ORIGINAL ARTICLE, MEDICINE

Role of Fecal Calprotectin as a Noninvasive Indicator for Ulcerative Colitis Disease Activity

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Received: 08 Sep 2018
Accepted: 18 Nov 2018
Published Online: 17 Dec 2018
Published: 30 June 2019

Key words: UCEIS, Mayo score, fecal calprotectin, ulcerative colitis

Citation: Nakov RV, Nakov VN, Gerova VA, Tankova LT. Role of fecal calprotectin as a noninvasive indicator for ulcerative colitis disease activity. Folia Med (Plovdiv) 2019;61(2):188-96.
doi: 10.2478/folmed-2018-0071

Background: It is essential in clinical management to determine the disease activity in ulcerative colitis (UC) patients. At present, the most accurate way of evaluating the UC severity is endoscopy with biopsy. Fecal calprotectin (FCP) is a non-invasive biomarker that is frequently used for monitoring of intestinal inflammation.

Aims: The purpose of our study was to assess the role of FCP as a noninvasive indicator for UC disease activity.

Materials and methods: This prospective study enrolled 116 patients with UC (56 with quiescent UC and 60 with active UC) and 36 controls, referred for colonoscopy to our Center. Colonoscopy was performed in all the patients and the findings were graded according to Mayo endoscopic score (EMS) and UC endoscopic index of severity (UCEIS). FCP was analyzed in stool samples by means of point-of-care desk-top Quantum Blue® method.

Results: There was no significant difference between mean FCP levels in controls and UC patients in remission (p=0.205). Mean FCP in patients with active UC was significantly higher than that in controls (p<0.001) and in patients in remission (p<0.001). FCP significantly correlated with UCEIS (r = 0.869, p<0.001) and EMS (r = 0.814, p<0.001).

Conclusion: The strong correlation with endoscopic disease activity suggests that FCP is a useful biomarker for noninvasive diagnosis and monitoring of disease activity in UC patients.

BACKGROUND

Ulcerative colitis (UC) is a chronic, idiopathic, inflammatory disease characterized by recurrent episodes of diffuse inflammation of the large intestine’s mucosa followed by periods of remission. In view of clinical management, it is essential to determine the disease activity. In order to evaluate it in a given patient, doctors rely on a combination of clinical and endoscopic findings as well as levels of laboratory biomarkers. At present, the most accurate way of evaluating the severity of UC and extent of intestinal inflammation is endoscopy with biopsy. However, this technique has the drawbacks of being invasive, time-consuming, and expensive. Moreover, this examination is painful, and requires both a skilled operator and an uncomfortable preparatory regimen. These limiting factors are often a burden to UC patients, and often prevent the frequent evaluation of UC activity by endoscopy. On the other hand, patients’ symptoms cannot reliably reflect the extent of disease and response to therapy, and their correlation with endoscopic activity is often limited. Therefore, for quantifying the disease activity, a combination of clinical examination, levels of laboratory biomarkers, endoscopic and microscopic findings is used in routine clinical practice.

Several laboratory biomarkers have been evaluated for the purpose of monitoring endoscopic UC activity. In UC, active inflammation is associated with an acute phase reaction and migration of leukocytes to the bowel lumen. As such, elevated levels of several proteins can be measured in serum and feces. Acute phase reactants (ESR, WBC, CRP) have been demonstrated to have very low sensitivity and specificity in correlation to intestinal inflammatory activity. Fecal markers, specifically, calprotectin may be more specific for assessing intestinal disease activity. Calprotectin is a small calcium-binding protein consisting of two heavy and one light polypeptide chains. It is found in abundance in neutrophilic granulocytes, in which it accounts for 60%
of the cytosolic fraction, as well as in monocytes and macrophages. Fecal calprotectin (FCP) is a biomarker that is frequently used for monitoring inflammatory bowel disease (IBD) activity.

There are several different scoring systems for the endoscopic evaluation of ulcerative colitis (UC) severity, such as the endoscopic Mayo score (EMS), the modified Baron score, etc. However, none of these instruments have been validated for a reliable assessment. The EMS (four-point scale with a maximum total score of 3) was developed in 1987 by Schroeder et al. Within EMS a score of 0 is given for normal mucosa or inactive UC, while a score of 1 is given for mild disease with evidence of mild friability, reduced vascular pattern, and mucosal erythema. A score of 2 is indicative of moderate disease with friability, erosions, complete loss of vascular pattern, and significant erythema, and a score of 3 indicates ulceration and spontaneous bleeding. Partly due to its simplicity, the EMS is the most commonly used endoscopic activity index in clinical trials for evaluating treatment efficacy in terms of endoscopic improvement. There is an overlap in the features of the different levels of this index, which causes high interobserver variation. The most troublesome component of EMS is friability, as this is subjective and leads to inconsistent results. This inconsistency has led to adaptation of the index to remove friability from level 1. More recently, the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) has been reported by Travis et al. This score was developed as an index that captures 90% of the variance in the overall assessment of endoscopic severity. It is the first validated endoscopic index of severity in UC. Basically, the UCEIS is calculated as the simple sum of three descriptors: vascular pattern (score 0–2), bleeding (score 0–3), and erosions and ulcers (score 0–3). Possible total scores range from 0 to 8, and UC activity can be graded as normal, mild, moderate or severe.

**AIM**
The purpose of our study was to assess the role of FCP as a noninvasive indicator for UC disease activity.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of ulcerative colitis patients enrolled in the study</th>
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<tr>
<td><strong>Characteristics</strong></td>
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<td>Number of patients</td>
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<td><strong>Sex</strong></td>
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<td>Female</td>
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<td><strong>Age, mean ± SD, range (years)</strong></td>
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<td><strong>Duration of the disease, mean ± SD (years)</strong></td>
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<td><strong>Smoking habit</strong></td>
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<tr>
<td>Never smoked</td>
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<td>Ex-smokers</td>
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<tr>
<td><strong>Disease location</strong></td>
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<tr>
<td>Proctitis – E1</td>
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<td>Left-sided colitis – E2</td>
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<td>Extensive colitis – E3</td>
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<td><strong>Medication at endoscopy</strong></td>
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<tr>
<td>None</td>
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<td>Topical 5-ASA</td>
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<td>Systemic 5-ASA</td>
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<td>Azathioprine</td>
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<td>TNF-alpha inhibitor</td>
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PATIENTS AND METHODS

Patients: This prospective observational case-control study enrolled 36 patients with irritable bowel syndrome (IBS) used as controls (group 1), 116 patients – 64 male and 52 female (mean age 38.9 ± 9 yrs, range 18-63) with UC, 56 of them with quiescent disease (group 2) and 60 with active UC (group 3), referred for colonoscopy to the Clinic of Gastroenterology of Queen Giovanna University Hospital in Sofia between May 2014 and February 2018. The diagnosis was made according to the ECCO Guidelines.20 As a remission we accepted EMS 0. UC patients with proctitis, left-sided colitis and extensive colitis were included in the study. Disease location was categorized using the Montreal Classification system.21 An overview of the demographic patient characteristics at baseline is provided in Table 1.

The inclusion criteria for this study were as follows: (1) age 18–85 years, (2) complete colonoscopy with intubation of the cecum (intubation of terminal ileum was not mandatory), (3) biopsies (at least six biopsies from the areas of the colon and rectum affected by UC), (4) completion of a written informed consent, and (5) fecal specimens collected within 1–2 days before colonoscopy.

Exclusion criteria were: (1) incomplete colonoscopy, (2) inadequate fecal sample, (3) colorectal cancer or colon polyps, (4) Crohn’s disease, (5) indeterminate colitis, (6) history of colorectal surgery, (7) urinary incontinence (due to the risk of contamination of fecal samples), (8) pregnancy, (9) history of active non-steroidal anti-inflammatory drugs (NSAID) intake (2 tablets/week), (10) having infectious colitis, (11) primary immunodeficiency, and (12) underlying chronic disease at the time of the study.

Endoscopies were performed by two experienced board-certified gastroenterologists who graded the findings according to EMS and UCEIS.

Ulcerative colitis endoscopic index of severity (UCEIS)

The UCEIS score is a tool based on a visual analogue scale (VAS) that provides a model accounting for UC endoscopic severity (Table 2). We evaluated the patients by total colonoscopy, whereas in the original determination of the UCEIS score Travis and colleagues17 used sigmoidoscopy. The worst affected part of the colon visualized by colonoscopy was identified and the ultimate score calculated by simply adding the scores from each component.
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ranging from 0 (normal) to 8 (the worst.) We stratified the UCEIS scores into four groups: remission (UCEIS 0–1); mild (UCEIS 1–3); moderate (UCEIS 4–5); and severe (UCEIS 6–8). It was assumed that UCEIS 1 in the remission group was a descriptor limited to vascular patterns and UCEIS 1 in mild activity group describes bleeding, erosions and ulcers.

**Fecal Calprotectin**

FCP was analyzed in stool samples by means of point-of-care desk-top Quantum Blue Reader® (POC Reader) method. It is a lateral flow technology based on ELISA techniques. We performed the test according to the manufacturer’s instructions (Quantum Blue® Calprotectin, Bühlmann Laboratories AG, Switzerland). The POC device uses internal standards within a range of 30–300 µg/g and a sensitivity of <10 µg/g, thus guaranteeing consistency in results. When we received results > 300 µg/g, we performed additional 1:10 dilution with extraction buffer according to the manufacturer’s instructions, allowing us to receive FCP levels up to 3000 µg/g. FCP values above the upper limit of the measurement ranges were registered as 3000 µg/g and FCP values below the lower limit were accordingly registered as 30 µg/g.

**Statistical analysis**

The statistical analysis was performed using SPSS for Windows, Version 25.0. (SPSS Inc., Chicago, USA). For data analysis the following statistical methods were used: descriptive statistics for tabular and graphical presentation of results, Kolmogorov-Smirnov test, Shapiro-Wilk test, Kruskal-Wallis test, Mann-Whitney U test and Spearman’s correlation coefficient. The obtained results were assessed as statistically reliable in threshold level of significance p<0.05.

**Ethics approval**

The study was approved by the Ethics Committee of Queen Giovanna University Hospital in Sofia, Bulgaria. Before initiating the study, written informed consent was obtained from all patients. Patients agreed to participate in this study after being informed of the study purpose and the nature of the procedures involved. Further, the investigation was conducted in accordance with the Principles of Good Clinical Practice and the ethics standards laid down in the 1964 Helsinki Declaration and its subsequent amendments.

**RESULTS**

The mean levels of FCP were 925.49 µg/g (30-3000; SD - 1102), the mean EMS was 1.25 (0-3; SD – 1.31) and the mean UCEIS was 2.13 (0-7; SD – 2.25). FCP levels were very closely associated with EMS subgroups (p<0.0001, H=91.053) (Fig. 1). We found significant difference between FCP values in patients with EMS 0 and EMS 1 (p<0.001; Z= -4.357) and between patients with EMS 1 and EMS 2 (p<0.001; Z= -3.799), but there was no significant difference between FCP values in EMS 2 and EMS 3 (p<0.331; Z= -0.973). Moreover, FCP levels were significantly associated with UCEIS subgroups (p<0.001, H=92.687) (Fig. 2). Patients with UCEIS 1-3 had significantly higher FCP levels than patients in remission (UCEIS 0-1) (p<0.001; Z= -6.543). Patients with moderate (UCEIS 1–3) activity had higher FCP levels than those with mild (UCEIS 4–5) activity (p<0.040; Z= -2.052). There wasn’t any significant difference in FCP levels between patients with moderate (UCEIS 4–5) and severe (UCEIS 6–8) activity (p=0.132; Z= -1.506). There was no statistically significant difference between mean FCP levels of controls (34.72 ± 7.43 µg/g) and UC patients in remission (47.10 ± 26.91 µg/g) (p=0.205). However, patients with active UC had significantly higher mean FCP levels (1933.08 ± 940.98 µg/g) than controls (p<0.001) and patients with quiescent UC (p<0.001) (Fig. 3). FCP significantly correlated with UCEIS (r = 0.869) and EMS (r=0.814). For both items, p<0.001 was found (Fig. 4). Moreover, UCEIS had very strong correlation with EMS (r = 0.922, p<0.001).

**DISCUSSION**

This study demonstrates the role of FCP as a noninvasive indicator for UC disease activity by showing an excellent correlation with endoscopic activity evaluated by UCEIS and EMS. Therefore, FCP can be used as an alternative to endoscopy and can be accepted as a direct biomarker of intestinal inflammation in UC patients. It is simple, less expensive and non-invasive compared to colonoscopy. Currently, there are sufficient data in the literature demonstrating relationship between FCP values and severity of intestinal inflammation in IBD. However, our results emphasize on the importance of FCP in evaluation of disease activity in relatively not small Bulgarian cohort of UC patients.

The main goal of the modern IBD treatment is not just symptom amelioration but also mucosal healing (MH) achievement. Endoscopy with biopsies is the best way to evaluate MH. However, because of the drawbacks of this technique, the correlation of endoscopic activity in IBD with biomarkers such as
Figure 1. Boxplot of fecal calprotectin concentrations in ulcerative colitis patients with endoscopic Mayo score (EMS) of 0, 1, 2 and 3. Data are presented as box and whisker plots showing median (horizontal line), interquartile range (box) and range of measurements (whisker).

Figure 2. Boxplot of fecal calprotectin concentrations in ulcerative colitis (UC) patients with UC endoscopic index of severity (UCEIS) of 0, 1, 2, 3, 4, 5, 6, and 7. Data are presented as box and whisker plots showing median (horizontal line), interquartile range (box) and range of measurements (whisker).
**Figure 3.** Comparison of fecal calprotectin values in controls, patients with quiescent ulcerative colitis (UC) and active UC.

**Figure 4.** Correlation between fecal calprotectin (FCP) concentration and A. Ulcerative Colitis Endoscopic Index of Severity (UCEIS) - \(r=0.869\) and (B.) Mayo endoscopic score (EMS) - \(r=0.814\). Spearman’s correlation test.
FCP has been provoking interest in gastroenterologist during the last decade. Most studies described that FCP concentrations correlate well with endoscopic and histological IBD activity and thus with MH. Therefore, FCP is increasingly being used as an alternative to MH assessment.

In our study, we did not find a statistically significant difference between FCP levels in controls and UC patients in remission, and thus confirmed the presence of MH. In a large study by Schoeppefer et al., the mean FCP value in UC patients in remission was 42.0 μg/g, which is largely consistent with the results in the present study - 47.10 μg/g. In some patients with inactive UC, low-grade intestinal inflammation might occur, with higher than normal FCP values, but usually not exceeding 150 μg/g. In line with this, in our group of patients with UC in remission, the highest FCP value was 136 μg/g. On the other hand, FCP concentrations in patients with active UC were so significantly higher than those of controls and patients in remission that we could not even detect a cut-off level between activity and remission in UC patients.

The UCEIS score is a relatively new tool to evaluate disease activity in UC patients and is closely correlated with former endoscopic activity scores. The original and modified Baron scores and the Mayo score have been widely used in the clinical practice and are easy to implement. However, recent studies have demonstrated that the rate of agreement is only 27% for endoscopic remission (Baron score 0) and 37% for moderate activity (Baron 2). Inter-observer disagreement has been reported for the severity of inflammation in UC. Therefore, the development of new methods for the assessment of severity is warranted. More recently, the UCEIS and the ulcerative colitis colonoscopic index of severity (UCCIS) were developed to address the issue of low inter-observer agreement for endoscopic scores. Close correlation between UCEIS and FCP levels was indicated by study of Taghvaei et al. (r = 0.607, p = 0.001). FCP levels in patients with an overall UCEIS >1 were considerably higher than in patients with normal colonoscopy findings. However, that study did not directly show the differences in FCP levels between the patients with a UCEIS of 0 or 1. Another recent investigation also indicated that FCP levels were correlated with EMS and UCEIS. In the current study we found very strong correlation between UCEIS and the widely used EMS (r = 0.922, p<0.001).

Moreover, FCP levels correlated quite well with both UCEIS (r=0.869, p<0.001) and EMS (r=0.814, p<0.001). Thus, FCP can be used as an alternative to endoscopy and can be accepted as a direct biomarker of intestinal inflammation in UC patients. It is less expensive and non-invasive compared to colonoscopy.

In the present study FCP was analyzed in stool samples by means of point-of-care Quantum Blue® method. We find this test really useful, because it is simple to use, can be done in doctor’s office and is quite fast (results can be obtained in less than 30 min including protein extraction). Another major advantage is the simplicity of sample preparation and analysis. No more than 80 mg of stool sample is required for the assessment and sample preparation and analysis is user friendly and does not involve the need of special equipment, which makes it ideally suited for every lab and even doctor’s office. Moreover, the point-of-care test can serve as a reliable alternative to ELISA. It has been shown that Quantum Blue® is the instrument of choice for fast and reliable determination of FCP levels.

CONCLUSION

FCP concentration demonstrated a very strong correlation with endoscopic activity in UC according to UCEIS and EMS. Our study shows the excellent role of FCP as a noninvasive indicator for UC disease activity in a relatively not small Bulgarian cohort.

ACKNOWLEDGMENTS

The authors received no financial support for the research, authorship, and/or publication of this article.

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Роль фекального кальпротектина в качестве неинвазивного индикатора активности язвенного колита

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Дата получения: 08 сентября 2018
Дата приемки: 18 ноября 2018
Дата онлайн публикации: 17 декабря 2018
Дата публикации: 30 июня 2019

Ключевые слова: UCEIS (эндоскопический индекс тяжести язвенного колита), Mayo-классификация оценки тяжести язвенного колита, фекальный кальпротектин, язвенный колит

Введение: С точки зрения клинического контроля заболевания, важно определить активность заболевания у пациентов с язвенным колитом (ЯК). В настоящее время наиболее точным способом оценки степени тяжести ЯК является эндоскопия и биопсия. Фекальный кальпротектин (ФКП) является неинвазивным биомаркером, который часто используется для мониторинга воспаления кишечника.

Цель: Целью нашего исследования было оценить роль ФКП в качестве неинвазивного индикатора активности ЯК.

Материалы и методы: Это проспективное исследование включало 116 пациентов с ЯК (56 с латентным и 60 с активным) и 36 контрольных групп, направленных на колоноскопию в наш центр. Колоноскопия была проведена пациентам, и результаты были оценены по эндоскопической классификации Mayo (EMS) и UCEIS (эндоскопический индекс тяжести язвенного колита). ФКП анализировали с помощью фекальных проб с использованием метода Quantum Blue® на месте.

Результаты: Не установлено значимой разницы между средними уровнями ФКП в контрольной группе и у пациентов с ЯК в ремиссии (p = 0,205). Средний ФКП у пациентов с активным ЯК была значительно выше, чем у контрольных (p <0,001) и у пациентов с ремиссией (p <0,001). ФКП значительно коррелирует с UCEIS (r = 0,869, p <0,001) и EMS (r = 0,814, p <0,001).

Выводы: Значительная зависимость от активности заболевания позволяет предположить, что ФКП является полезным биомаркером для неинвазивной диагностики и наблюдения за активностью заболевания у пациентов с ЯК.