

## Rapid fecal calprotectin testing predicts mucosal healing better than C-reactive protein and serum tumor necrosis factor $\alpha$ in patients with ulcerative colitis

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**Background and Aims.** Serum and fecal biomarkers have been used as noninvasive methods for assessing disease activity in ulcerative colitis. C-reactive protein, serum tumor necrosis factor- $\alpha$  and fecal calprotectin are among the most promising such biomarkers. However, their role in the management of ulcerative colitis patients remains to be clarified. We aimed to evaluate the accuracy of C-reactive protein, fecal calprotectin and tumor necrosis factor- $\alpha$  in detecting clinical and endoscopic activity and predicting disease outcome.

**Methods.** A cohort of ulcerative colitis patients was prospectively evaluated for clinical and endoscopic disease activity using the Mayo score. Serum C-reactive protein and tumor necrosis factor- $\alpha$  levels were measured and a point-of-care method was used for determining Calprotectin levels.

**Results.** Fifty-three patients with ulcerative colitis were followed for a median of 12 months. Fecal calprotectin and C-reactive protein levels were significantly higher in patients with clinically active disease at baseline, but only calprotectin levels correlated with endoscopic activity. Calprotectin values over 300  $\mu\text{g/g}$  had 60% sensitivity and 90% specificity for detecting active endoscopic disease and 61% sensitivity and 89% specificity for predicting mucosal healing.

**Conclusion.** Rapid calprotectin testing is a better predictor of mucosal healing than serum biomarkers and it could improve the management of ulcerative colitis patients by decreasing the need for invasive investigations.

**Key words:** ulcerative colitis, fecal calprotectin, C-reactive protein, mucosal healing.

### INTRODUCTION

Serum and fecal biomarkers have emerged as important noninvasive tools in the workup of inflammatory bowel disease (IBD) patients [1, 2]. The purpose behind developing accessible tests for the diagnosis and monitoring of IBD is to minimize the need for cumbersome and invasive investigations such as colonoscopy. As a result, most studies have focused on the relationship between biomarker levels and clinical and endoscopic disease activity in order to improve accuracy in the diagnosis of IBD.

C-reactive protein (CRP) and fecal calprotectin (FC) are among the most promising and intensely studied biomarkers, showing good diagnostic and prognostic accuracy in IBD [3-5]. The expanding role of such noninvasive tools includes assessing therapeutic response [6-8], detecting subclinical mucosal inflammation [9-13] and predicting disease relapse [14, 15].

Both CRP and FC are linked with the tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) pathway which plays an integral part in the pathogenesis of gut inflame-

mation. Data from experimental models shows that calprotectin stimulates the increase of TNF $\alpha$  secretion which, in turn, induces hepatic synthesis of proinflammatory cytokines including CRP [16-19]. The importance of TNF $\alpha$ -driven response in IBD is underlined by the efficiency of antiTNF $\alpha$  biologic agents in inducing and maintaining disease remission [20]. Despite the wide use of antiTNFs for the treatment of IBD, data regarding the diagnostic and prognostic accuracy of serum TNF $\alpha$  levels is conflicting [21, 22].

The aim of this study was to evaluate the diagnostic and prognostic accuracy of FC, serum CRP and TNF $\alpha$  in a cohort of ulcerative colitis patients.

### MATERIALS AND METHODS

#### PATIENTS

Consecutive patients with a confirmed diagnosis of ulcerative colitis from the Multimodal Approach in Inflammatory Bowel Disease (MAID) cohort

were included in this analysis. Briefly, this prospective observational study of IBD patients at “Colentina” Clinical Hospital (NCT01705522) includes ulcerative colitis and Crohn’s disease patients over 18 years of age, irrespective of disease location, clinical activity or medication. The study was conducted according to the principles of the Declaration of Helsinki (1975, last revised 2008). Study design was approved by the local ethics committee and all patients signed an informed consent form prior to study enrollment.

At enrollment a detailed history was obtained, current and past medication for IBD was reviewed and patients underwent a complete physical examination. Colonoscopy was performed using EXERA II 180 series Olympus colonoscopes by endoscopists with experience in IBD and the Mayo endoscopy score [23] was noted for each patient.

Scheduled study visits that included physical examination, serum and fecal biomarker assessment and colonoscopy were carried out at 12 month intervals when data about disease relapse and/or disease-related complications (i.e. – surgery) was also obtained.

#### DISEASE ACTIVITY AND FOLLOW-UP

Disease activity was assessed using the Mayo score; patients with a score of 2 or less were considered to be in clinical remission. Endoscopic activity was defined by an endoscopic Mayo subscore of at least 1 and disease extension was evaluated according to the Montreal classification [24].

#### BIOMARKER ASSESSMENT

Serum and fecal samples obtained prior to colonoscopy at each scheduled study visit were stored at -80°C.

Fecal calprotectin levels were determined using the Buhlmann Quantum Blue Reader®. This is a fast and accessible point-of-care method for determining calprotectin levels in the range of 30-300 µg/g. Serum levels of TNF α were determined using sandwich ELISA kit from Immunodiagnostik (Bensheim, Germany) according to the manufacturer’s instructions. All samples were assayed in duplicate. The absorbance was then measured at 450 nm on a spectrophotometer (Biorad Hercules, CA, USA)

Serum levels of C reactive protein (CRP) were measured using an immunoturbidimetric assay (Abbott Clinical Chemistry Diagnostics, Illinois, USA).

#### OUTCOME MEASURES

The main outcome measures for this study were clinical and endoscopic remission at 12 months follow-up.

#### DATA COLLECTION AND ANALYSIS

Results are expressed as frequencies for categorical variables (further analyzed by Fisher’s exact test), mean and standard deviation for normal continuous variables (analyzed by Student’s *t* test), and median and extremes for non-normal continuous variables (analyzed by the Mann-Whitney *U* and Kruskal Wallis tests). Each biomarker (CRP, fecal calprotectin and TNFα) was individually evaluated as a diagnostic test for clinical and endoscopic disease activity; area under the receiver operator characteristic curve (AUROC) was calculated as well as sensitivity (Sn) and specificity (Sp) with 95% confidence intervals (95% CI). Multivariate analysis was conducted using linear regression to evaluate the interaction between different biomarkers while adjusting for cofactors. Hypothesis testing was 2-tailed, with  $p < 0.05$  considered statistically significant. The statistical software package SPSS for Windows Version 16.0 (SPSS Inc., Chicago, IL) was used to analyze the data.

#### RESULTS

Fifty-three ulcerative colitis patients (23 female, mean age 37) were included in the study and followed for a median of 12 months (range 6-24 months), between November 2012 and February 2015, accounting for a total of 92 study visits (median 2, range 1-3). At baseline evaluation, 37 patients had clinically active disease with a Mayo score of at least 3 and 48 had active inflammation at endoscopy, with a Mayo sub-score of at least 1. The baseline characteristics of the included subjects are detailed in Table I. Clinical and endoscopic evaluation as well as serum samples were available for all visits, but only 63 out of 92 fecal samples provided were sufficient for analysis.

#### BIOMARKER ACCURACY IN DETECTING CLINICALLY ACTIVE DISEASE

CRP levels and FC levels were significantly higher in patients with clinically active disease than in patients in remission, but no difference was

observed for serum TNF $\alpha$  levels (Table II). At a cut-off of 300  $\mu$ g/g FC had a 67% Sn (95% CI 50-81%) and 70% Sp (95% CI 49-87%) in diagnosing clinically active UC, with an AUROC of 0.72 (CI 95% 0.58-0.86). At a cut-off value of 5mg/L CRP had a 57% Sn (95% CI 42-71%) and 88% Sp (95% CI 72-96%) for diagnosing clinically active UC, with an AUROC of 0.76 (95% CI 0.66-0.86).

#### BIOMARKER ACCURACY IN DETECTING ENDOSCOPIC ACTIVITY

Median FC levels were significantly increased in patients with ulcerative colitis who had endoscopically active disease compared to patients with mucosal healing (300  $\mu$ g/g vs. 30  $\mu$ g/g,  $p = 0.001$  Mann Whitney U). As a diagnostic test for mucosal inflammation, FC performed well, with an estimated AUROC of 0.79 (95% CI 0.61-0.98). At a cut-off value of 300  $\mu$ g/g FC had a 90% Sp (95% CI 55-98%) and a 60% Sn (95% CI 46-75%), with a positive predictive value of 96% (95% CI 83-99%).

None of the serum biomarkers correlated significantly with endoscopic disease activity (Table II).

#### BIOMARKER ACCURACY IN PREDICTING DISEASE COURSE

Clinical and endoscopic disease activity at 12 months follow-up were available for a total of 43 study subjects. Of these patients, 25 were in clinical remission (58%) but only 11 (26%) had

mucosal healing at endoscopy. Serum and fecal biomarker levels did not differ significantly at baseline between patients who were in clinical remission at 12 months compared to patients with active disease at follow-up.

However, baseline fecal calprotectin levels were significantly higher in patients who had active endoscopic disease compared to those of patients showing mucosal healing at follow-up (300  $\mu$ g/g vs. 37  $\mu$ g/g,  $p = 0.01$  Mann Whitney U) (Figure 1). At a cut-off value of 300  $\mu$ g/g FC had a 61% Sn (95% CI 38.5-80%) and a 89% Sp (95% CI 52-98%) for predicting mucosal healing at follow-up, with a positive predictive value of 93% (95% CI 68-98%) and an AUROC of 0.79 (95% CI 0.61-0.97) (Figure 2). Patients with a calprotectin level of 300 $\mu$ g/g or higher had an OR of 12.4 (95% CI 1.3-117) of having active mucosal inflammation at follow-up endoscopy.

None of the serum biomarkers were able to predict either clinical or endoscopic activity at follow-up (Table III).

#### MULTIVARIATE ANALYSIS

There was no statistically significant correlation between the levels of any serum or feces biomarkers at univariate analysis. On multivariate analysis with linear regression, using the other biomarkers and the type of treatment as covariates, there was no significant interaction between the biomarkers.

Table I

Baseline characteristics of the study group including on-going medication

<b>Gender (female / total)</b>	<b>23 / 53</b>
<b>Age (mean, SD)</b>	<b>37 (10)</b>
<b>Disease extension</b>	
<b>Proctitis</b>	9 (17%)
<b>Left-sided colitis</b>	12 (22%)
<b>Extended colitis</b>	32 (61%)
<b>Clinical activity at baseline</b>	35 (66%)
<b>Median Mayo clinical score (range)</b>	3 (0-11)
<b>Endoscopic activity at baseline</b>	47 (89%)
<b>Median Mayo endoscopic score (range)</b>	1 (0-3)
<b>Patient medication at baseline</b>	
<b>Corticosteroids</b>	8 (15%)
<b>5 Aminosalicylates</b>	32 (61%)
<b>Thiopurines</b>	5 (9%)
<b>Anti TNF<math>\alpha</math></b>	9 (17%)

TNF – tumor necrosis factor $\alpha$ ; SD – standard deviation

*Table II*  
Comparison of inflammatory biomarkers levels in ulcerative colitis patients  
according to clinical and endoscopic disease activity

Biomarker	Clinical activity	Remission	p	Endoscopic activity	Mucosal healing	p
CRP mg/L	6.5 (0-122)	2 (0.1-12.9)	<b>&lt;0.001<sup>¶</sup></b>	3.9 (0-122)	1.9 (0.5-12.9)	0.11
FC µg/g	300 (30-300)	97 (30-300)	<b>0.002<sup>¶</sup></b>	300 (30-300)	30 (30-300)	<b>0.001<sup>¶</sup></b>
TNFα pg/mL	2.9 (0-1439)	3 (0-364)	0.8	2.3 (0-1440)	8.8 (0-271)	0.16

CRP – C-reactive protein; FC – fecal calprotectin; TNFα – tumor necrosis factor α; <sup>¶</sup> statistically significant, p < 0.05 using Mann Whitney U

*Table III*  
Comparison of serum and fecal biomarker levels  
according to patient outcome at follow-up

Biomarker	Active disease	Remission	P	Endoscopic activity	Mucosal healing	p
CRP mg/L	4.4 (1-122)	6 (0.2-66)	0.6	4.2 (0.2-122)	8.8 (0.8-19)	0.8
FC µg/g	300 (30-300)	210 (30-300)	0.3	300 (30-300)	37 (30-300)	<b>0.01<sup>¶</sup></b>
TNFα pg/mL	2.6 (0-657)	3 (0-411)	0.9	0.6 (0-657)	8.8 (0-411)	0.17

CRP – C-reactive protein; FC – fecal calprotectin; TNFα – tumor necrosis factor α; <sup>¶</sup> statistically significant – Mann Whitney U

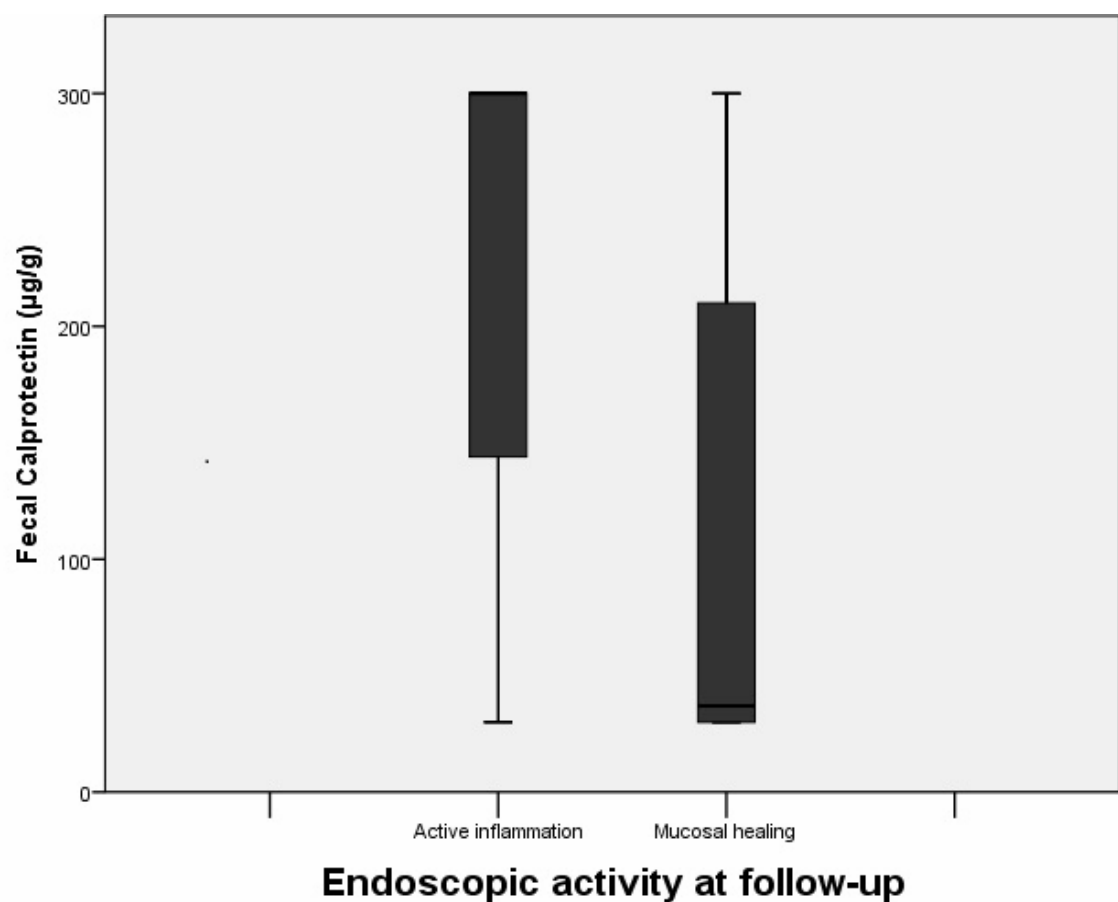


Figure 1. Baseline fecal calprotectin levels in patients with mucosal healing at 12 months follow-up compared to those with active endoscopic activity (median values 37µg/g and 300 µg/g, p = 0.01 Mann Whitney U).

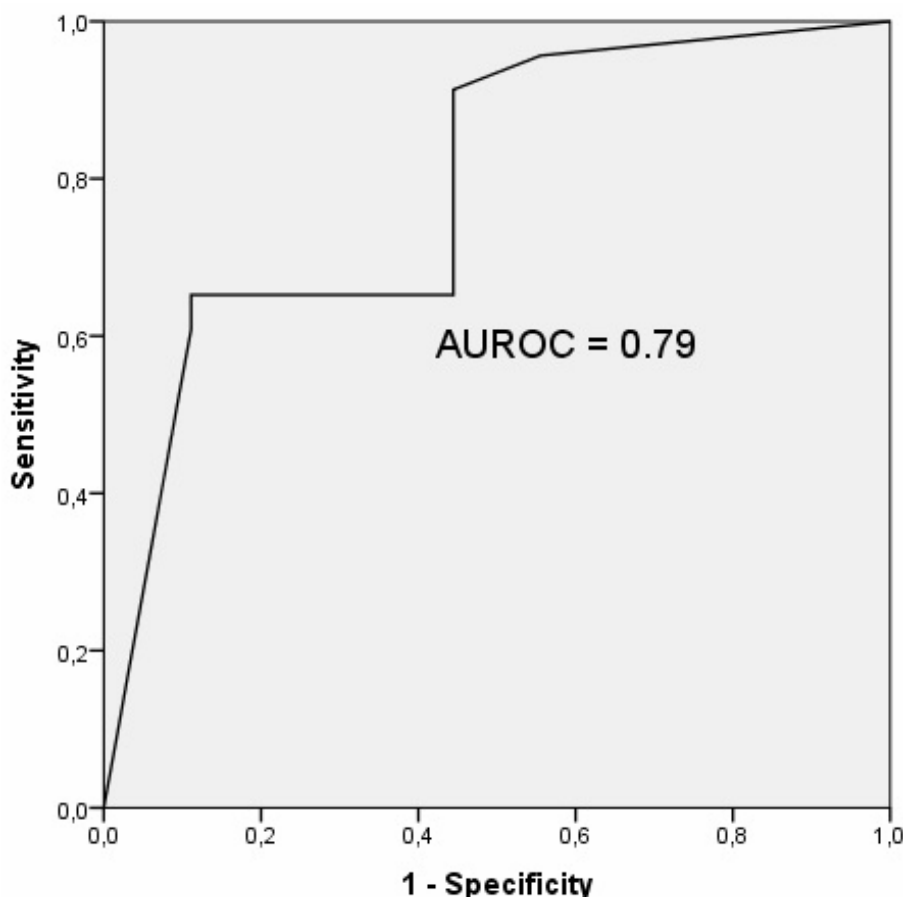


Figure 2. Area under the receiver-operator characteristic (AUROC) for rapid fecal calprotectin testing as a predictor of mucosal healing at 12 months follow-up.

## DISCUSSION

This study shows that rapid FC testing can accurately detect mucosal inflammation and predict mucosal healing in UC patients and it is a better diagnostic and predictive tool than serum biomarkers such as CRP and TNF $\alpha$ .

Developing diagnostic and predictive instruments based on serum and fecal biomarkers is an important challenge in the management of UC patients. Current guidelines rely mainly on clinical and endoscopic scores (i.e. the Mayo score) for assessing disease severity and predicting disease course in UC patients, and so far noninvasive biomarkers such as CRP or fecal calprotectin have found only limited use as either diagnostic or predictive markers [25, 26]. Recent studies have focused on biomarker discovery and validation [27], as well as finding new ways to combine non-invasive tests to increase diagnostic and predictive accuracy in IBD [28, 29]. The investigation of various cytokines involved in the TNF $\alpha$  inflammatory cascade is a promising endeavor since

antiTNF agents acting on this pathway represent one of the mainstays of therapy in IBD [22, 30-32]. Among these various markers, FC has emerged as a valuable diagnostic and prognostic biomarker, although it remains unclear how this tool will be integrated in everyday practice.

An important advantage of our study is the fact that we have employed a simple semi-quantitative method for determining FC levels in the stool of UC patients. This point-of-care method for determining FC has been compared with ELISA-based methods with satisfying results [33] but it is less costly and readily available in the physician's office. Our results validate earlier data from a pilot study of both Crohn's disease and UC patients showing a good accuracy for rapid FC in detecting active mucosal inflammation in IBD patients in clinical remission [34].

Both rapid FC levels and CRP correlated with clinical disease activity, however only FC accurately predicted endoscopic activity both at baseline and at follow-up. This finding can be explained by the fact that FC seems a better indicator of mucosal

inflammation than serum biomarkers, including CRP [35-37]. Moreover, FC can also predict important disease-related outcomes such as relapse or response to therapy [38].

In contrast, serum TNF $\alpha$  levels did not correlate with either disease activity or CRP and TNF $\alpha$  levels, irrespective of antiTNF treatment. Up-to-date studies evaluating the correlation between TNF $\alpha$  levels and disease activity or response to treatment have shown conflicting results, suggesting a wide variation of biomarker levels in both TNF $\alpha$ -treated and naïve patients [39-41]. Also, several studies analyzing biomarker panels consisting of proinflammatory serum cytokines have failed to show a correlation between TNF levels and disease activity or other biomarker levels, despite the widely accepted notion that TNF is central to the inflammatory process in IBD [21, 42].

The main limitations of our study are the limited cohort size and the relatively low compliance of patients in returning sufficient fecal samples. However, this appraisal of a cohort of consecutive patients with UC reflects the real-life setting of managing IBD patients and has the advantage of an adequate follow-up period. The data from our study is consistent with previous research and supports the use of simple, point-of-

care tests such as rapid FC for an easy and accurate assessment of mucosal inflammation.

An important question that still needs to be addressed is the optimal cut-off for FC. In our study FC levels above 300 $\mu$ g/g correlated well with endoscopic activity at baseline and were also accurate in predicting mucosal healing at 12 months. Using an ELISA-based method but the same threshold value for FC, De Vos also showed high specificity in predicting disease activity at 12 months [43]. However, other studies with different patient populations and outcome measures have proposed different cut-off values for FC [10, 34, 45]. It is likely that cut-off values need to be further explored according to the particular setting in which the test is used – i.e. for diagnosing IBD *versus* assessing disease activity in a patient with a confirmed diagnosis.

In conclusion, this study shows that FC provides better diagnostic and prognostic accuracy than serum biomarkers, and it should become a routine test in the management of UC patients, thus reducing the need for invasive investigations such as colonoscopy.

**Acknowledgement.** This research was partly supported by a Young Researcher Grant from the Carol Davila School of Medicine (Tineri Cercetători TC 2013 / 28339).

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**Introducere.** Biomarkerii serici și fecali au un rol în evaluarea noninvasivă a activității bolii la pacienții cu colită ulcerativă. Proteina C reactivă, factorul de necroză tumorală alfa și calprotectina fecală se numără printre cei mai promițători asemenea markeri. Cu toate acestea, rolul lor în managementul pacienților cu colită ulcerativă este incomplet definit.

**Obiective.** Ne-am propus evaluarea acurateții diagnostice a proteinei C reactive, calprotectinei fecale și TNF $\alpha$  în detecția activității clinice și endoscopice precum și în predicția evoluției pacienților cu colită ulcerativă.

**Metode.** Am evaluat prospectiv activitatea clinică și endoscopică a bolii într-o cohortă de pacienți cu colită ulcerativă folosind scorul Mayo. Am măsurat nivelurile serice ale PCR și TNF $\alpha$  și am cuantificat calprotectina fecală folosind o metodă rapidă semicantitativă.

**Rezultate.** 53 de pacienți cu colită ulcerativă au fost urmăriți pe o durată medie de 12 luni de zile. Calprotectina și PCR au fost semnificativ mai mari la pacienții cu boală clinic activă la înrolare dar numai calprotectina fecală s-a corelat cu activitatea clinică. Valori ale calprotectinei peste 300  $\mu$ g/g au avut o sensibilitate de 60% și o specificitate de 90% în detectarea activității endoscopice și o sensibilitate de 61% cu o specificitate de 89% în predicția vindecării mucosale.

**Concluzii.** Determinarea semicantitativă a proteinei fecale este un predictor superior al vindecării mucosale comparativ cu markerii serici și ar putea îmbunătăți managementul pacienților cu colită ulcerativă prin scăderea necesarului de examinări endoscopice invazive.

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Received May 31, 2015