Journal of Crohn's and Colitis (2010) 4, 144–152

available at www.sciencedirect.com

Does fecal calprotectin predict relapse in patients with Crohn's disease and ulcerative colitis?

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Received 24 June 2009; received in revised form 23 August 2009; accepted 24 September 2009

KEYWORDS
Crohn's disease;
Ulcerative colitis;
Inflammatory bowel disease;
Calprotectin;
Fecal marker

Abstract

Background and aims: An evaluation is made of the utility of fecal calprotectin in predicting relapse in patients with inflammatory bowel disease (IBD). The possible differences in its predictive capacity in Crohn's disease (CD) versus ulcerative colitis (UC), and the different phenotypes, are also examined.
Methods: This is a prospective study with 135 patients diagnosed with IBD in clinical remission for at least 3 months. The patients submitted a stool sample within 24 hours after the baseline visit, for the measurement of fecal calprotectin. All patients were followed-up on for one year.
Results: Sixty-six patients had CD and 69 UC. Thirty-nine (30%) suffered from relapse. The fecal calprotectin concentration was higher among the patients with relapse than in those that remained in remission: 444 μg/g (95% CI 34–983) versus 112 μg/g (95% CI 22–996); $p<0.01$. Patients with CD and calprotectin >200 μg/g relapsed 4 times more often than those with lower marker concentrations. In UC, calprotectin >120 μg/g was associated with a 6-fold increase in the probability of disease activity outbreak. The predictive value was similar in UC and CD with colon involvement and inflammatory pattern. In this group, calprotectin >120 μg/g predicted relapse risk with a sensitivity of 80% and a specificity of 60%. Relapse predictive capacity was lower in patients with ileal disease.
Conclusions: Fecal calprotectin may be a useful marker for predicting relapse in patients with IBD. Its predictive value is greater in UC and CD with colon involvement and inflammatory pattern, compared with ileal CD.

Abbreviations: CRP, C-reactive protein; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; ESR, erythrocyte sedimentation rate; IBD, inflammatory bowel disease; TW, Truelove Witts; UC, ulcerative colitis.

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doi:10.1016/j.crohns.2009.09.008

1. Introduction

The natural course of inflammatory bowel disease (IBD) is characterized by activity outbreaks and periods of remission. In most cases, relapses in Crohn's disease (CD) and in ulcerative colitis (UC) are unpredictable and despite effective medical treatment, a degree of subclinical inflammation may persist in the bowel wall, contributing to create a significant risk of relapse.[1,2]

The detection of such inflammation in patients under conditions of clinical remission would constitute an important advance in clinical practice. It would allow us to select and individualize the treatment of patients according to the existing risk of relapse, avoiding the generalized prescription of maintenance therapy.

Acute phase reactants have been demonstrated that their sensitivity and specificity in correlating to intestinal inflammatory activity are very low,[3–5] and their capacity to predict disease relapse is moreover poor and controversial.[6–15]

Fecal markers may be more specific for assessing intestinal disease activity. Specifically, calprotectin has been detected in stools in direct proportion to neutrophil migration through the gastrointestinal tract.[16–18] It has been suggested that this marker may increase even in the early stages of disease activity, when no patient symptoms, systemic increases in other reactants, or endoscopic macroscopic alterations have yet appeared.[19] To date, five studies have been published on the utility of calprotectin in this context.[7,9,20–22] These show differences and characteristics that limit the utility of the results obtained. The first three series involve few patients (79, 80 and 32 subjects, respectively); others report differences in predictive value between patients with CD and UC; only one study refers to the CD phenotype; and finally the cutoff points selected in each study differ.

The present study examines whether direct measurement of bowel inflammation based on fecal calprotectin is able to predict an outbreak of disease activity, and evaluates the possible existence of differences in predictive value of this biological marker in relation to the type of disease and the patient CD phenotype.

2. Methods

A prospective, single-center study was made. The good clinical practice guidelines were followed, and the study was approved by the local ethics committee.

2.1. Patients

We included 135 consecutive patients with IBD diagnosed on the basis of clinical, endoscopic, radiological, and histological criteria.[23] Sixty-six patients had CD and 69 UC. According to the Montreal classification,[24] the extent of CD was defined as ileal, colonic, ileocolonic or upper gastrointestinal, while UC was defined as distal colitis or extensive colitis. The disease behavior of CD was described as inflammatory, stenosing or penetrating.

All patients were in clinical remission for at least 3 months before inclusion in the study. Remission was defined as a Crohn's Disease Activity Index (CDAI) of less than 150 points in the case of patients with CD,[25] and as a modified Truelove Witts (TW) score of less than 11 points in the case of UC.[26,27] The patient characteristics are summarized in Table 1.

None of the patients was using nonsteroidal antiinflammatory drugs or antibiotics during the 3 months prior to inclusion. We excluded patients with cardiopulmonary, renal, liver, neurological, rheumatological or serious psychiatric disorders.

A venous blood sample was collected to determine laboratory test parameters, and a plain stool sample was requested within 24 h after the initial study visit. The stool samples were frozen (−20 °C) until calprotectin determination. Fecal calprotectin was quantitated using an ELISA test

Table 1 Clinical characteristics of the patients at the time of inclusion in the study.

Characteristics	CD (n=66)	UC (n=69)
Sex, n (%)		
Male	36 (54.5)	41 (59.4)
Female	30 (45.5)	28 (40.6)
Age, mean ± SD (years)	36.9 ± 9.2	40.4 ± 13.1
Duration of the disease, mean ± SD	6 ± 4.8	6.7 ± 5.4
Smoking habit, n (%)		
Smoker	15 (22.7)	12 (17.4)
Non-smoker	51 (77.3)	57 (82.6)
Never smoked	34 (66.6)	40 (70.1)
Ex-smoker	17 (33.3)	17 (29.8)
Activity index, mean ± SD		
CDAI	71.1 ± 20.8	
Modified TW		9 ± 0.2
Disease location, n (%)		
Ileal	20 (30)	
Colonic	20 (30)	
Ileocolonic	24 (37)	
Upper gastrointestinal	2 (3)	
Distal colitis		39 (55)
Extensive colitis		30 (45)
Disease behavior, n (%)		
Inflammatory	50 (76)	
Stenosing	3 (4)	
Penetrating	13 (20)	
Current treatment with some of the following drugs, alone or in combination, n (%)		
None	0	0
Mesalazine	36 (54)	63 (92)
Immunosuppressors (azathioprine or methotrexate)	39 (59)	19 (27)
Biological therapies (infliximab)	4 (6)	1 (1.4)
Prior surgery, n (%)	22 (33)	0 (0)
Extraintestinal manifestations, n (%)	11 (17.7)	11 (16.9)
Mean time in remission before inclusion, months (mean ± SD)	17 ± 15	15 ± 18

CD, Crohn's disease.
UC, ulcerative colitis.
CDAI, Crohn's Disease Activity Index.
TW, Truelove Witts.
SD, standard deviation.

(Calprest, Eurospital, Trieste, Italy), following the instructions of the manufacturer.

All patients were followed-up on in the clinic every two months for one year, or more often as required by the presence of symptoms. Clinical relapse was defined as a worsening of the symptoms, accompanied by a CDAI score of $\geq$150 points, or a modified TW score of $\geq$11 points. All relapses were of sufficient severity to warrant a change in treatment. All patients maintained a stable dose of medication during follow-up.

2.2. Statistical analysis

The SPSS version 13.0 was used to analyze the data. The laboratory test parameters and calprotectin values were compared between the patients remaining in remission and those who relapsed. The comparison of frequencies was based on the chi-square test with Yates correction where required. Continuous variables were reported as the medians and ranges. Kolmogorov–Smirnov test was used to evaluate whether fecal calprotectin and other laboratory parameters followed a normal (Gaussian) distribution. The Mann–Whitney test and Student-*t* test were used to compare these variables in relapsers and those that remained in remission depend on the values' distribution which did or did not fit the normal curve. The cumulative proportion of patients in remission was calculated with the Kaplan–Meier method, defining the time elapsed from the last remission until the relapse (not from inclusion in the study) as the variable "time". The patients were divided into two groups according to each continuous variable and considering a cutoff value, calculated from the receiver operating characteristic (ROC) curve. The selected cutoff point was that offering the greatest relapse predicting capacity, with the best combination of sensitivity and specificity. The cumulative proportion of patients in remission over time was compared between both groups using the log-rank test (univariate analysis). Those variables reaching statistical significance in the univariate analysis were included in a Cox regression model (multivariate analysis) in order to identify the independent relapse predicting factors. Statistical significance was accepted for $p<0.05$.

3. Results

Eighteen of the 66 (27%) patients with CD relapsed during the year of follow-up, with a mean time from last remission (not from inclusion in the study) of 17±12 months. In the UC group, 21 of the 69 (31%) patients relapsed after an average of 15±6 months.

No significant differences were found in laboratory test parameters between the relapsing patients and those maintaining remission (Table 2).

The median calprotectin concentration was 153 μg/g (95% CI 24–1037, range 19–1150) in patients with CD and 121 μg/g (95% CI 30–963, range 19–1029) in patients with UC. Kolmogorov–Smirnov test showed that calprotectin values' distribution did not fit the normal curve ($p<0.01$). The calprotectin concentration was significantly higher among the patients with relapse than in those that remained in remission: 444 μg/g (95% CI 34–983, range 34–983) versus 112 μg/g (95% CI 22–996, range 19–1150); $p<0.01$. These differences were found in both CD and in UC. In CD, calprotectin concentration was 524 μg/g (95% CI 101–983, range 101–983) in the relapsing patients and 123 μg/g (95% CI 20–1105, range 19–1150) in those that maintained remission ($p<0.01$). In UC, these values were 298 μg/g (95% CI 34–883, range 34–883) versus 105 μg/g (95% CI 24–1009, range 19–1028), respectively ($p<0.01$) (Fig. 1).

Table 2 Conventional laboratory test parameters and disease relapse: mean±SD, median (range).

	CD			UC		
	Relapse ($n=18$)	Remission ($n=48$)	p [a]	Relapse ($n=21$)	Remission ($n=48$)	p [a]
Platelet count ($\times10^4/mm^3$)	28±5 29.5 (18.6–36.7)	28±6 27.7 (17–58)	0.91	26±6 25.7 (13.1–38.1)	26±6 26 (13.7–41.4)	0.91
Hemoglobin (g/dl)	12.9±0.9 12.8 (11.5–14.8)	12.8±1 12.7 (9.4–17.2)	0.73	13.5±1 13.7 (9.7–15.4)	13.7±1 13.8 (10.4–16.5)	0.58
Albumin (g/dl)	4.4±0.3 4.5 (3.9–5.2)	4.4±0.3 4.4 (3.3–5)	0.57	4.5±0.2 4.5 (4.1–4.8)	4.5±0.1 4.6 (4–5.1)	0.35
Iron (μg/dl)	79.7±29	86±44 76 (25–197)	0.51	88.7±33 90 (24–145)	90.7±33 86 (26–204)	0.82
ESR (mm/h)	17.5±11 14 (5–51)	16.2±15 11 (11–53)	0.23	14.1±19 6 (1–65)	7.6±8 3 (1–34)	0.18
CRP (mg/l)	4.6±5 4.6 (0.7–21.4)	5.6±8 2.1 (0.1–38.6)	0.79	3.8±6 1.4 (0.3–28.7)	1.7±2 0.9 (2–8.6)	0.06
Orosomucoid (mg/dl)	29.2±17 99 (71–122)	94.2±27 96.5 (8–161)	0.56	85.5±21 84.5 (59–159)	82.5±22 82.5 (4–125)	0.63

CD, Crohn disease.
UC, ulcerative colitis.
CRP, C-reactive protein.
ESR, erythrocyte sedimentation rate.
SD, standar deviation.
[a] Mann–Whitney test (ESR and CRP). Student-*t* test (platelet, hemoglobin, albumin, iron, and orosomucoid).

The ROC curve showed calprotectin to offer good relapse predictive capacity in the overall patients (Fig. 2). The area under the curve (AUC) was 0.72 (standard error = 0.04), and the best cutoff point was 150 µg/g. This value yielded a sensitivity of 75%, a specificity of 68%, a positive predictive value (PPV) of 49%, and a negative predictive value (NPV) of

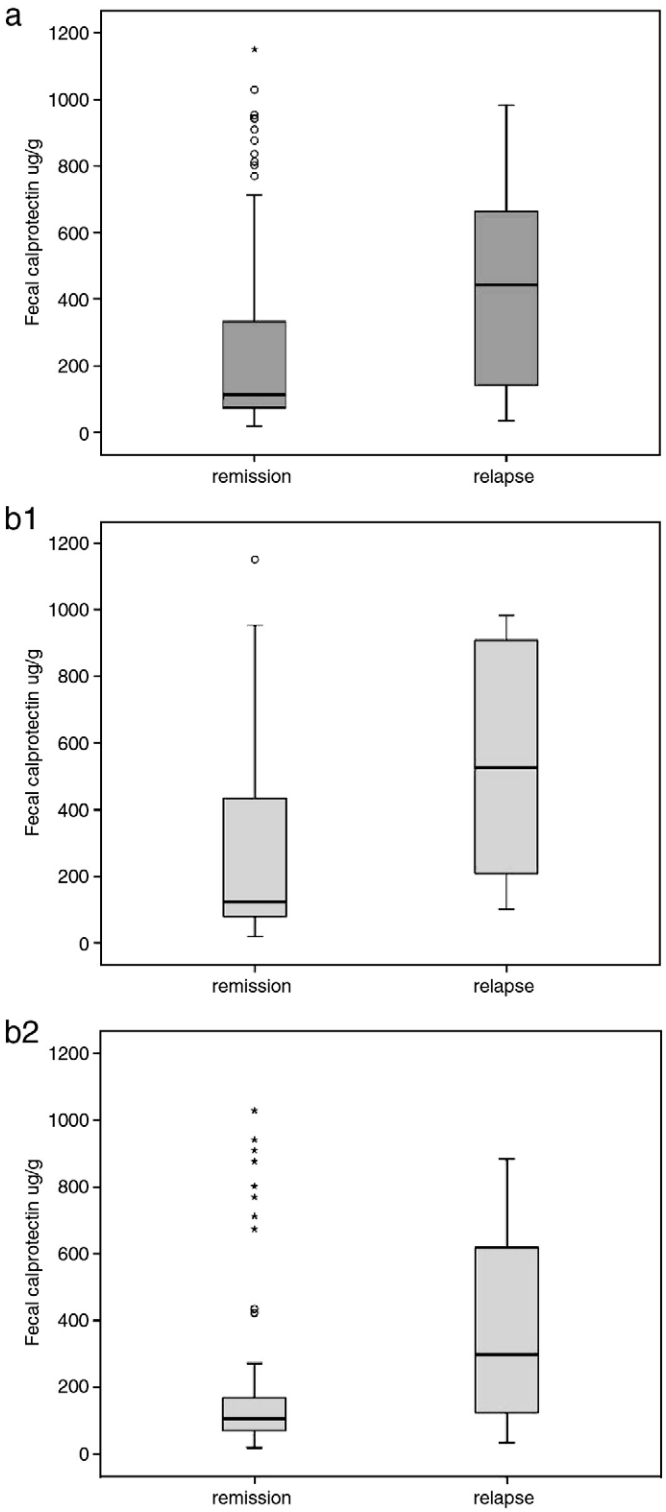

Figure 1 Box graph representation of calprotectin determinations. The lower and higher limits of the box represent the first and the third quartiles, respectively. The black crossbar line in the box represents the median. The top and the bottom black crossbar lines represent the highest and the lowest values, respectively. The symbol 'O' indicated an isolated or extreme value. a) Basal calprotectin concentration and probability of subsequent relapse in all patients ($p<0.01$). b 1) Basal calprotectin concentration and probability of subsequent relapse in patients with CD ($p<0.01$). b 2) Basal calprotectin concentration and probability of subsequent relapse in patients with UC ($p<0.01$).

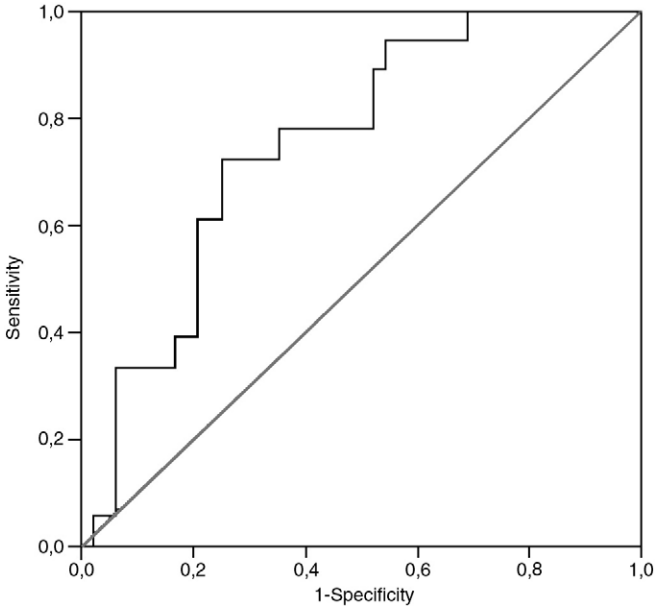

Figure 2 The area under the receiver operating characteristic (ROC) curve of calprotectin for predicting relapse in all patients was 0.72 (standard error = 0.04).

68%. The proportion of patients that relapsed with a concentration of over 150 μg/g was significantly greater than among those presenting lesser concentrations (75% versus 25%; $p<0.01$). Based on the Kaplan–Meier method (Fig. 3), the cumulative proportion of patients without relapse was greater among the subjects with calprotectin levels of less than 150 μg/g (chi-square = 11.2; $p<0.01$). This cutoff point was independently correlated to relapse risk, with an odds ratio (OR) of 5.53 (95% CI 2.40–12.73; $p<0.01$). In other words, patients with IBD in remission and levels in excess of 150 μg/g presented an almost 6-fold greater risk of relapse than those patients with lower concentrations.

In CD, the AUC for predicting a disease activity outbreak was 0.75 (standard error = 0.06). In this case, 200 μg/g yielded a sensitivity of 80%, a specificity of 65%, a PPV of 46% and a NPV of 88%. In the patients with UC, the AUC was 0.70 (standard error = 0.06), while 120 μg/g was found to be the best cutoff value (Fig. 4). The sensitivity, specificity, PPV and NPV for this value were 81%, 63%, 49% and 88%, respectively. Both cutoff points reached statistical significance in the multivariate analysis on considering both diseases separately, with OR = 4.35 (95% CI 1.14–16.58; $p=0.03$) and 6.48 (95% CI 1.89–22.16; $p<0.01$), respectively. In other words, the patients with CD and calprotectin in excess of 200 μg/g relapsed four times more often than those with lower concentrations. In turn, in UC, a concentration of over 120 μg/g implied a 6-fold greater risk of suffering from disease relapse.

In order to evaluate the possible influence of phenotype upon the predictive value of calprotectin in patients with CD, those with ileal disease and a penetrating disease pattern, were excluded from the analysis. A total of 105 patients were evaluated (36 with CD characterized by colon involvement and inflammatory pattern, and 69 with UC). In this case the AUC was 0.71 (standard error = 0.05), and 120 μg/g was the calprotectin cutoff value with the best relapse predictive capacity (Fig. 5). The sensitivity, specificity, PPV and NPV for this value were 80%, 60%, 41% and 89%, respectively. Based on the Kaplan–Meier method, the cumulative probability of

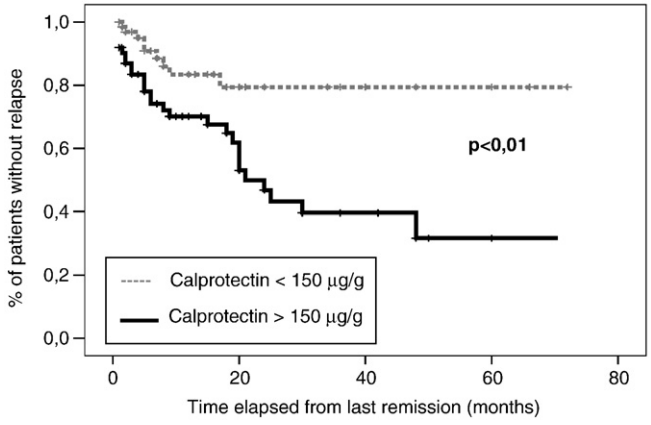

Figure 3 Kaplan–Meier plot for the overall patients in relation to calprotectin levels below and above 150 μg/g.

remission was found to be greater in the patients with concentrations of under 120 µg/g, compared with those presenting higher levels (chi-square = 11.3; $p < 0.01$) (Fig. 6). In these same patients, this cutoff point predicted relapse risk in the multivariate analysis, with OR = 5.03 (95% CI 1.82–13.85). Thus, the patients with UC or colonic or ileocolonic CD and inflammatory pattern exhibiting calprotectin levels in excess of 120 µg/g presented a 5-fold greater risk of relapse than the patients with lower concentrations.

Considering only the patients with ileal CD, the AUC decreased considerably, to 0.64 (standard error = 0.12). In this case, a higher cutoff point of 223 µg/g proved necessary to secure a sensitivity of 83% and a specificity of 50%.

In UC, no differences were observed in the predictive capacity of this marker according to disease extent.

4. Discussion

This study shows that fecal calprotectin may be a useful marker for predicting relapse in patients with IBD. Its predictive value proved greater in UC and CD with colonic

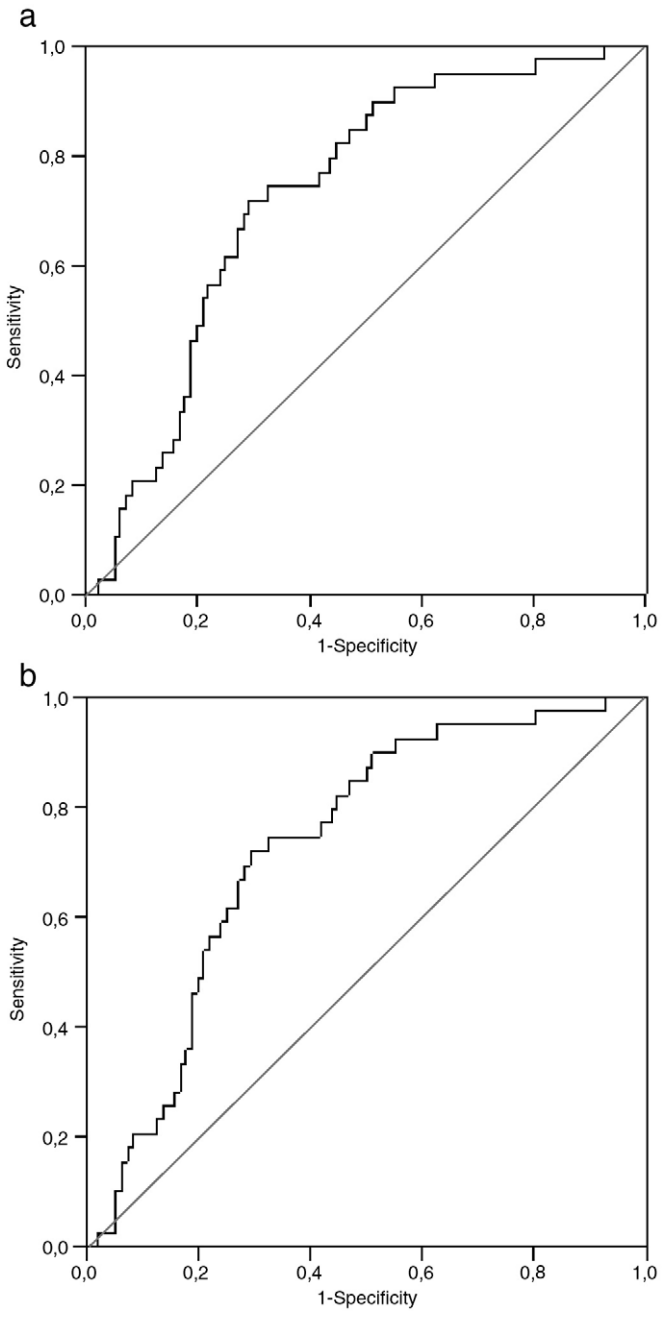

Figure 4 a) Receiver operating characteristic (ROC) curve of calprotectin for predicting relapse in patients with CD. The area under the curve was 0.75 (standard error = 0.06). The best cutoff value was 200 µg/g (sensitivity 80%, specificity 65%, PPV 46%, and NPV 88%). b) Receiver operating characteristic (ROC) curve of calprotectin for predicting relapse in patients with UC. The area under the curve was 0.70 (standard error = 0.06). The best cutoff value was 120 µg/g (sensitivity 81%, specificity 63%, PPV 49%, and NPV 88%).

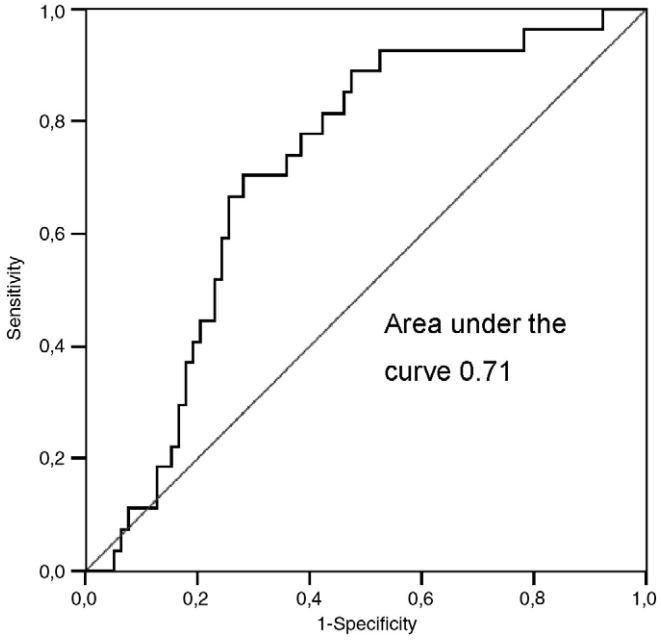

Figure 5 Receiver operating characteristic (ROC) curve of calprotectin for predicting relapse in patients with UC and CD with colon involvement and inflammatory pattern.

or ileocolonic involvement and inflammatory pattern. In this series, none of the laboratory test parameters was of use in predicting relapse.

To date, one study has proved that calprotectin seems to be a good marker of the risk of histological relapse in pediatric IBD patients.[28] Moreover, four studies in adults[7,9,21,22] and one in a pediatric population[20] have evaluated the capacity of this marker for predicting clinical relapse. Tibble et al.[7] found that 90% of their patients and elevated calprotectin levels at the start of the study relapsed in the course of a year, compared with only 10% of those with lower marker levels. In the second study, Costa et al.[9] reported a 2- and 14-fold greater risk of relapse in patients with CD and UC, respectively, among those subjects with higher concentrations of this marker at the time of inclusion in the study. Recently, D'Inca et al.[21] reported that 130 mg/kg predicts the appearance of an activity outbreak

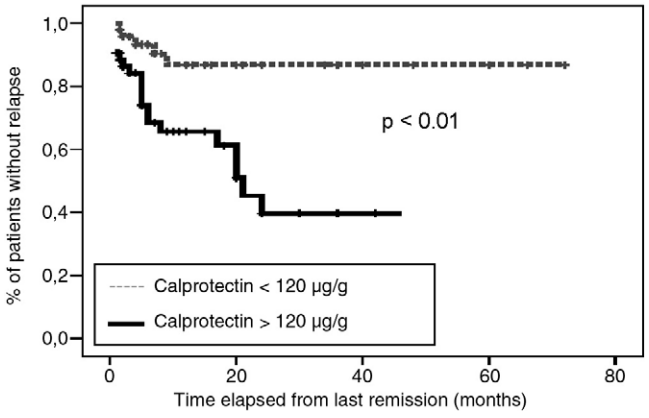

Figure 6 Kaplan–Meier plot for patients with UC and CD with colon involvement and inflammatory pattern, in relation to calprotectin levels below and above 120 µg/g.

with a sensitivity and specificity of 68% and 67%, respectively. Gisbert et al.[22] informed that 8% of the patients having calprotectin concentrations under 150 µg/g relapsed during follow-up, while this occurred in as many as 30% of the patients with calprotectin above 150 µg/g at baseline. Therefore, fecal calprotectin's (above 150 µg/g) sensitivity and specificity to predict relapse in IBD were approximately 70%. Lastly, Walkiewicz et al.[20] found that 90% of their children with CD and increased calprotectin levels relapsed, while 89% of those with lower levels remained in remission during the average 9 months of follow-up. In our series, calprotectin has been shown to be potentially useful for predicting relapse in patients with IBD. The calprotectin levels were significantly higher in those subjects that relapsed than in those who remained in remission.

Nevertheless, the capacity of calprotectin for predicting relapse according to the type of IBD is not clear. In effect, while Tibble et al.[7] found no differences between the two diseases, Costa et al.[9] considered this marker to offer a more reliable prediction of relapse in UC than in CD. The specificity in CD relapse was only 43%, compared with 83% in the first study. D'Inca et al.[21] reported no significant differences in baseline calprotectin levels between CD patients that relapsed and those that remained in remission. A concentration of 130 mg/kg proved unable to predict relapse in the overall group of patients with CD, in contrast to the situation among those with UC. The results obtained in our study show that calprotectin may be a useful marker for predicting relapse in patients with CD and UC, though the predictive capacity was found to be slightly lower in the former group. These discrepancies have a number of explanations. On one hand, CD and UC are diseases with different inflammatory patterns and their response to medical treatment for the induction of remission is not the same. This suggests that the degree of inflammation and its impact upon relapse may be different for these two diseases.[1,29] In UC, clinical remission is accompanied

by endoscopic and histological healing in up to 70% and 50% of cases, respectively.[29] However, in CD, this correlation is only observed in 13% of all patients.[30] For this reason, the cutoff point established for calprotectin in predicting relapse in CD should be higher than in the case of UC. Walkiewicz et al.[20] recently have proposed 400 μg/g of this marker as the best cutoff point for predicting relapse.

It had been suggested that CD patient stratification according to phenotype could influence the predictive value of calprotectin more than the different inflammatory patterns of the two types of disease. Calprotectin used as a marker directly measures the degree of bowel inflammation; it therefore seems logical that the molecule would prove more useful in UC and CD with colon involvement and inflammatory pattern.[9] To date, only the study by D'Inca et al.[21] refers to the possible influence of CD location upon the predictive usefulness of this marker. These authors showed that calprotectin predicts the appearance of activity outbreaks, particularly in patients with colonic CD, but not in those with ileal or ileocolonic disease. In this latter group, the AUC was only 0.54. These results are notorious, since patients with ileocolonic CD also has colon involvement, and it would be reasonable to assume that calprotectin behavior should be similar to that seen in patients with CD located exclusively in the colon. Possibly the length of the affected segment, or the disease behavior, may have influenced their results. In our series, the predictive capacity of this marker was greater in patients with colonic and ileocolonic CD and inflammatory pattern, than in those with exclusively ileal disease.

In conclusion, fecal calprotectin may be a useful marker for predicting relapse in patients with IBD. Its predictive value is greater in patients presenting UC and CD with colonic or ileocolonic involvement and inflammatory pattern.

Acknowledgment

All authors have made substantial contributions to all of the following: the conception and design of the study (VGS), acquisition of data (VGS, EIF), drafting the article (VGS), revising it critically for important intellectual content (JPG, FGC, JGV, JDV, JMR, RGO, AGG, and ANR), and final approval of the version to be submitted (FGC, JPG).

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