J Pediatr Res 2022;9(2):126-31
DOI: 10.4274/jpr.galenos.2021.99907

Original Article

Impact of Fecal Calprotectin Measurement for Inflammatory Bowel Disease in Children with Alarm Symptoms

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ABSTRACT

Aim: The differentiation of inflammatory bowel diseases (IBD) from other gastrointestinal diseases in pediatric patients is highly important and the definitive diagnosis of IBD is established by endoscopic examination. The use of non-invasive methods (clinical symptoms and laboratory tests) allows for the early and accurate referral of patients from first step health centers to advanced health centers. We aimed to investigate the effectiveness of fecal calprotectin (FC) in the differentiation of IBD from other gastrointestinal diseases in children.

Materials and Methods: This retrospective study included patients who had undergone FC testing and colonoscopy. The demographic characteristics, alarm symptoms (AS), and abnormal laboratory findings (ALF) were recorded for each patient. A negative calprotectin result was considered to be less than 50 µg/g, and a second cut-off value for FC was accepted as 150 µg/g. Definitive diagnosis was established by colonoscopy in each patient.

Results: The study included 88 consecutive patients (mean age, 10.2±6.1 years; 51.1% female). Of these, 20 (22.7%) patients were diagnosed with IBD. No significant difference was found between IBD and non-IBD patients with regard to the presence of AS except for involuntary weight loss ($p<0.001$). The prevalence of increased C-reactive protein and hypoalbuminemia was significantly higher in the IBD patients ($p=0.002$ and $p=0.026$, respectively). FC>50 µg/g [80.0 vs 39.7%, $p=0.044$, odds ratio (OR): 6.07, 95% confidence interval (CI) 1.83 to 23.42] and >150 µg/g (60.0 vs 16.2%, $p=0.002$, OR: 7.78, 95% CI 1.83 to 20.14) was significantly higher in the IBD patients compared to the non-IBD patients. AS combined with ALF and FC>150 µg/g had the highest specificity (95.12%).

Conclusion: Although primary care clinicians often use AS and laboratory parameters in the differentiation of IBD from non-IBD diseases, FC was found to have a relatively higher diagnostic value.

Keywords: Alarm symptoms, fecal calprotectin, pediatric

Introduction

Differentiation of inflammatory bowel diseases (IBD) from other gastrointestinal diseases in pediatric patients is highly important and the definitive diagnosis of IBD is made by endoscopic and histopathological examinations. Although the definite diagnosis of IBD is established by colonoscopy and histopathologic examinations, the use of non-invasive methods (clinical symptoms and laboratory tests) allows for the early and accurate referral of patients from family physicians or general pediatricians to pediatric gastroenterology centers. Additionally, the use of these methods will be helpful in the differentiation of IBD from

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Received: 16.06.2021 **Accepted:** 16.08.2021

other organic diseases such as polyps, solitary rectal ulcer, and allergic colitis (1).

Fecal calprotectin (FC), which is a neutrophil-derived protein released in the stool in response to mucosal inflammation, has recently emerged as a practical, simple, and non-invasive test in the diagnosis of IBD. FC plays an immunoregulatory role in the interaction with the zinc-dependent metalloproteinases responsible for the activation of proinflammatory cytokines. Moreover, its fecal excretion is highly correlated with the severity of intestinal inflammation (2). FC is resistant to colonic bacterial degradation and can be stored at -20 °C without decomposition and is stable for up to seven days at room temperature, which increases its use in clinical practice (3). Its sensitivity is remarkably high (usually 100% at <50 µg/g, ranging from 83% to 100% at 50 µg/g) since it has a broad range (0-3,000 µg/g) and increases as a result of numerous factors including celiac disease, infectious gastroenteritis, and the use of proton pump inhibitors and non-steroidal anti-inflammatory drugs (4-6). Moreover, its specificity ranges between 51% and 100% (6), with this being relatively lower in children (7) and thus, additional findings are needed to enhance its specificity in children.

In the present study, we aimed to investigate the effectiveness of FC measurement in the differentiation of IBD and other colonic diseases in children with clinical and laboratory findings.

Materials and Methods

This retrospective study included 88 patients who presented to Karadeniz Technical University Medical School Pediatric Gastroenterology outpatient clinic with gastrointestinal symptoms [chronic diarrhea (>14 days), rectal bleeding, weight loss of unknown origin, perianal lesions] and underwent colonoscopy with a pre-diagnosis or exclusion of organic gastrointestinal disease and who also had FC measurement. The demographic characteristics, alarm symptoms (AS), and abnormal laboratory findings (ALF) were recorded for each patient. AS included rectal bleeding, involuntary weight loss, chronic diarrhea, perianal lesions, extraintestinal findings, or a family history of IBD (8,9). ALF included anemia, hypoalbuminemia (<3.5 g/dL), increased erythrocyte sedimentation rate (ESR) (>20 mm/sec), or increased C-reactive protein (CRP) level (>1 mg/dL), thrombocytosis (>450,000/µL) (10). Anemia was defined as a hemoglobin (Hb) level of <-2 standard deviations (SD) from the mean for age and gender for the entire population (8).

The concentration of FC in the stool samples was measured semi-quantitatively using a CalFast XT immunochromatographic assay with a mixture of anticalprotectin monoclonal and polyclonal antibodies (Eurospital, Trieste, Italy). A negative calprotectin result was considered to be less than 50 µg/g (4), and a second cut-off value for FC was accepted as 150 µg/g (6). Its range varied from 0 to 300 µg/g. Within one-week, a definitive diagnosis of colonic disease was established by colonoscopy and histopathological examination in each patient.

Non-specific colitis was defined as an inflammatory condition of the colon which microscopically lacks the characteristic features of any specific form of colitis (11).

Statistical Analysis

Data were analyzed using SPSS version 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Quantitative variables were expressed as mean, SD, and minimum-maximum values. Categorical variables were expressed as frequencies (n) and percentages (%). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated for the diagnosis of IBD in patients with AS and FC using binary logistic regression. The Area under the curve (AUC) was used to assess the value of AS and FC in the prediction of IBD in children with gastrointestinal symptoms.

This study was approved by Karadeniz Technical University Medical School Ethical Committee (approval no: 2020-127).

Results

This study included 88 consecutive patients (mean age, 10.2±6.1 years; range, 2 months - 18 years; 51.1% female). Of these, 20 (22.7%) patients were diagnosed with IBD, including 12 (13.6%) patients with ulcerative colitis and 8 (9.1%) patients with Crohn's disease. The remaining 68 (77.3%) patients had no IBD, including 16 (18.2%) patients with lymphonodular hyperplasia, 11 (12.5%) patients with non-specific colitis and 10 (11.4%) patients with allergic colitis. Thirty-one (35.2%) patients had normal histopathological findings (Figure 1). The patients' endoscopic and histopathological findings are summarized in Table I. Five patients with non-specific colitis did not come for follow-up. The other 6 patients recovered with probiotics and diet.

AS was present in 55 (62.5%) patients and the most common AS was rectal bleeding (n=37; 42.0%), followed by

involuntary weight loss (n=31; 35.2%), family history of IBD (n=7; 7.79%) and perianal lesions (n=3; 3.4%, anal fissure in 2, skin tag in 1) (Table II). No extraintestinal symptoms were detected in any patient. No significant difference was found between IBD and non-IBD patients with regard to AS except for involuntary weight loss, which was significantly greater in IBD patients compared to non-IBD patients [75.0 vs 23.5%, p<0.001, odds ratio (OR): 9.75, 95%, confidence interval (CI) 3.0 to 31.00] (Table II).

ALF was present in 38 (43.2%) patients and the most common ALF was increased CRP (n=15; 17.0%), followed by increased ESR (n=12; 13.6%), hypoalbuminemia (n=9; 10.2%), and anemia (n=8; 9.1%) (Table II). The prevalence of increased CRP (40.0 vs 10.3%, p=0.002, OR: 5.81, 95% CI 1.77 to 19.06), hypoalbuminemia (25.0 vs 5.9%, p=0.026, OR: 5.33, 95% CI 1.28 to 22.29), FC>50 µg/g (80.0 vs 39.7%, p=0.044, OR: 6.07, 95% CI 1.83 to 23.42) and >150 µg/g (60.0 vs 16.2%, p=0.002, OR: 7.78, 95%, CI 1.83 to 20.14) was significantly higher in the IBD patients compared to the non-IBD patients. The sensitivity, specificity, PPV, NPV, accuracy and AUC of the presence of AS, ALF, and FC>50 µg/g, FC>150 µg/g and their combinations are shown in Table III. AUC for FC>150 µg/g and AS+ FC>150 µg/g were significant in predicting IBD (AUC=0.715, p=0.011, 95% CI: 0.566-0.865 and AUC=0.702, p=0.016, 95% CI: 0.541-0.863, respectively).

FC was revealed to be a significant predictor in the differentiation of IBD and non-IBD diseases at a cut-off value of 207 µg/g (AUC=0.794, p<0.05, 95% CI: 0.658-0.930), with a sensitivity and specificity of 70.6% and 82.5%, respectively (Figure 2).

Discussion

The present study aimed to investigate the effectiveness of clinical and laboratory findings in the differentiation of IBD from other gastrointestinal diseases in pediatric patients. As proposed by Waugh et al. (6), two distinct cut-off values were determined for FC (50 µg/g and 150 µg/g) (6). FC<50 µg/g is known to rule out IBD (12). Holtman et al. (10) reported that FC had a high sensitivity (0.99; 95% CI, 0.81-1.00) and suggested that negative FC (50 µg/g) safely rules out IBD. Another study indicated that clinical follow-up is recommended in those patients with an FC level of 50-150 µg/g and those patients with these levels may develop IBD as well as diseases which do not require endoscopy such as irritable bowel syndrome (6). In such patients, additional findings are needed to make a decision for invasive procedures such as endoscopy.

The present study investigated the predictive role of AS and ALF in the diagnosis of IBD, among which involuntary weight loss was found to be the most significant predictor of IBD among AS cases. Heida et al. (8) reported that rectal bleeding and perianal lesions were accepted as high-risk factors for the diagnosis of IBD, while a family history of IBD, extraintestinal findings, and weight loss were accepted as low-risk factors. However, these low-risk factors were accepted as high-risk factors when combined with FC>50 µg/g. In our study, AS had a specificity of 39.71% and this level increased to 87.5% for AS combined with FC>150 µg/g.

Among the ALFs analyzed, CRP and hypoalbuminemia were found to be more effective than other ALFs in the diagnosis of IBD. Caviglia et al. (13) reported that CRP was significantly higher in IBD patients compared to controls, and FC was revealed to be the only significant factor on multivariate regression analysis. Holtman et al. (4) indicated

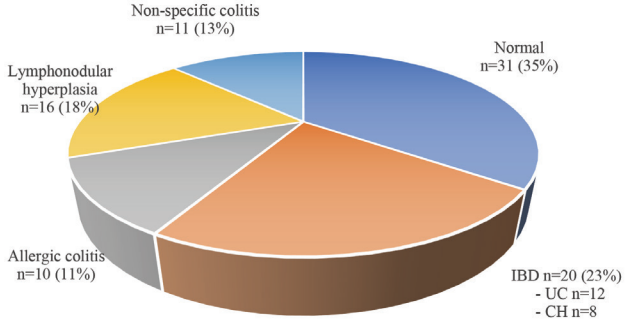

Figure 1. Final diagnosis of patients after colonoscopic examination

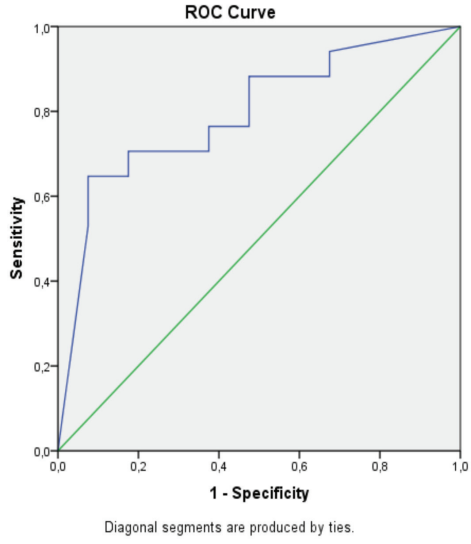

Figure 2. Fecal calprotectin for predicting IBD (AUC=0.794, p<0.05, 95% CI: 0.658-0.930) at a cut-off value of 207 µg/g

ROC: Receiver operating characteristic, IBD: Inflammatory bowel diseases, AUC: Area under the curve, CI: Confidence interval

that AS in combination with CRP had no remarkable benefit in the diagnosis of IBD, while AS in combination with FC had the highest benefit. In our study, AS had a specificity of 72.06% when combined with ALF and a specificity of 87.5% when combined with FC>50 µg/g. Moreover, the accuracy rates and AUC indicated that ALF had no significant effect in the differentiation of IBD and non-IBD diseases.

In a previous meta-analysis, Degraeuwe et al. (14) found the AUC value for FC 212 µg/g to be 0.94 (95% CI, 0.92-0.95) in the differentiation of IBD and non-IBD diseases. Another meta-analysis conducted in 2017 evaluated a large cohort of 1,120 pediatric patients and revealed that FC improved the AUC more than other laboratory markers (ESR, CRP, platelet count, Hb, albumine). Additionally, the pooled AUC of FC (6 studies) was 0.95 (95% 0.93-0.98) in this meta-analysis (9). In our study, the AUC for FC 207 µg/g was 0.794, which was lower than those of other studies. As proposed by Holtman et al. (9), there are numerous factors playing a role in the differentiation of IBD and non-IBD diseases. FC has a remarkably high AUC value, which could be associated with an overestimation of FC levels. Moreover, the contribution

Table I. Patients' endoscopic and histopathological findings

		IBD (n=20) n (%)	Non-IBD (n=68) n (%)	Total (n=88) n (%)
Endoscopic findings	Normal	0 (0)	22 (32.3)	22 (25)
	Nodularity (colon)	2 (10)	9 (13.2)	11 (12.5)
	Erosions	4 (20)	6 (8.8)	10 (11.3)
	Ulcers	10 (50)	2 (2.9)	12 (13.6)
	Nodularity (terminal ileum)	2 (10)	18 (26.5)	20 (22.7)
	Edema	6 (30)	4 (5.9)	10 (11.3)
	Hyperemia	10 (50)	7 (10.3)	17 (19.3)
Histopathological findings	Normal	0	31 (45.6)	31 (35.2)
	Chronic active colitis	4 (20)	0 (0)	4 (4.5)
	Acute active colitis	11 (55)	10 (14.7)	21 (23.9)
	Lymphoid aggregates and plasma cells	3 (15)	10 (14.7)	13 (14.8)
	Eosinophilia	0 (0)	10 (14.7)	10 (11.3)
	Noduler lymphoid hyperplasia	0 (0)	4 (5.9)	4 (4.5)
	Non-specific colitis	0 (0)	11 (16.2)	11 (12.5)
	Terminal ileitis	4 (20)	0 (0)	3 (3.4)

IBD: Inflammatory bowel diseases

Table II. Demographic and clinical characteristics

Variables	Total 88 (100)	IBD (+) 20 (22.7)	IBD (-) 68 (77.3)	p-value
Demographic characteristics				
Age, (years) mean ± SD (range)	10.26±6.15	10.66±4.99	10.14±6.47	0.740
Gender, female	45 (51.1)	10 (50.0)	35(51.2)	0.908
Alarm symptoms	55 (62.5)	14(70.0)	41(46.6)	0.431
Rectal bleeding	37 (42.0)	11 (55.0)	26 (38.2)	0.182
Involuntary weight loss	31 (35.2)	15 (75.0)	16 (23.5)	**<0.001**
Family history for IBD	7 (7.9)	4 (20.0)	3 (4.4)	0.073
Perianal lesions	3 (3.4)	2 (10.0)	1 (1.5)	0.251
Abnormal laboratory findings	38 (43.2)	11 (55.0)	27 (39.7)	0.225
CRP (>1 mg/dL)	15 (17.0)	8 (40.0)	7 (10.3)	**0.002**
ESR (>20 mm/hour)	12 (13.6)	5 (25.0)	7 (10.3)	0.216
Hypoalbuminemia (<3.5 g/dL)	9 (10.2)	5 (25.0)	4 (5.9)	**0.026**
Anemia (hemoglobin <-2 SD for age and gender)	8 (9.1)	4 (20.0)	4 (5.9)	0.075
Thrombocytosis (>450,000/µL)	14 (15.9)	3 (15.0)	11 (16.2)	1.000
FC positivity				
>50 µg/g	43 (48.9)	16 (80.0)	27 (39.7)	**0.044**
>150 µg/g	23 (26.1)	12 (60.0)	11 (16.2)	**0.002**

IBD: Inflammatory bowel diseases, SD: Standard deviation, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, FC: Fecal calprotectin

Table III. Impact of fecal calprotectin, alarm symptoms and abnormal laboratory findings on the diagnosis of IBD

Parameters	Sensitivity (%) (Lower - Upper CI)	Specificity (%) (Lower - Upper CI)	PPV (%)	NPV (%)	Accuracy (%)	AUC (%) (Lower - Upper CI)	p-value
AS (n=55)	70 (45.72-88.11)	39.71 (28.03-52.3)	25.75	81.59	46.67	0.439 (0.276-0.602)	0.469
ALF (+) (n=38)	55 (31.53-76.94)	60.29 (47.7-71.97)	29.27	81.77	59.08	0.418 (0.256-0.581)	0.333
AS+ALF (+) (n=25)	30 (11.89-54.28)	72.06 (59.85-82.27)	24.28	77.15	62.39	0.522 (0.356-0.688)	0.794
FC>50 µg/g (n=43)	94.12 (71.31-99.85)	32.5 (18.57-49.13)	37.41	92.80	50.99	0.367 (0.219-0.515)	0.114
AS+FC>50 µg/g (n=24)	58.82 (32.92-81.56)	65 (48.32-79.37)	41.87	78.65	63.15	0.619 (0.458-0.781)	0.158
FC>150 µg/g (n=23)	70.59 (44.04-89.69)	72.50 (56.11-85.4)	52.38	85.19	71.93	0.715 (0.566-0.865)	**0.011**
AS+FC>150 µg/g (n=14)	52.94 (27.81-77.02)	87.50 (73.2-95.81)	64.48	81.27	77.13	0.702 (0.541-0.863)	**0.016**
AS+ALF+FC>50 µg/g (n=12)	23.53 (6.81-49.9)	80.49 (65.13-91.18)	33.0	72.04	63.97	0.518 (0.351-0.684)	0.834
AS+ALF+FC>150 µg/g (n=6)	23.53 (6.81-49.9)	95.12 (83.47-99.4)	66.33	75.28	74.36	0.593 (0.422-0.764)	0.272

CI: Confidence interval, PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, AS: Alarm symptoms, ALF: Abnormal laboratory findings, FC: Fecal calprotectin

of ALF to the AUC value is relatively low due to the greater AUC values of AS and FC. On the other hand, the lower number of pediatric patients in the meta-analyses further complicates this evaluation (9), which was a limitation of our study as well.

Given that FC testing is not available in most primary healthcare centers despite being a useful marker of IBD, the present study also aimed to provide a basis which could aid primary care clinicians in the referral of patients with suspicious IBD to pediatric gastroenterology clinics. Accordingly, AS and ALF are more important than FC, although they were found to be inadequate for the prediagnosis of IBD in the present study. In a previous study, 17 (19%) out of 90 patients who had AS and were referred from a primary healthcare center were diagnosed with IBD (10). Similarly, in our patients, IBD was diagnosed in 25.4% of the patients with AS. Unfortunately, there are limited studies on this topic and thus further studies are needed to substantiate our findings.

Study Limitations

Our study was limited in several ways. Firstly, the number of IBD patients was significantly less than the number of non-IBD patients. Another limitation was that the age of the patients ranged from 2 months to 18 years. FC cut-off levels have been well established in children older than 4 years of age but FC values vary widely in infants and high FC levels levels may be normal in infancy (15). These limitations may have affected our results. Therefore, future larger scale randomized trials with different age ranges are needed.

Conclusion

In conclusion, although primary care clinicians often use AS and laboratory parameters in the differentiation of IBD and non-IBD diseases, FC was found to have a relatively higher diagnostic value. Moreover, although there are varying cut-off values of FC reported in the literature, a cut-off value of >150 µg/g was found to be highly effective in the diagnosis of IBD.

Ethics

Ethics Committee Approval: The study was approved by Karadeniz Technical University Medical School Ethical Committee (approval no: 2020-127).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: B.G., M.Ç., Design: K.B., E.S., Supervision: F.İ., Data Collection and/or Processing: B.G., E.S., Analysis or Interpretation: F.İ., Literature Review: B.G., E.S., Writing: B.G., M.Ç.

Conflict of Interest: The author(s) indicated no potential conflicts of interest.

Financial Disclosure: No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

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