ORIGINAL ARTICLE

Fecal Calprotectin for Assessment of Inflammatory Bowel Disease Activity

Radislav V. Nakov[1], Ventsislav N. Nakov[1], Lyudmila T. Tankova[1], Vanya A. Gerova[1], Todor G. Kundurzhiev[2]

[1]Clinic of Gastroenterology, "Tsaritsa Yoanna" University Hospital, Medical University of Sofia, Sofia, Bulgaria, [2]Department of Social Medicine and Health Management, Section of Biostatistics, Medical University of Sofia, Sofia, Bulgaria

ABSTRACT

Background: Fecal calprotectin (FCP) is frequently used for monitoring inflammatory activity and prediction of relapse in inflammatory bowel disease (IBD). We aimed to assess the usefulness of FCP as a marker of disease activity in patients with IBD. **Methods:** This prospective study enrolled 174 patients (84 with Crohn's disease [CD] and 90 with ulcerative colitis [UC]) referred for colonoscopy to our center. FCP was analyzed in stool samples by means of a point-of-care Quantum Blue® method. **Results:** Mean FCP values in patients with colonic or ileocolonic CD were significantly higher than in patients with ileal CD (<0.001) and quiescent CD (<0.001). Mean FCP levels in quiescent CD were higher than those of controls (P = 0.001). Patients with active ileal CD had significantly higher FCP values than quiescent CD patients (<0.001). A cutoff value of 315 μg/g predicted mucosal inflammation in CD patients with 94% sensitivity and 98% specificity. 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 in controls (P < 0.001) and in patients in remission (P < 0.001). **Conclusion:** FCP is a reliable surrogate marker of endoscopic activity in IBD. FC could be useful in the prediction of mucosal healing. Moreover, the rapid point-of-care method allows an easier assessment of FCP in clinical practice.

Key words: Fecal calprotectin, inflammatory bowel disease, mucosal healing

INTRODUCTION

Inflammatory bowel disease (IBD), which includes both Crohn's disease (CD) and ulcerative colitis (UC), is a chronic idiopathic inflammatory disorder affecting the gastrointestinal tract.

In view of clinical management, it is essential to determine the disease activity and to achieve mucosal healing (MH) which is reported to be associated with sustained remission and a reduced risk of surgery in IBD.[1,2] To evaluate activity in a given patients, doctors rely on a combination of clinical and endoscopic findings as well as levels of laboratory biomarkers.[3]

Nowadays, a gold standard for assessing the site, extension, and severity of the intestinal inflammation is the endoscopy with biopsy.[4] However, endoscopic procedures are invasive, with a substantial risk of complications.[5] Such procedures can be painful and frequently require use under general anesthesia, which may be dangerous for the patient. Bowel preparation for colonoscopy is unpleasant and could be hazardous in some situations.[6] Moreover, the standard criteria for the evaluation of disease severity as well as the definitions of MH on colonoscopy are still unclear.

On the other hand, patient symptoms cannot reliably reflect the extent of disease and response to therapy, and their correlation with endoscopic activity is often limited.[7]

Address for correspondence:
Radislav V. Nakov, Clinic of Gastroenterology, "Tsaritsa Yoanna" University Hospital, 1527 Sofia, 8 Byalo More Str., Bulgaria. Tel.: +359889807558. Fax: +35929432323. E-mail: radislav.nakov@gmail.com

Therefore, for quantifying the disease activity, a combination of clinical examination, levels of laboratory biomarkers, and endoscopic and microscopic findings are used in routine clinical practice.[8]

Several laboratory biomarkers have been evaluated for the purpose of monitoring endoscopic UC activity.[9] 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 (erythrocyte sedimentation rate, white blood cell, and C-reactive protein) have been demonstrated that their sensitivity and specificity in correlating to intestinal inflammatory activity are very low.[10,11]

Fecal markers, specifically, and calprotectin may be more specific for assessing intestinal disease activity.[12] 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.[13]

Fecal calprotectin (FCP) is a biomarker that is frequently used for monitoring IBD activity.[14] Moreover, increased concentrations of FCP have been reported to be indicative of clinical relapse in IBD patients.[15]

In the current study, we aimed to assess the usefulness of FCP as a marker of disease activity in patients with IBD.

MATERIALS AND METHODS

Study design, inclusion and exclusion criteria of enrolled subjects

This prospective observational case–control study enrolled 174 patients - 80 males and 94 females at an average age of 39.8 ± 9 (19–68) years with IBD (84 with CD and 90 with UC), referred for colonoscopy to the Clinic of Gastroenterology of "Tsaritsa Yoanna" University Hospital in Sofia between May 2015 and April 2017.

We included patients who responded to all inclusion and exclusion criteria. Inclusion criteria for were: (1) Age 18–85 years, (2) known UC or CD diagnosed according to the ECCO Guidelines,[16,17] and (3) completion of a written informed consent.

Exclusion criteria were: (1) Colorectal cancer or colon polyps, (2) indeterminate colitis, (3) history of colorectal surgery, (4) urinary incontinence (due to the risk of contamination of fecal samples), (5) pregnancy, (6) history of active nonsteroidal anti-inflammatory drugs intake (2 tablets/week), (7) oral steroids or steroid enemas intake in the past 3 months, or initiation of azathioprine treatment within the past 3 months, (8) infectious colitis, and (9) primary immunodeficiency.

We defined clinical remission in UC as a Lichtiger Clinical Activity Index of 3 points or less[18] and endoscopic remission - as UC Endoscopic Index of Severity of 0 or 1 if the descriptor was limited to vascular pattern.[19] Clinical remission in CD was determined as CD activity index <150, and endoscopic remission - as lack of mucosal lesions (erosions, ulcers, and aphthous lesions) on ileocolonoscopy.

All enrolled patients underwent a full medical assessment including a detailed medical history and physical examination. All therapies taken by the patients before enrollment were recorded. All patients underwent ileocolonoscopy. Indications for colonoscopy were clinically active disease (flare), evaluation of disease activity after treatment, having unexplained symptoms, and dysplasia surveillance for the longstanding disease.

Fecal samples were collected within 1–2 days before the colonoscopy. FCP levels were assessed in all the patients and 18 subjects, taken as a control group.

Fecal calprotectin

Calprotectin was analyzed in stool samples by means of point-of-care desktop 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).[20] The POC device uses internal standards within a range of 30–300 μg/g and sensitivity of <10 μg/g, thus, guarantying 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 the tabular and graphical presentation of results, Kolmogorov-Smirnov test, Shapiro-Wilk test, Mann-Whitney test, and receiver operating characteristic (ROC) curve analysis - to determine the cutoff of the quantitative variables. The obtained results were assessed as statistically reliable in the threshold level of significance $P < 0.05$.

Ethics approval

The study was approved by the Ethics Committee of "Tsaritsa Yoanna" University Hospital in Sofia, Bulgaria. Before initiating this study, written informed consent was obtained from all patients and healthy controls included in the study.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's human research committee.

RESULTS

A total of 84 patients with CD, 90 with UC and 18 controls (Group 1) were enrolled in this study. 41 of CD patients were with quiescent disease (Group 2), 20 with active ileal disease (Group 3), and 23 with active colonic or ileocolonic CD (Group 4). 40 of UC patients were with quiescent disease and 45 with active.

An overview of the demographic patient characteristics at baseline is provided in Table 1.

Mean FCP levels of patients with quiescent CD (97.12 ± 85.48 μg/g) were significantly higher than those of controls (34.72 ± 7.43 μg/g) (P = 0.001). There was a statistical significance between mean FCP levels of controls and patients with active ileal CD (958.44 ± 694.25 μg/g) (P < 0.001), patients with active colonic or ileocolonic CD (2143.35 ± 882.29 μg/g) (<0.001), and patients with active CD irrespective of location (Group 3 and 4) - (1533.97 ± 984.05 μg/g) (<0.001). There was a significant difference between mean FCP levels of quiescent CD and active ileal CD (<0.001), colonic or ileocolonic CD (<0.001), and active CD irrespective of location (<0.001). Furthermore, FCP concentrations of patients with active ileal CD were significantly lower than those with active colonic or ileocolonic CD (<0.001) [Table 2 and Figure 1].

The ROC analysis found that a cutoff FCP level of 315 μg/g differentiates quiescent CD from active ileal disease with 94 % sensitivity, 98 % specificity, and area under the curve (AUC) 0.984 [Figure 2]. The same cutoff FCP level (315 μg/g) differentiates quiescent CD from active CD irrespective of disease location with the same sensitivity (84%), and specificity (98%), and AUC 0.989 [Figure 3].

In UC patients, we did not found statistically significant difference between mean FCP levels of controls (34.72 ± 7.43 μg/g) and quiescent UC (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) [Table 3 and Figure 4].

DISCUSSION

At present, there are sufficient data in the literature demonstrating relationship between FCP concentrations and severity of

Table 1: Patient demographics

Characteristic	Crohn's disease	Ulcerative colitis
Number of patients	84	90
Females (%)	45 (53.6)	49 (54.4)
Age	40.2±9 (19–60)	39.4±9 (18–63)
Disease duration, mean±SD (years)	4.78±3.8	4.2±3.5
Current smoking (%)	20 (23.8)	17 (18.8)
Location of disease (%)	L1 (ileal) - 26 (30.9) L2 (colonic) - 25 (29.8) L3 (ileocolonic) - 33 (39.3)	E1 (proctitis) - 13 (14.4) E2 (left-sided colitis) - 42 (46.7) E3 (extensive colitis) - 35 (38.9)
Disease phenotype	B1 (inflammatory) - 71 (84.5) B2 (stricturing) - 8 (9.5) B3 (penetrating) - 5 (6)	-

Table 2: Fecal calprotectin (FCP) values in controls (Group 1), quiescent Crohn's disease - CD (Group 2), active ileal CD (Group 3), active colonic or ilecolonic CD (Group 4), and in all active CD patients (Groups 3 and 4)

Group	*n*	Mean FCP	SD	Median	Minimum	Maximum
Group 1 (controls)	18	34.72	7.43	31.00	30.00	58.00
Group 2 (quiescent CD)	41	97.12	85.48	60.00	30.00	360.00
Group 3 (ileal CD)	20	958.44	694.25	800.00	136.00	3000.00
Group 4 (colonic or ilecolonic CD)	23	2143.35	882.29	2150.00	247.00	3000.00
Group 3 and Group 4	43	1533.97	984.05	1460.00	136.00	3000.00

CD: Crohn's disease

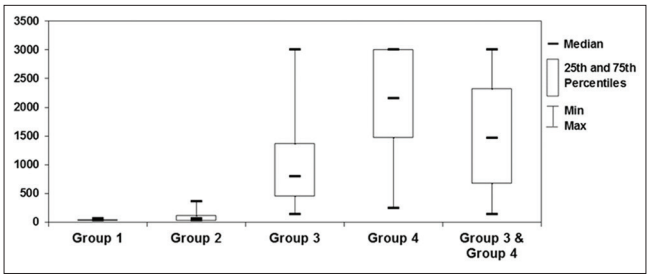

Figure 1: Comparison of fecal calprotectin values in controls (group 1), quiescent Crohn's disease - Crohn's disease (CD) (Group 2), active ileal CD (Group 3), active colonic or ileocolonic CD (Group 4), and in all active CD patients (Group 3 and 4)

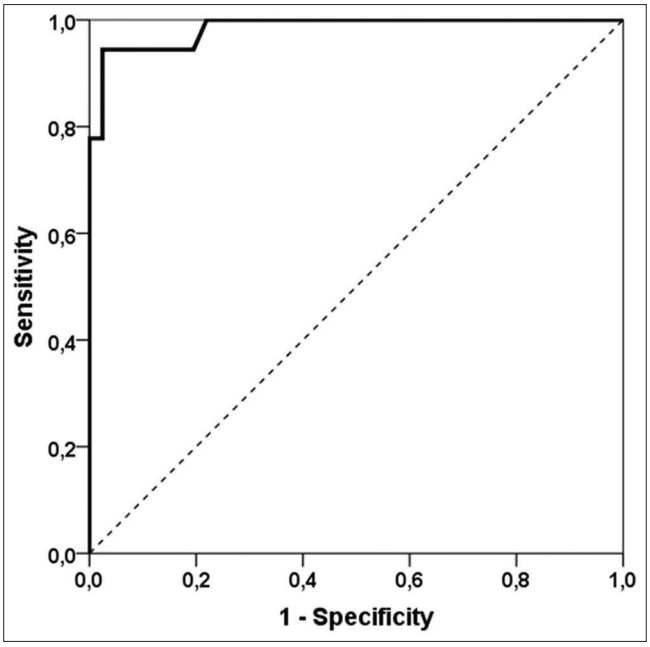

Figure 2: Receiver operating characteristic curve analysis with area under the curve comparing FCP values in patients with quiescent Crohn's disease (CD) with those in the active ileal CD

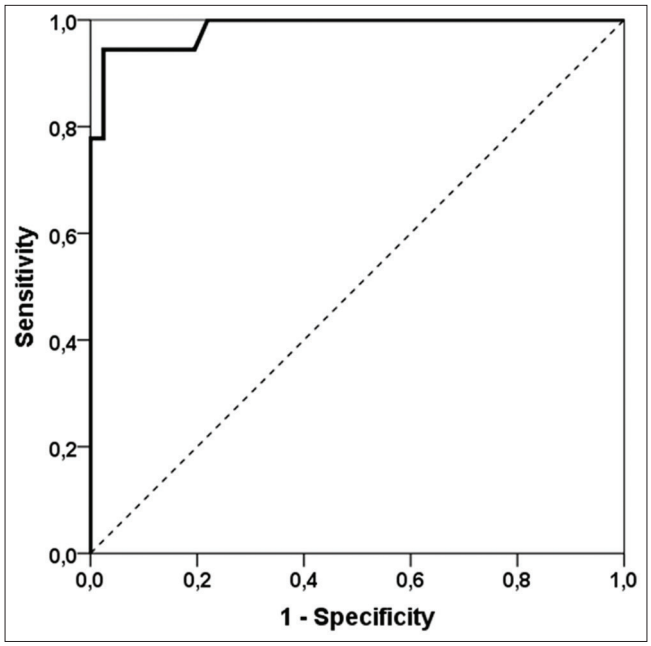

Figure 3: Receiver operating characteristic curve analysis with area under the curve comparing FCP values in patients with quiescent Crohn's disease (CD) with those in the active CD

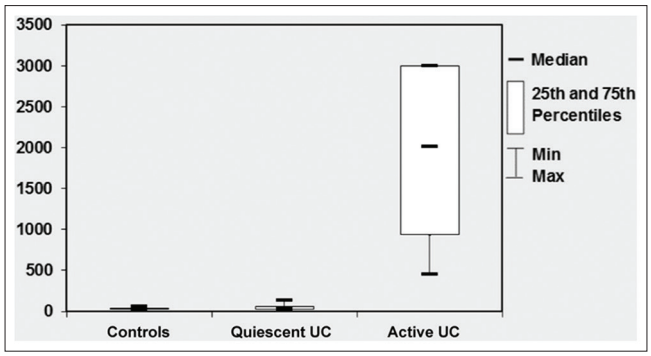

Figure 4: Comparison of fecal calprotectin values in controls, patients with quiescent ulcerative colitis (UC), and active UC

Table 3: Fecal calprotectin levels (μg/g) in controls, patients with ulcerative colitis in remission and with activity

Group	*n*	Mean	SD	Median	Min	Max
Controls	18	34.72	7.43	31.00	30.00	58.00
Quiescent UC	40	47.10	26.91	36.50	30.00	136.00
Active UC	45	1933.08	940.98	2010.00	450.00	3000.00

UC: Ulcerative colitis

intestinal inflammation in IBD. Therefore, FCP assessment is essential for the determination of remission and activity in these patients. The main goal of the modern IBD treatment is not only symptom amelioration but also MH achievement.

Even though endoscopy with biopsies is the best way to evaluate MH,[3] repetitive endoscopic examinations cannot be an integral part of any management strategy for IBD because of the well-known drawbacks of this technique. Therefore, FCP is increasingly being used as an alternative for MH assessment.

Several studies have previously suggested FCP as a marker for endoscopic disease activity in IBD patients. However,

some studies have shown contrary results.[7,21,22] Several studies showed a significant correlation between FCP levels and endoscopic activity in both UC and CD.[22-24] Røseth *et al*. performed colonoscopy with biopsies on 45 patients with IBD in clinical remission and found that 38 of them had both normal FCP values and normal histology.[21] Lamb *et al*. reported that patients with UC in endoscopic and histological remission had similar FCP values to controls.[25] This proved the presence of MH and therefore suggested that FCP could be considered as a surrogate marker of MH.

In the current 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 Schoepefer *et al*., the mean FCP value in UC patients in remission was 42.0 μg/g, which was very close to the results in the present study - 47.10 μg/g.[26] 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.[26] In line with that, 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 cutoff level between activity and remission in UC patients.

In our study, we demonstrated that a cutoff value of 315 μg/g predicts mucosal inflammation in CD patients with 94% sensitivity and 98% specificity. Similar results are shown by other authors. D'Haens *et al*. reported that a cutoff of 250 μg/g could predict mucosal inflammation with 94% sensitivity and 62% specificity in CD patients.[27] Vázquez-Morón *et al*. showed that a cutoff concentration of ≥170 μg/g (sensitivity 77.6% and specificity 95.5%) predicts a high probability of endoscopic activity.[28]

Some authors believe that FCP levels may depend on the location of inflammatory changes in CD.[7,29] However, the data are currently insufficient and controversial. In the present study, we demonstrated that FCP values in patients with the colonic or ileocolonic location were significantly higher than those with ileal disease and the latter were significantly higher than those with quiescent disease.

Although one of the most commonly reported drawbacks of FCP is its decreased diagnostic value in patients with ileal disease, in our study FCP values of all patients with active ileal CD were increased. Furthermore, a cutoff value of 315 μg/g distinguishes with the same sensitivity (94%) and specificity (98%) quiescent CD from active ileal disease and active CD regardless of location.

Probably, the biggest strength of the study is that this is one of the first observations of a large cohort of patients with IBD examined with 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 <30 min including protein extraction). Another major advantage is the simplicity of sample preparation and analysis. No more than 80 mg 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.[30,31] It has been shown that Quantum Blue® is the instrument of choice for fast and reliable determination of FCP levels.[24,30,31]

CONCLUSIONS

FCP is a reliable surrogate marker of endoscopic activity in IBD. FCP could be useful in the prediction of MH. Therefore, FCP has the potential to replace colonoscopy for the serial assessment of mucosal inflammation in IBD patients. Moreover, the rapid point-of-care method allows an easier assessment of FCP in clinical practice.

REFERENCES

1. Sandborn WJ, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, *et al.* Certolizumab pegol for the treatment of Crohn's disease. N Engl J Med 2007;357:228-38.
2. Sakuraba A, Annunziata ML, Cohen RD, Hanauer SB, Rubin DT. Mucosal healing is associated with improved long-term outcome of maintenance therapy with natalizumab in Crohn's disease. Inflamm Bowel Dis 2013;19:2577-83.
3. Fefferman DS, Farrell RJ. Endoscopy in inflammatory bowel disease: Indications, surveillance, and use in clinical practice. Clin Gastroenterol Hepatol 2005;3:11-24.
4. Konikoff MR, Denson LA. Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. Inflamm Bowel Dis 2006;12:524-34.
5. Limburg PJ, Ahlquist DA, Sandborn WJ, Mahoney DW, Devens ME, Harrington JJ, *et al.* Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. Am J Gastroenterol 2000;95:2831-7.
6. Paduchova Z, Durackova Z. Fecal calprotectin as a promising marker of inflammatory diseases. Bratisl Lek Listy 2009;110:598-602.
7. Schoepfer AM, Beglinger C, Straumann A, Trummler M, Renzulli P, Seibold F, *et al.* Ulcerative colitis: Correlation of the rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. Inflamm Bowel Dis 2009;15:1851-8.
8. Nikolaus S, Schreiber S. Diagnostics of inflammatory bowel disease. Gastroenterology 2007;133:1670-89.
9. Zauber AG. Cost-effectiveness of colonoscopy. Gastrointest Endosc Clin N Am 2010;20:751-70.
10. Sipponen T, Savilahti E, Kolho KL, Nuutinen H, Turunen U, Färkkilä M, *et al.* Crohn's disease activity assessed by fecal calprotectin and lactoferrin: Correlation with Crohn's disease

activity index and endoscopic findings. Inflamm Bowel Dis 2008;14:40-6.
11. Langhorst J, Elsenbruch S, Mueller T, Rueffer A, Spahn G, Michalsen A, *et al.* Comparison of 4 neutrophil-derived proteins in feces as indicators of disease activity in ulcerative colitis. Inflamm Bowel Dis 2005;11:1085-91.
12. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: Useful, magic, or unnecessary toys? Gut 2006;55:426-31.
13. Bjerke K, Halstensen TS, Jahnsen F, Pulford K, Brandtzaeg P. Distribution of macrophages and granulocytes expressing L1 protein (calprotectin) in human peyer's patches compared with normal ileal lamina propria and mesenteric lymph nodes. Gut 1993;34:1357-63.
14. Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. Gastroenterology 2011;140:1817-2600.
15. Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. Gastroenterology 2000;119:15-22.
16. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, *et al.* Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: Definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. J Crohns Colitis 2017;11:649-70.
17. Gomollón F, Dignass A, Annese V, Tilg H, Van Assche G, Lindsay JO, *et al* 3rd European evidence-based consensus on the diagnosis and management of Crohn's disease 2016: Part 1: Diagnosis and medical management. J Crohns Colitis 2017;11:3-25.
18. Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, *et al.* Cyclosporine in severe ulcerative colitis refractory to steroid therapy. N Engl J Med 1994;330:1841-5.
19. Travis SP, Schnell D, Krzeski P, Abreu MT, Altman DG, Colombel JF, *et al.* Developing an instrument to assess the endoscopic severity of ulcerative colitis: The ulcerative colitis endoscopic index of severity (UCEIS). Gut 2012;61:535-42.
20. Rosenfeld G, Greenup AJ, Round A, Takach O, Halparin L, Saadeddin A, *et al*. FOCUS: Future of fecal calprotectin utility study in inflammatory bowel disease. World J Gastroenterol 2016;22:8211-8.
21. Røseth AG, Aadland E, Grzyb K. Normalization of faecal calprotectin: A predictor of mucosal healing in patients with inflammatory bowel disease. Scand J Gastroenterol 2004;39:1017-20.
22. D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, *et al.* Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis 2012;18:2218-24.
23. Ricanek P, Brackmann S, Perminow G, Lyckander LG, Sponheim J, Holme O, *et al.* Evaluation of disease activity in IBD at the time of diagnosis by the use of clinical, biochemical, and fecal markers. Scand J Gastroenterol 2011;46:1081-91.
24. Lee YW, Lee KM, Lee JM, Chung YY, Kim DB, Kim YJ, *et al*. The usefulness of fecal calprotectin in assessing inflammatory bowel disease activity. Korean J Intern Med 2018; doi: 10.3904/kjim.2016.324.
25. Lamb CA, Mohiuddin MK, Gicquel J, Neely D, Bergin FG, Hanson JM, *et al.* Faecal calprotectin or lactoferrin can identify postoperative recurrence in Crohn's disease. Br J Surg 2009;96:663-74.
26. Schoepfer AM, Trummler M, Seeholzer P, Seibold-Schmid B, Seibold F. Discriminating IBD from IBS: Comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. Inflamm Bowel Dis 2008;14:32-9.
27. D'Haens GR, Fedorak R, Lémann M, Feagan BG, Kamm MA, Cosnes J, *et al.* Endpoints for clinical trials evaluating disease modification and structural damage in adults with Crohn's disease. Inflamm Bowel Dis 2009;15:1599-604.
28. Vázquez Morón JM, Pallarés Manrique H, Machancoses FH, Ramos Lora M, Ruiz Frutos C. Accurate cut-offs for predicting endoscopic activity and mucosal healing in Crohn's disease with fecal calprotectin. Rev Esp Enferm Dig 2017;109:130-6.
29. Damms A, Bischoff SC. Validation and clinical significance of a new calprotectin rapid test for the diagnosis of gastrointestinal diseases. Int J Colorectal Dis 2008;23:985-92.
30. Coorevits L, Baert FJ, Vanpoucke HJ. Faecal calprotectin: Comparative study of the quantum blue rapid test and an established ELISA method. Clin Chem Lab Med 2013;51:825-31.
31. Sydora MJ, Sydora BC, Fedorak RN. Validation of a point-of-care desk top device to quantitate fecal calprotectin and distinguish inflammatory bowel disease from irritable bowel syndrome. J Crohns Colitis 2012;6:207-14.

How to cite this article: Nakov RV, Nakov VN, Tankova LT, Gerova VA, Kundurzhiev TG. Fecal Calprotectin for Assessment of Inflammatory Bowel Disease Activity. Clin Res Immunol 2018;1(1):1-6.