Nobel Med 2020; 16(3): 13-21

# THE COMBINED PERFORMANCE OF FECAL CALPROTECTIN WITH FECAL OCCULT BLOOD, LYMPHOCYTE AND NEUTROPHIL PERCENTAGES IN DISCRIMINATING ULCERATIVE COLITIS

**Esma Sürmen Gür, Özge Cindemir**

*Uludağ Üniversitesi Tıp Fakültesi Tıbbi Biyokimya Anabilim Dalı, Bursa*

## ABSTRACT

***Objective:*** *Fecal calprotectin (FC) is a promising marker for discrimination of irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). The search for non-invasive tools for identification of ulcerative colitis (UC), in IBD patients, is still an issue. We aimed to evaluate if combination with other parameters improves the predictive value of FC in UC diagnosis.*

***Material and Method:*** *Patients who underwent FC analysis and that were diagnosed with IBS (n=121) and IBD (UC, n=186 and Crohn's disease (CD), n=101) were selected for this methodologic study. Logistic regression analysis was used to model the prediction of UC using FC individually or in combination with fecal occult blood (FOB), lymphocyte percent (LP) and neutrophil percent (NP) values.*

***Results:*** *FC, FOB, LP, and NP were significantly different in UC patients compared to both CD and IBS patients. The AUCs of "FC+FOB+LP+NP" and "FC+FOB" models were significantly greater than that of FC for predicting UC in the entire patient population (AUC=0.789, 0.774 and 0.705, respectively, p<0.05) and in IBD patients (AUCs=0.755, 0.708 and 0.607, respectively, p<0.05). AUCs of "FC+LP"(0.800) and "FC+LP+NP" (0.800) models were significantly greater compared to that of FC (0.756) in predicting IBD in the entire patient population (p<0.05).*

***Conclusion:*** *The combination of inflammatory blood markers and stool biomarkers may provide valuable, non-invasive tools for the identification of UC in IBS and IBD patients.*

***Keywords:*** *Fecal calprotectin, fecal occult blood, ulcerative colitis, inflammatory bowel disease, ırritable bowel syndrome, lymphocyte percent, neutrophil percent.*

**CORRESPONDING AUTHOR:** Esma Sürmen Gür Uludağ Üniversitesi Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı,16059 Bursa, Turkiye esma@uludag.edu.tr

**ESG https:**//orcid.org/0000-0001-7377-9682 **ÖC https:**//orcid.org/0000-0002-2973-6682

**DELIVERING DATE:** 04 / 07 / 2019 • **ACCEPTED DATE:** 13 / 01 / 2020

## FEKAL KALPROTEKTİNİN, GAİTADA GİZLİ KAN, LENFOSİT VE NÖTROFİL YÜZDELERİ İLE BİRLİKTE DEĞERLENDİRİLMESİNİN ÜLSERATİF KOLİTİN AYIRICI TANISINA KATKISI


## ÖZET

**Amaç:** Fekal kalprotektin (FC) irritabl bağırsak sendromu (IBS) ve inflamatuvar bağırsak hastalığının (IBD) ayırımında ümit vaat eden yeni bir belirteçtir. Ancak, IBD vakalarında ülseratif kolitin (UC) ayırdedilmesi için yeterli görülmemektedir. Bu çalışmada FC'nin başka parametreler ile birleştirildiğinde UC tanısı için prediktif değerinin ne kadar değişebileceğini değerlendirmeyi amaçladık.

**Materyal ve Metot:** Bu metodolojik çalışmaya, FC ölçümü yapılmış hastalar arasından IBS (n=121) ve IBD (UC, n=186 ve Crohn hastalığı (CD), n=101) tanısı alanlar dahil edildi. Lojistik regresyon analizi ile FC'nin tek başına ve gaitada gizli kan (FOB), lenfosit yüzdesi (LP) ve nötrofil yüzdesi (NP) ile birlikte modellendiğinde UC tahminindeki değeri incelendi.

**Bulgular ve Sonuç:** UC hastalarında FC, FOB, LP ve NP değerleri hem CD hem de IBS hastalarına göre anlamlı olarak farklıydı. Bütün hasta popülasyonunda UC tahmini için "FC+FOB+LP+NP" ve "FC+FOB" modellerinin eğri altı alanları (AUC) (sırasıyla, 0,789 ve 0,774), FC'ninkinden (0,705) anlamlı olarak büyüktü ($p$<0,05). Benzer şekilde, IBD hastalarında UC tahmini için de "FC+FOB+LP+NP" ve "FC+FOB" modellerinin AUC'leri (sırasıyla, 0,755 ve 0,708) FC'ninkinden (0,607) anlamlı olarak büyüktü ($p$<0,05). Bütün hasta popülasyonunda IBD tahmini için "FC+LP"ve "FC+LP+NP" modellerinin AUC'leri (0,800 ve 0,800), FC'ninkinden (0,756) anlamlı olarak yüksekti ($p$<0,05). Bulgular, inflamatuvar belirteçler ile gaita belirteçlerinin birleştirilerek değerlendirilmesinin IBS ve IBD vakalarında UC'nin tanınması için yararlı, non-invaziv yöntemler sağlayabileceğini düşündürmektedir.



**Anahtar kelimeler:** Fekal kalprotektin, gaitada gizli kan, ülseratif kolit, inflamatuvar bağırsak hastalığı, irritabl bağırsak sendromu, lenfosit yüzdesi, nötrofil yüzdesi.


## INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon characterized by mucosal ulceration with increasing incidence rate worldwide in individuals of all ages.[1] It is one of the forms of chronic intestinal inflammation named inflammatory bowel disease (IBD). IBD is frequently confused with irritable bowel syndrome (IBS) because they share many common clinical presentations. While IBS is a common gastrointestinal functional disorder with a benign course, IBD has two major forms: Crohn's disease (CD) and ulcerative colitis (UC), whose progress alternate between inflammatory active and remission periods causing severe complications, that may need hospitalization. Of these two IBD forms, UC presents continuous inflammation in colon mucosa whereas CD shows a discontinuous involvement in any part of the intestinal tract, mostly terminal ileum and colon. Like the difficulties in IBD and IBS discrimination, UC and CD discrimination is also hard because of the similar clinical manifestations of the two diseases; and is equally important because of different medical and surgical treatment strategies of the two disorders.[2] It is emphasized that the importance of the distinction between UC and CD would grow as the disease-specific therapies evolve in clinical practice.[2]

Blood tests, stool analysis, and endoscopic evaluations are common studies for proper diagnosis of IBD in the practice. Although endoscopy is the gold standard to confirm UC and CD diagnosis, its use remains limited because the procedure is operator-dependent, invasive and expensive as well as discomforting for the patient.[3] Therefore, various screening tests to guide for the need for endoscopy are widely studied recently.[2,4] Diagnostic value of blood tests such as inflammatory biomarkers like erythrocyte sedimentation rate, C-reactive protein or leukocyte-derived proteins like eosinophilic cationic protein, elastase, esterase, myeloperoxidase, lysozyme, lactoferrin, whole blood count or fecal occult blood (FOB) has been studied in this respect, but the sensitivity and specificity of none of these parameters were sufficient for this purpose.[5] Over the past years, calprotectin has been studied in stool as a biological marker of intestinal inflammation and has been indicated to be a better marker for active IBD.[6-9] This neutrophil-derived cytosolic protein is shown to be closely correlated with clinical activity of both UC and CD and is reported to be the most promising marker to help diagnose IBD, however, it is not sensitive and specific enough to differentiate UC and CD.[7,9,10] Therefore, the quest for new tests to discriminate between UC, CD, and IBS is still a matter in hand.

In this retrospective study, we aimed to evaluate the combined diagnostic strength of fecal calprotectin (FC) with some inflammatory biomarkers and FOB in differentiating UC from CD and IBS.

## MATERIAL AND METHOD

### Study Design

This was a methodologic single-center study including all patients with gastrointestinal complaints, who underwent FC analysis in our hospital from May 2016 to June 2017. The study protocol was approved by the local institutional ethics review board (Uludag Universitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu; protocol number 2017-16/5; date of approval: 07 November 2017). It is in accordance with the Declaration of Helsinki. Clinical and demographic data on age, gender, laboratory results, and diagnosis were extracted from electronic hospital records.

### Study Population

Among the patients that referred to the clinics of our hospital with gastrointestinal complaints during the study period, 726 patients underwent FC analysis (Figure 1). FC test was requested for differential diagnosis of irritable bowel from chronic inflammatory bowel diseases and for the exclusion of an organic disease of the intestinal tract in the presence of signs of a functional bowel disease. Among these patients, those who were investigated endoscopically and had a final diagnosis of IBS or IBD (UC and CD) whose diagnoses were confirmed by endoscopic imaging were included in this study. Rome III criteria and endoscopic evaluation were used together to diagnose IBS. Cases without any recorded diagnosis in the hospital information system (HIS), with diagnoses of unclassified IBD, with a diagnosis other than IBS, UC or CD, and the IBD cases of which the active IBD diagnoses were not confirmed by endoscopic imaging and/or pathological evaluation were not included in the study (n=318). Differential diagnosis for UC and CD were made by clinical evaluation integrated with a combination of endoscopic, and/or histological investigations according to the European Crohn's and Colitis Organization (ECCO) guidelines.[11] Intestinal tuberculosis is a very common clinical entity that needs to be differentiated from CD; traditional tuberculin skin test or interferon-gamma release assay (QuantiFERON-TB Gold In-Tube) or Polymerase Chain Reaction analysis of endoscopic biopsy specimens were used for discrimination of intestinal tuberculosis.

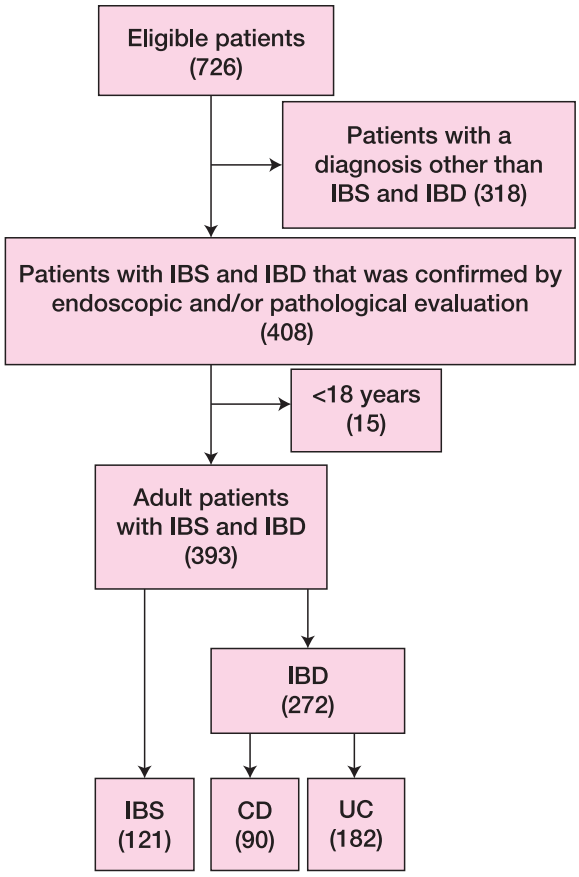


**Figure 1.** Flow diagram of patients who underwent fecal calprotectin analysis and were included in the study
**UC:** Ulcerative colitis, **IBS:** irritable bowel syndrome, **IBD:** inflammatory bowel disease

Frequencies of patient ages in study groups showed that there were no patients under 18 years in the IBS group. When age frequency was assessed in IBD group, 4 of cases under 18 years were diagnosed with ulcerative colitis and 11 with Crohn's disease, while the number of 18 years and older patients were 182 for UC and 90 for CD. It is stated that FC concentrations are significantly higher in younger patients compared to adults and therefore should be evaluated separately.[12] In the present study, because of the small sample sizes in younger patient groups, results of cases 17 years and younger were excluded from the study.

### Laboratory Tests

Results of inflammatory biomarkers erythrocyte sedimentation rate, C-reactive protein, whole blood count and fecal markers FC and FOB were used for evaluation. These tests were performed at the time of referral to the clinics with gastrointestinal complaints. Those parameters that were significantly different in UC patients compared to both IBS and CD patients were selected to evaluate if they provide any additional predictive value for UC in a combined model with FC. Accordingly, combined models were

**Table 1.** Age and Gender Distribution of Study Groups.

| | IBS (n=121)(31)[a] | IBD (n=272)(69)[a] | UC (n=182)(67)[b] | CD (n=90)(33)[b] |
|---|---|---|---|---|
| **Female** | 59 | 133 | 88 | 45 |
| **Male(%)** | 62 (51) | 139 (51) | 94 (52) | 45 (50) |
| **Age (SD)** | 39.5 (13.8) | 41.5 (13.9) | 43.1 (13.9)[c] | 38.1 (13.3) |

[a]: percent ratio in study population
[b]: percent ratio in IBD
[c]: significantly different from IBS ($p$<0.05) and from CD ($p$<0.01)
**IBS:** Irritable bowel syndrome, **IBD:** inflammatory bowel disease, **UC:** ulcerative colitis, **CD:** Crohn's disease, **SD:** standard deviation.

**Table 2.** Diagnostic Characteristics of the FC, FC+FOB and FC+FOB+LP+NP Models in the Diagnosis of UC and IBD.

| | | Cut-off | AUC | Sensitivity | Specificity | +LR | -LR | +PV | -PV |
|---|---|---|---|---|---|---|---|---|---|
| **UC in IBS+IBD** | FC | >67 | 0.705 | 61.54 | 73.46 | 2.32 | 0.52 | 66.7 | 68.9 |
| | FC+FOB | >0.253[a] | 0.774[b] | 66.07 | 82.20 | 3.71 | 0.41 | 63.8 | 83.6 |
| | FC+FOB+LP+NP | >0.351[a] | 0.789[b] | 58.18 | 90.35 | 6.03 | 0.46 | 74.4 | 81.7 |
| **UC in IBD** | FC | >170 | 0.607 | 37.91 | 85.56 | 2.62 | 0.73 | 84.1 | 40.5 |
| | FC+FOB | >0.439[a] | 0.708[b] | 71.43 | 67.39 | 2.19 | 0.42 | 72.7 | 66.0 |
| | FC+FOB+LP+NP | >0.517[a] | 0.755[b] | 63.64 | 80.00 | 3.18 | 0.45 | 79.5 | 64.3 |
| **IBD in IBS+IBD** | FC | >55 | 0.756 | 63.60 | 84.30 | 4.05 | 0.43 | 90.1 | 50.7 |
| | FC+LP | >0.730[a] | 0.800[b] | 57.62 | 92.31 | 7.49 | 0.46 | 94.5 | 48.6 |
| | FC+LP+NP | >0.693[a] | 0.800[b] | 65.43 | 85.47 | 4.50 | 0.40 | 91.2 | 51.8 |

**FC:** Fecal calprotectin, **FOB:** fecal occult blood, **LP:** lymphocyte percent, **NP:** neutrophil percent, **UC:** ulcerative colitis, **IBS:** irritable bowel syndrome, **IBD:** inflammatory bowel disease, **AUC:** area under the curve, **LR:** likelyhood ratio, **PV:** predictive value.
[a]: the cut-offs for the combined models are estimated by individual predicted probabilities.
[b]: significantly different from that of FC, $p$<0.05.

constructed as: "FC+FOB", "FC+ lymphocyte percent (LP)", "FC+ neutrophil percent (NP)", "FC+FOB+LP", "FC+FOB+NP", "FC+FOB+LP+NP", "FC+LP+NP". FC was measured by immunochromatographic assay using Calfast (Eurospital, Trieste, Italy ) and FOB was determined by colloidal gold agglutination measured as optical change, using Hemo Techt NS-Plus C system (Alfresa Pharma Co., Osaka, Japan ) and whole blood counts were determined by the multi-angle polarized light scattering separation method on the Cell-Dyn 3700 (Abbott-USA), while the rest of the biochemical parameters (not used for combined models in the present study) were measured by the automated systems in our routine laboratory.

### Statistical Evaluation

Statistical analysis was performed using statistical software IBM SPSS v.23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. ) and MedCalc Statistical Software version 18.10.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018). After assessing normality of data, all continuous variables were summarized in terms of means (standard error). Groups were compared using one-way analysis of variance (ANOVA) and Bonferroni test was used as post hoc test. Value of $p$<0.05 was considered statistically significant.

Logistic regression analysis was used to model the prediction of UC using FC in combination with FOB, LP and NP values. Receiver operating characteristics (ROC) curves were plotted for the variables and cutoff values were determined. The gold standard investigation was endoscopic imaging and histopathological examination for all IBD and IBS cases and merely endoscopic evaluation for IBS cases when a biopsy specimen was not available for histopathological analysis. The sensitivity, specificity, positive likelihood ratio (+LR), negative (-) LR, positive predictive value (PPV) and negative predictive value (NPV) were determined for each cut-off value. The performance of the variables in predicting UC was determined using ROC curves, with the area under the curve (AUC) being of primary interest. AUCs were compared to determine the best model of predictors for UC in IBS and IBD patients.

## RESULTS

### Characteristics of Patients

The study population consisted of 121 IBS patients and 272 IBD patients of which 182 were UC and 90 were CD patients. Age and gender distribution among study groups are given in Table 1.

### Results of Laboratory Tests

Of the parameters evaluated, only FC, FOB, LP and NP values were significantly different in UC patients compared to both CD and IBS patients (Figure 2). FC was 60 µg/g in IBS, 101 µg/g in CD and 143 µg/g in UC patients; FOB was 83 ng/mL in IBS, 303 ng/mL in CD and 1458 ng/mL in UC patients; LP was 33% in IBS, 25% in CD and 27% in UC patients; NP was 57% in IBS, 66% in CD and 62% in UC patients. While FC and FOB in UC patients were significantly higher compared to both CD ($p$<0.001) and IBS ($p$<0.001) groups; LP were significantly lower in both UC and CD patients compared to those of IBS ($p$<0.001), with significantly greater decrease in CD patients compared to UC ($p$<0.05), whereas NP were significantly higher in both UC and CD patients compared to those of IBS ($p$<0.001) with greater increase in CD patients compared to UC ($p$<0.01).

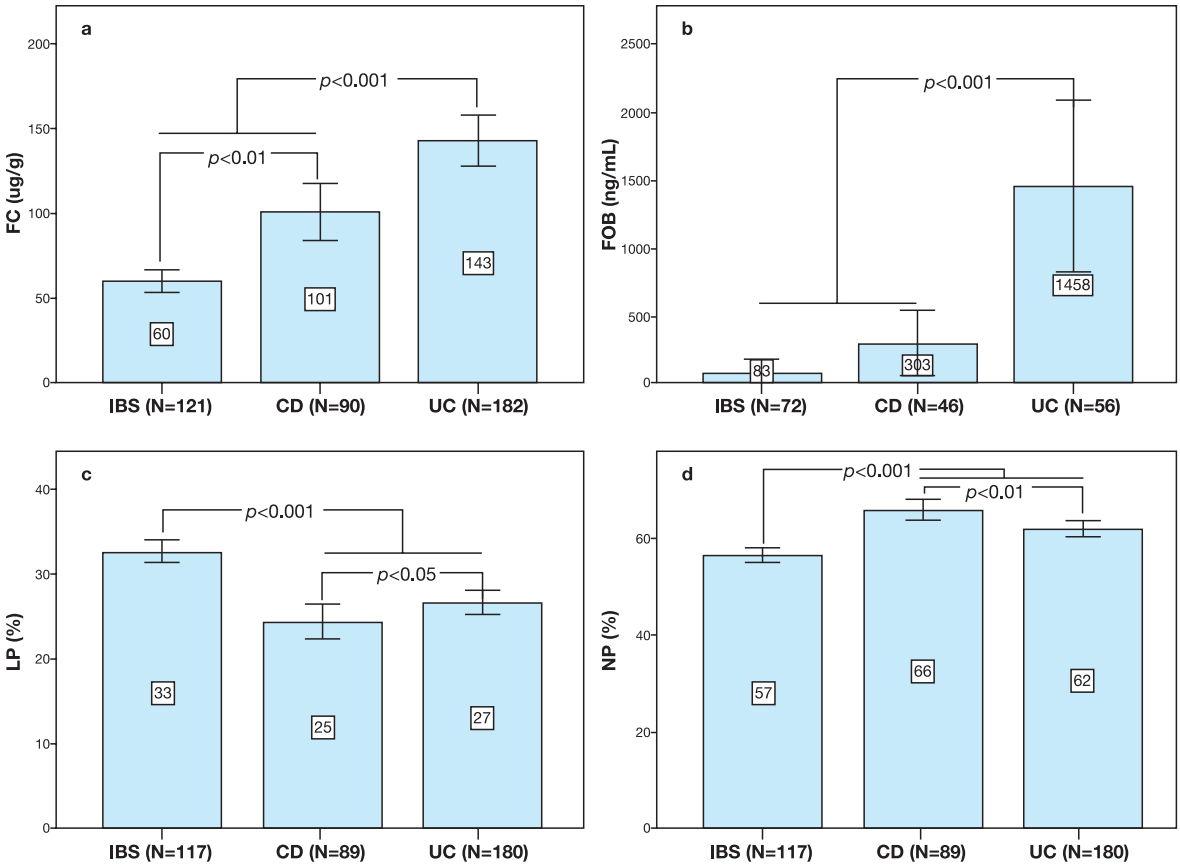


**Figure 2. (a)** Fecal calprotectin (FC), **(b)** Fecal occult blood (FOB), **(c)** Lymphocyte percentage (LP), and **(d)** Neutrophil percentage (NP) in irritable bowel syndrome (IBS), Crohn's disease (CD) and ulcerative colitis (UC) patients. Bars represent means (SE).

### ROC Analysis of Combined Models

Among the AUCs of FC (0.705), "FC+FOB" (0.774), "FC+LP" (0.707), "FC+NP" (0.702), "FC+FOB+LP" (0.761), "FC+FOB+LP+NP" (0.789), "FC+FOB+NP" (0.753) and "FC+LP+NP" (0.729) for prediction of UC in the entire study population (IBS + IBD), AUCs of combined models "FC+FOB" and "FC+FOB+LP+NP" were significantly greater than that of FC ($p<0.05$) (Figure 3-a). Similarly, among the AUCs of FC (0.607), "FC+FOB" (0.708), "FC+LP" (0.653), "FC+NP" (0.671), "FC+FOB+LP" (0.722), "FC+FOB+LP+NP" (0.755), "FC+FOB+NP" (0.727) and "FC+LP+NP" (0.688) for prediction of UC in solely IBD patients, only AUCs of combined models "FC+FOB" and "FC+FOB+LP+NP" were significantly greater than that of FC ($p<0.05$) (Figure 3-b). "FC+FOB+LP+NP" model displayed the largest AUC for predicting UC in the entire patient population and in the IBD patients, however, was not statistically different from that of "FC+FOB" (Figure 3-a and b). AUCs of these models were also assessed for prediction of IBD in the entire IBD and IBS patients: FC (0.756; 0.000), "FC+FOB" (0.746; 0.000), "FC+LP" (0.800; 0.000), "FC+NP" (0.793; 0.000), "FC+FOB+LP" (0.757; 0.000), "FC+FOB+LP+NP" (0.760; 0.000), "FC+FOB+NP" (0.761; 0.000) and "FC+LP+NP" (0.800; 0.000). In predicting IBD in the entire patient population, AUCs of "FC+LP" and "FC+LP+NP" models were significantly greater compared to that of FC ($p<0.05$) (Figure 3-c). The sensitivity, specificity, +LR, - LR, PPV, and NPV for the models that show significantly different performance are given in Table 2.

## DISCUSSION

FC is suggested as a promising marker for discriminating IBD from IBS, however, need of new non-invasive tools for differentiating the two forms of IBD, namely UC and CD, is still a point at issue.[3,9,13] This retrospective study was conducted to investigate if the combination of some blood tests with stool analysis strengthens the diagnostic power of FC. The study population constituted of adults with gastrointestinal complaints that underwent FC analysis and diagnosed with IBS or IBD. Only the cases of which the diagnoses were confirmed by endoscopic imaging and/or pathological evaluation were included in the study (Figure1). The reason of the fewer number of IBS patients included in this study may be that most of the IBS patients did not undergo endoscopic imaging or FC analysis, possibly due to their sufficient clinical signs for IBS diagnosis

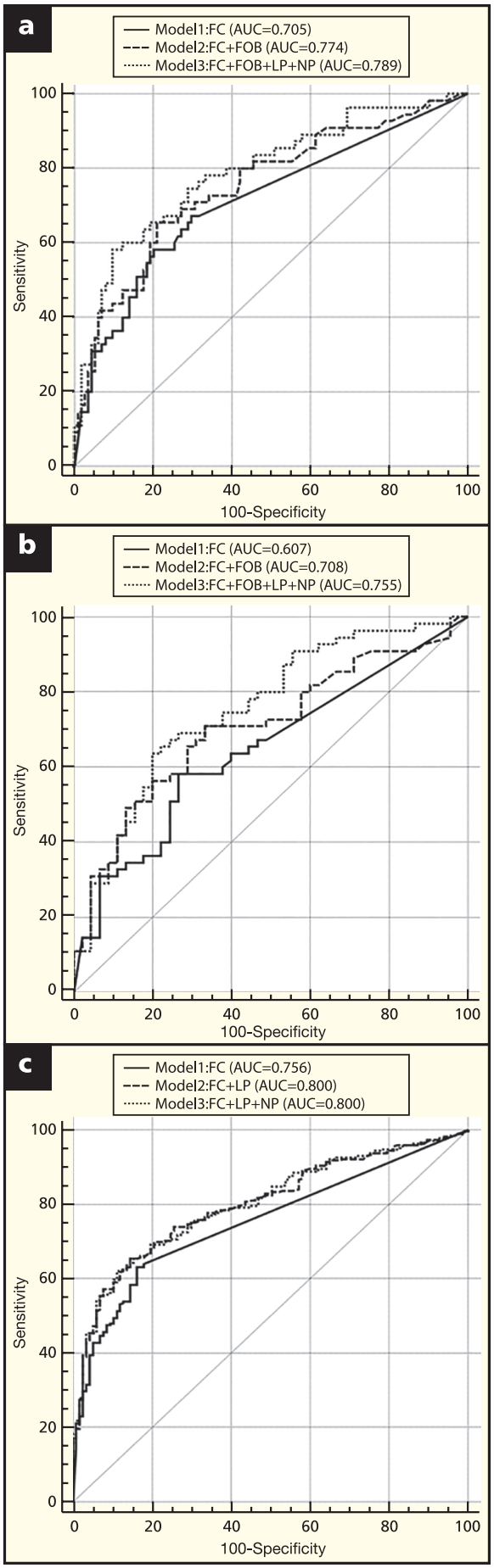


**Figure 3.** Receiver operating characteristic curves of differentiating models (a) for UC in IBD+IBS; (b) for UC in IBD; and (c) for IBD in IBD+IBS.

**UC:** Ulcerative colitis, **IBS:** Irritable bowel syndrome, **IBD:** Inflammatory bowel disease

at the referral to the hospital. The significantly higher FC values in UC ($p<0.001$) and CD ($p<0.01$) patients compared to those of IBS patients in the present study are in accordance with a large body of literature.[7-9,14] Also, the significantly higher FC results in UC patients compared to CD patients ($p<0.001$) are in agreement with the previous studies.[15,16] In the current study, ROC curve analysis was used to establish the optimal cut-off values for discrimination of UC. Higher cut-off values were calculated for discrimination of UC in IBD patients compared to that calculated for discrimination of UC in IBD+IBS patients (Table 2). High FC concentrations of CD and UC patients contribute to the higher mean FC concentration in the IBD group. On the other hand, lower FC concentrations of IBS patients decrease the mean FC concentration in the whole patient population (IBD+IBS). This influences the different cut-off values computed for UC discrimination in the two groups. Distinct cut-off levels for FC were reported in discrimination of UC in previous studies with different settings.[3,17-21] As D'Angelo *et al.* indicated, a clear cut-off level of FC for differential diagnosis is still lacking.[7] In their review, Tontini *et al.* suggested that FC alone, like various other fecal biomarkers, cannot be considered useful to refine the differential diagnosis in subjects with IBD colitis.[2] Therefore we considered combining FC results with FOB measurements. While FC is accepted as a biomarker of intestinal inflammation, FOB is a well-known indicator of mucosal damage in the intestine.[22] Several investigators mentioned the value of FOB quantification in IBD diagnosis.[21,23] In the present study, both FC and FOB levels were significantly higher in UC patients compared to both CD and IBS patients leading us to evaluate their possible combined strength in differentiating UC from CD and IBS.

Kok *et al.* have quantified the diagnostic accuracy of FC and FOB tests for the inclusion or exclusion of organic bowel diseases in patients with persistent lower-abdomen complaints who needed colonoscopy referral and suggested that these tests could rule out organic bowel disease to a reasonable extent.[24] In our patient population, different from theirs, patients with UC and CD among organic bowel diseases and patients with IBS among non-organic bowel diseases were included. Therefore this study examines similar parameters in a refined cross-sectional population, focusing on identification of UC in IBS and IBD patients. According to the ROC analysis, the combined model of FC and FOB demonstrates significantly better diagnostic performance in discriminating UC (Figure

3-a and b). With higher sensitivity and specificity, our results indicate that in a combined model with FOB test, the strength of FC for discriminating UC in this patient cohort improves significantly when compared to the single FC assessment (Table 2). When evaluated for discrimination of UC in IBD patients, the combined model with FOB displays a higher sensitivity with a decreased specificity compared to that of single FC test. This suggests that the combined model has greater ability to correctly identify UC but is not equally good in identifying individuals without UC.

The two other parameters which were significantly different in UC patients compared to both CD and IBS were LP and NP (Figure 2-c and d). Decreased LP levels observed in IBD patients are in accordance with the previous reports.[25,26] It is known that circulating lymphocyte apoptosis increases in systemic inflammatory response causing a decrease in LP in IBD.[25-27] It is also well known that the decrease in the percentages of lymphocyte is accompanied by an increase in circulating neutrophils in systemic inflammation.[28] Wera *et al.* suggested that the role of neutrophils in the pathogenesis of IBD would be dual and may differ between CD and UC.[29] While neutrophil accumulation in colonic mucosa is increased in UC, CD seems to result from an impaired recruitment of these cells. Pawlica-Gosiewska *et al.* reported increased neutrophil counts in both UC and CD patients in a recent study and in line with our findings, the increase was significantly higher in CD patients compared to that of UC group.[30] The difference in the level of circulating NP increment in UC and CD patients may be due to the distinct role of neutrophils in the two clinical states. In the present report, higher fecal concentrations of calprotectin, which is a neutrophilic protein, indicate a larger number of neutrophil accumulation in colonic mucosa of UC patients, leading to greater tissue damage and consequently a greater increase in FOB concentration compared to CD cases, probably because of the different inflammatory patterns of the two diseases.

According to the results of this study, combining LP and NP with FOB and FC increased the diagnostic performance for UC in the whole patient population (AUC=0.789), however, this did not significantly improve the performance achieved by the FC+FOB model (Table 2, Figure 3-a). FC+FOB+LP+NP model increased the specificity compared to FC+FOB model, however, did not influence the sensitivity equally. Comparably, this model demonstrated better performance for differentiating UC from CD, with higher sensitivity compared to single FC assessment and higher specificity compared to that of FC+FOB model.

When the performance of combined models in discriminating IBD and IBS were evaluated, combining FC and FOB did not affect the diagnostic performance of FC. In a prospective study by Högberg *et al.*, the performance of combining FC and fecal haemoglobin were evaluated for identification of colorectal carcinoma and IBD in patients that received an FC or fecal haemoglobin test.[23] Although the patient populations and the designs of the two studies differ, with findings similar to ours, they suggested that combining the FC test showed no improvement over fecal haemoglobin alone. In the present study, addition of either LP or LP+NP to FC measurement improved the diagnostic performance for IBD significantly (AUC=0.800; $p<0.05$)(Table 2, Figure 3-c). These two models, with equal AUCs, were not superior to one another.

Although there are studies defining the role of combined models of FC in various colorectal diseases, to our knowledge, this is the first study questioning the combined performance of FC with other laboratory tests in discriminating UC from CD and IBS patients.[21,23,24,31] The key conclusion of this study is that the combination of FC concentration with FOB measurement and lymphocyte and neutrophil percentages improves diagnosis of UC, and combining FC with lymphocyte and neutrophil percentages influences the diagnostic performance of FC in identification of IBD. These results bring in mind that although classified one within the other, UC discrimination and IBD discrimination may benefit from different parameters, and we suggest that future prospective studies assessing the diagnostic performance of combined models of various inflammatory and/or intestinal tissue damage markers with FC would contribute to the need for non-invasive means to improve differential diagnosis in IBD patients.

The present work has several limitations, and they are mostly due to its retrospective design. In this study, available data on the HIS database were evaluated in a selected patient population. Therefore, some data like records on disease sub-typing, disease severity or Bristol stool form notes, that would be valuable to discuss, were not available on HIS. Another limitation was the lack of radiological investigation records available for all cases. The small size of our population is also a weakness. Of the 726 patients who underwent

FC analysis within the study interval, we could only include 393. The reason for this was mainly that our strict inclusion criteria limited the number of eligible patients. Also, the retrospective nature of the study restricted the variety of parameters to be evaluated in combined models. Finally, other factors, such as current medications or the use of immunosuppressive agents, which may affect the level of inflammatory markers were not evaluated in the present study. Therefore, future prospective studies with a more clear and defined patient cohort of IBD and IBS would provide more detailed data for a better interpretation.

Ethical statement: The study protocol was approved by the local institutional ethics review board (Uludağ Üniversitesi Tıp Fakültesi Klinik Araştırmalar Etik Kurulu; protocol number 2017-16/5; date of approval: 07 November 2017).


## Acknowledgments

Authors are grateful to Assoc. Prof. Güven Özkaya for his great assistance in statistical evaluation.

*The authors declare that there are no conflicts of interest.


## REFERENCES


1. Muthas D, Reznichenko A, Balendran CA, et al. Neutrophils in ulcerative colitis: a review of selected biomarkers and their potential therapeutic implications. Scand J Gastroenterol 2017; 52:125-135.
2. Tontini GE, Vecchi M, Pastorelli L, Neurath MF, Neumann H. Differential diagnosis in inflammatory bowel disease colitis: State of the art and future perspectives. World J Gastroenterol 2015; 21:21-46.
3. Lozoya Angulo ME, de Las Heras Gómez I, Martinez Villanueva M, Noguera Velasco JA, Aviles Plaza F. Faecal calprotectin, an useful marker in discriminating between inflammatory bowel disease and functional gastrointestinal disorders. Gastroenterol Hepatol 2017; 40: 125-131.
4. Mortensen JH, Manon-Jensen T, Jensen MD, et al. Ulcerative colitis, Crohn's disease, and irritable bowel syndrome have different profiles of extracellular matrix turnover, which also reflects disease activity in Crohn's disease. PLoS ONE. 2017; 12:e0185855.
5. Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic, or unnecessary toys? Gut 2006; 55: 426-431.
6. Canani RB, Rapacciuolo L, Romano MT, et al. Diagnostic value of faecal calprotectin in paediatric gastroenterology clinical practice. Digestive and Liver Disease 2004; 36: 467-470.
7. D'Angelo F, Felley C, Frossard JL. Calprotectin in daily practice: where do we stand in 2017? Digestion 2017; 95: 293-301.
8. Reenaers C, Bossuyt P, Hindryckx P, et al. Expert opinion for use of faecal calprotectin in diagnosis and monitoring of inflammatory bowel disease in daily clinical practice. United European Gastroenterology Journal 2018; 6: 1117–1125.
9. McMahon CW, Chhabra R. The role of fecal calprotectin in investigating digestive disorders. J Lab Precis Med 2018; 3: 19.
10. Sipponen T, Kolho K-L. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. Scand J Gastroenterol 2015; 50: 74-80.
11. Annese V, Daperno M, Rutter MD, et al. European evidence based consensus for endoscopy in inflammatory bowel disease. J Crohns Colitis 2013; 7: 982-1018.
12. Rheenen PF, Vijver EV, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ 2010; 341: c3369.
13. Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. Clin Exp Gastroenterol 2016; 9: 21-29.
14. Tibble J, Teahon K, Thjodleifsson B, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000; 47: 506-513.
15. Lin J-F, Chen JM, Zuo J-H, et al. Meta-analysis: Fecal calprotectin for assessment of ınflammatory bowel disease activity. Inflamm Bowel Dis 2014; 20:1407-1415.
16. Manz M, Burri E, Rothen C, et al. Value of fecal calprotectin in the evaluation of patients with abdominal discomfort: an observational study. BMC Gastroenterol 2012; 12: 5-13.
17. Onal IK, Beyazit Y, Sener B, et al. The value of fecal calprotectin as a marker of intestinal inflammation in patients with ulcerative colitis. Turk J Gastroenterol 2012; 23: 509-514.
18. Costa F, Mumolo MG, Bellini M, et al. Role of faecal calprotectin as non-invasive marker of intestinal inflammation. Dig Liver Dis 2003; 35: 642-647.
19. Vieira A, Fang CB, Rolim EG, et al. Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin: correlation with laboratory parameters, clinical, endoscopic and histological indexes. BMC Res Notes 2009; 2: 221-228.
20. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis 2012; 18: 2218-2224.
21. Fu Y, Wang L, Xie C, et al. Comparison of non-invasive biomarkers faecal BAFF, calprotectin and FOBT in discriminating IBS from IBD and evaluation of intestinal inflammation. Sci Rep 2017; 7: 2669-2678.
22. Foell D, Wittkowski H, Roth J. Monitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage. Gut 2009; 58: 859-868.
23. Högberg C, Karling P, Rutegård J, Lilja M. Diagnosing colorectal cancer and inflammatory bowel disease in primary care: The usefulness of tests for faecal haemoglobin, faecal calprotectin, anaemia and iron deficiency. A prospective study. Scand J Gastroenterol 2017; 52: 69-75.
24. Kok L, Elias SG, Witteman BJM, et al. Diagnostic Accuracy of Point-of-Care Fecal Calprotectin and Immunochemical Occult Blood Tests for Diagnosis of Organic Bowel Disease in Primary Care: The cost-effectiveness of a decision rule for abdominal complaints in primary care (CEDAR) Study. Clinical Chemistry 2012; 58: 989-998.

**25.** El-Hodhod MA, Aly RH, Youssef SR, Mohamed SI. Enhanced blood lymphocytes apoptosis in children with inflammatory bowel disease. ISRN Gastroenterol 2013; 2013: 415-417.
**26.** Schulze-Koops H. Lymphopenia and autoimmune diseases. Arthritis Res Ther 2004; 6: 178-180.
**27.** Neurath MF, Finotto S, Fuss I, Boirivant M, Galle PR, Strober W. Regulation of T-cell apoptosis in inflammatory bowel disease: to die or not to die, that is the mucosal question. Trends Immunol 2001; 22: 21-26.
**28.** Schneider C, Zanetti M, Romeo D. Surface-reactive stimuli selectively increase protein phosphorylation in human neutrophils. FEBS Lett 1981; 127: 4-8.
**29.** Wéra O, Lancellotti P, Oury C. The Dual Role of Neutrophils in Inflammatory Bowel Diseases. J Clin Med 2016; 5: 118-141.
**30.** Pawlica-Gosiewska D, Solnica B, Gawlik K, et al. The use of selected neutrophil protein plasma concentrations in the diagnosis of Crohn's disease and ulcerative colitis – a preliminary report. Postepy Hig Med Dosw (Online) 2017; 71: 243-253.
**31.** Elias SG, Kok L, de Wit NJ, et al. Is there an added value of faecal calprotectin and haemoglobin in the diagnostic work-up for primary care patients suspected of significant colorectal disease? A cross-sectional diagnostic study. BMC Med 2016; 14: 141-151.