justifies endoscopy to evaluate the presence of inflammatory bowel disease (IBD).(1-2) When the indication for endoscopy is less obvious, as in the case of patients with chronic abdominal pain and non-bloody diarrhoea, a triage test may help to distinguish who are in need of immediate referral to endoscopy.

Several meta-analyses (3-6) have shown that measuring a single faecal calprotectin level can help to distinguish IBD from functional abdominal disorders. Calprotectin concentrations above 50 µg/g predict the presence of IBD with high sensitivity [99% (range 92 to 100%)], but the mediocre specificity [65% (range 54 to 74%)](6) is the reason that a substantial number of children are wrongly exposed to endoscopy. A refinement of the cut-point to 250 µg/g was insufficient to reduce the rate of unnecessary endoscopies.(7-9)

Complications of endoscopy, related to the invasiveness of the procedure itself (colonic perforation or tear) or to anaesthesia, may be rare but could cause severe morbidity.(10-12) A diagnostic strategy that includes a combination of tests would potentially further reduce the number of children exposed to this invasive and costly procedure.

We evaluated four diagnostic strategies to predict the presence of IBD: [1] symptoms alone, [2] symptoms plus blood markers, [3] symptoms plus faecal calprotectin, and [4] symptoms plus blood markers plus faecal calprotectin.

**PATIENTS AND METHODS**

**Study design**

This international multi-centre study was a planned ancillary study of the prospective CACATU cohort (clinicaltrials.gov NCT02197780). The cohort and the calprotectin results have previously been described (11) and are replicated here for the subgroup of previously undiagnosed children and teenagers presenting with persistent or recurrent non-bloody diarrhoea and abdominal pain. Patients were assessed by a local clinician and data collected during history taking and physical examination were entered on a secured study website (www.cacatustudie.eu). Blood tests were performed at the local hospital and the results were uploaded to the study website. Stool samples were sent to the

Department of Laboratory Medicine of the University Medical Centre Groningen. Immediately after arrival, the faecal calprotectin concentration was measured and the result was made visible to the local clinician by an e-mail notification that included an automated advice on the next best move. Patients with a faecal calprotectin concentration ≥250 μg/g moved on to endoscopy with biopsies (reference standard). Patients with a faecal calprotectin concentration <250 μg/g were re-evaluated at 6 month follow-up after inclusion for possible latent IBD to become visible (alternative reference standard). Deviation from the automated advice on the next best move was considered a protocol violation. The study protocol has been published in BMJ Open.(13)

**Participants**

Patients were recruited from paediatric outpatient clinics of sixteen general hospitals and three tertiary care hospitals in the Netherlands and Belgium. The clinicians at the various sites were general paediatricians or paediatric gastroenterologists. Six participating centres had a paediatric endoscopy unit. Patients eligible for inclusion in this ancillary study were aged 6 to 18 years, showing persistent or recurrent non-bloody diarrhoea and abdominal pain. Patients with rectal bleeding or perianal disease were not analysed in this ancillary study, as their symptoms prompted colonoscopy regardless of any biomarker result.(1)

**Outcome**

Primary outcome was IBD confirmed by endoscopy of the upper and lower gastrointestinal tract, or IBD ruled out by either endoscopy or uneventful clinical follow-up for 6 months. In case of macroscopic and histological absence of inflammation, imaging of the small intestine was encouraged.

**Statistical methods**

Dichotomous data collected at baseline (including presence of chronic non-bloody diarrhoea, weight loss, first degree relatives with IBD and extra-intestinal symptoms) were used to construct a basic logistic regression model to predict the presence of IBD. The incremental value of blood markers (increased C-reactive protein (CRP) and haemoglobin (Hb) below -2 standard deviations) and increased faecal calprotectin (≥250 μg/g) were evaluated by adding them to the basic prediction model (**table 1**).

**Table 1:** Overview of predictors

| Test | Measurement | Definition of positive result |
|---|---|---|
| **Symptoms** | | |
| Persistent non-bloody diarrhoea | History | Duration ≥4 weeks |
| Recurrent non-bloody diarrhoea and abdominal pain | History | ≥2 episodes in 6 months |
| Unintended weight loss | History and physical examination | > 1 kg |
| First degree relative with IBD | History | Affected father, mother, sibling |
| Extra-intestinal symptoms | Physical examination | Episcleritis, uveitis, erythema nodosum, psoriasis, finger clubbing, arthritis |
| **Blood markers** | | |
| Increased C-reactive protein | Local laboratory | >10 mg/L |
| Anaemia (haemoglobin <−2 SD for age and gender) | Local laboratory | 4-12 years < 7.1 mmol/l<br>boys 12-18 years < 8.1 mmol/l<br>girls 12-18 years < 7.4 mmol/l |
| **Stool markers** | | |
| Increased faecal calprotectin | Central laboratory[1] | ≥250 μg/g |

[1] fCAL enzyme-linked immunosorbent assay, BÜHLMANN Laboratories AG, Schönenbuch, Switzerland

We estimated the performance of the four diagnostic strategies by calculating (1) the area under the receiver-operating-characteristics curve (AUC), and (2) the net benefit of each strategy through decision curve analysis. Net benefit combines the number of children that were correctly triaged for endoscopy (true positives) and the number of children exposed to an unnecessary endoscopic procedure (false positives) into a single number. We show the net benefit of each strategy through a range of risk thresholds. Finally, we calculated sensitivity and specificity with 95% confidence intervals (CIs) of the optimal diagnostic strategy. Computations were carried out with R (version 3.5.1).

**Human Subjects Protection**

The study was conducted according to the principles of the Declaration of Helsinki. The Medical Ethics Review Committee of the University Medical Center in Groningen (METc 2013/503) and Antwerp University Hospital (14/40/407) approved the study protocol. The legal guardian(s) of all participants, as well as children aged 12 and above, gave informed consent to use data generated by routine medical care. The investigators collected and recorded data in such a manner that subjects could not be identified, directly or through identifiers linked to the subjects.

**RESULTS**

Between September 2014 and September 2016, we prospectively included 354 children and teenagers in the CACATU cohort. Of these, 135 had overt rectal bloodloss or perianal disease, which justified immediate endoscopic evaluation for the presence of IBD. Fifteen patients were excluded as their stool samples arrived at the hospital laboratory after an unacceptable delay that may have caused calprotectin degradation.(14) A total of 204 patients were included for this ancillary study, of which 193 continued down the decision tree until a final diagnosis was made (**figure 1**).

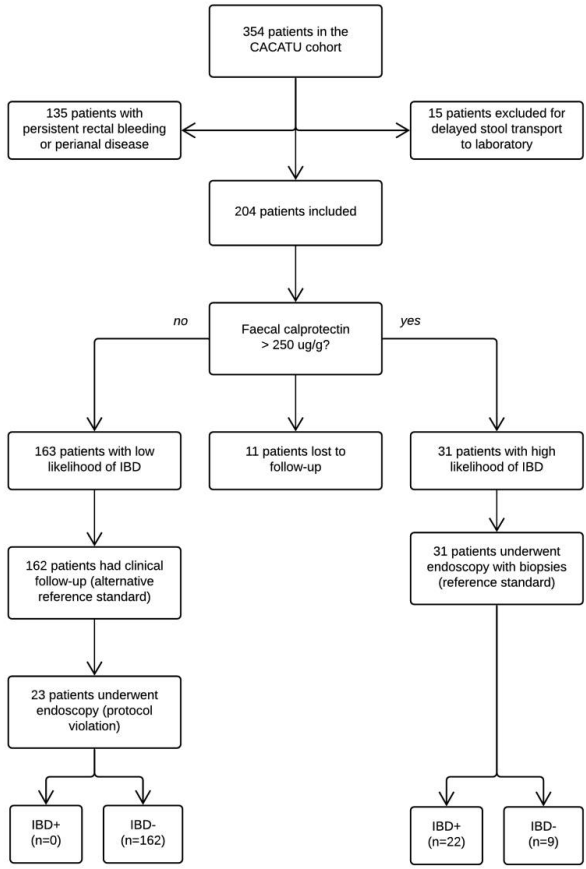


**Figure 1 I** Flow of participants

Baseline characteristics are shown in **table 2**. IBD was confirmed in 22 of 193 patients (11%), of whom 8 had ulcerative colitis and 14 Crohn's disease.

**Table 2:** Baseline characteristics of 193 patients

| Characteristics | IBD (n=22) | non-IBD (n=171) | p-value |
|---|---|---|---|
| **Demographics** | | | |
| Median (IQR) age in years | 14 (8-17) | 12(6-17) | |
| Male gender | 9 (41%) | 98 (57%) | |
| **Symptoms** | | | |
| Persistent non-bloody diarrhoea (>4 weeks) | 15 (68%) | 58 (34%) | 0.004 |
| Recurrent non-bloody diarrhoea and abdominal pain | 15 (68%) | 149 (87%) | 0.043 |
| Unintended weight loss | 10 (46%) | 48 (28%) | 0.154 |
| First degree relative with IBD | 1 (5%) | 17 (10%) | 0.667 |
| Extra-intestinal symptoms | 3 (14%) | 11 (6%) | 0.430 |
| **Blood markers** | | | |
| Increased C-reactive protein | 13 (59%) | 9 (5%) | <0.001 |
| Anaemia | 15 (68%) | 14 (8%) | <0.001 |
| **Stool markers** | | | |
| Faecal calprotectin ≥250 μg/g | 22 (100%) | 18 (11%) | <0.001 |

Data are number (%) of patients unless stated otherwise.
Abbreviations: IQR, inter quartile range; SD, standard deviation

**Area under the receiver-operating-characteristics curve (AUC)**

Receiver-operating-characteristics (ROC) curve analysis revealed an AUC of 0.718 [95% CI: 0.604-0.832] for the basic model to predict IBD. In comparison, the ROC curve analyses of strategy 2 (symptoms + blood markers), strategy 3 (symptoms + calprotectin) and strategy 4 (symptoms + blood markers + calprotectin) revealed AUCs of 0.930 [95% CI: 0.884-0.977], 0.967 [95%CI: 0.945-0.990] and 0.997 [95%CI: 0.993-1.000], respectively (**Figure 2**).

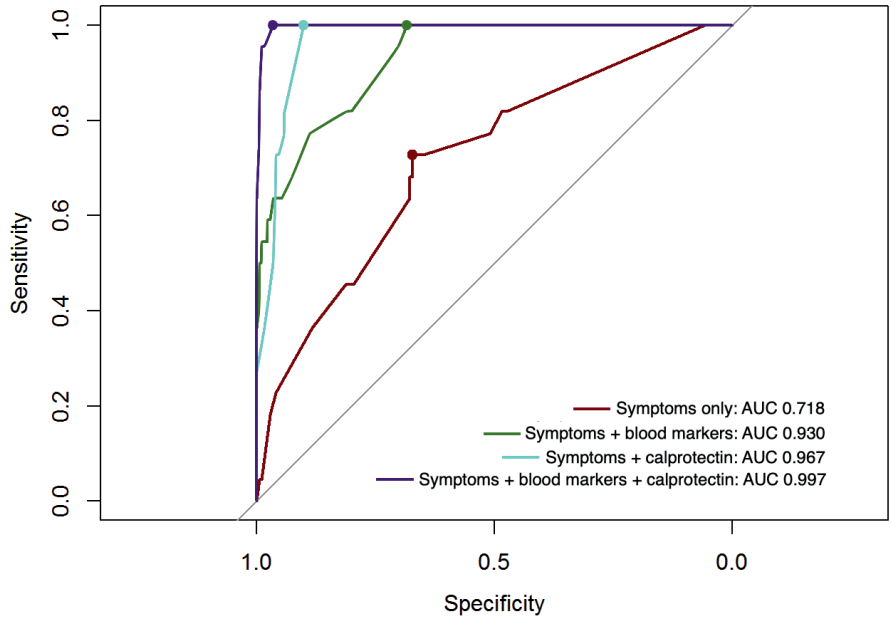


**Figure 2 I** ROC-curves representing the accuracy for detecting IBD in children with chronic non-bloody diarrhoea. Abbreviation: AUC, area under the curve.

The accompanying changes in sensitivity and specificity are shown in table 3. The sensitivity was 100% for strategy 2, 3 and 4, and the specificity increased from 68.4% to 90.1% and 96.5%, respectively.

**Table 3:** Accuracy measures for four diagnostic strategies to predict inflammatory bowel disease.

| Diagnostic strategy | Sens | Spec | Number per 100 patients (IBD prevalence 11%) | | | |
|---|---|---|---|---|---|---|
| | | | **TP** | **TN** | **FP** | **FN** |
| 1. Symptoms only | 72.7% | 67.3% | 8 | 60 | 29 | 3 |
| 2. Symptoms + blood markers | 100% | 68.4% | 11 | 61 | 28 | 0 |
| 3. Symptoms + calprotectin | 100% | 90.1% | 11 | 80 | 9 | 0 |
| 4. Symptoms + blood markers + calprotectin | 100% | 96.5% | 11 | 86 | 3 | 0 |

Abbreviations: Sens, sensitivity; Spec, specificity; TP, true positives; TN, true negatives; FP, false positives; FN, false negatives

Regardless of whether strategy 2, 3 or 4 was used, all IBD-affected patients were correctly exposed to endoscopy. Strategy 2, 3 and 4 correctly advised against referring 61%, 80% and 86% of patients for endoscopy, respectively.

The pre-test probability of IBD in the study cohort was 11%; a positive result of strategy 4 produced a post-test probability of IBD of 78% [95% CI: 60-87%]. The probability of IBD, if strategy 4 was negative, was reduced to 0% [95% CI 0-4%].

**Decision curve analysis**

In the decision curve analysis, strategy 4 (symptoms + blood markers + calprotectin) had the greatest net benefit for predicting IBD across the range of risk thresholds up to 70% (**figure 3**).

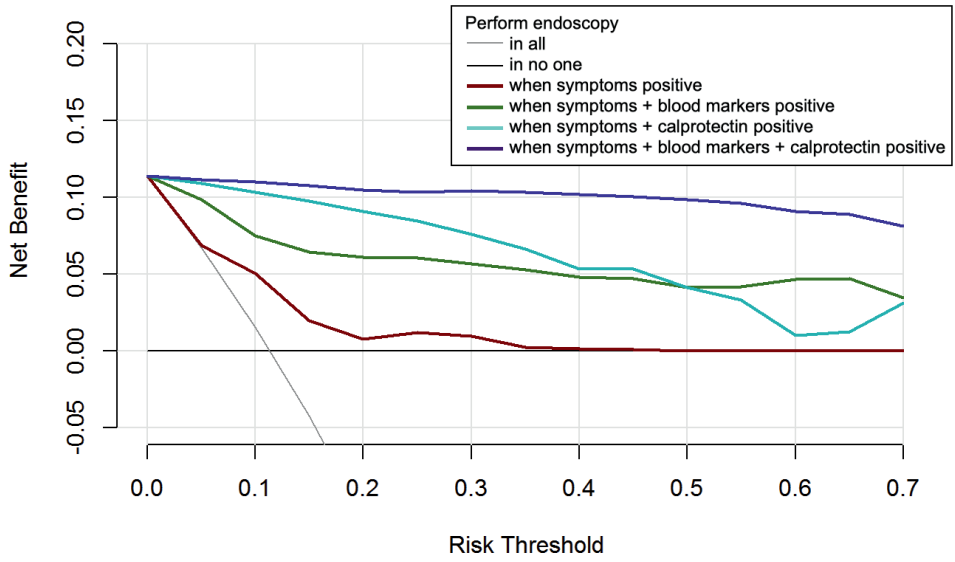


**Figure 3 I** Decision curves for four diagnostic strategies to predict IBD.
The default strategies were to perform endoscopy in all patients or in none. A diagnostic strategy is clinically useful if it has a greater net benefit than the default strategies.

Strategy 3 (symptoms + calprotectin) provided greater net benefit than strategy 2 (symptoms + blood markers) up to a risk threshold of 50%. When the risk threshold was 50 to 70%, strategy 2 had greater net benefit. The basic model (symptoms only) provided hardly any greater net benefit than performing endoscopy in all patients, or alternatively, performing endoscopy in no one.

**Box. How to read figure 3?**

Assume that a clinician does not want to expose more than 2 children to endoscopy to detect one case with IBD. In this instance the "harm-to-benefit" ratio is 1:1 (or a risk threshold of 50%). At this risk threshold the net benefit of 0.10 means that strategy 4 leads to exposing 100 per 1000 children at risk, with all of the exposed having IBD.

**DISCUSSION**

In this international prospective, multicentre cohort study, we demonstrate that a decision strategy based on symptoms, c-reactive protein, haemoglobin and faecal calprotectin offers physicians an opportunity to reliably screen children and teenagers with abdominal pain and non-bloody diarrhoea for IBD before referring them for endoscopy. This strategy indicates with high reliability which patients are at negligible risk for IBD and therefore should not undergo endoscopy. Prompt and accurate prediction of IBD enables paediatricians to efficiently allocate resources in endoscopy units, by reassuring those with a low risk for IBD, and at the same time prioritize those with a high risk for IBD. The time saved by refraining from unnecessary endoscopies may be better used elsewhere in the health care system, such as for offering gut-directed hypnotherapy to those with functional abdominal pain.(15, 16)

**Comparison with other studies**

The outcome – IBD – identified by strategy 4 was assessed in a large group of previously undiagnosed children and teenagers presenting with persistent or recurrent non-bloody diarrhoea and abdominal pain. They represented a spectrum of patients that is commonly seen in general paediatric practice. Previous studies on calprotectin included patients with perianal symptoms or overt rectal bleeding.(3,4,17) These red flag symptoms give sufficient reasons for immediate endoscopic evaluation. Inclusion of these patients causes overestimation of the discriminating power relative to the practical situation, where a test or diagnostic strategy is necessary to distinguish those with functional abdominal pain from those with IBD who lack the red flag symptoms.

**Study limitations**

Although the estimated sensitivity of strategy 4 to predict IBD was 100%, the 95% confidence interval suggests that IBD may occasionally be missed. Performing careful physical and laboratory examinations and arranging for follow-up will protect the patient from the sequelae of missing a case. For children and teenagers who are categorized as “low-risk” patients, but whose abdominal pain and non-bloody diarrhoea have not improved after one month, we recommend to repeat the faecal calprotectin test.

We did not demonstrate yet that following diagnostic strategy 4 has an impact on actual clinical practice. A randomised controlled trial is necessary to measure the impact of

applying the decision strategy in a clinical setting in terms of patient outcome, health professionals' behaviour, and resource use.

In this study we used the enzyme-linked immunosorbent assay of one manufacturer. Although other test kits have an acceptable agreement in the lower range (below 250 µg/g),(18) inter-assay variability is considerable above this cut-off point. We emphasize the need for assay standardisation, but in its absence assay-specific cut-offs may improve diagnostic performance.

In the strategy with blood markers, we relied on a subgroup of commonly used laboratory data, that is CRP and Hb. We did not include erythrocyte sedimentation rate (ESR), as an inverse correlation exists between Hb and ESR that could hamper the interpretation of our statistical model. Neither did we include albumin, which is known to be abnormal in a considerable proportion of paediatric patients with severe IBD,(19,20) but was a highly unusual clinical presentation in our study cohort.

**Implications for practice**

In many decision curves there is a trade-off in net benefit when risk thresholds increase. This is hardly the case with the optimal decision strategy in this study, where the graph takes an almost horizontal course. Paediatricians can be reassured that properly evaluating children using clinical findings, CRP, haemoglobin and calprotectin is a highly accurate non-invasive approach to investigation of possible IBD in any clinical setting.

## CONCLUSION

Evaluating symptoms plus blood and stool markers in patients with non-bloody diarrhoea is the optimal test strategy that allows paediatricians to reserve a diagnostic endoscopy for children at high-risk for IBD.

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