Diagnostic performance of rapid tests for detection of fecal calprotectin and lactoferrin and their ability to discriminate inflammatory from irritable bowel syndrome

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Abstract

Background: Ruling out somatic bowel disease, such as inflammatory bowel disease (IBD), is an important goal in the management of abdominal complaints. Endoscopy is commonly used but is invasive and expensive. Mucosal inflammation in IBD can be detected through fecal biomarkers, though the present enzyme-linked immunoabsorbent assay (ELISA) tests require laboratory facilities. We validated the diagnostic performance of two new fecal rapid tests (FRTs) for the detection of calprotectin and lactoferrin and assessed their potential to differentiate IBD from irritable bowel syndrome (IBS).

Methods: The calprotectin and lactoferrin FRTs and ELISA tests were performed on the fecal samples of 114 patients referred for endoscopy, 80% of whom had IBS and 20% IBD, and validated against the endoscopic diagnosis.

Results: The sensitivity and negative predictive value of the calprotectin FRT were both 100%, whereas they were 78% and 95%, respectively, for the lactoferrin FRT. The specificity and positive predictive value were slightly higher for the lactoferrin FRT. Both FRTs had similar diagnostic accuracy as the corresponding ELISA tests.

Conclusions: The calprotectin and lactoferrin rapid tests are as good as the ELISA tests in detecting colonic inflammation. Given their simple use, FRTs can support the non-invasive exclusion of IBD, notably in primary care.

Keywords: biomarkers; enzyme-linked immunosorbent assay (ELISA); fecal calprotectin; fecal lactoferrin; inflammatory bowel disease; irritable bowel syndrome.

Introduction

Chronic abdominal complaints are highly prevalent in primary care practice. In the majority of patients, they have a functional background. Irritable bowel syndrome (IBS) is the most prevalent functional intestinal condition. Most patients with IBS can be managed adequately in primary care. Despite expert based diagnostic criteria, such as the Rome criteria, it remains difficult to differentiate IBS from organic and more severe colonic abnormalities, such as inflammatory bowel disease (IBD) and even colon carcinoma, using symptoms and signs only. Existing diagnostic items are insufficient to rule out organic disorders due to a substantial overlap in symptomatology and other test results with non-organic or functional diseases (1). Hence, at least 20% of patients with chronic abdominal complaints are still referred to hospital for invasive tests, such as endoscopy, to rule out organic disorders (2). In approximately 70% of these referred patients, no severe abnormalities are found at endoscopy (3). Thus, endoscopy is not indicated in all primary care patients with chronic abdominal complaints. Less invasive and cheaper diagnostic tests that could more effectively rule out IBD in primary care patients with chronic abdominal complaints are needed to reduce the number of unnecessary referrals for endoscopy.

Calprotectin and lactoferrin are degradation products of neutrophil granulocytes in the mucosal layer of the colon. Neutrophil granulocytes play an important role in the inflammation process in IBD. Patients suffering from IBD have a larger intestinal permeability than healthy individuals, resulting in an increased transport of neutrophil granulocytes into the gut (4, 5). Granulocytes reaching the lumen lead to apoptosis, releasing lactoferrin and calprotectin into the gut (6). This explains the increased concentration of calprotectin and lactoferrin in the feces in the case of inflammation. Both calprotectin and lactoferrin resist enzymatic degradation, in vivo and in vitro, and show a high stability in feces (more than 1 week at room
temperature) (7–10). Bowel inflammation can thus be diagnosed by measuring calprotectin and lactoferrin in fecal samples, using enzyme-linked immunosorbent assay (ELISA) methods in the laboratory. Several studies have demonstrated significantly different concentrations of both biomarkers in the feces of patients with IBD and IBS (11–13). Increased fecal concentrations of calprotectin and lactoferrin after exacerbation of Crohn’s disease and colitis ulcerosa can also be measured by ELISA methods, as well as mucosal healing after the start of treatment for IBD (4, 14–18).

Unfortunately, ELISA methods detecting fecal biomarkers are relatively time consuming and expensive. They require laboratory facilities and cannot be performed in the general practitioner’s office. Recently, two new point-of-care or fecal rapid tests (FRTs) for calprotectin and lactoferrin have been developed that can support non-invasive differentiation of colonic inflammation from other (functional) diagnoses in primary care patients with chronic abdominal complaints.

Our aim was to evaluate the diagnostic accuracy of these two new rapid calprotectin and lactoferrin fecal tests in assessing colonic inflammation in patients with chronic abdominal complaints. In addition, the association of the results of the two rapid tests is compared with the results of the calprotectin and lactoferrin ELISA tests.

Patients and methods

Design and patients

This diagnostic study involved a cross-sectional design. Consecutive patients with lower gastro-intestinal abdominal complaints, including bloating, change in defecation frequency or consistency, or blood and mucus in the feces, referred for endoscopy or sigmoidoscopy to the endoscopy unit of the Gelderse Vallei Hospital, a major regional hospital in The Netherlands, were included in the study. Patients younger than 18 years, patients with a history of colonic surgery and those with iron deficiency were excluded from the study. All patients referred between May and June 2007 who met the inclusion criteria were invited by phone to participate in the study. After consenting, participants were sent a fecal sample tube and an information leaflet on how to collect and store the sample. On the day of endoscopy, patients returned their fecal sample and informed consent form. FRTs were performed at the endoscopy unit before the procedure. Fecal samples were frozen for ELISA determination at a later stage. Both calprotectin and lactoferrin are reported to be stable at a temperature of –20°C for 3–6 months (19). The study protocol was approved by the local Ethical Committee.

Index tests

The calprotectin rapid test (Prevent ID®, CalDetect Preventis, Bensheim, Germany) is a semi-quantitative immunochromatographic test to detect the presence of calprotectin in feces. The test was performed according to the manufacturer’s instruction (www.preventis-online.de). Briefly, the collection stick of the sample collection device was dipped in the fecal sample and then into the extraction buffer of the sample collection device and shaken thoroughly. This was repeated once. Two drops of the diluted fecal sample were added to the calprotectin rapid test device. The diluted feces were allowed to migrate laterally, thus providing a control line indicating the test worked properly. In the presence of calprotectin, one, two or three test lines appeared, corresponding with calprotectin concentrations of <15 mg/kg, between 15 and 60, or >60 mg/kg. In line with the instructions of the manufacturer, the test was evaluated positive when at least the second test line appeared, i.e., concentration ≥15 mg/kg. The test results were interpreted after 10 min.

The lactoferrin rapid test (IBD EZ VUE®, TECHLAB, Blacksburg, VA, USA) is an immunochromatographic test for the qualitative detection of lactoferrin. According to the manufacturer’s instruction (www.techlab.com/product_details), a portion of 0.05 g feces was weighted on an analytic balance and mixed with 2.5 mL diluent (10× concentration of a buffered protein solution containing 0.2% thimerosal). Four drops of this diluted feces were added to the test device and allowed to migrate on the absorbent strip. If the test worked properly, a control line appeared. The test result could be read from the test line, which was positive at concentrations of lactoferrin ≥128 ng/mL. The results were read after 3 min.

Both FRTs were performed and interpreted by two of the authors (C.O. and L.K.) at the endoscopy department, before endoscopy.

ELISA test

Fecal calprotectin (Phical test®, CALPRO AS, Oslo, Norway) and lactoferrin (IBD-SCAN®, TECHLAB, Blacksburg, VA, USA) concentrations were also measured by ELISA methods (19, 20). Both ELISA tests were carried out by the laboratory staff of the hospital. Until analysis, the fecal samples were stored at –20°C for a maximum of 1 month. According to the manufacturer’s cut-off value, the ELISA calprotectin test was considered positive if the concentration was higher than 50 mg/kg, and for the ELISA lactoferrin test if the concentration was higher than 7.25 mg/mL. The two rapid tests and the two ELISA tests were all performed and interpreted without knowledge of the other test results, and blinded for patient history, physical examination and endoscopy results.

Diagnostic outcome (reference standard)

The outcome of the present study was defined as the presence or absence of IBD. To this aim, all patients underwent colonoscopy or sigmoidoscopy according to routine procedure, performed by experienced gastroenterologists (more than 700 colonoscopies annually, cecal intubation rate 97%) at the endoscopy department. According to routine clinical practice, the diagnosis was based on the endoscopic picture, biopsies were taken if necessary to confirm the diagnosis. The endoscopists were blinded for the results of the rapid tests and ELISAs.

Statistical analysis

We calculated for both rapid tests the sensitivity, specificity, positive and negative predictive values (PPVs and NPVs), and positive and negative likelihood ratios (LR+ and LR–) with 95% confidence intervals for the detection of IBD, in comparison with the result of the colonoscopy or sigmoidoscopy. For the calprotectin rapid test, these characteristics were calculated for the two possible cut-off points, i.e., ≥15 mg/kg and ≥60 mg/kg. This was not possible for the lactoferrin rapid test, as this is a qualitative test.

We also calculated these diagnostic accuracy parameters for the two ELISA tests using the standard applied cut-off...
points of the manufacturer, i.e., >50 mg/kg for the calprotectin ELISA test and >7.25 μg/mL for the lactoferrin ELISA test.

The correlation between the results of the ELISA tests and the two rapid tests were also assessed, using the kappa statistic.

Results

In total, 180 consecutive patients referred for colonoscopy or sigmoidoscopy by either the general practitioner (GP) (80%) or the gastroenterologist (20%) were invited to participate in the study. Of these, 36 refused participation; 144 patients (80%) were included in the study. The endoscopy procedure had to be aborted in two patients because of discomfort during the procedure. In addition, three other patients were excluded because they did not have active bowel complaints at the time of feces collection and endoscopy. From the remaining 139 patients (77%), a diagnosis at endoscopy was available. In 25 of these patients, somatic bowel disorders other than IBD were diagnosed: polyps (n = 16), cancer (n = 4, aged 64–58–61–75 years) or other (n = 5). As our aim was to study whether the rapid tests could differentiate IBD from IBS, we analyzed the 114 patients (Table 1), of whom 109 (96%) underwent colonoscopy, and five (4%) sigmoidoscopy. Of these 114 patients, 23 were diagnosed with IBD (20%) and 91 with IBS (80%). In half of the patients with IBD, biopsies were taken; six patients had Crohn’s disease, five had ulcerative colitis, and 12 had unspecified colitis.

Patients with IBS were older (mean age 52.3 years) than those with IBD (44.5 years). Of the patients with IBD, 56% presented with rectal blood loss and 87% with diarrhea, compared to 26% and 67% of patients with IBS, respectively.

The sensitivity of the calpro- tectin FRT was 100% at the cut-off point ≥15 mg/kg and 60.6% at the cut-off point ≥60 mg/kg (Table 2). The corresponding specificities were 94.5% and 97.8%, respectively. The PPVs for detecting IBD were 82.1% and 87.5% for the cut-off points ≥15 and ≥60 mg/kg, respectively, and the NPVs were 100% and 90.8%, respectively. For the lactoferrin rapid test, sensitivity was 78%, specificity 99%, and the PPV and NPV were both 94.7%.

The sensitivity value of the calprotectin and lactoferrin ELISA tests were 95.7% and 78.3%, with specificity values of 86.8% and 90.1%, respectively. The PPVs for diagnosing IBD were 64.7% for the calprotectin ELISA and 66.7% for the lactoferrin ELISA, with NPVs of 98.8% and 94.3%, respectively.

The correlation between the calprotectin and lactoferrin rapid tests showed a kappa statistic of 0.76 (Table 3), whereas this was 0.67 between the two ELISA tests. The correlation between the lactoferrin FRT and lactoferrin ELISA test was 0.68, and between the calprotectin FRT and ELISA test 0.69.

Finally, the diagnostic performance of the combination of the two FRTs at endoscopy was determined (Table 4). If both test results were positive (with calprotectin FRT at the cut-off point ≥15 mg/kg), the probability of the presence of IBD was 95% (18/19). If the calprotectin test was positive, but the lactoferrin test negative, the probability of IBD was 44% (4/9). There were no patients with a positive lactoferrin test and negative calprotectin test, and if both tests were negative, the probability of IBD was also 0%. If either

Table 1 Patients referred for lower gastrointestinal endoscopy (n = 114).

<table>
<thead>
<tr>
<th>n = 114</th>
<th>IBS</th>
<th>IBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>91</td>
<td>23</td>
</tr>
<tr>
<td>Age (mean), years</td>
<td>52.3</td>
<td>44.5</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>49 (53.8)</td>
<td>12 (52.2)</td>
</tr>
<tr>
<td>Symptomatology (n = 113)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectal blood loss</td>
<td>24 (26.4)</td>
<td>13 (55.8)</td>
</tr>
<tr>
<td>Constipation</td>
<td>51 (56.0)</td>
<td>8 (34.8)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>61 (67.0)</td>
<td>20 (87.0)</td>
</tr>
<tr>
<td>Pain</td>
<td>68 (74.7)</td>
<td>17 (73.9)</td>
</tr>
<tr>
<td>Bloating</td>
<td>49 (53.8)</td>
<td>16 (69.6)</td>
</tr>
</tbody>
</table>

Patients’ characteristics and presenting symptoms are in absolute numbers and %.

Table 2 Diagnostic accuracy parameters (with 95% confidence intervals) of the calprotectin FRT and lactoferrin FRT and the two ELISA tests for distinguishing IBS from IBD as determined by colonoscopy or sigmoidoscopy (n = 114 patients).

<table>
<thead>
<tr>
<th>Calprotectin FRT</th>
<th>Calprotectin FRT</th>
<th>Lactoferrin FRT</th>
<th>ELISA calprotectin</th>
<th>ELISA lactoferrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity, %</td>
<td>94.5 (78.1–98.0)</td>
<td>97.8 (91.5–99.6)</td>
<td>99.0 (93.0–99.9)</td>
<td>86.3 (77.7–92.7)</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>100 (78.1–98.0)</td>
<td>80.6 (38.8–79.5)</td>
<td>78.0 (65.0–82.0)</td>
<td>95.7 (76.0–99.8)</td>
</tr>
<tr>
<td>PPV, %</td>
<td>82.1 (62.4–93.2)</td>
<td>87.5 (60.4–97.8)</td>
<td>94.7 (71.9–99.7)</td>
<td>64.7 (46.5–89.0)</td>
</tr>
<tr>
<td>NPV, %</td>
<td>100 (94.7–100)</td>
<td>90.8 (82.9–95.5)</td>
<td>94.7 (87.6–98.0)</td>
<td>98.8 (92.3–99.9)</td>
</tr>
<tr>
<td>LR+</td>
<td>18.2 (7.7–42.7)</td>
<td>27.7 (6.7–113.3)</td>
<td>75.1 (9.5–534.2)</td>
<td>7.3 (4.3–12.4)</td>
</tr>
<tr>
<td>LR−</td>
<td>0</td>
<td>0.4 (0.2–0.7)</td>
<td>0.2 (0.1–0.5)</td>
<td>0.5 (0.007–0.8)</td>
</tr>
</tbody>
</table>

Table 3 Correlation between the calprotectin and lactoferrin FRT and ELISA test results (n = 114 patients).

<table>
<thead>
<tr>
<th>Calprotectin FRT vs. lactoferrin FRT</th>
<th>Calprotectin ELISA vs. lactoferrin ELISA</th>
<th>Calprotectin FRT vs. calprotectin ELISA</th>
<th>Lactoferrin FRT vs. lactoferrin ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohen’s kappa</td>
<td>0.76</td>
<td>0.67</td>
<td>0.69</td>
</tr>
</tbody>
</table>
one of the two tests was positive, the probability of the presence of IBD was 79% (22/28), and if either one of the two tests was negative, the presence of IBD was 4% (4/95).

Discussion

Recently introduced rapid tests for the detection of calprotectin and lactoferrin in fecal samples have a good diagnostic performance to differentiate patients with IBD from those with IBS, which is at least comparable to that of the ELISA tests. The calprotectin rapid test (at the cut-off value of ≥ 15 mg/kg) seems to have better diagnostic accuracy than the lactoferrin rapid test, notably to rule out IBD, viewing the higher sensitivity and NPV and the lower LR−. Addition of the lactoferrin rapid test result did not improve the exclusion of IBD; in our study, a negative calprotectin test correctly ruled out IBD already.

The mission of the GP is to select those patients who require specialist attention because of a high risk of serious disease. If the GP performs this gatekeeper function adequately, the referred population will have a high risk for severe gastrointestinal (GI) disease, while the risk in the non-referred group will be below average. Ruling out IBD is the most important diagnostic goal in patients with non-alarming abdominal complaints, especially in the case of diarrhea predominant complaints. A fecal biomarker test can support the GP in this selection process, as in many countries the primary care physician is the first to encounter patients with these symptoms. The calprotectin rapid test could be particularly useful as an initial test to rule out IBD in subjects with chronic lower abdominal complaints, and thus to limit the number of (unnecessary) referrals for endoscopy.

To our knowledge, this is the first study to quantify the accuracy of the rapid fecal tests for discriminating IBD (usually diagnosed with endoscopy) from IBS. We specifically included patients referred for endoscopy for suspicion of presence of IBD, to determine to what extent the tests can discriminate between IBD and IBS. Accordingly, we also included in this initial study patients referred for endoscopy by the gastroenterologist. A substantial number of the patients referred for endoscopy by their GP had rectal blood loss as part of their abdominal complaints. Rectal blood loss can be indicative for colorectal cancer and IBD. This was probably one of the main reasons for the GP to consider referral, even though the predictive value of rectal blood loss for colorectal cancer in primary care is low (21). Of those patients who had IBD at endoscopy, more than half had rectal blood loss as a presenting symptom. Of those who were diagnosed as having IBS after a normal endoscopy, 26.4% had rectal blood loss as a presenting symptom. Hemorrhoids, which are reported to be prevalent in 20% of patients with IBS (22), are the most likely explanation for this blood loss.

We chose a reference diagnostic workup that matches with daily clinical practice. Ileocolonoscopy was considered as reference standard, with biopsies only in the case of clinical suspicion of IBD. Patients were not routinely checked for the rarer causes of upper GI disease, such as gluten intolerance, lactose intolerance or peptic ulcer, unless this was indicated on clinical suspicion.

A strong point of our study – and in line with the STARD (Standards for reporting of diagnostic accuracy) guidelines – is that we included patients on their indication for referral for endoscopy rather than on their true presence or absence of IBD. However, the rapid tests are, as said, particularly useful in a primary care setting to be applied to all patients with non-alarming chronic colonic complaints. Given the promising results of the present study in referred patients with IBS and IBD only, we believe it is timely to quantify the accuracy and cost-effectiveness of the calprotectin test in a primary or family care population. Such research should preferably be larger to allow for a more precise estimation (i.e., with smaller confidence intervals) of the diagnostic accuracy parameters, notably the sensitivity, NPV and LR−. It is very likely that in the primary care setting the prevalence of IBD and organic disorders will be lower than in our referred population. As found for other disorders, this would potentially result in higher NPV and sensitivity, and lower LR− (23).

A number of studies have also reported changes in fecal calprotectin and lactoferrin in patients with colorectal cancer and polyps (24). In a meta-analysis, the pooled sensitivity and specificity of (non-rapid) fecal calprotectin for diagnosing colorectal neoplasia was 36% and 71%, respectively (15). Hence, subsequent studies should not only quantify the ability of these rapid tests to exclude IBD from IBS but rather on their accuracy to discriminate between organic disorders (including diverticulitis and cancer) and functional dis-

<table>
<thead>
<tr>
<th></th>
<th>IBD+ (n=23)</th>
<th>IBD− (n=91)</th>
<th>Total (n=114)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calpro FRT + and lacto FRT +</td>
<td>18 (95%)</td>
<td>1 (5%)</td>
<td>19</td>
</tr>
<tr>
<td>Calpro FRT + and lacto FRT −</td>
<td>4 (44%)</td>
<td>5 (56%)</td>
<td>9</td>
</tr>
<tr>
<td>Calpro FRT– and lacto FRT +</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Calpro FRT– and lacto FRT–</td>
<td>0 (0%)</td>
<td>86 (100%)</td>
<td>86</td>
</tr>
<tr>
<td>At least one of the two FRTs positive</td>
<td>22 (79%)</td>
<td>6 (21%)</td>
<td>28</td>
</tr>
<tr>
<td>At least one of the two FRTs negative</td>
<td>4 (4%)</td>
<td>91 (96%)</td>
<td>95</td>
</tr>
</tbody>
</table>

Calpro FRT, calprotectin fecal rapid test; lacto FRT, lactoferrin fecal rapid test; −, negative result; +, positive result.
orders. Many guidelines recommend an age threshold for a safe non-endoscopic diagnosis of IBS, usually >50 years (25, 26). The patients in our study who were excluded because of colorectal cancer were all older than 50 years. In subsequent studies, the diagnostic contribution of other patient characteristics (age), (blood) test results (such as fecal occult blood test) and lifestyle habits should also be taken into account.

Some studies reported factors that may influence the diagnostic capacity of biomarker tests: marginally elevated fecal calprotectin concentration in patients with physical inactivity, obesity and increasing age, a lower concentration in the case of high fiber intake and vegetable consumption and considerable day-to-day variability in some patients (27). The diagnostic value of calprotectin may be limited in patients with collagenous colitis, as up to 40% of the patients with active collagenous colitis are reported to have a normal calprotectin excretion (28).

Hence, subsequent studies should not only quantify the value of the rapid tests in isolation, but rather in combination with, e.g., the Rome III criteria, family and medical history, physical examination and blood test results (29). Such analysis could result in a multi-variable diagnostic model to differentiate between functional and somatic bowel disease without endoscopy (30).

As in all diagnostic tests, the procedure and interpretation of the FRTs need proper instruction and performance improves with experience. This could interfere with the application of the test in primary care, because the tests may be used less frequently than in a hospital setting. We experienced some minor problems with the procedure of the rapid tests. The calprotectin FRT yielded some interpretation problems, as the brightness of the test lines may vary. Although the reading of the lactoferrin FRT is easier than of the calprotectin FRT, the test procedure for calprotectin is easier, because less laboratory devices are required.

In conclusion, in this sample of patients referred for endoscopy, the recently developed fecal calprotectin and lactoferrin rapid tests have good diagnostic performance, comparable to that of the more expensive and time-consuming ELISA tests. The calprotectin test shows better performance to rule out IBD, while the lactoferrin test seems better to detect the presence of IBD. These rapid tests for fecal biomarkers have a potential role to easily and non-invasively determine the absence of IBD in patients with chronic colonic symptoms and signs that seem ideal for use in primary care. The cost-effectiveness of their use in primary care is yet unknown and is a topic for further study.

Acknowledgements

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References
