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A prospective study of fecal calprotectin and lactoferrin as predictors of small bowel Crohn's disease in patients undergoing capsule endoscopy

Ariella Bar-Gil Shitrita, Benjamin Koslowskya**, Dan M. Livovskya, David Shitrita, Kalmans Paz, Tomer Adara, Samuel N. Adlera and Eran Goldina

**Digestive Diseases Institute, Shaare Zedek Medical Center, Jerusalem, Israel; The Department of Pulmonology, Meir Medical Center, Kfar Saba, Israel

**These authors contributed equally to this study.

Abstract

Background: Capsule endoscopy (CE) is often used to investigate small bowel Crohn's disease (CD). The aim of this study is to prospectively assess the value of fecal calprotectin and lactoferrin to predict CE findings.

Patients and methods: Sixty-eight consecutive patients that were referred for CE were included. Stool samples for calprotectin and lactoferrin and blood samples were collected for relevant parameters. CE findings compatible with CD were found in 23 (33%) patients and 45 (67%) were negative for CD. The average age of the CD group was 34 compared to 46 in the non-CD group (p = .048). Median calprotectin and lactoferrin in the CD group and in the control group were 169 mg/kg vs. 40 (p = .004) and 6.6 mg/kg vs. 1 (p = .051), respectively. The area under the ROC curve was 0.767 for calprotectin and 0.70 for lactoferrin. A fecal calprotectin concentration of 95 mg/kg and fecal lactoferrin of 1.05 mg/kg had a sensitivity, specificity, positive predictive value and negative predictive value of 77 and 73%, 60 and 65%, 50 and 50%, and 84 and 84% in predicting CE findings compatible with CD.

Conclusions: Fecal markers are simple and noninvasive surrogates for predicting CE findings compatible with CD. Fecal markers can help determine which patients should be referred for CE.

ClinicalTrials.gov Identifier: NCT01266629

Introduction

Crohn's disease (CD) may affect any part of the GI tract. The small bowel is involved in up to 60% of patients.[1–3] The diagnosis of isolated small bowel CD remains a challenge, thus its true prevalence is difficult to establish and there is a paucity of literature on the matter. Data from the French EPIMED reported that 20.6% of CD patients in their cohort had 'pure' small bowel involvement.[4] Nevertheless, this number may be an underestimation since in that study the diagnosis was done by small bowel follow through X-ray and not by capsule endoscopy (CE).

CE, a novel, wireless method of investigating the small bowel uses a remote instrument that is swallowed and propelled through the gastrointestinal tract by peristalsis.[5] The capsule contains an imaging device that transmits images of the intestine to sensors placed on the abdominal wall. It can detect diffuse mucosal disease without radiation exposure. The value of CE for the investigating CD is well established based on numerous studies comparing CE to small bowel radiologic studies.[6,7] Moreover, a recent meta-analysis showed that CE is superior to small bowel radiology, CT enterography, and ileo-colonoscopy in diagnosing suspected CD.[8] Indeed do to its high sensitivity, CE is recommended by the ECCO guidelines for patients in whom the suspicion of CD remains high despite negative evaluation with other modalities.[9] However, CE is expensive and its results can be influenced by the operator's skills.

Among the available fecal biomarkers for the diagnosis and monitoring of inflammatory bowel disease (IBD), only calprotectin and lactoferrin have translated into useful clinical tools. Calprotectin (MRP8/14) is a calcium-binding heterodimer of the S100 protein family, and it is present in granulocytes, macrophages, and epithelial cells of humans and other mammals.[10] It is believed to play an essential role in immunity.[11,12] Calprotectin is released upon neutrophil/monocyte activation, where it can be detected in serum and body fluids, including stool.[13] It is poorly degraded during passage through the gastrointestinal tract and remains stable in refrigerated storage. Taken together, these features make calprotectin a potentially ideal marker of clinical inflammatory and neoplastic states.[14] Recently, several studies suggested that a high fecal calprotectin concentration might...
distinguish patients with IBD from patients with irritable bowel syndrome (IBS).[15]

Lactoferrin is an iron-binding glycoprotein secreted by most mucosal membranes and a major constituent of secondary granules of neutrophils, a component of the inflammatory response.[16,17] A number of studies have investigated the use of fecal lactoferrin as a noninvasive marker to distinguish between IBD and non-inflammatory GI conditions. Lactoferrin has been reported to be highly sensitive for active IBD compared to IBS; the distinction between inactive IBD and IBS is less clear.[18–21] Lactoferrin can be detected using simple, inexpensive techniques and it has excellent stability in feces over a long period. It has a good diagnostic precision for differentiating between organic and functional intestinal disease.

The aim of this prospective study was to assess the value of fecal calprotectin and lactoferrin in predicting findings of CD in patients undergoing CE.

Methods

The study group consisted of 68 consecutive patients referred to the Digestive Diseases Institute at Shaare Zedek Medical Center for CE for any indication from January 2013 through February 2014. All the patients had a normal colonoscopy and gastroscopy prior to CE referral.

Study protocol

After obtaining informed consent, data were collected on patient’s symptoms, including the presence of abdominal pain, weight loss, diarrhea, abdominal mass, extra-intestinal manifestations, or family history of IBD. Stool samples for calprotectin and lactoferrin as well as blood samples were obtained the day prior to the CE procedure before starting the bowel preparation.

Exclusion criteria

The patients who were using non-steroidal agents and/or antibiotics during the three months preceding the study were excluded. Additional exclusion criteria were concomitant serious illness, pregnancy, alcohol abuse, or evidence of a respiratory tract infection.

Stool analysis

Prior to CE preparation, patients were asked to collect a fresh stool specimen, which they were to store in a household refrigerator and bring with them on the day of examination. The specimens were stored at 4°C until assayed. Fecal calprotectin levels were determined with a commercially available quantitative enzyme-linked immunoassay (Calpreset; Eurospital, Trieste, Italy and IBD SCAN). Normal levels of calprotectin were defined as 25 mg/kg stool. Fecal lactoferrin test was performed on each sample, as previously described.[22] We were unable to perform lactoferrin analysis for all the patients but this was due a technical problem that caused thirty samples to be terminated. This fault was completely arbitrary and not related to any bias.

Capsule endoscopy examination

The CE procedure has been well-described in the literature (Pillcam SB, Given Imaging Ltd., Yoqneam, Israel).[5] Briefly, the patients had a soft diet on the day before examination, 12 h before swallowing the capsule two sachets of polyethylene glycol solution (Kleen-Prep, Norgine) were administered, followed by fasting until capsule ingestion. The patients were allowed to drink 2 h after and eat a light snack 4 h after ingestion of the endoscopy capsule. The sensor array and recorder pack were disconnected after 8 h and images were downloaded to the workstation. All the videos were analyzed by an experienced consulting gastroenterologist who was blinded to the results of the stool analyses. CE findings were classified as normal or abnormal. Abnormal CE results were categorized as CD, bleeding angiodysplasia or others.

Recently, two scoring systems for CE findings have been proposed. The Lewis score was introduced by Gralnek et al. in 2008 and includes three parameters, villous edema, ulcers and stenosis.[23] Another scoring system proposed by Niv et al includes three other parameters, namely, inflammation, disease extent and stricture.[24] Both these systems, although published, have not yet been widely accepted and validated, and are complicated to perform. Furthermore, these scores intended to score the severity of patients with known CD, and not to diagnose CD. Therefore, we decided to use the more traditional definition of more than three linear ulcers seen on CE as the defining criteria for a diagnosis of CD.[25]

Blood markers

Complete blood count, C-reactive protein (CRP) level, and erythrocyte sedimentation rate (ESR) (normal values ESR < 12 mm/h; CRP < 5 mg/l), were measured in all the patients.

Statistical analysis

Results are shown as mean ± standard deviation. Pearson correlation coefficient (r) was calculated between the continuous variables. To analyze differences in the distribution of categorical data, chi-square test or Fisher exact test was used, as appropriate. Mean differences in continuous variables between the two patient groups (normal and abnormal CE) were analyzed by t-test. A p-value of .05 or less was considered statistically significant. The sensitivity and specificity of calprotectin and lactoferrin in predicting CE findings were calculated at different cutoff values, as determined by construction of a receiver operating characteristic (ROC) curve (sensitivity plotted against 1-specificity at different levels of calprotectin) including the entire study population. The ROC curve was formulated using IBM statistics SPSS v20, Chicago, IL.

Ethical considerations

The study was approved by the Ethics Committee Review Board of Shaare Zedek Medical Center, Jerusalem, Israel (No. 4907). ClinicalTrials.gov Identifier: NCT01266629
Results

Study population

A total of 74 consecutive, eligible patients were referred for outpatient CE during the study period. Six patients were excluded due to inadequate stool specimens; thus 68 patients were included for the analysis. Indications for CE were clinical suspicion of CD in 47 (69%) patients, unexplained anemia in 18 (26%) patients, and bleeding or suspicious pathological findings on CT in 3 (4%) patients.

Abnormal findings on CE were detected in 37 (54%) patients, 23 (33%) were compatible with CD, 11 (16%) had bleeding angiodysplasias and three (4%) had small bowel polyps. The remaining 31 (46%) patients had a normal CE. The characteristics and test indications for all the patients who were diagnosed with CE are summarized in Table 1. Sample pictures from all CD patients are provided in Supplemental Figure 1.

Correlations between laboratory parameters and capsule endoscopy

Table 2 summarizes the clinical and laboratory characteristics of the patients in the CD group compared to the patients in the group without CD. The average age of the CD group was 34 (95% CI 17–51) compared to 46 (95% CI 28–62) in the non-CD group (p = .048). Suspected CD as indication for CE was significantly associated with a final diagnosis of CD, 20 (45%) patients referred for this indication were diagnosed as having CD compared to only 3 (12%) of the patients who were sent to CE for other reasons (p = .006). Median calprotectin and lactoferrin in the study group compared to the controls were 169 mg/kg vs. 40 and 6.6 mg/kg vs. 1, respectively, p = .004 and .051 for each comparison (Figure 1(A,B)).

All other laboratory parameters were similar between the two groups. A statistically significant positive correlation was noted between the two fecal markers, calprotectin and lactoferrin, Pearson correlation = .53 (p = .0001).

The ROC curve

The ROC curves for fecal calprotectin and lactoferrin as a predictor of CE findings of CD are shown in Figure 2(A) and (B), respectively. The area under the curve (AUC) was 0.767 and 0.7, respectively. A fecal calprotectin concentration of 95 mg/kg had a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 77%, 60%, 50%, and 84% in predicting CE findings compatible with CD.

A fecal lactoferrin concentration of 1.05 mg/kg had sensitivity, specificity, PPV and NPV of 73%, 65%, 50%, and 84% in predicting CE findings of CD.

When combining the factors of age under 40 together with a clinical suspicion of CD and a calprotectin of 150 or above, the PPV of finding CD on the CE was 71% compared to patients who had none of these risk factors, who had a NPV of 0 (p = .008).

<table>
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<th>Table 1. Characteristics of patients with a diagnosis of Crohn’s disease.</th>
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<th>Table 2. Clinical and laboratory characteristics of both groups.</th>
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<td>Age, years, mean (range)</td>
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<td>Calprotectin, µg/ml, range</td>
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<td>Study indication</td>
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<td>Suspected CD, [n = 44] (n (%)</td>
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<td>Anemia/GI bleeding, [n = 24] (n (%)</td>
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ESR: erythrocyte sedimentation rate; CRP: C reactive protein; WBC: white blood count.


Discussion

The patients with CD can present with a variety of symptoms including altered bowel habits or nonspecific abdominal pain.[26] These symptoms can be similar to those of IBS and can even be confused with it. Thus, the reported mean delay of 1–7 years for the diagnosis of CD is not surprising.[27] The identification of small bowel involvement in patients with suspected CD is crucial to establish the diagnosis and provide effective treatment. CE offers a noninvasive technique with a high-diagnostic yield [28,29] for detecting small bowel CD, while avoiding radiation exposure to the patients who are often young and of childbearing age. However, it is time-consuming, expensive and results are largely influenced by the operator’s skill. Therefore, we assessed the predictive value of fecal biomarkers, calprotectin, and lactoferrin in patients undergoing CE for suspected small bowel CD.

A recent meta-analysis has shown fecal calprotectin to be a reliable marker for active small bowel CD.[30] Previous retrospective studies have also shown biomarkers to correlate positively with CE findings.[31,32]

In this prospective study we found that both markers correlated well with abnormal CE findings. Moreover, we found useful cutoffs for both markers that lead to a relatively high area under the ROC curve. The diagnostic yield of the fecal markers was statistically significant when comparing patients with CD vs. all other patients.

Previous studies have shown the utility of fecal markers in IBD. Langhorst et al. compared the performance of fecal lactoferrin, calprotectin, polymorphonuclear neutrophil elastase (PMN-e), as well as serum CRP in patients with IBD to address whether these markers can differentiate IBD patients with and without inflammation and from IBS and whether they correlate with endoscopic severity of inflammation.[18] They found that IBD patients with active inflammation demonstrated significantly higher levels of fecal lactoferrin and calprotectin, and PMN-e when compared to patients with inactive inflammation, as well as to patients with IBS. Calprotectin showed the highest diagnostic accuracy in CD (81.4%), whereas lactoferrin was superior to the other markers in ulcerative colitis (83.3%). None of these three stool markers was consistently superior in its ability to reflect endoscopic inflammation, but all three were superior to CRP in their diagnostic accuracy. Dai et al. showed similar results and reported that lactoferrin is a sensitive and specific marker for measuring the activity of IBD.[33]

A parallel between fecal lactoferrin levels and IBD activity estimated with clinical, endoscopic, and histological parameters has been confirmed.[33] However, this correlation seems

![Figure 1](image1.png)

Figure 1. (A) Box plot distribution of the median calprotectin in patients with Crohn’s disease compared to patients without Crohn’s disease. (B) Box plot distribution of the median lactoferrin in patients with Crohn’s disease compared to patients without Crohn’s disease.

![Figure 2](image2.png)

Figure 2. (A) Receiver operating curve for fecal calprotectin in predicting capsule endoscopy abnormal findings. (B) Receiver operating curve for fecal lactoferrin in predicting capsule endoscopy findings of Crohn’s disease. Area under the curve =0.66.
to be lower in CD than in ulcerative colitis, especially in CD patients with purely ileal disease. Fecal lactoferrin may also be useful in predicting impending clinical relapse in IBD and it may be a helpful, noninvasive diagnostic tool for monitoring therapeutic efficacy, primarily in regard to mucosal healing, as decreasing lactoferrin concentration can be interpreted as a marker of therapeutic response.[33] In addition, in patients with CD who have undergone ileo-colic resection, those with higher fecal levels of lactoferrin might be more prone to post-surgical recurrence.

However, it is believed that fecal makers should not be thought of as markers of organic disease; rather, they are markers of ‘neutrophil intestinal inflammation’.[34] This is an important distinction, as many common organic intestinal diseases such as celiac disease, diverticular disease, and colorectal carcinoma are not uniformly characterized by significant neutrophilic infiltrates. Thus, using lactoferrin as a nonspecific test for all organic intestinal diseases will lead to lower sensitivity and will compromise its usefulness as a screening test. Nevertheless, typical histologic hallmarks of enteritis or colitis are polymorphonuclear infiltration and ulceration, and it is unknown how much mucosal ulceration contributes to high fecal lactoferrin levels. Therefore, these markers can provide important information to the clinician.[34]

Our study has strengths and limitations. The most important strength is the prospective fashion in which our study was performed. To the best of our knowledge, this is the first prospective study to assess the value and usefulness of fecal markers together with CE in the assessment of adult patients with suspected isolated small bowel abnormalities. Despite the relative small number of participants we were able to observe encouraging results among this group of patients with suspected intestinal inflammation, but with normal serological markers of inflammation and no findings on upper and lower endoscopy. These patients represent a diagnostic challenge even to experienced clinicians. On the other hand, the relatively low sample size limits the power of our conclusions.

All the study patients had normal colonoscopy and gastroscopy and therefore, the markers assessed the small bowel. This can explain why the diagnostic yield of the fecal markers was relatively low when comparing our results to other studies that assessed the role of calprotectin and lactoferrin in patients with suspected colonic abnormalities. The proposed cutoff level for calprotectin of 95 mg/kg is lower than the levels shown for CD patients in remission.[35] This difference is probably due to lower calprotectin levels in healthy participants than in patients with CD in remission. Both calprotectin and lactoferrin were used and compared in this study. Although calprotectin was significantly more predictive, both markers performed good, had relatively good PPV and NPV of 73–84% and correlated to each other. Lactoferrin may have been underpowered in this study due to small amount of patients who completed the exam.

In our study, a single, morning, freshly refrigerated stool sample was requested. All stool samples that were collected over the prior three days but were adequately refrigerated were accepted. Recently, some studies have questioned the reliability of fecal biomarkers due to the large daily intra-individual variability of fecal calprotectin levels. Factors contributing to this variability have to do with the technique of stool collection and storage, the stool consistency, the time of the day the stool was collected and the amount of time interval between the bowel movements.[36,37] In contrast a prospective controlled study did not show a significant intra-individual variability of calprotectin in CD,[38] and this question is still unresolved.[39]

This study used both fecal calprotectin and lactoferrin as surrogate fecal biomarkers. Both biomarkers were taken from the small stool sample. This technique was previously reported to be reliable.[40] Although fecal calprotectin has been studied more rigorously than lactoferrin, both have shown to be reliable fecal markers. Some studies have shown calprotectin to be superior, but the unique role of lactoferrin has also been reported in many studies,[41–43] and currently its role is yet to be determined.

Our findings advocate for the utility of these markers in the investigation of suspected small bowel CD; but equally important our results provide evidence that CE should be avoided in patients with normal fecal markers. If confirmed in further studies, inexpensive, easily available fecal markers may be a major change in the investigation of subtle IBD patients, avoiding unnecessary, expensive tests.

In summary, this prospective study showed that fecal calprotectin and lactoferrin have the power to tell apart between the patients with normal and CD findings on capsule endoscopy.

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Dr. Benjamin Koslowsky is providing this manuscript as the guarantor. Drs. Bar-Gil Shitrit (1), Koslowsky (2) and Adler (7) have designed the research, collected the data and performed the analysis. Drs. Livovsky (3) and Shitrit (4) have added major statistical and writing contributions. Drs. Paz (5) and Adar (6) have helped recruit the participants and collect and analyze the final data. Dr. Goldin (8) was the primary supervisor of this manuscript. All authors have approved the final version of the manuscript.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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References


Kronborg O, Ugstad M, Fuglerud P, et al. Faecal calprotectin lev-

Limburg PJ, Devens ME, Harrington JJ, et al. Prospective evalu-

Hoff G, Grotmol T, Thiis-Evensen E, et al. Testing for faecal calpro-

Tone H, Brandsnes, Dale S, et al. Improved assay for fecal calpro-


Langhorst J, Elsenbruch S, Koelzer J, et al. Noninvasive markers in


Liangpunsakul S, Maglinte DD, Rex DK. Comparison of wireless

Voderholzer WA, Beinhoezl J, Rogalla P, et al. Small bowel involvement in Crohn’s disease: a prospective comparison of wire-


Hoff G, Grotmol T, Thii-Evensen E, et al. Testing for faecal calpro-

Ton H, Brandsnes, Dale S, et al. Improved assay for fecal calpro-

Kronborg O, Ugstad M, Fuglerud P, et al. Faecal calprotectin lev-


Langhorst J, Jelsenbruch S, Koelzer J, et al. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-


Kane SV, Sandborn WJ, Rufo PA, et al. Fecal lactoferrin is a sensitiv-


Kopylov U, Yung DE, Engel T, et al. Fecal calprotectin for the pre-


Egea VJ, Pereniquez LA, Perez FV, et al. Fecal calprotectin and C-


Dai J, Liu WZ, Zhao YP, et al. Relationship between fecal lactofe-


Lasson A, Stotzer PO, Ohman L, et al. The intra-individual variabil-


