Patient-relevant outcomes of stool testing in pediatric inflammatory bowel disease.
Heida, Anke

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TRIAGING CHILDREN WITH CHRONIC ABDOMINAL PAIN AND DIARRHEA FOR ENDOSCOPY: COMPARISON OF DIAGNOSTIC ACCURACY OF CALGRANULIN C AND CALPROTECTIN STOOL TEST


CACATU consortium

Submitted
ABSTRACT

**Background and objectives:** Calgranulin C (S100A12) is a relatively unknown fecal marker of inflammation that is potentially more specific for IBD than calprotectin, since it is only released by activated granulocytes. We compared calprotectin and calgranulin C to see which of the two markers best predicted IBD in children and teenagers with chronic abdominal pain and diarrhea.

**Methods:** We performed a multicenter prospective study in 19 pediatric clinics in the Netherlands and Belgium. Eligible patients with chronic abdominal pain and diarrhea, aged between 6 and 18 years, sent a stool sample to the coordinating laboratory for calprotectin (ELISA, BÜHLMANN Laboratories) and calgranulin C (Inflamark® ELISA, CisBio Bioassays) analysis. Patients with a high likelihood of IBD underwent upper and lower endoscopy (i.e. preferred reference test), while those with a low likelihood were followed for 6 months for latent IBD to become visible (i.e. alternative reference test). We used a Bayesian method that takes into account the two reference standards to correct for differential verification bias. Test accuracy measures were evaluated for predefined and optimal cut-off points (calculated from ROC-curve).

**Results:** We included 337 patients of which 142 underwent endoscopy and 195 clinical follow up. Eventually a total number of 93 patients were diagnosed with IBD. When common thresholds were used (calprotectin 50 µg/g; calgranulin C 0.75 µg/g) calgranulin C had better specificity (resp. 71% (95% credible interval (CI) 63-79%) vs. 97% (95% CI 94-99%)). When the optimal thresholds were used (calprotectin 400 µg/g; calgranulin C 0.75 µg/g), both tests performed equally well (specificity 98% (95% CI 95-100%) vs. 97% (95% CI 94-99)).

**Conclusions:** Both calprotectin and calgranulin C have excellent test characteristics to select children and teenagers with high likelihood for IBD for endoscopy.
INTRODUCTION

Irritable bowel syndrome (IBS) is a common disorder in children and teenagers, causing abdominal pain with a change in stool frequency and form.\textsuperscript{1,2} The majority of patients with mild complaints improve with reassurance and time, and the condition causes no permanent bowel damage. A distinct subset of patients with one or more alarm symptoms (see table 1) is difficult to distinguish from those with inflammatory bowel disease (IBD). IBD comprises two main disorders, ulcerative colitis and Crohn’s disease, which are characterized by inflammation of the gastro-intestinal tract and can cause serious complications that may lead to bowel resections. Identification of patients with low likelihood of IBD justifies a non-invasive “watchful waiting” strategy, while on the other hand a high likelihood of IBD would justify referral to specialist services for endoscopy to make a final diagnosis,\textsuperscript{3} start appropriate therapy and prevent progressive bowel damage.

**Table 1. Alarm symptoms in children and teenagers with chronic abdominal pain and diarrhea**

<table>
<thead>
<tr>
<th>Gastrointestinal blood loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent diarrhea</td>
</tr>
<tr>
<td>Family history of IBD</td>
</tr>
<tr>
<td>Arthritis</td>
</tr>
<tr>
<td>Perianal disease</td>
</tr>
<tr>
<td>Involuntary weight loss or deceleration of linear growth</td>
</tr>
<tr>
<td>Delayed puberty</td>
</tr>
</tbody>
</table>

In recent years the stool calprotectin test has been promoted as a safe and easy interpretable triage tool for endoscopy. Calprotectin is mainly released by neutrophil granulocytes, but other cells including monocytes and epithelial cells do also excrete this protein.\textsuperscript{4} To date, a calprotectin level below 50 µg/g has been proposed to rule out IBD and not to proceed to endoscopy.\textsuperscript{5,6} However, there are concerns about the mediocre specificity of the test at this threshold, which may give rise to a considerable proportion of children and teenagers proceeding to a pointless invasive procedure.

Calgranulin C is a less frequently investigated marker of intestinal inflammation that is exclusively released by activated granulocytes.\textsuperscript{4} In previous case-control studies calgranulin C showed diagnostic promise with better specificity compared to calprotectin,\textsuperscript{7–10} but large studies in a prospective cohort with chronic abdominal pain and diarrhea are lacking.
The aim of this study was to compare calprotectin and calgranulin C to see which of the two markers best predicted IBD in children and teenagers with chronic abdominal pain and diarrhea.

**METHODS**

**Design**

This was an international multicentre, prospective diagnostic accuracy study with a paired design. Previously undiagnosed children and teenagers with chronic abdominal pain and diarrhea were screened with the calprotectin stool test (existing test) and with the calgranulin C test (new test). Confirmation of the target condition (IBD) was based on endoscopy with biopsies (reference standard) or clinical follow-up (alternative reference standard). The study was registered before recruitment of the first participant, and the study protocol has been published in BMJ Open.\(^\text{11}\)

**Patients**

Patients were recruited from sixteen secondary and three tertiary level hospitals in the Netherlands and Belgium. Eligibility criteria were described previously.\(^\text{11}\) Patients were invited for participation by their attending pediatrician. Baseline characteristics, date of birth, alarm symptoms, use of non-steroidal anti-inflammatory drugs and blood tests (hemoglobin, C-reactive protein and erythrocyte sedimentation rate) were entered on the study website (www.cacatustudie.eu). A stool specimen was collected at home and sent to the hospital laboratory of the coordinating study center, where it was tested for calprotectin and colon pathogens (including *Shigatoxin-producing Escherichia coli*, *E. coli O157* gen, *Salmonella*, *Shigella/EIEC* and *Campylobacter*) and parasites (*Giardia lamblia*, *Cryptosporidium spp*, *Dientamoeba fragilis* and *Entamoebe histolytica*) with the real-time multiplex PCR. One tube was stored at \(-80^\circ\text{C}\) for calgranulin C batch testing at a later stage.

**Assays**

Stool calprotectin levels (μg/g) were measured with the fCAL ELISA (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland), and stool calgranulin C levels (μg/g) with the commercially available Inflamark ELISA (CisBio Bioassays, Codolet, France), both on a Dynex DS2 Automated ELISA System (Alpha Labs, Easleigh, UK) in the same laboratory. The extraction and measuring technique of calgranulin C was previously described in detail.\(^\text{10}\) In discordant pairs (i.e. increased calprotectin and normal calgranulin C, or vice versa) we did a posthoc analysis of potential viral causes (adeno-, entero-, astro-, rota,
noro-, parecho- and sapovirus). Laboratory technicians were blinded for symptoms filled in on the website. The attending pediatricians were informed of the calprotectin and PCR result for bacteria and parasites, but they were blinded for the calgranulin C and PCR result for viruses. Calprotectin levels ≤50 µg/g and calgranulin levels ≤0.75 µg/g were considered normal.10

Reference tests
The attending pediatricians risk-stratified their patients for IBD using alarm symptoms, blood tests, stool calprotectin and PCR results. In general, children and teenagers with increased calprotectin levels without colon pathogens proceeded to endoscopy (the preferred reference standard) for verification of IBD. Patients with a normal stool calprotectin test were considered to have a low probability of IBD and it was regarded as unethical to expose this specific group to endoscopy. Instead, these patients were followed up clinically for possible latent IBD to become visible (the alternative reference standard). Pediatricians were free to use any other diagnostic test, such as celiac disease screening, breath test or ultrasonography (whichever was deemed suitable).

Endoscopy was performed under general anesthesia by an experienced pediatric gastroenterologist in one of six participating centers. Both upper and lower gastrointestinal tract were evaluated according to the revised Porto criteria,3 and biopsies were taken from every bowel segment. Histopathological examination was performed by experienced histopathologists. Endoscopists and histopathologists had access to clinical information and calprotectin results, but were blinded for the results of the calgranulin C test.

In case the alternative reference standard was used, the patient was evaluated for the presence of new alarm symptoms and calprotectin six months after inclusion. Blood tests were repeated when the pediatrician indicated this as clinically useful. If these results increased the probability of IBD, endoscopy was performed in second instance.

Statistical analysis
Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 22.0 for Windows (Chicago, Illinois, USA)) and presented with GraphPad Prism (version 5 for Windows (San Diego, California, USA)). Diagnostic accuracy measures (sensitivity, specificity, positive predictive value, negative predictive value) were calculated for both the high-risk and low-risk stratum using predefined thresholds, as well as optimal thresholds (defined as the most upper left data point in the receiver operating characteristic (ROC) curve). Since it is methodologically incorrect to combine
the results of the two reference standards in the traditional (‘frequentist’) statistical method, we used a Bayesian correction method to adjust for differential verification bias.12–14 We assumed that endoscopy had 95–100% sensitivity and 95–100% specificity to diagnose IBD, and that the alternative reference standard (clinical follow-up) had a sensitivity of 80–100% and a specificity of 60–80% to diagnose latent IBD. Our interferences are based on the posterior distributions calculated using JAGS ('Just Another Gibbs Sampler'), a free program licensed under GNU General Public License.15 The R-package script is provided in supplementary data 2. The sample size calculation was previously described.11

**Human Patients Protection**

This study was performed according to the Declaration of Helsinki. This study was conducted with the approval of the Medical Ethical Committee of the University Medical Center in Groningen and Antwerp University Hospital. All participants aged 12 and above and their legal guardians gave informed consent to use data generated by routine medical care. The data were collected and recorded by the investigators in such a manner that subjects could not be identified, directly or through identifiers linked to the subjects.

![Figure 1: Study flow diagram](image)
RESULTS

A total number of 337 children and teenagers with chronic abdominal pain and diarrhea sent in a stool specimen between September 2014 and September 2016. Of those, 142 proceeded to endoscopy in first instance, while 195 were followed for 6 months for the appearance of additional alarm symptoms or an increase in stool calprotectin level. In second instance, nineteen children from the low-risk group were referred for endoscopy. Eventually a total of 93 patients (27.6%) were diagnosed with IBD. The patient study flow is shown in figure 1. Baseline characteristics are presented in table 2. The group of patients with a high risk of IBD were older, had more alarm symptoms and higher calprotectin levels than those with a low risk of IBD. The distributions of calprotectin and calgranulin C values per diagnosis are shown in figure 2.

Figure 2: Box- and whisker plot for calprotectin (A) and calgranulin C (B) levels per diagnosis. Whiskers represent the 95% confidence interval. Number of cases in brackets.
Table 2: Baseline characteristics of patients with chronic gastrointestinal symptoms classified as “high risk” or “low risk” for inflammatory bowel disease. Values are number (%) unless otherwise stated.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>High risk (N=142)</th>
<th>Low risk (N=195)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choice of reference test</td>
<td>Endoscopy</td>
<td>Clinical follow up</td>
<td></td>
</tr>
<tr>
<td>Median age in years (interquartile range)</td>
<td>14 (11-15)</td>
<td>12 (9-14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender</td>
<td>67 (47%)</td>
<td>112 (57%)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Alarm symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent diarrhea for more than 4 weeks</td>
<td>77 (54%)</td>
<td>60 (31%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rectal blood loss</td>
<td>90 (63%)</td>
<td>32 (16%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peri-anal disease</td>
<td>20 (14%)</td>
<td>8 (4%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Involuntary weight loss</td>
<td>52 (37%)</td>
<td>47 (24%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Extra-intestinal symptoms (including arthritis)</td>
<td>20 (14%)</td>
<td>13 (7%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Family history of IBD</td>
<td>12 (9%)</td>
<td>18 (9%)</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Blood tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia (hemoglobin &lt;2 SD for age and gender)</td>
<td>56 (39%)</td>
<td>19 (10%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Increased erythrocyte sedimentation rate or C-reactive protein (resp. &gt;20 mm/hour or &gt;10 mg/L)</td>
<td>58 (41%)</td>
<td>10 (5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Stool tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased calprotectin (&gt;50 μg/g)</td>
<td>125 (88%)</td>
<td>76 (39%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Predefined thresholds**

Table 3 shows the diagnostic accuracy measures based on predefined thresholds for calprotectin (50 μg/g) and calgranulin C (0.75 μg/g). We calculated these measures per risk group (i.e. per reference standard) with both the ‘frequentist’ and the ‘Bayesian’ method. The overall diagnostic accuracy measures were, irrespective of what reference standard was used, calculated with the Bayesian correction method. In this analysis calgranulin C had significantly better specificity (97.3% (95% credible interval (CI): 94.1 to 99.4) vs. 71.3% (CI: 63.3 to 79.0)) and better positive predictive value (92.7% (CI: 84.6 to 98.4) vs. 72.7% (CI: 63.8 to 81.0). A graphical representation of these differences is shown in figure 3a.
Table 3: Diagnostic accuracy measures of the calprotectin and calgranulin C test to diagnose IBD in children using predefined thresholds (respectively 50 µg/g and 0.75 µg/g) and optimal thresholds (respectively 400 µg/g and 0.75 µg/g).

<table>
<thead>
<tr>
<th>Patient spectrum</th>
<th>Diagnostic accuracy characteristics</th>
<th>Calprotectin (50 µg/g)</th>
<th>Calgranulin C (0.75 µg/g)</th>
<th>Calprotectine (400 µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>‘Frequentist’ method</td>
<td>‘Bayesian’ method</td>
<td>‘Frequentist’ method</td>
<td>‘Bayesian’ method</td>
</tr>
<tr>
<td>High risk¹</td>
<td>Sensitivity</td>
<td>100 (96.0-100)</td>
<td>97.4 (94.1-99.1)</td>
<td>86.7 (77.9-92.9)</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>32.7 (20.3-47.1)</td>
<td>69.9 (62.2-77.1)</td>
<td>86.5 (74.2-94.4)</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>100 (100-100)</td>
<td>97.2 (93.7-99.0)</td>
<td>79.0 (68.7-86.5)</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>72.0 (68.0-75.7)</td>
<td>71.6 (63.2-79.0)</td>
<td>91.8 (84.8-95.7)</td>
</tr>
<tr>
<td>Low risk²</td>
<td>Sensitivity</td>
<td>100 (29.2-100)</td>
<td>88.7 (83.8-92.5)</td>
<td>66.7 (9.4-99.2)</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>62.0 (54.7-68.9)</td>
<td>65.3 (56.5-73.8)</td>
<td>96.9 (93.3-98.8)</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>100 (100-100)</td>
<td>87.2 (82.2-91.3)</td>
<td>99.5 (97.4-99.9)</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>4.0 (3.3-4.7)</td>
<td>68.5 (57.5-77.8)</td>
<td>25 (9.8-50.6)</td>
</tr>
<tr>
<td>Complete cohort³</td>
<td>Sensitivity</td>
<td>-</td>
<td>99.5 (97.2-100)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
<td>-</td>
<td>71.3 (63.3-79.0)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NPV</td>
<td>-</td>
<td>99.4 (97.0-100)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PPV</td>
<td>-</td>
<td>72.7 (63.8-81.0)</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

¹ verification with endoscopy; ² verification by follow up; ³ verification with either reference test.

Estimates calculated with the frequentist statistical method are shown with the 95% confidence interval, estimates calculated with the Bayesian statistical method with the 95% credible interval.
Optimal (ROC-based) thresholds

The optimal (ROC-based) threshold for calprotectin was 400 μg/g. The corresponding diagnostic accuracy measures are shown in the right-column of table 3. The optimal threshold of calgranulin C was equal to the pre-defined threshold (0.75 μg/g). The difference in specificity and positive predictive value disappeared when optimal thresholds were compared. A graphical representation of the equivalence between calprotectin and calgranulin C for the complete study cohort (verified with either reference test) is shown in figure 3b.

Markers of intestinal inflammation versus PCR results

Bacterial and parasitic microorganisms were detected with real-time multiplex PCR in the stool of 142 of 337 (42%) patients. Dientamoeba fragilis, whose potential pathogenicity has increasingly been questioned,\textsuperscript{16–18} was the most prevalent microorganism. The distributions of calprotectin and calgranulin C values per microorganism are shown in table 4. Forty-one of 141 patients (29%) with a positive stool PCR for bacteria or parasites fulfilled the criteria to proceed to endoscopy in first or second instance. Twenty-two were ultimately diagnosed with IBD.

Concordant vs. discordant pairs

Figure 4 shows that 306 of 337 pairs of calprotectin and calgranulin C results were concordant (91%). Discordant pairs (9%) are described in detail in supplementary table 1. Thirteen children with a discordant result were diagnosed with IBD. Two cases were missed with the calprotectin test (threshold 400 μg/g) and 11 cases were missed with the calgranulin C test (threshold 0.75 μg/g).
Table 4: Presence of bacterial and parasitic microorganisms detected in the stool of 142 patients with real-time multiplex PCR, and categorized per diagnosis.

|                      | IBD                                                                 | Non-IBD                                                               |
|----------------------|----------------------------------------------------------------------|                                                                      |
|                      | Number of cases | Calprotectin (µg/g) | Calgranulin C (µg/g) | Number of cases | Calprotectin (µg/g) | Calgranulin C (µg/g) |
| Dientamoeba Fragilis | 15             | 1800 (1340-3760)    | 2.85 (1.03-26.43)    | 96              | 40 (40-60)           | 0.22 (0.22-0.22)    |
| Shigatoxin producing E. Coli | 6 | 1285 (494-1868) | 8.43 (0.73-10.54)    | 12              | 79 (40-480)          | 0.22 (0.22-0.22)    |
| Giardia Lamblia    | 0               | -                   | -                     | 7               | 100 (45-900)         | 0.24 (0.22-0.99)    |
| Salmonella         | 0               | -                   | -                     | 1               | 355                  | 0.22                 |
| Campylobacter      | 1               | 4920                | 38.53                 | 3               | 1910 (120-)          | 1.78 (0.23-)        |

Values of calprotectin and calgranulin C are median (interquartile range)

Figure 4: Scatter plot showing concordant and discordant pairs of calprotectin and calgranulin C measurements. The broken lines represent the ROC-based optimal thresholds for calprotectin (400 µg/g) and calgranulin C (0.75 µg/g). White fields represent concordant pairs (91%), grey fields represent discordant pairs (9%).

DISCUSSION

The clinical presentation of pediatric IBD is often nonspecific and overlaps with IBS. Early differentiation is important to avoid delay in proceeding to endoscopy on the one hand and to avoid unnecessary invasive procedures on the other. The mere existence of this trade-off means that a noninvasive and highly discriminative test is needed. We compared the calprotectin and calgranulin C stool test to see which of the two markers best predicted IBD in children and postulated that the latter probably had better specificity. In the largest pediatric diagnostic accuracy study ever on markers
of intestinal inflammation we show that calgranulin C has better specificity for IBD than calprotectin, provided the use of common thresholds. When optimal (ROC-based) thresholds are used (i.e. calprotectin 400 µg/g; calgranulin C 0.75 µg/g), both tests have exceptionally high sensitivity and specificity to diagnose IBD.

**Comparison with existing literature**

Well-designed studies on the discriminative power of calgranulin C are scarce. An Australian research team previously reported on a study comparing calprotectin and calgranulin C.⁸ They obtained stool samples from 61 children (2-16 years old) who presented with gastrointestinal symptoms prior to admission for gastrointestinal endoscopy. The predefined threshold used for calgranulin C in their study cohort (10 µg/g) was substantially higher than the one we used (0.75 µg/g).⁷,¹⁰ The difference is likely to be explained by differences in assays and selection of patients. We included a fair amount of patients that did not proceed to endoscopy, which increases the applicability of our results for populations seen in non-specialized centers. An important methodological flaw in the Australian study was the omission of a fair comparison of optimal thresholds for both markers, which may have resulted in an overinterpretation of calgranulin C test accuracy. Several recently published meta-analyses have shown that the calprotectin stool test has good negative predictive (“rules out”) value at the commonly used threshold (50 µg/g).⁵,¹⁹-²¹ A large share of the studies included in these meta-analyses had a case control design which gives rise to spectrum bias and overestimation of test accuracy relative to the real-life practice.²² We avoided spectrum bias and therefore expected to find more modest accuracy measures than previously reported. Contrary to our expectations, we found that the good ‘rule-out’ value of calprotectin still holds in a heterogeneous study population with chronic abdominal pain and diarrhea. Likewise, the specificity of the calprotectin test in our study population (71%) was comparable with previously reported values.

**Strengths and limitations**

This large multicenter cross-border accuracy study better reflects ‘real-life’ practice than other previously published study on stool tests for screening and selecting children for endoscopy. Decisions to proceed to endoscopy were based on a combination of symptoms, blood and stool tests. The cooperation of both secondary and tertiary level hospitals in this study promotes the generalizability of our results and conclusions. The downside of this ‘real-life’ design was that the attending pediatricians were not blinded for the calprotectin results, but, on the contrary, partly based their decision to proceed
to endoscopy on these results. This diagnostic work-up bias (syn. sequential-ordering bias) is usually the case in screening studies where only patients with a positive index test result move on to the reference standard. We minimized this bias by following the low-risk patients for 6 months for possible latent IBD to become visible. One might argue that this observation period was not sufficiently long, but we are confident that the majority of initially missed cases with IBD would become apparent within this time.

**Clinical implications**

Both calprotectin and calgranulin C have excellent test characteristics to select children and teenagers with high likelihood for IBD for endoscopy, but previously reported thresholds need to be adjusted. We observed that many pediatricians participating in this study already took into consideration that the commonly used threshold (50 μg/g) leads to many false positives. Figure 5 shows that only 33% of patients with calprotectin levels between 50 and 400 μg/g were referred to specialist services for endoscopy.

This observation underlines the relevance of a so-called ‘two-threshold strategy’ as proposed in several publications. The grey zone between the commonly used threshold of 50 μg/g to demarcate the normal range, and 400 μg/g as the action threshold (see figure 2A and 5) gives room for shared decision making with the patient and his/her parents, in which severity of symptoms and impact on daily functioning of the child may additionally guide management. One can opt for watchful waiting with monthly monitoring of stool calprotectin or decide to move on to endoscopic evaluation. When calprotectin levels are truly out of range (>400 μg/g), and gastrointestinal infections and non-steroidal anti-inflammatory drug use are excluded, the patient should proceed to endoscopy to rule in IBD. A two-threshold strategy does not seem to be of added value when the calgranulin C stool test is picked as the triaging tool of preference. Despite the
undeniable evidence that stool markers are of great help to distinguish children with IBD from those with IBS, physicians should take note that different patient populations and different test assays may lead to variations in thresholds.\textsuperscript{23,26}

**CONCLUSIONS**

Measuring calprotectin or calgranulin C levels in stool is a useful triage tool for identifying patients who are most likely to need endoscopy for suspected inflammatory bowel disease. The discriminative power to safely exclude the disease (specificity) is significantly better than previously reported. When the optimal ROCs based thresholds are used (calprotectin 400 µg/g; calgranulin C 0.75 µg/g), both tests perform equally well in secondary and tertiary level hospitals.
REFERENCES


Supplementary data 1: ROC curves of calprotectin (red dots) and calgranulin C (blue dots).
Chapter 5

Supplementary data 2: JAGS and R script (available online)

Supplementary data 3: Detailed description of discordant pairs of calprotectin and calgranulin C results (n=31).

### DISCORDANT RESULTS LOW CALPROTECTIN - HIGH CALGRANULIN C

<table>
<thead>
<tr>
<th>Calprotectin (µg/g)</th>
<th>Calgranulin C (µg/g)</th>
<th>Bacterial and parasite PCR</th>
<th>Risk-stratification IBD</th>
<th>Diagnosis</th>
<th>Post hoc viral PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>315</td>
<td>1.22</td>
<td>negative</td>
<td>High</td>
<td>Crohn's disease</td>
<td>negative</td>
</tr>
<tr>
<td>340</td>
<td>1.03</td>
<td>D. Fragilis</td>
<td>High</td>
<td>Crohn's disease</td>
<td>negative</td>
</tr>
<tr>
<td>150</td>
<td>1.06</td>
<td>Giardia Lamblia</td>
<td>High</td>
<td>GI-infection</td>
<td>negative</td>
</tr>
<tr>
<td>165</td>
<td>3.17</td>
<td>D. Fragilis</td>
<td>High</td>
<td>GI-infection</td>
<td>negative</td>
</tr>
<tr>
<td>105</td>
<td>1.98</td>
<td>negative</td>
<td>High</td>
<td>Irritable bowel syndrome</td>
<td>negative</td>
</tr>
<tr>
<td>220</td>
<td>1.11</td>
<td>negative</td>
<td>High</td>
<td>Irritable bowel syndrome</td>
<td>negative</td>
</tr>
<tr>
<td>255</td>
<td>1.82</td>
<td>D. Fragilis</td>
<td>Low</td>
<td>Irritable bowel syndrome</td>
<td>negative</td>
</tr>
<tr>
<td>40</td>
<td>8.04</td>
<td>negative</td>
<td>Low</td>
<td>Hemolytic uremic syndrome</td>
<td>negative</td>
</tr>
<tr>
<td>345</td>
<td>1.62</td>
<td>D. Fragilis</td>
<td>High</td>
<td>Solitary rectal ulcer</td>
<td>negative</td>
</tr>
</tbody>
</table>

### DISCORDANT RESULTS HIGH CALPROTECTIN - LOW CALGRANULIN C

<table>
<thead>
<tr>
<th>Calprotectin (µg/g)</th>
<th>Calgranulin C (µg/g)</th>
<th>Bacterial and parasite PCR</th>
<th>Risk-stratification IBD</th>
<th>Diagnosis</th>
<th>Post hoc viral PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>480</td>
<td>0.42</td>
<td>D. Fragilis</td>
<td>High</td>
<td>Ulcerative colitis</td>
<td>negative</td>
</tr>
<tr>
<td>510</td>
<td>0.23</td>
<td>Shigatoxin producing E. Coli</td>
<td>High</td>
<td>Crohn's disease</td>
<td>negative</td>
</tr>
<tr>
<td>855</td>
<td>0.49</td>
<td>negative</td>
<td>High</td>
<td>Crohn's disease</td>
<td>negative</td>
</tr>
<tr>
<td>980</td>
<td>0.24</td>
<td>negative</td>
<td>High</td>
<td>Crohn's disease</td>
<td>negative</td>
</tr>
<tr>
<td>1170</td>
<td>0.54</td>
<td>negative</td>
<td>High</td>
<td>Ulcerative colitis</td>
<td>negative</td>
</tr>
<tr>
<td>1270</td>
<td>0.33</td>
<td>negative</td>
<td>High</td>
<td>Crohn's disease</td>
<td>negative</td>
</tr>
<tr>
<td>1280</td>
<td>0.22</td>
<td>negative</td>
<td>High</td>
<td>Crohn's disease</td>
<td>negative</td>
</tr>
<tr>
<td>1410</td>
<td>0.22</td>
<td>D. Fragilis</td>
<td>High</td>
<td>Ulcerative colitis</td>
<td>negative</td>
</tr>
<tr>
<td>1440</td>
<td>0.22</td>
<td>negative</td>
<td>High</td>
<td>Crohn's disease</td>
<td>negative</td>
</tr>
<tr>
<td>1660</td>
<td>0.26</td>
<td>negative</td>
<td>High</td>
<td>Crohn's disease</td>
<td>negative</td>
</tr>
</tbody>
</table>
## Head-to-head comparison Calgranulin C and Calprotectin

<table>
<thead>
<tr>
<th>Calprotectin (µg/g)</th>
<th>Calgranulin C (µg/g)</th>
<th>Bacterial and parasite PCR</th>
<th>Risk-stratification IBD</th>
<th>Diagnosis</th>
<th>Post hoc viral PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>2080</td>
<td>0.68</td>
<td>negative</td>
<td>High</td>
<td>Crohn’s disease</td>
<td>negative</td>
</tr>
<tr>
<td>645</td>
<td>0.22</td>
<td>Shigatoxin producing E. Coli</td>
<td>High</td>
<td>Crohn’s disease</td>
<td>negative</td>
</tr>
<tr>
<td>1120</td>
<td>0.53</td>
<td>negative</td>
<td>High</td>
<td>GI-infection</td>
<td>negative</td>
</tr>
<tr>
<td>1370</td>
<td>0.24</td>
<td>Giardia Lamblia</td>
<td>Low</td>
<td>GI-infection</td>
<td>negative</td>
</tr>
<tr>
<td>1380</td>
<td>0.22</td>
<td>Shigatoxin producing E. Coli</td>
<td>Low</td>
<td>GI-infection</td>
<td>negative</td>
</tr>
<tr>
<td>2550</td>
<td>0.22</td>
<td>negative</td>
<td>Low</td>
<td>GI-infection</td>
<td>negative</td>
</tr>
<tr>
<td>580</td>
<td>0.22</td>
<td>negative</td>
<td>Low</td>
<td>Allergy</td>
<td>negative</td>
</tr>
<tr>
<td>585</td>
<td>0.58</td>
<td>Shigatoxin producing E. Coli, D. Fragilis,</td>
<td>High</td>
<td>Irritable bowel syndrome</td>
<td>Parechovirus</td>
</tr>
<tr>
<td>1160</td>
<td>0.22</td>
<td>D. Fragilis</td>
<td>High</td>
<td>Irritable bowel syndrome</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>685</td>
<td>0.22</td>
<td>negative</td>
<td>Low</td>
<td>Refluxoesophagitis</td>
<td>Adenovirus</td>
</tr>
<tr>
<td>1150</td>
<td>0.22</td>
<td>negative</td>
<td>High</td>
<td>No abnormalities</td>
<td>Norovirus</td>
</tr>
<tr>
<td>2510</td>
<td>0.24</td>
<td>D. Fragilis</td>
<td>Low</td>
<td>Spontaneous recovery of symptoms</td>
<td>Norovirus</td>
</tr>
</tbody>
</table>
PART II: MONITORING