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Prediction of Endoscopic Disease Activity in Ulcerative Colitis by Two Different Assays for Fecal Calprotectin

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Abstract

Background and aims: As mucosal healing is the goal of treatment in inflammatory bowel disease, defining a fecal [f-] calprotectin cut-off level for mucosal healing is crucial. Previous studies have presented different cut-off levels. The aim of this study was to investigate the ability of two f-calprotectin assays to differentiate mucosal healing from inflammation in ulcerative colitis.
Methods: Sixty-two patients with ulcerative colitis underwent colonoscopy for classification of mucosal inflammation [Mayo endoscopic subscore]. The patients also submitted a fecal sample for f-calprotectin analysis using two different assays, Calpro ELISA and Bühlmann ELISA.
Results: The two assays correlated significantly, with a Spearman rank correlation coefficient of 0.86. Both assays showed significantly different f-calprotectin levels in patients with a Mayo endoscopic subscore of 0 [mucosal healing] and 1–3 [inflamed mucosa] [p <0.001]. Using ROC curve analyses, we selected the best cut-off levels for both assays with responding sensitivity and specificity [presented with 95% confidence intervals]; Calpro ELISA cut-off 61 µg/g, sensitivity 84.1 % [75.0–93.2%], specificity 83.3 % [74.0–92.6%], and Bühlmann ELISA cut-off 96 µg/g, sensitivity 90.9 % [83.7–98.1%], specificity 83.3 % [74.0–92.6%]. Defining mucosal healing as a Mayo endoscopic subscore ≤1, cut-off levels increased: Calpro ELISA cut-off 110 µg/g, sensitivity 80.0 % [70–90%], specificity 66.6 % [54.9–78.3%]; and Bühlmann ELISA cut-off 259 µg/g, sensitivity 83.3 % [74–92.6%], specificity 71.9 % [60.7–83.1%].
Conclusions: The study demonstrates the need for assay specific cut-off levels in clinical practice, as the f-calprotectin cut-off level for endoscopic disease activity differed in these two assays.

Keywords: Inflammatory bowel disease; ulcerative colitis; fecal calprotectin; mucosal healing; assay-dependent fecal calprotectin

1. Introduction

Ulcerative colitis [UC] is a chronic inflammatory bowel disease [IBD] with a relapsing and remitting course. Evaluating relapses can be difficult due to poor correlation between clinical symptoms and mucosal inflammation.[1] Moreover, irritable bowel syndrome [IBS]-like symptoms are more prevalent in UC than in healthy individuals and may be misinterpreted as a relapse.[2,3] Mucosal healing is an important predictor of the course of disease and has therefore become the aim of UC treatment.[4–9] Repeated endoscopies to identify mucosal inflammation are expensive, time-consuming and

unpleasant from the patient's point of view. Hence there is a need for an inexpensive, simple, fast-to-perform and reliable surrogate marker of intestinal inflammation. Fecal [f-] calprotectin has become increasingly used as such a biomarker, and numerous studies have shown a close correlation between mucosal inflammation in IBD and high levels of f-calprotectin.[10–15]

Therefore, the aim of this study was to investigate two different ELISA assays for f-calprotectin available in Norway and their correlation with mucosal inflammation evaluated by Mayo endoscopic subscore activity index in patients with ulcerative colitis. Further, we aimed to determine the assays' cut-off levels associated with mucosal healing [MH].

2. Materials and Methods

2.1 Study design and patients

A total of 62 UC patients were included in this study from: [1] a prospective colonoscopy surveillance program for dysplasia in UC patients [n = 29]; [2] a prospective cohort where patients were enroled immediately after colonoscopy for a newly diagnosed or an acute flare of UC [n = 17]; and [3] a cohort of UC patients having colonoscopy as a part of a clinical routine follow-up and where the patients additionally had sent a fecal sample for f-calprotectin analysis [n = 16]. Montreal classification for disease extent was registered.[16] All patients submitted a fecal sample at the time of the colonoscopy.

2.2 Fecal calprotectin

The fecal samples were analysed with two different commercially available calprotectin enzyme-linked immunosorbent assays [ELISA], Calpro Calprotectin ELISA, Calpro AS, Norway [*Calpro ELISA*], and EK-CAL, Bühlmann Laboratories AG, Switzerland [*BM ELISA*] according to the manufacturers' instructions. The two assays had slightly different measurement ranges, 20–2500 µg/g for *Calpro ELISA* and 10–1800 µg/g for *BM ELISA*. F-calprotectin values above the upper limit of the measurement ranges were registered as 2500 µg/g and 1800 µg/g, respectively. F-calprotectin values below the lower limit were accordingly registered as 20 µg/g and 10 µg/g.

2.3 Endoscopic disease activity

The mucosal inflammation was classified according to the Mayo endoscopic subscore,[1] ranging from 0 to 3. The segment with the most severe disease activity was chosen to set the score. All the colonoscopies were performed by one senior gastroenterologist [AR]. The endoscopist did not have information about the f-calprotectin levels at hand prior to the procedure.

2.4 Statistical analyses

Data are presented as medians with ranges. Log-transformed data followed normal distribution and are therefore presented as point estimates of the mean with 95% confidence intervals.

Due to a limited sample size and a skewed distribution of data, correlation between the assays was calculated using the non-parametric Spearman rank correlation and with Passing Bablok regression analysis. F-calprotectin levels across groups defined by endoscopic disease activity were compared using one way ANOVA, with *post hoc* analysis using Least Significant Difference (LSD) adjustment. This analysis was done using log-transformed f-calprotectin values. Receiver operating characteristics [ROC] curve analyses were performed, and from these the presented cut-off levels for endoscopic remission were derived. A p-value <0.05 was considered statistically significant.

All statistical analyses were performed using IBM SPSS Statistics version 22.0, except the Passing-Bablok analysis, which was performed using Analyse-it for Microsoft Excel version 3.76.

2.5 Ethical considerations

All three part-studies were approved by the Norwegian South East Regional Committee for Medical and Health Research Ethics.

The study was performed in accordance with the Declaration of Helsinki and all the study participants signed informed consents.

3. Results

3.1 Patient characteristics

During 2010–2013, 62 UC patients were included in this study. Characteristics of the study population are presented in Table 1.

3.2 Correlation between assays

There was a high and statistically significant correlation between the two assays, with a Spearman rank correlation coefficient of 0.86, p <0.001. Passing Bablok regression, with *Calpro ELISA* as method X and *BM ELISA* as method Y, concluded with a slope of 1.66 and an intercept of -15. Figure 1 demonstrates the relationship between the f-calprotectin values for each individual analysed with both assays. Due to different measurement ranges, some values are not directly comparable.

3.3 Correlation between endoscopic disease activity and f-calprotectin

In Figure 2 we present the relationship between the different subgroups of the Mayo endoscopic subscore and log [f-calprotectin]. There was a trend towards increasing log [f-calprotectin] values with increasing Mayo score. Moreover, there was a statistically significant difference between Mayo subgroup 0 and the other Mayo subgroups for both assays [p <0.001].

3.4 ROC curve analysis

ROC curve analyses were performed for both assays' ability to identify mucosal inflammation [Figure 3]. Data for Mayo endoscopic

Table 1. Clinical and demographic data of the study population.

Number of patients – *n*	62
Male – *n* [%]	29 [47]
Age – median years [range]	35.5 [18–72]
Time between f-calprotectin and endoscopy mean days [95% CI]	9.7 [-2.8–22.3]
F-calprotectin µg/g *Calpro ELISA* – median [range]	208 [20–2500]
F-calprotectin µg/g *BM ELISA* – median [range]	331 [10–1800]
Mayo endoscopic subscore – *n* [%]	
0 – normal or inactive disease	18 [29]
1 – mild disease [erythema, decreased vascular pattern, mild friability]	14 [22.6]
2 – moderate disease [marked erythema, absent vascular pattern, friability, erosions]	19 [30.6]
3 – severe disease [spontaneous bleeding, ulceration]	11 [17.7]
Montreal classification – *n* [%]	
Proctitis	2 [3.2]
Left-sided colitis	11 [17.7]
Extensive colitis	49 [79]

CI, confidence interval.

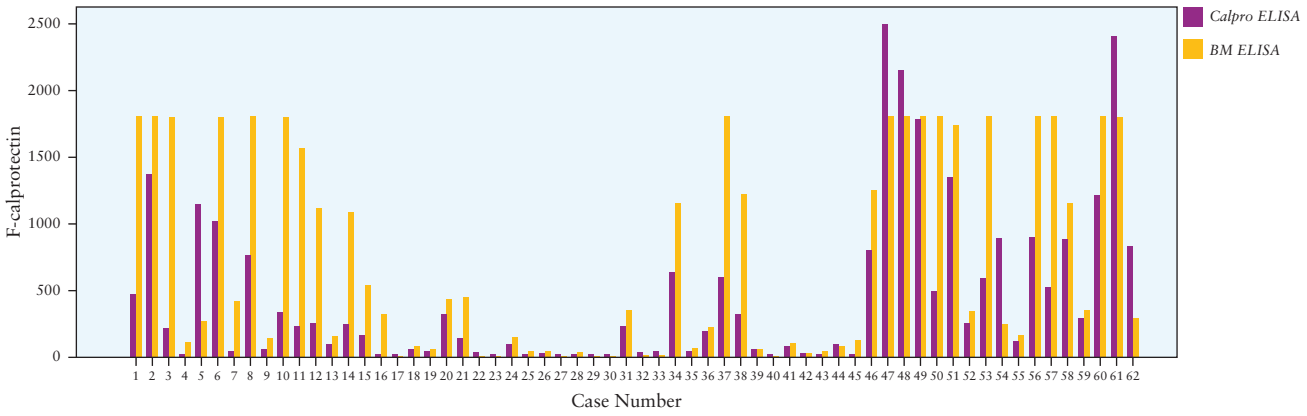

Figure 1. The relationship between f-calprotectin values for each study subject analysed with *Calpro ELISA* [black columns] and *BM ELISA* [grey columns]. *BM ELISA* has a lower measurement range [10–1800 µg/g] than *Calpro ELISA* [20–2500 µg/g], and f-calprotectin values <20 or >1800 µg/g are not comparable.

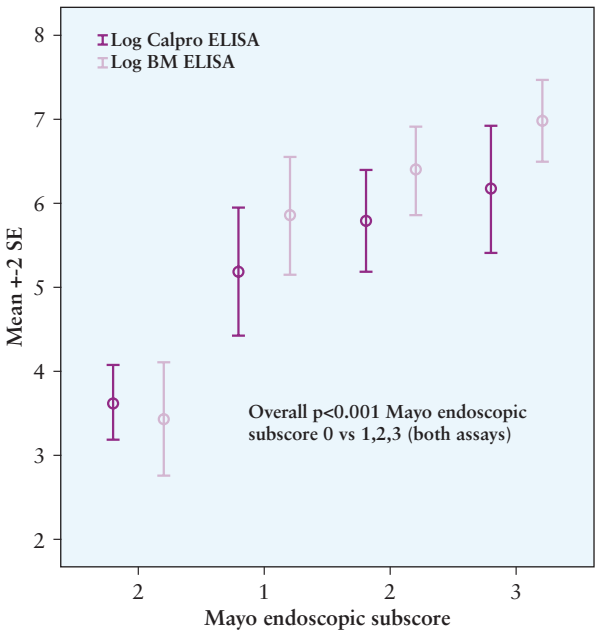

Figure 2. Log-transformed f-calprotectin [point estimates of the mean with 95% confidence interval] for each of the Mayo endoscopic subgroups 0,1,2,3. Both assays shown.

subscore 0 vs 1–3 and for Mayo endoscopic subscore 0–1 vs 2–3 are shown. *Calpro ELISA* shows generally lower cut-off levels for MH than *BM ELISA*. Clinical performance characteristics are listed in Tables 2 and 3.

4. Discussion

The present study demonstrates that f-calprotectin is a reliable predictor of endoscopic disease activity in UC, with an accuracy ranging from 73.1 % to 88.7 % depending on ELISA assay and definition of endoscopic disease activity. Two assays were studied, and their f-calprotectin measurements correlated significantly. There were no significant differences in the diagnostic performance of the two assays regarding sensitivity or specificity, but the optimal f-calprotectin cut-off levels for MH differed for the two assays.

Feces is a heterogeneous material, and f-calprotectin is measured in a spot sample. A slight difference in the f-calprotectin measurements should therefore be expected. Systematic differences between assays are, however, important to recognize. A recent study found that commercially available ELISA kits used for measuring f-calprotectin, namely *BM ELISA*, *Phical* [Immunodiagnostik AG], and *Calprest* [Eurospital], demonstrated adequate intra-assay variability, but *BM ELISA* reported up to 3.8 times higher f-calprotectin concentrations than the other two assays.[17] The conclusion was that verification of the diagnostic accuracy of different assays by comparing f-calprotectin concentrations with endoscopy and additionally histology was needed. As demonstrated by the present study, *BM ELISA* measured higher f-calprotectin levels than *Calpro ELISA* for the majority of the study subjects [Figure 1], which also resulted in a higher f-calprotectin cut-off level for differentiation between endoscopic MH [Mayo subscore 0] and inflamed and ulcerated mucosa [Mayo subscore ≥1].

In the European guidelines for management of UC, endoscopic remission is considered the goal of maintenance therapy.[18] However, endoscopic remission is not defined in these guidelines, and both complete normalization of mucosa as well as mild endoscopic inflammation have been treatment end-points in clinical trials.[4,9,19] Together with a poor validation of different endoscopic activity indices,[1] this represents a challenge in the search for an f-calprotectin cut-off level for MH.

D'Haens *et al.* defined endoscopic remission as Mayo endoscopic subscore of 0, and found an f-calprotectin cut-off level of 250 µg/g the optimal combination of sensitivity and specificity.[20] Defining endoscopic remission as Mayo endoscopic subscore 0–1, Lobatón *et al.* found 250 µg/g the optimal f-calprotectin cut-off level.[21] However, if only Mayo endoscopic subscore 0 was considered mucosal healing, the optimal cut-off level decreased to 160 µg/g. Using the Rachmilewitz Endoscopic index [not comparable to the Mayo endoscopic subscore] and defining a score of ≤3 as endoscopically inactive disease, Schoepfer *et al.* found the optimal cut-off at 50 µg/g.[22] Using the Modified Baron Score, rating endoscopic remission as a score ≤ 1[comparable to a Mayo endoscopic subscore of 0], the same authors later confirmed these findings, concluding with the optimal cut-off at 57 µg/g.[23]

Due to various endoscopic activity indices, the definition of MH in these studies is not uniform. This may explain the relatively wide range of f-calprotectin cut-off levels. Various f-calprotectin ELISA assays [respectively *PhiCal ELISA assay* [Genova Diagnostics Laboratory], *BM ELISA*, and *Calpro ELISA*] have been used in these studies, but inter-assay variability may also, as demonstrated by our study, contribute to the difference in cut-off levels for MH.

Our findings are in accordance with the conclusions of Labaere *et al.*, who studied six f-calprotectin assays, including ELISA assays

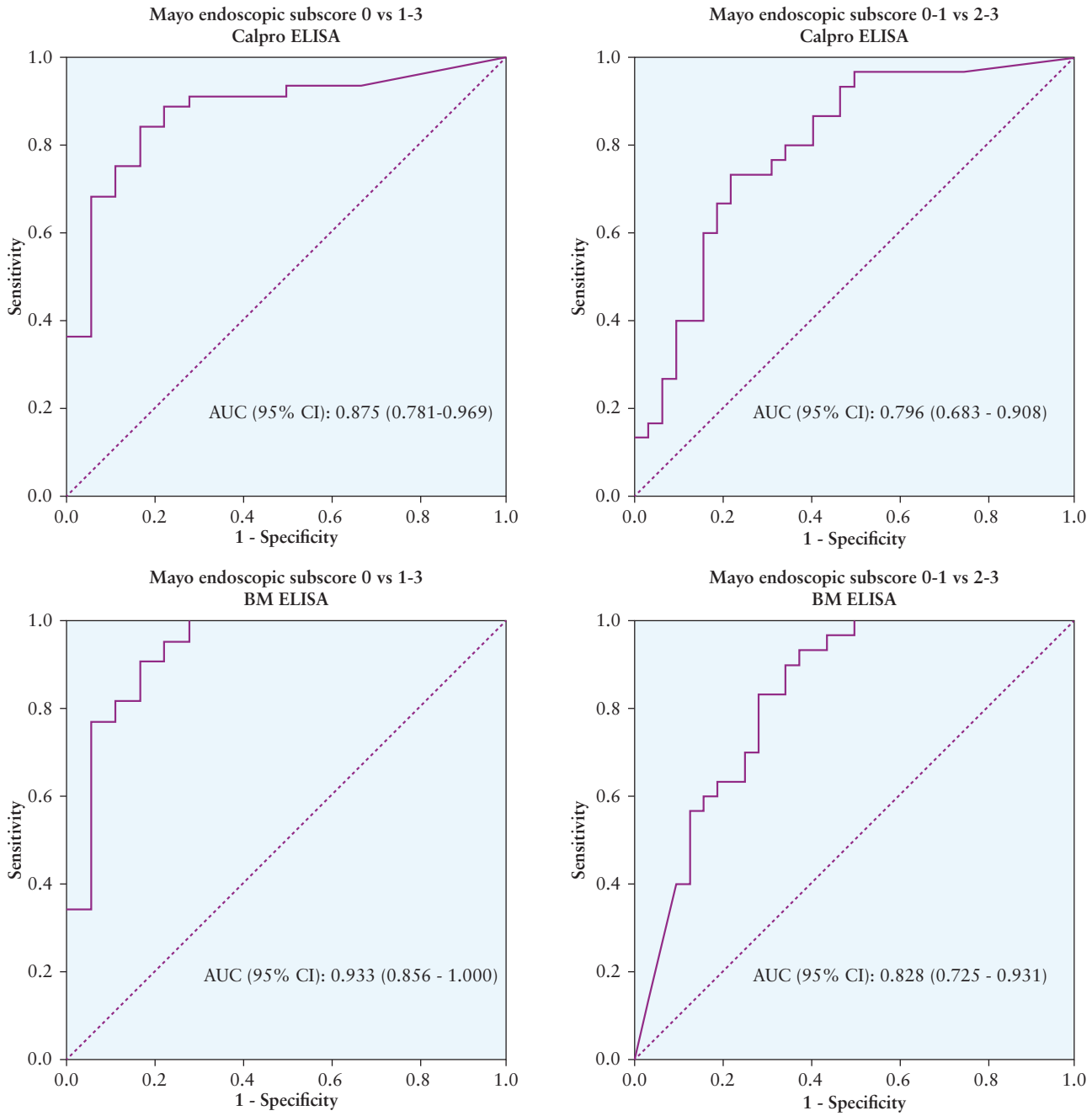

Figure 3. Receiver operating characteristics [ROC] curve analyses for both assays' ability to predict mucosal inflammation. [a] and [b] Mucosal inflammation defined as Mayo endoscopic subscore of ≥1 for assays *Calpro ELISA* and *BM ELISA*, respectively. [c] and [d] Mucosal inflammation defined as Mayo endoscopic subscore of 2–3 for assays *Calpro ELISA* and *BM ELISA*, respectively.

and point-of-care tests, and found variable cut-off levels for endoscopic remission in both UC [n = 22] and Crohn's disease [n = 21], ranging from 31 to 129 µg/g.[24] Subgroups analyses [endoscopic activity vs endoscopic remission] with very few cases led the authors to the conclusion that larger studies were needed.

C-reactive protein [CRP] has been proposed as a surrogate marker of MH in UC,[25,26] but has a poor overall correlation due to a weak CRP response,[22,27] and the specificity of CRP as a surrogate marker of MH is low.[21]

F-calprotectin is much more closely related to endoscopic findings than clinical activity scores.[20–23] Moreover, f-calprotectin is proposed as a marker of subclinical intestinal inflammation in patients in clinical remission,[28] as elevated f-calprotectin in UC patients in clinical remission is known to predict future relapse of disease.[29–32] Monitoring UC patients with consecutive f-calprotectin measurements may therefore be of substantial clinical importance, but requires a reliable cut-off level for intervention.

The importance of histopathology is still uncertain, as the absence of endoscopic inflammation may not necessarily mean that no microscopic inflammation is present.[4,19] Bitton *et al.* have demonstrated that histological changes [basal plasmacytosis in rectal biopsy specimens] in UC patients in endoscopic remission were

Table 2. Clinical performance of the f-calprotectin ELISA assays when defining mucosal healing as a Mayo endoscopic subscore of 0.

Assay	Cut-off [µg/g]	Sensitivity [95% CI] [%]	Specificity [95% CI] [%]	PPV [%]	NPV [%]	Accuracy [%]
Calpro ELISA	61	84.1 [75.0–93.2]	83.3 [74.0–92.6]	92.5	68.2	83.9
BM ELISA	96	90.9 [83.7–98.1]	83.3 [74.0–92.6]	93.0	78.9	88.7

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 3. Clinical performance of the f-calprotectin ELISA assays when defining mucosal healing as a Mayo endoscopic subscore of ≤1.

Assay	Cut-off [µg/g]	Sensitivity [95% CI] [%]	Specificity [95% CI] [%]	PPV [%]	NPV [%]	Accuracy [%]
Calpro ELISA	110	80.0 [70.0–90.0]	66.6 [54.9–78.3]	69.2	78.0	73.1
BM ELISA	259	83.3[74.0–92.6]	71.9 [60.7–83.1]	73.5	82.1	77.4

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

significantly predictive of early relapse,[33] indicating that histopathology is important in the evaluation of MH.

In the presented study, even discrete mucosal inflammation affected the levels of f-calprotectin, and a significant difference between f-calprotectin levels in the subgroup with a Mayo endoscopic subscore of 0 and all other subgroups were proven for both assays. Performing ROC curve analysis with both Mayo endoscopic subscores of 0–1 as the definition of endoscopic remission, clinical performance for both f-calprotectin assays was lower than if endoscopic remission only included Mayo 0 [complete normal mucosa], see Figure 3 and Tables 2 and 3. This observation makes us speculate whether microscopic inflammation in an otherwise apparently normal mucosa would give an elevated f-calprotectin. If this is the case, adding histopathology as a variable would potentially improve diagnostic accuracy. The clinical significance of this is unclear and further investigations are needed.

We have no information regarding the study patients' current medical treatment. Pharmaceuticals relevant for treatment of inflammatory bowel disease have previously not been shown to interfere with f-calprotectin ELISA.[34] Hence, there is no reason to expect that medical treatment should interfere with the results of the presented study.

In conclusion, f-calprotectin was introduced and validated as a diagnostic marker separating IBD from IBS. The European guidelines for diagnosis and management of UC now recommend the use of f-calprotectin as a tool for diagnosis and assessment of disease severity.[35] Reliable cut-off levels for mucosal healing are then crucial, and as demonstrated by our study, must be tailored and validated for each assay individually.

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VK participated in design of the study, data collection, interpretation of data and statistical analysis, and drafted the manuscript. PK participated in collection of data and helped to draft the manuscript. MC performed the statistical analyses and helped to draft the manuscript. AR participated in design of the study and data collection. VS participated in design of the study and interpretation of data. BM participated in design of the study and interpretation of data and helped to draft the manuscript. All authors revised the manuscript for intellectual content and approved the final manuscript.

Conflict of Interest

AR is a shareholder in Calpro AS, Oslo, Norway, and is a medical consultant for Bühlmann Laboratories AG, Basel, Switzerland.

The other authors state no conflicts of interest.

References

1. D'Haens G, Sandborn WJ, Feagan BG, *et al.* A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology* 2007;**132**:763–86.
2. Simren M, Axelsson J, Gillberg R, Abrahamsson H, Svedlund J, Bjornsson ES. Quality of life in inflammatory bowel disease in remission: the impact of IBS-like symptoms and associated psychological factors. *Am J Gastroenterol* 2002;**97**:389–96.
3. Jelsness-Jorgensen LP, Bernklev T, Moum B. Fatigue and disease-related worries among inflammatory bowel disease patients in remission; is it a reflection of coexisting IBS-like symptoms? A short report. *J Psychosom Res* 2012;**73**:469–72.
4. Travis SP, Higgins PD, Orchard T, *et al.* Review article: defining remission in ulcerative colitis. *Aliment Pharmacol Ther* 2011;**34**:113–24.
5. Meucci G, Fasoli R, Saibeni S, *et al.* Prognostic significance of endoscopic remission in patients with active ulcerative colitis treated with oral and topical mesalazine: a prospective, multicenter study. *Inflamm Bowel Dis* 2012;**18**:1006–10.
6. Ardizzone S, Cassinotti A, Duca P, *et al.* Mucosal healing predicts late outcomes after the first course of corticosteroids for newly diagnosed ulcerative colitis. *Clin Gastroenterol Hepatol* 2011;**9**:483–9.
7. Froslie KF, Jahnsen J, Moum BA, Vatn MH. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. *Gastroenterology* 2007;**133**:412–22.
8. Feagan BG, Reinisch W, Rutgeerts P, *et al.* The effects of infliximab therapy on health-related quality of life in ulcerative colitis patients. *Am J Gastroenterol* 2007;**102**:794–802.
9. Lichtenstein GR, Rutgeerts P. Importance of mucosal healing in ulcerative colitis. *Inflamm Bowel Dis* 2010;**16**:338–46.
10. Roseth AG, Aadland E, Jahnsen J, Raknerud N. Assessment of disease activity in ulcerative colitis by fecal calprotectin, a novel granulocyte marker protein. *Digestion* 1997;**58**:176–80.
11. Berni CR, Rapacciuolo L, Romano MT, *et al.* Diagnostic value of fecal calprotectin in paediatric gastroenterology clinical practice. *Dig Liver Dis* 2004;**36**:467–70.
12. von Roon AC, Karamountzos L, Purkayastha S, *et al.* Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol* 2007;**102**:803–13.
13. Sipponen T, Savilahti E, Karkkainen P, *et al.* Fecal calprotectin, lactoferrin, and endoscopic disease activity in monitoring anti-TNF-alpha therapy for Crohn's disease. *Inflamm Bowel Dis* 2008;**14**:1392–8.
14. Wagner M, Peterson CG, Ridefelt P, Sangfelt P, Carlson M. Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. *World J Gastroenterol* 2008;**14**:5584–9.
15. Sipponen T, Savilahti E, Kolho KL, Nuutinen H, Turunen U, Farkkila M. 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.

16. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut* 2006;**55**:749–53.
17. Whitehead SJ, French J, Brookes MJ, Ford C, Gama R. Between-assay variability of fecal calprotectin enzyme-linked immunosorbent assay kits. *Ann Clin Biochem* 2013;**50**[Pt 1]:53–61.
18. Dignass A, Lindsay JO, Sturm A, *et al.* Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *J Crohns Colitis* 2012;**6**:991–1030.
19. Mazzuoli S, Guglielmi FW, Antonelli E, Salemme M, Bassotti G, Villanacci V. Definition and evaluation of mucosal healing in clinical practice. *Dig Liver Dis* 2013;**45**:96977.
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**:2218–24.
21. Lobatón T, Rodriguez-Moranta F, Lopez A, Sánchez E, Rodriguez-Alonso L, Guardiola J. A new rapid quantitative test for fecal calprotectin predicts endoscopic activity in ulcerative colitis. *Inflamm Bowel Dis* 2013;**19**:1034–42.
22. Schoepfer AM, Beglinger C, Straumann A, Trummler M, Renzulli P, Seibold F. 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.
23. Schoepfer AM, Beglinger C, Straumann A, *et al.* Fecal calprotectin more accurately reflects endoscopic activity of ulcerative colitis than the Lichtiger Index, C-reactive protein, platelets, hemoglobin, and blood leukocytes. *Inflamm Bowel Dis* 2013;**19**:332–41.
24. Labaere D, Smismans A, Van Olmen A, *et al.* Comparison of six different calprotectin assays for the assessment of inflammatory bowel disease. *United European Gastroenterology Journal* 2014;**2**:30–7.
25. Beigel F, Deml M, Schnitzler F, *et al.* Rate and predictors of mucosal healing in patients with inflammatory bowel disease treated with anti-TNF-alpha antibodies. *PLoS One*. 2014;**9**:e99293.
26. Arai Y, Arihiro S, Matsuura T, *et al.* Prostaglandin E major urinary metabolite as a reliable surrogate marker for mucosal inflammation in ulcerative colitis. *Inflamm Bowel* Dis 2014;**20**:1208–16.
27. Henriksen M, Jahnsen J, Lygren I, *et al.* C-reactive protein: a predictive factor and marker of inflammation in inflammatory bowel disease. Results from a prospective population-based study. *Gut* 2008;**57**:1518–23.
28. 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.
29. 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.
30. Gisbert JP, Bermejo F, Perez-Calle JL, *et al.* Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm Bowel Dis* 2009;**15**:1190–8.
31. D'Incà R, Dal Pont E, Di Leo V, *et al.* Can calprotectin predict relapse risk in inflammatory bowel disease? *Am J Gastroenterol* 2008;**103**:2007–14.
32. Vos MD, Louis EJ, Jahnsen J, *et al.* Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. *Inflamm Bowel Dis* 2013;**19**:2111–7.
33. Bitton A, Peppercorn MA, Antonioli DA, *et al.* Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis. *Gastroenterology* 2001;**120**:13–20.
34. Ton H, Brandsnes, Dale S et al. Improved assay for fecal calprotectin. *Clin Chim Acta* 2000;**292**:41–54.
35. Dignass A, Eliakim R, Magro F, *et al.* Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 1: definitions and diagnosis. *J Crohns Colitis* 2012;**6**:965–90.