Improving Relapse Prediction in Inflammatory Bowel Disease by Neutrophil-Derived S100A12

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Background: Prediction of inflammatory bowel disease relapse has important implications for therapeutic strategies. Fecal S100A12 has been reported as a novel marker of intestinal inflammation. The objective was to investigate the utility of S100A12 as a marker for the confirmation of stable remission and prediction of relapses.

Methods: We consecutively included 147 adults and 34 children with Crohn's disease (n = 61) or ulcerative colitis (n = 120). Over a 3-year period, we collected 686 stool samples and 861 serum samples during regular follow-up visits. S100A12 and calprotectin levels were measured by an enzyme-linked immunoassay.

Results: Fecal S100A12 correlated with S100A12 serum levels, other laboratory markers, as well as disease activity, location, and behavior. Fecal S100A12 levels in the relapse group differed significantly from those of the nonrelapse group. A baseline fecal S100A12 level of >0.5 mg/kg was significantly associated with disease relapse within 18 months. Time course analysis of fecal S100A12 before and after relapse showed a clear increase of S100A12 concentrations up to 6 months before clinical relapse. At 0.43 mg/kg, the sensitivity and specificity of S100A12 for predicting relapse already 8 to 12 weeks earlier were 70% and 83%, respectively.

Conclusions: Regular measurements of fecal S100A12 levels reliably detect inflammatory bowel disease relapse at an early stage, which makes the test a promising noninvasive tool for monitoring and optimizing therapy, and may reduce the need for invasive investigations during disease follow-up.

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Key Words: Crohn's disease, ulcerative colitis, fecal marker, serum, calprotectin

The natural clinical course of inflammatory bowel disease (IBD) is characterized by unpredictable episodes of relapse and remission. The main treatment goal in Crohn's disease (CD) and ulcerative colitis (UC) is to induce and maintain remission. Despite successful medical treatment, subclinical intestinal inflammation may still exist, leading to a significant risk of relapse.[1] Therefore, monitoring of disease activity is the mainstay of clinical decision making. At present, accurate monitoring of intestinal inflammation relies on clinical indices (based on symptoms and clinical examination) and endoscopy, in conjunction with histologic investigation and imaging techniques. However, these diagnostic options have a number of drawbacks because they are time consuming, costly, invasive, and/or not necessarily objective. Indirect, yet reliable, measures of biologic disease activity are of utmost importance. Blood tests, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), are in common use but have insufficient sensitivity and specificity for intestinal inflammation.[1] However, fecal markers are a noninvasive way of specifically measuring intestinal inflammation and to assess disease activity in CD and UC. In recent decades, a number of fecal markers have been evaluated for their ability to differentiate and monitor IBD disease activity. The most important among them is calprotectin (S100A8/S100A9), a prominent neutrophil and monocyte-derived protein complex. Many studies have investigated the role of fecal calprotectin in IBD, but the reported results have been inconsistent. Thus, an ideal fecal marker for IBD is yet to be identified.

More recently, neutrophil-derived S100A12 (calgranulin C) has been reported as fecal marker of intestinal inflammation. S100A12 is a member of the damage-associated molecular pattern proteins. These endogenous molecules are released by activated or damaged cells under conditions of cell stress. Phagocyte-

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specific damage-associated molecular pattern proteins of the S100 family are released from neutrophils or monocytes, followed by proinflammatory activation of pattern recognition receptors.[2–8] S100A12 is remarkably resistant to degradation by fecal bacteria, and a stability of fecal specimens is acceptable for at least 7 days at room temperature.[9]

S100A12 has been suggested as a suitable marker of IBD, and several studies have shown a correlation between mucosal inflammation and S100A12 levels in blood[10–13] and feces[9,13–15] of patients with IBD. However, data on the role of S100A12 as a predictive marker of future relapses in pediatric or adult patients with IBD are lacking.[16,17] Therefore, in this study, we followed up fecal and serum S100A12 in pediatric and adult patients with CD and UC to determine its role as a biomarker of intestinal inflammation in the prediction of outcome, especially regarding disease relapse.

PATIENTS AND METHODS

Study Design and Patients

In a prospective multicenter study pediatric and adult patients with CD and UC in remission (as defined by clinical disease activity scores, see below) were consecutively recruited and followed up between April 2008 and June 2011 in 4 independent German outpatient clinics specialized in IBD: (1) Department of General Pediatrics, University Children's Hospital Münster; (2) Department of Medicine B, University Hospital Münster; (3) Department of Integrative Gastroenterology, Internal and Integrative Medicine, Kliniken Essen-Mitte, University of Duisburg-Essen; and (4) Gastroenterology Clinic, Germania Campus Münster, Münster, Germany. Patients with coexisting cardiopulmonary, hepatic, renal, neurologic, psychiatric, and rheumatologic disease, a history of HIV and/or viral hepatitis were excluded from the study. Patients were assessed at 3-month intervals or when relapse occurred. Serum and stool samples were collected at each visit when available. In addition to baseline characteristics, symptoms, medication, clinical signs, and standard laboratory results (full blood count, ESR, CRP) were recorded throughout the study. Ethical approval was obtained from the Ethics Committee of the University of Münster, and fully written informed consent was obtained from all patients or legal guardians.

Clinical Classification and Assessment of Disease Activity

The Montreal classification of CD based on age at diagnosis (16 years or younger [A1], 17–40 years [A2], older than 40 years [A3]), location (ileal [L1], colonic [L2], ileocolonic [L3], upper gastrointestinal [L4]), and behavior (nonstricturing, nonpenetrating [B1], stricturing [B2], penetrating [B3]) was used. For UC, the Montreal classification based on the extent of the disease was used: proctitis (E1), left-sided or distal colitis (E2), and extensive colitis (E3).[18] Disease activity was determined by a disease activity assessment based on the (pediatric) CD activity index ([P]CDAI) for patients with CD[19,20] and the (pediatric) ulcerative colitis activity index ([P]UCAI) for patients with UC.[21,22] Remission was defined as a CDAI <150, PCDAI <11, UCAI <5, and PUCAI <10. Relapse was defined as follows: CDAI >250 over 2 consecutive weeks or a CDAI >150 with an at least 70-point increase within 2 weeks as compared with CDAI at the previous study visit; PCDAI >20 over 2 consecutive weeks or a PCDAI >10 with an at least 5-point increase within 2 weeks as compared with PCDAI at the previous study visit; UCAI >6 over 2 consecutive weeks or a UCAI >4 with an at least 3-point increase within 2 weeks as compared with UCAI at the previous study visit; PUCAI >40 over 2 consecutive weeks or a PUCAI >10 with an at least 5-point increase within 2 weeks as compared with PUCAI at the previous study visit. Mildly active disease was defined as a CDAI between 150 and 219, PCDAI between 11 and 30, and PUCAI between 10 and 34. Moderately active disease was defined as a CDAI between 220 and 450, PCDAI >30, and PUCAI between 35 and 64. Very severe disease was defined as a CDAI >450 and PUCAI >64. Severity of UC was determined by disease activity assessment based on the Montreal classification: mild UC (S1), moderate UC (S2), and severe UC (S3).[18]

Stool and Serum Analysis

Stool and serum samples were coded and stored at $-80°C$ before analysis. Concentrations of S100A12 were determined by a double-sandwich ELISA established in our laboratory, as described previously.[11,14] Fecal calprotectin concentrations were also determined by ELISA (Immundiagnostik AG, Bensheim, Germany). The readers of the assays were blinded for diagnosis and disease stage. The upper limit of normal ranges for fecal and serum S100A12 have previously been defined.[11,14]

Statistical Analysis

For continuous variables, median and range were documented except when otherwise stated. For categorical variables, percentages are provided. Statistical comparisons of data between groups were tested by 2-sided Mann–Whitney U test. The correlations between S100A12 levels and clinical disease activity indices, full blood count parameters, CRP, and ESR were calculated using Spearman's rho correlation coefficient. Time-to-relapse analyses were performed using Kaplan–Meier curves, and differences between the groups were evaluated with the logrank test. To determine the accuracy of S100A12 measurements as a prognostic test receiver operating characteristics (ROC) curves were drawn by plotting sensitivity against 1-specificity. Overall accuracy of the marker in detecting IBD relapse was represented by area under the curve (AUC) with 95% confidence interval. Best cutoff point is defined as the maximum sum of sensitivity and specificity. A P value <0.05 was considered statistically significant. All calculations were performed by using the Statistical Package for the Social Sciences (version 14; SPSS, Inc, Chicago, IL).

RESULTS

Patients and Samples

In total, 181 patients with IBD (61 with CD and 120 with UC; 45% were males) were prospectively included in the study

with a median follow-up period of 45 weeks (4–147 weeks). The median age was 37.4 years (3.5–74.6 years), 19% were younger than 16 years, 47% were older than 40 years, and the remaining 34% of patients were between 17 and 40 years old. The median time since diagnosis of IBD was 1.3 years (0–6.3 years) in pediatric patients and 11.6 years (0.1–37.3 years) in adult patients. Throughout the study follow-up consisting of 937 visits, a total of 686 stool samples and 861 serum samples were collected.

At baseline, 39% of the patients received immunosuppressive therapy. Of these, 20% were taking oral corticosteroids, 18% were using 5-aminosalicylates, 18% were receiving azathioprine, 12% were receiving anti–tumor necrosis factor α agents, and 2% were using methotrexate. Sixty-one patients (34%) relapsed during the follow-up, after a median time of 28 weeks (3–118 weeks). At relapse, the median CDAI, PCDAI, UCAI, and PUCAI scores were 193 (155–231), 23 (15–38), 7 (5–13), and 48 (45–50), respectively. Relapses were classified as mild in 88% of patients with CD and 32% of patients with UC. Baseline characteristics of patients with CD and UC are summarized in Table 1.

TABLE 1. Characteristics of Patients With CD and UC at the Beginning of the Study

	CD	UC
Patients (n)	61	120
Age		
All, yrs (range)	23.4 (3.5–53.9)	44.7 (9.2–74.6)
<16, n (%)	24 (39.3)	10 (8.3)
17–40, n (%)	28 (45.9)	34 (28.3)
>40, n (%)	9 (14.8)	76 (63.3)
At diagnosis, yrs (range)	14.6 (3.4–35.2)	31.2 (6.5–72.1)
Sex		
Male, n (%)	27 (44)	55 (46)
Female, n (%)	34 (56)	65 (54)
Ratio (male/female)	0.79	0.85
Body mass index, (kg/m^2) (range)	20.5 (12.9–38.1)	25.7 (17.1–46.0)
Localization (%)		
Ileal	10.7	—
Colonic	19.6	—
Ileocolonic	69.6	—
Upper gastrointestinal disease	12.5	—
Ulcerative proctitis	—	18.2
Left-sided colitis	—	49.1
Pancolitis	—	32.7
Patients with relapse, n (%)	20 (32.8)	41 (34.2)
Observation period		
Duration, wks (range)	31 (0–147)	46 (0–120)
Visits (n)	280	657
Stool samples (n)	179	507
Serum samples (n)	232	629

S100A12 and Disease Activity in IBD

Fecal S100A12 was significantly higher in patients with active CD (1.5 mg/kg; 0.1–175 mg/kg; n = 57) compared with patients with CD in remission (0.7 mg/kg; 0.1–97 mg/kg; n = 122; $P < 0.01$) (Fig. 1A). Likewise, serum levels of S100A12 were higher during active CD (82 ng/mL; 9–500 ng/mL; n = 60) compared with inactive CD (54 ng/mL; 1–9500 ng/mL; n = 172; $P < 0.005$) (Fig. 1B). Analogously, fecal S100A12 was higher in active UC (2.5 mg/kg; 0.1–264 mg/kg; n = 74) than in inactive UC (0.4 mg/kg; 0.1–308 mg/kg; n = 432; $P < 0.0001$) (Fig. 1A). At the same time, serum levels of S100A12 were also higher in patients with active UC (98 ng/mL; 6–1,370 ng/mL; n = 95) than in those with UC who were in remission (73 ng/mL; 8–1,120 ng/mL; n = 534; $P < 0.001$) (Fig. 1B). Overall, medication had no influence on serum and fecal S100A12 levels (data not shown).

Correlation of S100A12 With Disease Location and Behavior in CD

Fecal S100A12 was significantly higher in patients with active CD and isolated colonic disease (1.0 mg/kg; 0.1–175 mg/kg; n = 53) than in those who had ileal disease (0.5 mg/kg; 0.1–2 mg/kg; n = 17; $P < 0.01$) or ileocolonic disease (0.8 mg/kg; 0.1–27 mg/kg; n = 102; $P < 0.05$). Likewise, levels of serum S100A12 were significantly higher in patients with active CD and isolated colonic disease (78 ng/mL; 12–500 ng/mL; n = 56) than in those who had ileal disease (28 ng/mL; 11–150 ng/mL; n = 24; $P < 0.001$) or ileocolonic disease (57 ng/mL; 1–9500 ng/mL; n = 145; $P < 0.01$).

Serum levels of S100A12 differed also significantly with respect to disease behavior: Serum levels of S100A12 were highest in penetrating disease (94 ng/mL; 22–250 ng/mL; n = 30) compared with stricturing disease (62 ng/mL; 5–1095 ng/ml; n = 77; $P < 0.05$) or nonstricturing/nonpenetrating disease (49 ng/mL; 1–9500 ng/mL; n = 120; $P < 0.001$). However, no statistically significant differences were detected in fecal S100A12 between stricturing disease (1.0 mg/kg; 0.1–175 mg/kg; n = 51), penetrating disease (0.3 mg/kg; 0.1–16 mg/kg; n = 15; $P = 0.089$), and nonstricturing/nonpenetrating disease (0.8 mg/kg; 0.1–27 mg/kg; n = 107; $P = 0.233$).

Correlation of S100A12 With Extent and Severity of UC

Levels of fecal S100A12 increase with increasing severity of UC: Fecal S100A12 was significantly higher in patients with severe UC (3.5 mg/kg; 0.1–107 mg/kg; n = 39) than in those who had moderate disease (1.3 mg/kg; 0.1–308 mg/kg; n = 51; $P < 0.05$), mild disease (0.5 mg/kg; 0.1–76 mg/kg; n = 98; $P < 0.01$), or no signs of disease activity (0.4 mg/kg; 0.1–72 mg/kg; n = 302; $P < 0.0001$). Similarly, levels of serum S100A12 were significantly higher in patients with severe UC (100 ng/mL; 6–390 ng/mL; n = 52) than in those who had moderate disease (82 ng/mL; 7–1370 ng/mL; n = 66; $P < 0.05$), mild disease (74 ng/mL; 12–

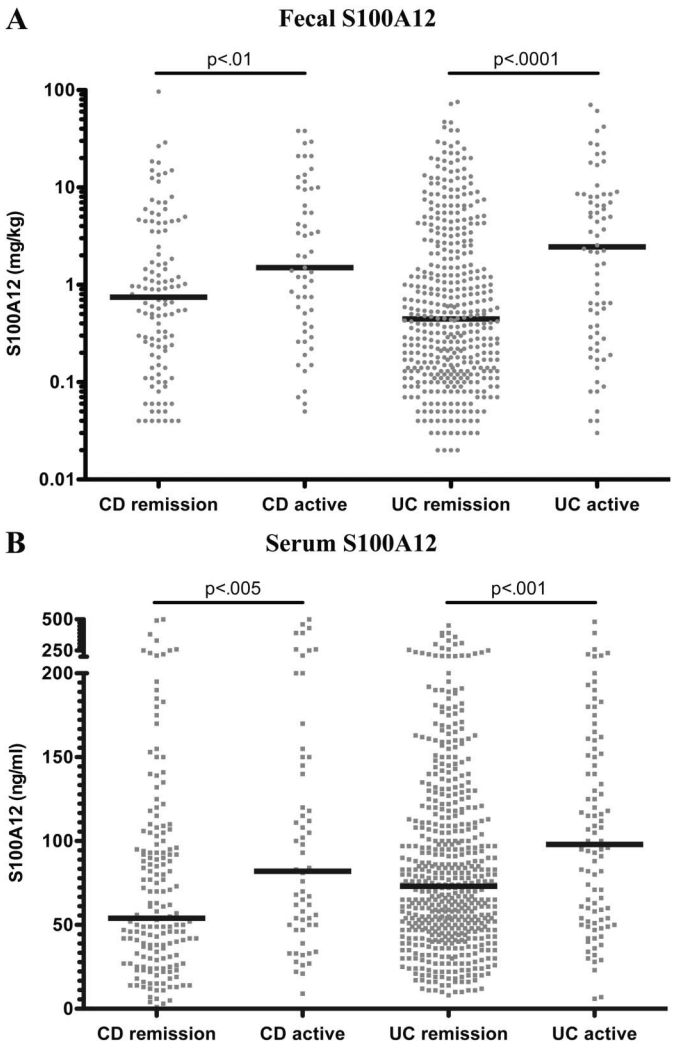


FIGURE 1. S100A12 levels in patients with IBD. The scatter plots show the median levels (central horizontal line) of S100A12 in stool samples (n = 686; A) and serum samples (n = 861; B) of pediatric and adult patients with CD (n = 61; CD) and UC (n = 120; UC). S100A12 concentrations are shown in correlation with disease activity (active disease versus remission) as assessed by IBD disease activity indices for children and adults (CDAI, PCDAI, UCAI, PUCAI). *P* values are shown.

270 ng/mL; n = 129; $P < 0.05$), or no signs of disease activity (71 ng/mL; 8–1120 ng/mL; n = 368; $P < 0.001$).

Furthermore, fecal S100A12 was highest in patients with active UC and pancolitis (0.7 mg/kg; 0.1–76 mg/kg; n = 169) compared with those with ulcerative proctitis (0.4 mg/kg; 0.1–107 mg/kg; n = 92; $P < 0.01$) and those with distal colitis (0.5 mg/kg; 0.1–308 mg/kg; n = 229; $P = 0.191$). However, serum levels of S100A12 were not related to the extent of the disease: Serum levels were comparable between patients with active UC and ulcerative proctitis (73 ng/mL; 11–1120 ng/mL; n = 113), left-sided UC (80 ng/mL; 7–1370 ng/mL; n = 297), and pancolitis (74 ng/mL; 6–390 ng/mL; n = 205).

Association of S100A12 With Disease Activity Indices and Inflammatory Markers

A significant correlation was found between fecal and serum S100A12 and fecal calprotectin levels. Both fecal and serum S100A12 levels correlated well with disease activity scores CDAI and UCAI, whereas there was no correlation of fecal/serum S100A12 and PCDAI and PUCAI. Furthermore, fecal and serum S100A12 correlated positively with CRP, ESR, white blood cell count, and platelets. Fecal but not serum S100A12 levels correlated negatively with hemoglobin. Neither fecal S100A12 levels nor serum S100A12 levels correlated with erythrocyte counts and hematocrit. The correlation of S100A12 and disease activity indices and serum inflammatory markers is summarized in Table 2. Standard laboratory markers (full blood count, CRP, and ESR) in the IBD relapse group did not significantly differ from that in the nonrelapse group (data not shown).

Accuracy of S100A12 in Predicting Clinical Relapse

Fecal S100A12 concentrations of patients who had a relapse during follow-up were higher at inclusion (1.10 mg/kg; 0.1–308 mg/kg; n = 277) than in those who were continuously in remission (0.43 mg/kg; 0.1–96 mg/kg; n = 409; $P < 0.0001$). This difference was noticeable both in patients with CD and UC. In contrast, no differences were observed in serum S100A12 levels of patients with IBD with relapse during follow-up (75 ng/mL; 1–1370 ng/mL; n = 315) and patients without relapse (72 ng/mL; 3–9500 ng/mL; n = 546; $P = 0.229$).

ROC curve analyses were performed to analyze the sensitivity and specificity of S100A12 in differentiating patients with IBD with relapse from those in remission at 1 study visit before the relapse becomes clinically apparent. Although the AUC to predict IBD relapse using serum S100A12 determination showed no satisfying results (graphs not shown), the AUC to predict IBD relapse using fecal S100A12 determination, however, was 0.78 (95% confidence interval, 0.68–0.88; $P < 0.0001$) (Fig. 2A). Results were compared by analogous ROC curve analyses examining the suitability of fecal calprotectin and serum CRP to predict IBD relapse (Fig. 2B, C).

Indeed, the percentage of patients with IBD relapsing out of a status of disease remission during follow-up (CD, n = 20; UC, n = 41) was higher in patients having high fecal S100A12 concentrations at the time of study enrollment in disease remission compared with those with low levels of fecal S100A12. A baseline fecal S100A12 level of >0.5 mg/kg was significantly associated with clinical IBD relapse within 18 months (Fig. 3A). Similar results were obtained when analysis was performed using the best previously selected cutoff point (0.43 mg/kg). Analogously, results were compared by Kaplan–Meier time-to relapse analyses using fecal calprotectin (baseline levels > or < 15 mg/kg) and serum CRP (baseline levels > or < 4.5 mg/L). In contrast to fecal S100A12, the respective survival curves for calprotectin and CRP were not significantly different (Fig. 3B, C). Differences in fecal S100A12, calprotectin, and CRP levels of the relapse and the nonrelapse groups were not influenced by differences in patient adherence to treatment (data not shown).

TABLE 2. Correlation Between S100A12, Fecal Calprotectin, Disease Activity, and Serum Inflammatory Markers

	Fecal S100A12				Serum S100A12			
	n	*r*	95% Confidence Interval	*P*	n	*r*	95% Confidence Interval	*P*
fA12	—	—	—	—	610	0.157	0.076 to 0.235	<0.001
sA12	610	0.157	0.076 to 0.235	<0.001	—	—	—	—
fCP	343	0.566	0.487 to 0.635	<0.0001	307	0.184	0.070 to 0.293	<0.005
CDAI	106	0.401	0.227 to 0.554	<0.0001	163	0.259	0.105 to 0.401	<0.001
PCDAI	73	−0.052	−0.285 to 0.187	0.664	68	0.163	−0.086 to 0.392	0.186
UCAI	479	0.259	0.171 to 0.343	<0.0001	606	0.154	0.073 to 0.233	<0.001
PUCAI	28	0.084	−0.310 to 0.452	0.673	23	−0.007	−0.428 to 0.418	0.976
CRP	594	0.270	0.191 to 0.345	<0.0001	783	0.304	0.237 to 0.368	<0.0001
ESR	459	0.251	0.160 to 0.337	<0.0001	565	0.177	0.094 to 0.258	<0.0001
White blood cells	590	0.184	0.102 to 0.263	<0.0001	776	0.470	0.412 to 0.525	<0.0001
Platelets	588	0.234	0.152 to 0.310	<0.0001	774	0.157	0.085 to 0.227	<0.0001
Hemoglobin	586	−0.214	−0.292 to −0.133	<0.0001	776	−0.005	−0.077 to 0.068	0.901
Red blood cells	147	0.113	−0.054 to 0.275	0.172	231	0.122	−0.011 to 0.251	0.064
Hematocrit	162	−0.099	−0.253 to 0.061	0.212	246	−0.090	−0.217 to 0.039	0.158

n, number of Pairs; r, Spearman's Correlation; fA12, fecal S100A12; sA12, serum S100A12; fCP, fecal calprotectin.

Utility of S100A12 in Monitoring Clinical Relapse

Time course analysis of fecal S100A12 showed an increase of S100A12 concentrations starting up to 6 months before clinical relapse (Fig. 4A). More specifically, fecal S100A12 concentrations were below the cutoff value of 0.43 mg/kg to predict relapse 9 months before relapse (0.1 mg/kg; 0.1–0.8 mg/kg; n = 30) and the levels increased significantly until the visit 6 months before relapse (0.5 mg/kg; 0.1–18 mg/kg; n = 30; $P < 0.0001$). Fecal S100A12 was even higher within 3 months before relapse (0.9 mg/kg; 0.1–42 mg/kg; n = 46; $P < 0.0001$) and reached a peak during relapse (5.5 mg/kg; 0.2–200 mg/kg; n = 60; $P < 0.0001$). After relapse, fecal S100A12 levels decreased within 3 months (0.75 mg/kg; 0.1–308 mg/kg; n = 41; $P < 0.0001$), followed by a further decrease at the time of the visit 6 months after relapse (0.58 mg/kg; 0.1–21 mg/kg; n = 20; $P < 0.001$). In the further course, fecal S100A12 levels decrease below the cutoff value 9 months after relapse (0.26 mg/kg; 0.1–1 mg/kg; n = 22; $P < 0.0001$). Simultaneously, comparative time course analysis using fecal calprotectin and serum CRP was performed (Fig. 4B, C). The overall level of agreement of fecal S10012 (cutoff value, 0.5 mg/kg) and fecal calprotectin (cutoff value, 15 mg/kg) was 73.1%. Thus, fecal levels of S100A12 but not calprotectin were elevated over the cutoff value in 14% of stool samples during relapse, 12% of stool samples before and after relapse, and 12% of stool samples during remission. On the other hand, fecal levels of calprotectin but not S100A12 were elevated over the cutoff value in 5% of stool samples during relapse, 17% of stool samples before and after relapse, and 20% of stool samples during remission.

In parallel, time course analysis of serum S100A12 before and after IBD relapse was performed. In contrast to fecal S100A12 levels, serum levels of S100A12 remained mainly unchanged during a 9-month period before relapse (71 ng/mL; 14–1370 ng/mL; n = 99). Nevertheless, serum levels increased significantly at the time of relapse (110 ng/mL; 6–500 ng/mL; n = 59; $P < 0.05$) and dropped promptly to baseline levels just within 3 months after relapse (71 ng/mL; 5–260 ng/mL; n = 76).

DISCUSSION

Others and we recently described S100A12 as a novel marker for gut inflammation, which correlated even better with intestinal inflammation than fecal calprotectin in adult patients[14] and pediatric patients[9,10,15,23] with IBD. In previous studies, fecal calprotectin was shown to be a specific and sensitive marker for gut inflammation in IBD.[15,23–34] In addition, a potential role for fecal calprotectin in predicting relapse of quiescent IBD was suggested.[1,35,36] The present study examined for the first time whether S100A12 is also a suitable marker for the confirmation of stable remission or the prediction of relapses during long-term follow-up.

The current results confirm previous findings of elevated fecal and serum S100A12 levels in patients with active CD or active UC compared with those with inactive IBD.[6,10,11,14] In addition, we found a significant correlation between fecal and serum S100A12 concentrations and the disease activity scores CDAI and UCAI in adult patients with CD and UC. Serum and fecal S100A12 also correlated with the severity of UC, as assessed by the Montreal classification. These results confirm that S100A12 is

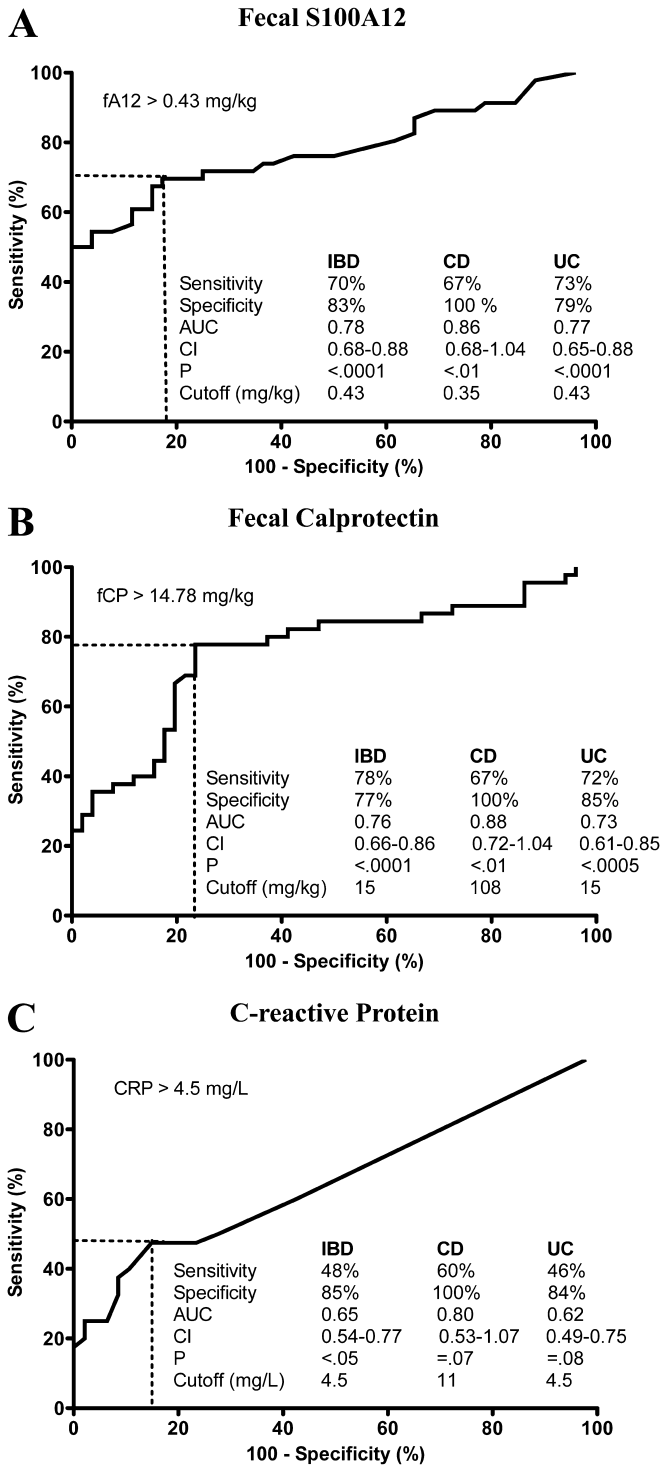


FIGURE 2. ROC curve for the prediction of IBD relapse. ROC curve analyses were performed to analyze the sensitivity and specificity of fecal S100A12 (A), fecal calprotectin (B), and serum CRP (C) in differentiating patients with IBD with disease relapse from patients with IBD in remission during the visit before the relapse becomes clinically apparent. Shown are the AUC, 95% confidence interval, and the *P* value. The value of fecal S100A12 (fA12), fecal calprotectin (fCP), and CRP that gives the best accuracy is shown.

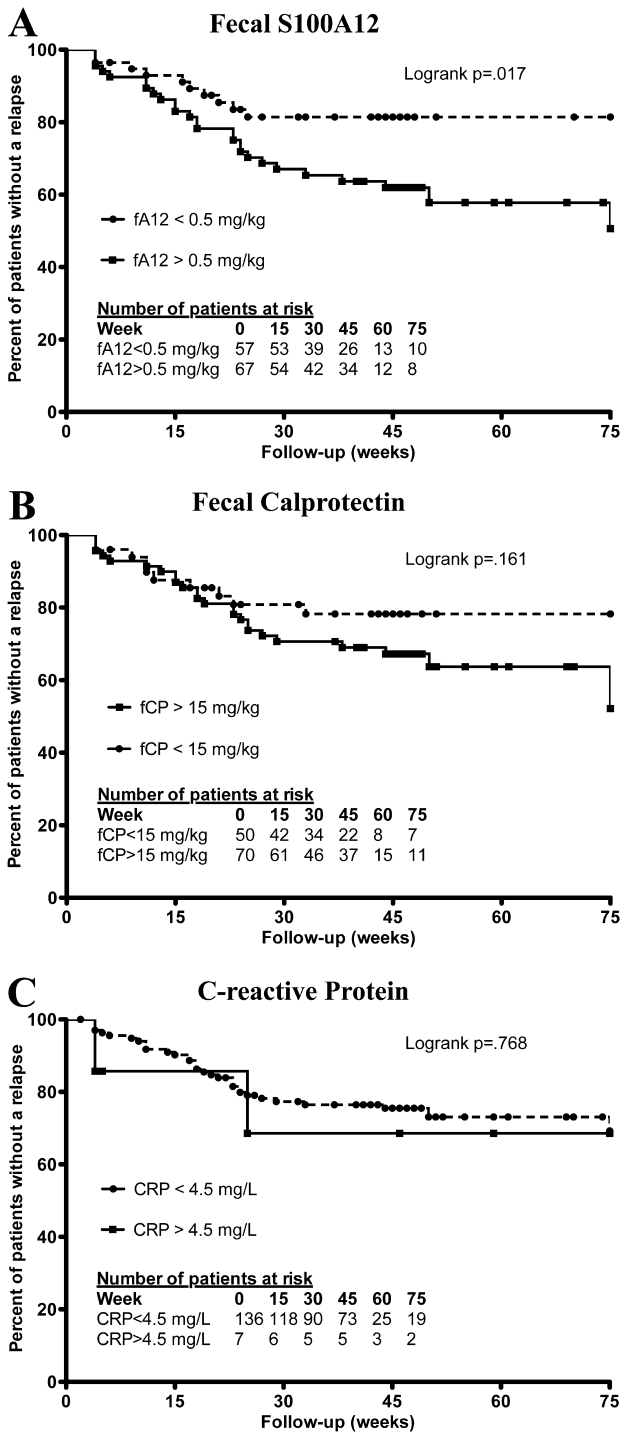
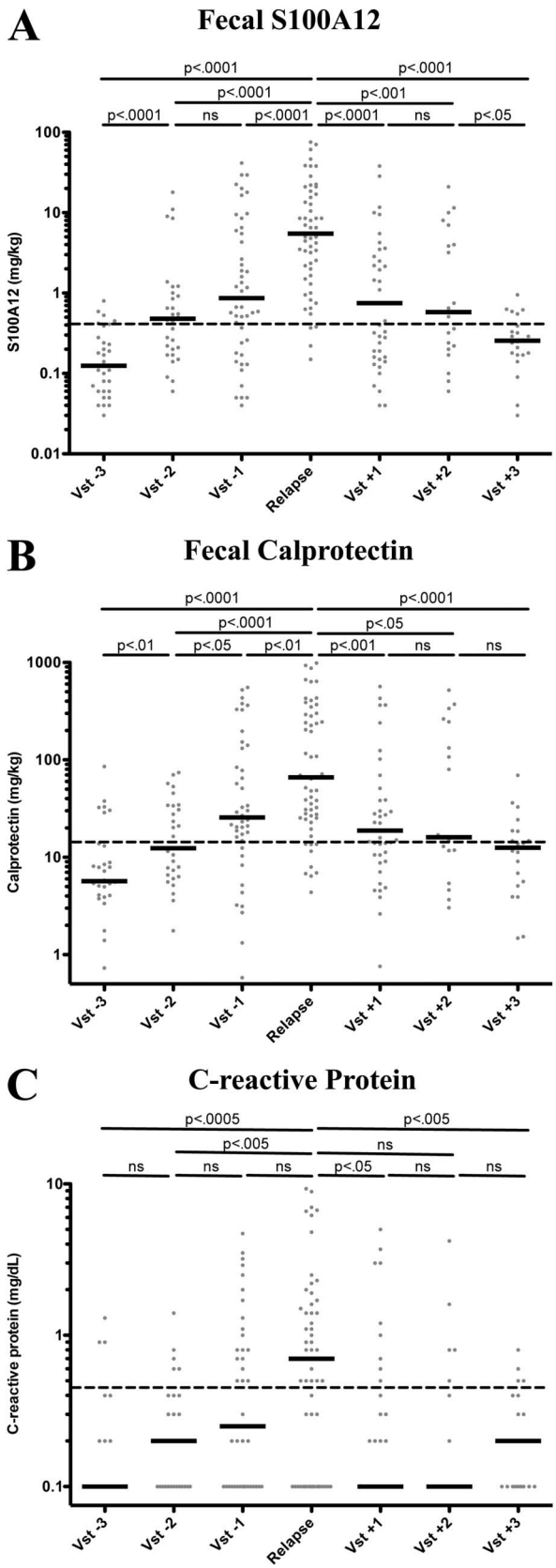


FIGURE 3. Kaplan–Meier time-to-relapse curves for patients with IBD. In all included patients with CD (n = 61) and UC (n = 120), there is a significant difference ($P < 0.05$, log rank) in the proportion of patients who relapsed over a 1.5-year period depending on the fecal S100A12 (fA12) concentration (> or < 0.5 mg/kg) at the time of inclusion into the study (A). The difference in the proportion of patients who relapsed over a 1.5-year period was not significant when fecal calprotectin (fCP) (B) (> or < 15 mg/kg) or serum CRP (C) (> or < 4.5 mg/L) concentrations were considered.



a valuable marker of intestinal inflammation and for monitoring disease activity. Interestingly, we found no correlation between S100A12 levels and pediatric disease activity scores. This may be because of the relative small number of included children or, more likely, as a result of the fact that clinical disease activity scores cannot sufficiently reflect subclinical intestinal inflammation and are hindered by inaccuracy as a result of subjective components. Accordingly, Sidler et al[15] also found no correlation between fecal S100A12 and disease activity, measured by the PCDAI, and another study showed only a correlation between fecal S100A12 and PCDAI in children with a continuous, but not noncontinuous, distribution of intestinal inflammation in CD.[9]

In the present study, fecal and serum S100A12 correlated positively with CRP, ESR, white blood cell count, and platelets. Fecal but not serum S100A12 levels correlated negatively with hemoglobin. We have previously reported that fecal S100A12 levels correlate with ESR, CRP, platelet and white blood cell count, hematocrit, and hemoglobin in adult patients with IBD.[14] Another study showed that although elevated S100A12 serum levels correlated with CRP, it could not discriminate between active and inactive states of IBD.[12] In contrast, fecal S100A12 levels were found to correlate significantly with CRP, platelet count, and albumin only in pediatric patients with CD with noncontinuous distribution of intestinal inflammation. However, children with pancolonic CD exhibited levels of fecal S100A12 that correlated with ESR and platelets but not with CRP or albumin.[9] In the study of Sidler et al,[15] fecal S100A12 did not correlate with ESR, CRP, platelet count, or serum albumin in children with CD.

In the present study, S100A12 performed not only as a promising marker for disease activity but also as a good marker for disease location and behavior in CD as well as for the extent of UC. Indeed, in a previous study, we were able to show that in active CD, the release of S100A12 is strongly dependent on localization, with little release from sites of active ileal inflammation compared with colonic inflammation.[6] A trend toward higher fecal S100A12 levels in distal inflammation compared with more proximal inflammation has been reported.[14] Thus, our present results confirm previous assumptions and underpin the utility of (in particular fecal) S100A12 in assessing the location of and disease extent in CD and UC.

Furthermore, serum S100A12 levels correlated with disease behavior in CD and were highest in patients with penetrating

FIGURE 4. Time course analysis before and after IBD relapse. A total of 249 stool samples of 61 patients with IBD (CD, n = 20; UC, n = 41) were analyzed and levels of fecal S100A12 (A), fecal calprotectin (B), and serum CRP (C) are shown for different time points before, during, and after disease relapse (Vst ± 3: study visit 7 to 9 months before/after relapse; Vst ± 2: study visit 4 to 6 months before/after relapse; Vst ± 1: study visit 2–3 month before/after relapse). The scatter plots show the median (central horizontal line) of fecal/serum concentrations. The cutoff point to differentiate patients with IBD with and without disease relapse (fecal S100A12, 0.5 mg/kg; fecal calprotectin, 15 mg/kg; CRP, 0.45 mg/dL) is represented by the dashed line. *P* values are shown (ns, not significant).

disease followed by stricturing and nonstricturing/nonpenetrating disease. Interestingly, fecal S100A12 concentrations did not significantly correlate with disease behavior in CD. This observation may reflect the fact that penetrating disease leads to a more pronounced systemic inflammatory process because of the presence of abscesses and (chronic) fistulas. Consequently, systemic inflammatory markers including S100A12 might be elevated, whereas the levels of more specific markers of gut wall inflammation (ie, fecal S100A12) do not reflect this condition.

Although it is unlikely that fecal biomarkers will ever replace invasive tests, such as endoscopy, they could be useful as inflammatory markers filtering for the need of invasive investigations while monitoring the disease course of the patients. To further investigate the accuracy of fecal S100A12 in predicting relapse of IBD, we determined S100A12 levels in prospectively collected serum and stool samples of patients with CD and UC. A baseline fecal S100A12 level of >0.5 mg/kg was significantly associated with disease relapse within 18 months. At 0.43 mg/kg, the sensitivity and specificity of fecal S100A12 for predicting relapse already 8 to 12 weeks earlier were 70% and 83%, respectively. Time course analysis of S100A12 up to 9 months before and after relapse showed a clear increase of fecal but not serum S100A12 concentrations up to 6 months before clinical relapse followed by a steady decrease, likely indicating the success of the intensified therapies. Thus, an elevated fecal S100A12 in patients with IBD in clinical remission as defined by clinical disease activity indices may represent a stage of enhanced or active mucosal inflammation, which progresses to cause an eventual clinical relapse of the disease. Conversely, our results suggest that measuring fecal S100A12 may serve as a tool for measuring the effects of treatment as also implied in previous reports.[9,11] Consequently, it may be that treatment of IBD could be tapered at a point where fecal S100A12 levels suggest that the relapse of disease is unlikely to occur within a defined period. However, these issues are beyond the scope of our present study, and further studies are needed to address these questions in more detail.

Previous studies on the role of S100A12 in IBD mainly examined its usefulness as a screening test for patients with suspected IBD and in assessing disease activity. Therefore, comparable data on long-term follow-up fecal S100A12 levels in patients with IBD have not been published so far. Standard laboratory parameters (eg, CRP) did not prove to be useful predictors of clinical relapse in IBD as a whole,[25] although they might me helpful in special clinical issues.[35] However, a recently published meta-analysis assessing the overall capacity of fecal calprotectin in predicting relapse of quiescent IBD demonstrated that the pooled sensitivity and specificity were 0.78 and 0.73, respectively. Evidence suggests that fecal calprotectin is a stronger predictor of relapse in UC than in CD.[25] Our ROC curve analyses showed also a lower sensitivity of fecal S100A12 in predicting relapse in CD compared with UC (67% versus 73%), but the specificity for predicting relapse was higher in CD than in UC (100% versus 79%). CD and UC are diseases with distinct inflammation patterns, and our results clearly show that the S100A12 levels are greatly dependent on the location of the disease in CD and UC. Likewise, some studies showed that fecal calprotectin predicts relapse particularly in patients with CD with colonic and ileocolonic disease but not in those with ileum disease.[26,31] The used fecal calprotectin cutoffs for relapse prediction ranged from 50 to 340 mg/kg.[1] Our comparative analyses of fecal S100A12, calprotectin, and serum CRP suggest that the diagnostic utility of fecal S100A12 in predicting IBD relapse is at least equivalent. We hereby provide cutoff values for predicting relapse of known IBD during disease follow-up for fecal S100A12 (0.5 mg/kg), fecal calprotectin (15 mg/kg), and serum CRP (4.5 mg/L). These cutoff values are below conventional reference ranges because they are adapted to a specific clinical setting in patients with known IBD but inactive disease. In our analyses, we found no age dependency in S100A12 levels, which makes our proposed cutoffs valid both in the pediatric and adult population.

In conclusion, if specific cutoffs for the prediction of the relapse risk out of a status of remission are applied, fecal S100A12 is a promising marker of intestinal inflammation with improved specificity compared with fecal calprotectin for the prediction of IBD relapse. Higher baseline levels of fecal S100A12 are significantly associated with disease relapse during the course of the disease. Fecal S100A12 levels are already increased up to 6 months before clinical relapse. Thus, fecal S100A12 is a suitable noninvasive tool for monitoring disease activity and predicting disease relapse both in CD and UC.

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