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УНИВЕРЗИТЕТ У БЕОГРАДУ UNIVERSITY OF BELGRADE
МЕДИЦИНСКИ FACULTY OF
ФАКУЛТЕТ MEDICINE

ORIGINAL ARTICLE

Is fecal calprotectin a dependable indicator of activity in inflammatory bowel diseases?

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Summary

Introduction/Aim: Fecal calprotectin (FCP) is an S100 protein biomarker used in diagnostic and monitoring algorithms of inflammatory bowel diseases (IBD). The role of FCP is established in differentiating inflammatory from functional bowel diseases, predicting relapse of IBD, and monitoring response to IBD therapy. The therapeutic strategy "treat-to-target" includes the normalization of laboratory biomarkers including FCP to attain mucosal healing (MH) as a result of effective Crohn's disease (CD) and ulcerative colitis (UC) treatment. Our research aimed to assess the relationship of FCP values in IBD patients with endoscopic and histological scores of disease activity.

Material and methods: We performed a cross-sectional study at the Clinic for Gastroenterohepatology, University Clinical Center of Serbia, encompassing 223 diagnosed IBD patients (110 CD and 113 UC). The concentration of FCP was analyzed from the first morning stool. The endoscopic activity of IBD was evaluated using the endoscopic Mayo score for UC, Simple Endoscopic Score (SES-CD) for CD, and Rutgeerts score in case of a prior operation. The Geboes grading score was used to evaluate IBD histological activity. Due to discontinuous bowel involvement in CD, histopathological grading was limited.

Results: Our results did not identify any statistically significant relationship between FCP and histological scores in patients with Crohn's disease (FCP median 950.98, PH median 3.57; p= 0.22). While FCP values did not show a correlation with the Rutgeerts score, we did observe a notable correlation between FCP and the SES-CD. In UC patients, values of FCP strongly correlated with endoscopic and histological grading (FCP median 1162.62, PH median 3.67; p = 0.011).

Conclusion: FCP has shown to be a useful and reliable biomarker for assessing UC disease activity, while its applicability is restricted when it comes to CD.

Keywords: fecal calprotectin, ulcerative colitis, Crohn's disease

INTRODUCTION

Fecal calprotectin (FCP) is a commonly used biomarker of inflammation in the management of IBD (1, 2). It is a cytosolic S100 protein distinguished by a heteromeric two-subunit A8/A9 complex (1). Calprotectin as an innate immune protein has antimicrobial properties affecting immunomodulation (1, 3, 4). Namely, FCP is used to distinguish inflammatory and functional bowel diseases, predict relapse in IBD patients, and monitor treatment efficacy (1, 5, 6). The concentration of FCP in stool samples reflects the presence of calprotectin, which is released during an inflammatory process by recruited immune cells that infiltrate and damage the intestinal wall. Nevertheless, the optimal cut-off values of FCP have been controversial and vary among several measurement kits (2,7).

Mucosal healing (MH) in IBD was highlighted in the treat-to-target era, with the preferable use of noninvasive biomarkers in everyday practice. Nevertheless, the evaluation of mucosal healing in Crohn's disease (CD) and ulcerative colitis (UC) is still based on colonoscopy and histopathologic examinations. Current research data indicate a strong association between the endoscopic grading of UC activity and FCP levels (7, 8). Furthermore, the FCP value has been reported as a valuable predictor of disease recurrence in UC patients and an important marker for therapy algorithms (6, 7). Published data indicate that endoscopically active CD can be identified by elevated FCP with sensitivity of up to 97%, while specificity ranges from 45% to 98% (9).

Owing to the challenging scoring of Crohn's disease histologic activity, most studies have concentrated on ulcerative colitis (10). According to previous research, FCP showed the ability to predict histological MH due to an identified notable correlation with histological activity in UC patients. The aim of our study was to investigate the utility of FCP as an activity marker in Crohn's disease and ulcerative colitis.

MATERIAL AND METHODS

A cross-sectional research conducted at the Clinic for gastroenterohepatology, University Clinical Center of Serbia was in accordance with the Helsinki Declaration and approved by the Ethics Committee (decision number 1393/12).

Patients

The study enrolled a total of 223 patients diagnosed with IBD, including 110 cases of CD and 113 cases of UC. The IBD diagnosis was determined by conventional clinical, laboratory, endoscopic, and histological findings in accordance with the European recommendations from 2019 (11). Out of 110 CD patients, 9 were intraoperatively diagnosed.

Stool samples

Stool samples of the patient's first-morning stool were analyzed in the laboratory of the Clinic for Gastroenterohepatology. The concentration of FCP was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland). As per the manufacturer's specifications, FCP is less than 50 mg/kg in general population. The cut-off value for elevated FC in our study was 200mg/kg.

Endoscopic and histological assessment of IBD activity

All patients encompassed in our research underwent colonoscopy with terminal ileoscopy using Olympus endoscopes, specifically the CF-H180AL video colonoscope with High-Definition Television (HDTV). Successive intestinal biopsies were collected and separately analyzed by a pathologist.

Endoscopic evaluation was performed using the Simple Endoscopic Score for Crohn's disease (SES-CD) and the endoscopic Mayo score for UC (13). The score values were determined by experienced endoscopists. SES-CD graded the extent of ulceration, inflammation, and stenosis in five defined sections of the bowel with values less than 2 indicating remission, mild disease suggested by the range 3 to 6, moderate nature ranging from 7 to 15, and scores ≥15 representing severe endoscopic findings. The postoperative risk of CD relapse was established using the Rutgeerts score categorizing remission or disease recurrence (14). Endoscopic findings after ileocolic anastomosis were described as remission in cases of lesion absence or less than 5 aphthous lesions (score 0-1), while recurrence was denoted with more than 5 aphthous or larger lesions, diffuse ileitis, or presence of ulcerations and stenosis (score 2-4) (14). Endoscopic Mayo score in UC cases assessed the colon for erythema, vascular mucosa, vulnerability, lesions, and spontaneous bleeding with values from 0 to 3 (13).

The histological activity of UC was evaluated using Geboes scores ranging from 0 to 5.4 with elevated values suggesting a more significant presence of chronic inflammation. Although target biopsies of endoscopically active CD were analyzed, grading by the Geboes system was limited by the discontinuous nature of the disease.

Statistics

Data analyses were conducted using SPSS software version 23 (Chicago, IL). The Gaussian distribution of variables was assessed using the Shapiro-Wilk test. Summary statistics were applied encompassing measures such

as mean, median, and standard deviation. Spearman's corelation test was applied to investigate the association between analyzed variables, with p-values < 0.05 considered statistically significant.

RESULTS

Features of the IBD patients included in our research are outlined in **Table 1**.

Table 1. Characteristics of Study Participants

Subjects' parameters	CD (n = 110)	UC (n = 113)
Age, y	37 ± 11	44 ± 14
Females, n (%)	53 (48.2)	55 (48.7)
Smoking, n (%)	44 (40.0)	39 (34.5)
IBD family history, n (%)	16 (14.5)	14 (12.4)
NSAID use, n (%)	25 (22.7)	30 (26.5)
FCP, mg/kg (median)	1200.0	2000.0
Localization of disease		
[a]L1/[b]E1 (%)	32 (29.1)	18 (15.9)
[a]L2/[b]E2 (%)	28 (25.4)	35 (30.9)
[a]L3/[b]E3 (%)	50 (45.5)	60 (53.2)
Phenotype of disease	60 (54.5)	45 (39.8)
[c]B1/[d]S1 (%)	26 (23.6)	28 (24.8)
[c]B2/[d]S2 (%)	24 (21.9)	40 (35.4)
[c]B3/[d]S3 (%)		

Localization of CD according to Montreal classification, L1— ileum, L2—colon, L3—ileocolonicic; [b]Extent of UC according to Montreal classification, E1—ulcerative proctitis, E2—left side UC, E3—extensive UC; [c]Behavior of CD according to Montreal classification, B1— nonstricturing, nonpenetrating disease, B2—stricturing, B3–penetrating.; [d]Severity of UC according to Montreal classification, S1—mild, S2—moderate, S3—severe. CD, Crohn's disease; UC, ulcerative colitis; NSAID, non-steroid anti-inflammatory drugs

There was no notable statistical correlation observed between the degree of histological activity in CD and levels of fecal calprotectin **(Figure 1)**. During our analysis of the CD group of patients, we found a significant positive correlation between the degree of endoscopic activity assessed by SES-CD and FCP values (p = 4.9e-) **(Figure 2)**. Nevertheless, our results did not find a correlation of statistical importance regarding endoscopic activity in operated CD patients and FCP (p = 0.7) **(Figure 3)**

In patients with UC, a significant correlation displayed in **Figure 4** was detected between FCP and mucosal histological activity (p = 0.011). Moreover, results regarding the UC group of patients indicated a strong correlation between fecal calprotectin and endoscopic activity (p = 0.00013) **(Figure 5)**, highlighting the importance of FCP as an activity marker in UC.
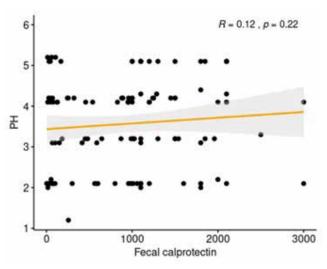

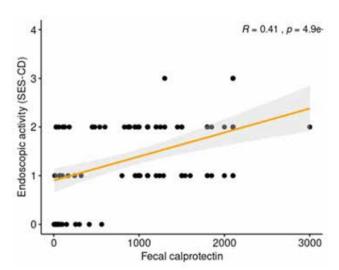**Figure 1.** Correlation of FCP levels and histological activity in CD

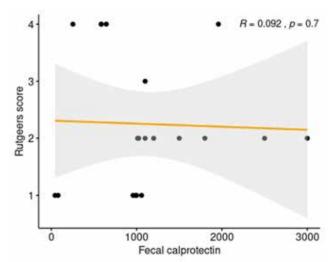**Figure 2.** Correlation of FCP levels and endoscopic activity (SES-CD score) in CD

Figure 3. Correlation of FCP and Rutgeerts values in operated CD patients

DISCUSSION

Calprotectin is an antimicrobial protein with elevated values detected in a variety of immunological and immunopathological conditions (10). A significant over-
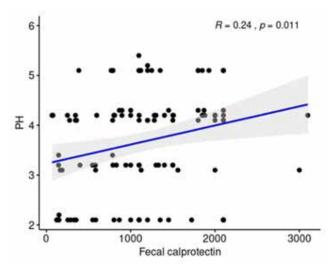
Figure 4. Correlation of FCP values and histological activity in the UC
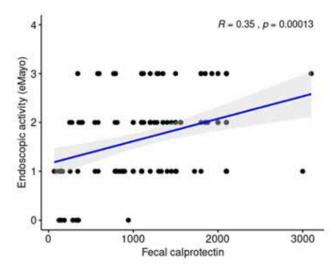

Figure 5. Correlation of FC values and endoscopic activity (eMayo score) in the UC

lap between symptoms of functional and inflammatory disorders has increased the colonoscopy rate. Hence, non-invasive diagnostic markers are needed in everyday clinical practice.

As early as 1997, Roseth et al. highlighted the utility of FCP in IBD diagnosis with elevated values registered in UC compared to controls (15). Nevertheless, consistent challenges occurred across different research studies regarding variability in the sensitivity and specificity of the FCP threshold. Published data such as studies by Jensen MD et al. and Diamanti et al. indicated that the interpretation of fecal calprotectin levels can be demanding, underscoring the complex nature of diagnosing gastrointestinal disorders, especially IBD (16, 17). Possible reasons for the diverse cut-off values of FCP in IBD might be in different clinical settings and demographic and clinical characteristics of IBD patient cohorts. Our study's threshold value of FCP was 200 mg/kg in IBD patients.

Examining the status of MH in individuals with IBD is based on endoscopic and histologic activities. So far, several scoring systems have been developed based on endoscopic and histopathologic findings. Furthermore, re-

cent studies reported a correlation between these scores and FCP levels (10, 15, 18).

According to our results, we found a notable correlation between FCP and the score of endoscopic activity in the UC study group. Based on our findings, we have used a suitable threshold for FCP in assessing endoscopically active UC individuals. Furthermore, a strong correlation was observed linking FCP and mucosal histological activity in UC as evaluated by the Geboes grading system. According to the outcomes derived from our study, FCP represents an important and valuable biomarker in the management of UC patients. These results align with previous studies that have also demonstrated the importance of FCP in diagnosing and following disease activity in ulcerative colitis (10, 15, 18). Moreover, Hart L. et al. and Mak WY et al. reported different FCP thresholds for distinguishing endoscopically "silent" ulcerative colitis (18, 19). Additionally, Schoepfer AM et al. found that FCP had greater efficacy as an indicator of endoscopic activity in ulcerative colitis in comparison with C-reactive protein and clinical presentation of the disease (20)

The management of CD and postoperative courses has a crucial role in optimizing long-term outcomes with regular endoscopic evaluation for monitoring disease activity. Nevertheless, the field of endoscopy faces significant challenges in standardizing scoring systems for consistent and reliable evaluation (21, 22, 23). The results of our study did not observe the link between levels of FCP and endoscopic activity in operated CD patients assessed with a scoring system developed by Rutgeerts. However, interpreting these results might be constrained due to the small number of operated CD patients included in our study. Nevertheless, SES-CD showed a notable correlation with FCP in our CD study group. In line with our results, Sipponen et al. showed a strong link between CD endoscopic activity using CD Endoscopic Index of Severity and FCP, while suggesting FCP values of 200 µg/g as a significant threshold for predicting endoscopic relapse (21). In alignment with previous findings, Moein S. et al. found FCP to be an important and applicable biomarker in predicting endoscopic activity both in UC and CD patients (22).

The IBD histologic activity has also been associated with FCP, but the majority of studies have been focused on UC (23) So far, conducted studies have a relatively low number of UC patients. Nevertheless, our study included 223 IBD patients. Although we reported a correlation between FCP and histologic activity in UC using the Geboes scoring system, CD histologic activity showed no correlation with the values of FCP. However, recently published research data reported that FCP can be used as a reliable predictor of histological remission in IBD (10, 24).

CONCLUSIONS

FCP represents a valuable non-invasive biomarker in the routine clinical practice of IBD patients. The disease activity in patients with UC is strongly linked with levels of FCP. However, its application in CD is currently limited.

Future studies on IBD management should be focused on investigating the monitoring ability of FCP in different personalized therapy algorithms and its prognostic significance in the disease course such as the need for hospitalization or surgery.

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DA LI JE FEKALNI KALPROTEKTIN POUZDAN MARKER KOD INFLAMATORNIH BOLESTI CREVA?

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Sažetak

Uvod/Cilj: Fekalni kalprotektin (FCP) je S100 protein koji se koristi kao biomarker u algoritmima dijagnostike i praćenja inflamatornih bolesti creva (IBC). FCP ima ulogu u diferencijaciji inflamatornih od funkcionalnih bolesti creva, predikciji relapsa IBC i praćenju odgovora na IBC terapiju. Terapijska strategija *"treat-to taget"* za IBC podrazumeva i normalizaciju laboratorijskih biomarkera uključujući FCP u cilju postizanja mukoznog zaceljenja kao rezultata efikasnog lečenja Kronove bolesti (KB) i ulceroznog kolitisa (UK). Cilj rada je ispitivanje korelacije vrednosti FCP sa endoskopskom i histološkom aktivnosti bolesti kod pacijenata sa IBC.

Metodologija: U studiju preseka koja je sprovedena na Klinici za gastroenterohepatologiju Univerzitetskog Kliničkog Centra Srbije uključeno je 223 IBC pacijenta (110 KB, 113 UK). Nivo vrednosti FCP detektovane su iz uzorka prve jutarnje stolice. Endoskopska aktivnost određena je primenom endoskopskog Mayo skora za UK i Pojednostavljenog Endoskopskog skora za KB (SES-CD) . Rutgeerts skor je korišćen kod prethodno operisanih KB pacijenata. Histološka aktivnost opisana je primenom Geboes grading sistema. Primena histološkog skoring sistema u KB je limitirana diskontinuiranom lokalizacijom u crevu.

Rezultati: Nije uočena statistički značajna korelacija između FCP i histološke aktivnosti KB (FCP median 950.98; PH median 3.57, p= 0.22). Značajna korelacija detektovana je između FCP i endoskopske aktivnosti KB, dok je ista izostala sa Rutgeerts skorom. FCP je značajno korelisao sa histološkom i endoskopskom aktivnosti kod UK (FCP median 1162.62; PH median 3.67, p = 0.011).

Zaključak: FCP je praktičan, neinvazivan marker u proceni endoskopske i histološke aktivnosti kod pacijenata sa UK, dok postoji limitiranost primene kod KB.

Ključne reči: fekalni kalprotektin, ulcerozni kolitis, Kronova bolest