Granulocyte Macrophage Colony-Stimulating Factor Auto-Antibodies and Disease Relapse in Inflammatory Bowel Disease

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OBJECTIVES: **Along with others, we have reported that neutralization of granulocyte macrophage colony-stimulating factor (GM-CSF) increases intestinal permeability and bacterial translocation, and reduces neutrophil bacterial killing and anti-microbial seroreactivity. The objective was to investigate the utility of serum GM-CSF auto-antibody (Ab) as a marker for confirmation of stable remission and prediction of relapses in patients with inflammatory bowel disease (IBD).**

METHODS: **We consecutively included 181 adults and children with Crohn's disease (CD, *n*=61) or ulcerative colitis (UC, *n*=120). Over a 3-year period, we collected 861 serum samples and 610 stool samples during regular follow-up visits. GM-CSF Abs and fecal S100 proteins were measured by an enzyme-linked immunoassay.**

RESULTS: **Serum GM-CSF Ab levels correlated with disease activity, location, and extent. Time course analysis before and after relapse showed a clear increase of GM-CSF Ab concentrations up to 6 months before clinical relapse. At 1.7 μg/ml (CD) and 0.5 μg/ml (UC), the sensitivity and specificity of GM-CSF Ab for predicting relapse already 2–6 months earlier were 88% and 95% in CD and 62% and 68% in UC, respectively. A baseline GM-CSF Ab level of >1.7 μg/ml was significantly associated with relapse of CD within 18 months.**

CONCLUSIONS: **As GM-CSF is required for myeloid cell antimicrobial functions and homeostatic responses to tissue injury, serum GM-CSF Ab levels might reflect the degree of bowel permeability and bacterial translocation. Therefore, GM-CSF Ab might identify IBD patients at risk of disease relapse at an early stage, which makes the test a potential tool for monitoring disease activity and optimizing therapy.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

The clinical course of the inflammatory bowel diseases (IBDs) is quite variable. The best outcomes include only a 65% rate of sustained remission—in other words, at least 35% of patients receiving standardized care will experience at least one relapse over the course of a year (1,2). Approximately 15% ultimately do not respond to medical therapy and require surgery for stricturing/penetrating behavior within 3 years of diagnosis (3). These varied outcomes are likely due to substantial genetic, microbial, and immune heterogeneity in disease pathogenesis (4). Clinical tools and biomarkers to define important patient subsets are lacking. Although earlier use of anti-tumor necrosis factor (TNF) therapy is likely to improve rates of sustained remission and reduce rates of surgery, there are currently no diagnostic tools with sufficient accuracy to predict either relapse or stricturing and thereby guide introduction of anti-TNF when it is likely to provide the greatest benefit (3,5).

A recent bioinformatics analysis identified a central role for granulocyte macrophage colony-stimulating factor (GM-CSF) signaling in the pathogenesis of Crohn's disease (CD) (6). GM-CSF

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is a cytokine that promotes myeloid cell development and maturation, and dendritic cell differentiation and survival *in vitro*. Growing evidence supports the notion that GM-CSF exerts pleotropic effects in the gut and has an important role in the regulation of mucosal injury, intestinal immune and inflammatory responses (7). GM-CSF reduces chemically induced gut injury in mice, and clinical trials of GM-CSF in CD have demonstrated a reduction in disease activity in some patients (6,8–13). Furthermore, endogenous cytokine auto-antibodies (Abs) have been described in healthy individuals and those with chronic inflammatory disorders (14). Cytokine Ab may exert neutralizing, activating, or no effect upon the target cytokine (15–17). Although the therapeutic use of anti-cytokine monoclonal Abs in IBD has been the focus of intense investigation over the past decade, the role of endogenous cytokine Ab in regulating mucosal immunity and patient outcomes has received relatively little attention. In one study, IBD patients with higher TNFα neutralizing capacity due to endogenous TNFα Ab exhibited a lower rate of clinical response to infliximab administration (18). Therefore, studies of cytokine Ab in IBD are quite likely to provide fundamental new insights into both mechanisms of pathogenesis, and variation in response to biologic therapy. Our previous studies have examined the role of endogenous GM-CSF Ab as a regulator of myeloid cell function, and biomarker for disease activity and stricturing in CD. We found that GM-CSF Ab production is enriched within the strictured ileum in CD, and high titers of GM-CSF Ab are associated with reduced GM-CSF bioactivity and neutrophil bacterial killing as well as increased intestinal permeability and anti-microbial seroreactivity (19–23). This suggests that variation in GM-CSF Ab and thereby GM-CSF bioactivity might also be associated with clinical relapse in IBD patients. In order to validate these findings, we followed up serum GM-CSF Ab levels in pediatric and adult patients with CD and ulcerative colitis (UC) to determine their role as a biomarker of intestinal inflammation in the prediction of outcome, especially regarding disease relapse (24).

METHODS

Subject enrollment

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. Two study centers included adult patients with CD and UC, one study center recruited adult patients with UC, and one study center enrolled pediatric patients with CD and UC. Study design and clinical classifications have been reported in detail before (25). Ethical approval was obtained from the Ethics Committee of the University of Münster (reference no. 2006-267-f-S), and fully written informed consent was obtained from all patients or legal guardians.

Assessment of disease activity

Disease activity was determined by a disease activity assessment based on the (pediatric) CD activity index for patients with CD (26,27) and the (pediatric) UC activity index for patients with UC (28,29). The definitions of disease remission and relapse are summarized in **Table 1**.

Table 1. Definition of disease remission and relapse

	Remission	Relapse
CDAI	<150 And <Δ70 points/2 weeks	≥Δ70 Points/2 weeks
PCDAI	<11 And <Δ5 points/2 weeks	≥Δ5 Points/2 weeks
UCAI	<5 And <Δ3 points/2 weeks	≥Δ3 Points/2 weeks
PUCAI	<10 And <Δ5 points/2 weeks	≥Δ5 Points/2 weeks

(P)CDAI, (pediatric) Crohn's disease activity index; (P)UCAI, (pediatric) ulcerative colitis activity index.

Stool and serum analysis

Stool and serum samples were coded and stored at −80 °C before analysis. Serum concentrations of GM-CSF Abs were quantified by enzyme-linked immunosorbent assay (ELISA) as previously described (19). Concentrations of fecal S100A12 were determined by a double-sandwich ELISA established in our laboratory, as described previously (30). Fecal calprotectin (S100A8/A9) concentrations were also determined by ELISA (Immundiagnostik AG, Bensheim, Germany). The readers of the assays were blinded for diagnosis and disease stage.

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 two-sided Mann–Whitney *U*-test. The correlations between serum GM-CSF Ab levels and clinical disease activity indices, full blood count parameters, C-reactive protein, and erythrocyte sedimentation rate 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 log-rank test. To determine the accuracy of serum GM-CSF Ab measurements as a prognostic test receiver operating characteristics 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 with 95% confidence interval (CI). 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 GraphPad Prism Version 5.00 for Windows (GraphPad Software, La Jolla, CA). Logistic regression analyses were performed using Stata statistical packages Version 12.0 (StataCorp, College Station, TX).

RESULTS

Clinical and demographic characteristics

In total, 181 patients with IBD (61 with CD and 120 with UC) were prospectively included in the study. Clinical and demographic characteristics of the study subjects have been reported

in detail before (25) and are summarized in **Table 2**. The median time since diagnosis at the time of enrolment into the study was 3.2 years (range 0.1–35.6 years) in patients with CD and 7.5 years (range 0.1–37.3 years) in patients with UC. Nine patients with CD (15%) and 47 patients with UC (39%) were in long-term remission and on no medication at the time of the inclusion into the study (**Table 2**). The majority of these patients (75%) remained in remission during the study follow-up and received no immunosuppressants, immunomodulators, or biologics.

Table 2. Characteristics of patients with CD and UC at the beginning of the study

	CD	UC
Patients, *n* (%)	61 (34)	120 (66)
Age		
All, years (range)	23.4 (3.5–53.9)	44.7 (9.2–74.6)
<16, *n* (%)	24 (39)	10 (8)
17–40, *n* (%)	28 (46)	34 (28)
>40, *n* (%)	9 (15)	76 (63)
At diagnosis, years (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
Localization (%)		
Ileal	11	—
Colonic	20	—
Ileo-colonic	70	—
Upper gastrointestinal disease	13	—
Ulcerative proctitis	—	18
Left-sided colitis	—	49
Pancolitis	—	33
Patients with relapse		
All, *n* (%)	37 (61)	53 (44)
Time, weeks (range)	38 (3–99)	20 (4–118)
Severe disease, *n* (%)	8 (22)	33 (62)
Observation period		
Duration, weeks (range)	31 (0–147)	46 (0–120)
Visits, *n*	286	657
Serum samples, *n*	233	628
Stool samples, *n*	132	478
Medication, n (%)		
No medication	9 (15)	47 (39)
Oral corticosteroids	23 (38)	12 (10)
5-Aminosalicylates	17 (28)	64 (53)
Azathioprine	22 (36)	8 (7)
Anti-TNFα agents	19 (31)	3 (3)
Methotrexate	3 (5)	1 (1)

CD, Crohn's disease; TNF, tumor necrosis factor; UC, ulcerative colitis.

Serum GM-CSF Ab and monitoring of disease activity

GM-CSF Abs were significantly higher in patients with active CD (8.5 μg/ml; 0.1–576 μg/ml; $n=150$) compared with patients with CD in remission (1.3 μg/ml; 0.2–61 μg/ml; $n=83$; $P<0.0001$; **Figure 1a**). Similarly, GM-CSF Abs were higher in active UC (0.8 μg/ml; 0.1–38 μg/ml; $n=253$) than in inactive UC (0.5 μg/ml; 0.02–21 μg/ml; $n=374$; $P<0.0005$; **Figure 1a**). GM-CSF Abs were also significantly higher in patients with endoscopic evidence of active UC (1.2 μg/ml; 0.2–19 μg/ml; $n=14$) compared with patients with UC in endoscopic remission (0.5 μg/ml; 0.1–8 μg/ml; $n=49$; $P<0.03$; **Supplementary Figure S1** online). Furthermore, GM-CSF Ab levels were higher in CD (both during active disease and remission) compared with GM-CSF Ab levels in serum samples of patients with UC ($P<0.0001$; **Figure 1a**).

Time course analysis of GM-CSF Ab in patients with IBD and disease relapse showed an increase of neutralizing Ab concentrations starting up to 6 months before clinical relapse (**Figure 2a**). More specifically, GM-CSF Ab concentrations were at low levels 9 months before relapse and the levels increased steadily until the visit 6 months before relapse. GM-CSF Ab levels were even higher within 3 months before relapse and reached a peak during relapse. After relapse, GM-CSF Ab levels decreased within 2–6 months. In the further course, GM-CSF Ab levels decreased to baseline levels 9 months after relapse (**Table 3**). Head-to-head comparison showed that in contrast to serum GM-CSF Ab titers, fecal calprotectin levels remained mainly unchanged during a 9-month period before relapse. Nevertheless, fecal levels increased significantly at the time of clinical relapse and dropped promptly to baseline levels just within 3 months after relapse (**Figure 2b**).

It is conceivable that the observed overlap in serum GM-CSF Ab levels of patients with active IBD and those with quiescent IBD (**Figure 1a**) is caused by limitations in the accuracy of clinical disease activity indices. To address this issue in more detail we have calculated changes in serum levels (ΔGM-CSF Ab) of individual patients with IBD. We found that individual GM-CSF Ab levels increased significantly within 6 months before and during relapse and that individual serum titers decreased within the following 6 months. In addition, we observed no significant change in individual GM-CSF Ab levels at the time interval 7–9 months before or after clinical relapse (**Supplementary Figure S2** online).

Serum GM-CSF Ab in patients with IBD in stable remission

GM-CSF Ab levels in serum samples ($n=290$) of patients with IBD who had no clinical disease relapse during the study follow-up showed a low intra-individual variation (Δ0.4 μg/ml; 0.06–1.62 μg/ml). Moreover, GM-CSF Ab levels were below the cut-point where GM-CSF Ab start to inhibit neutrophil anti-bacterial function (<5 μg/ml) (23) in these patients

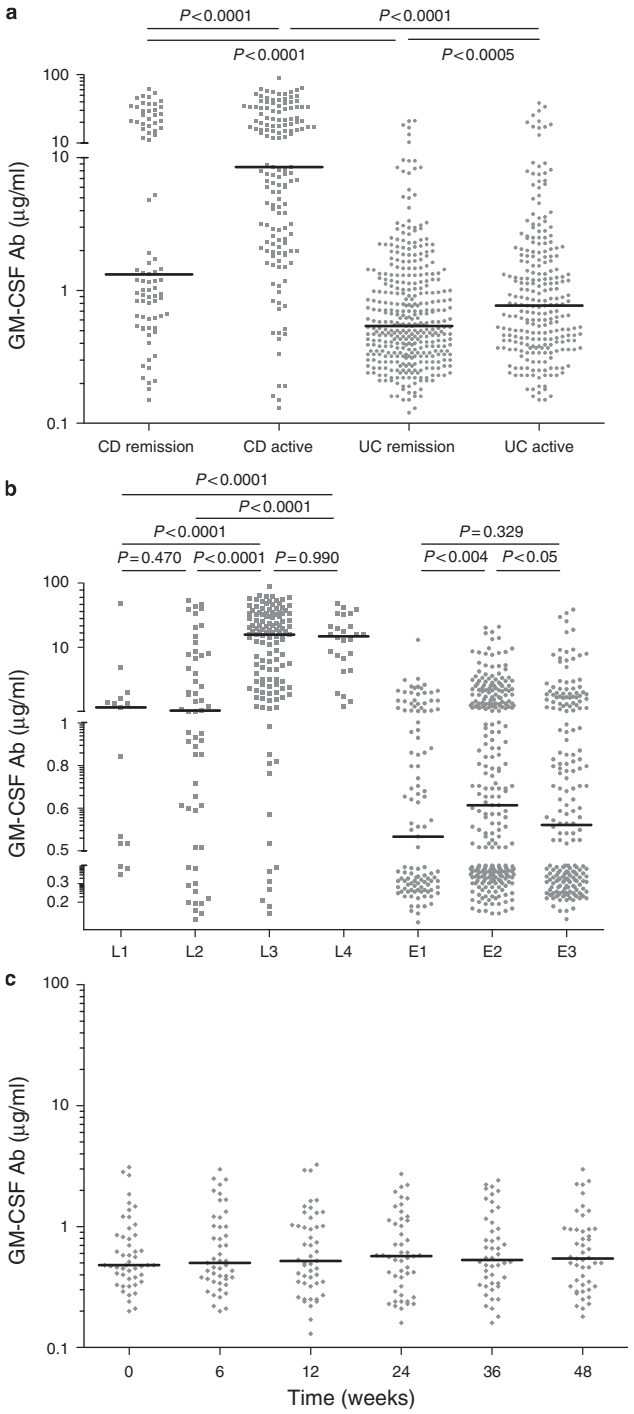

Figure 1. Correlation of serum granulocyte macrophage colony-stimulating factor (GM-CSF) auto-antibody (Ab) with activity, location, and extent of inflammatory bowel disease (IBD). The scatter plots show the median levels (central horizontal line) of GM-CSF Ab levels in serum samples of pediatric and adult patients with Crohn's disease (CD) and ulcerative colitis (UC). *P* values are shown. (**a**) GM-CSF Ab concentrations are shown in correlation with disease activity (active disease versus remission). (**b**) GM-CSF Ab concentrations are shown in correlation with disease location in CD and extent of UC as assessed by the Montreal classification (ileal CD (L1), colonic CD (L2), ileo-colonic CD (L3), concomitant upper gastrointestinal disease (L4); ulcerative proctitis (E1), left-sided or distal colitis (E2), and extensive colitis (E3)). (**c**) A total of 290 serum samples of 49 patients with quiescent IBD were analyzed and levels of GM-CSF Ab are shown for different time points after study enrollment.

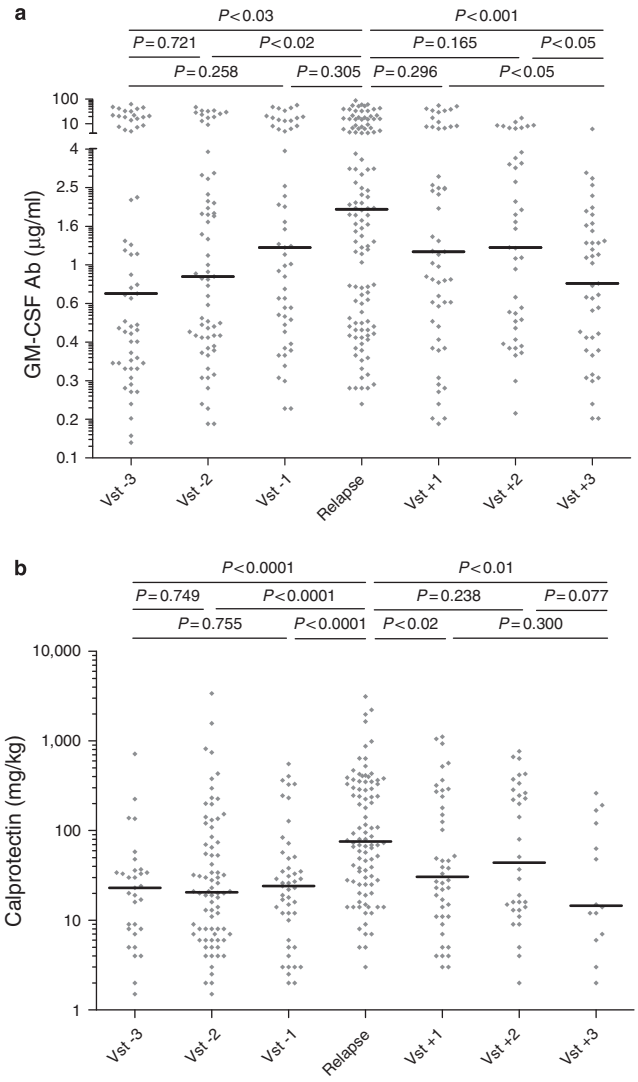

Figure 2. Monitoring of clinical relapse. Serum granulocyte macrophage colony-stimulating factor (GM-CSF) auto-antibody (Ab) levels (**a**) and fecal calprotectin concentrations (**b**) in the same patients are shown for different time points before, during, and after disease relapse (Vst±3: study visit 7–9 months before/after relapse; Vst±2: study visit 4–6 months before/after relapse; Vst±1: study visit 2–3 months before/after relapse).

(**Figure 1c**). Accordingly, GM-CSF Ab levels were significantly lower in serum samples of patients without medication (0.6 µg/ml; 0.02–43 µg/ml; $n = 599$) compared with GM-CSF Ab levels in serum samples of IBD patients who were treated with oral corticosteroids (2.0 µg/ml; 0.1–576 µg/ml; $n = 92$; $P < 0.0001$), 5-aminosalicylates (1.7 µg/ml; 0.2–88 µg/ml; $n = 78$; $P < 0.0001$), azathioprine (6.3 µg/ml; 0.2–63 µg/ml; $n = 81$; $P < 0.0001$), anti-TNFα agents (8.8 µg/ml; 0.4–576 µg/ml; $n = 131$; $P < 0.0001$), and/or methotrexate (1.8 µg/ml; 0.2–42 µg/ml; $n = 19$; $P < 0.03$). In all, 58% of patients on medical therapy at the time of the inclusion into the study had low GM-CSF Ab serum levels (< 1.7 µg/ml) and 38% of serum samples collected during the study follow-up

Table 3. Serum GM-CSF Ab levels at different time points before, during and after relapse of IBD

	Crohn's disease				Ulcerative colitis			
Time interval	Median (μg/ml)	Range (μg/ml)	*n*	*P* value	Median (μg/ml)	Range (μg/ml)	*n*	*P* value
7–9 Months before	2.9	0.1–63	22	<0.01	0.42	0.1–8	38	=0.689
4–6 Months before	12.8	3–47	11	<0.0001	0.52	0.2–25	52	<0.05
2–3 Months before	16.0	2–57	6	<0.0005	0.67	0.1–21	47	<0.01
During relapse	21.5	0.2–576	54	<0.0001	0.79	0.2–38	64	<0.0001
2–3 Months after	8.0	0.2–528	9	<0.05	0.69	0.2–34	35	<0.05
4–6 Months after	4.7	0.5–51	16	<0.0001	0.64	0.2–30	36	<0.01
7–9 Months after	2.1	1–33	10	<0.0001	0.60	0.2–8	31	=0.104

Ab, auto-antibody; GM-CSF, granulocyte macrophage colony-stimulating factor; IBD, inflammatory bowel disease.

of patients on medical therapy showed elevated GM-CSF Ab concentrations (>1.7 μg/ml).

Correlation of serum GM-CSF Ab with disease location and behavior in CD

GM-CSF Abs were significantly higher in patients with CD and ileo-colonic disease (15.7 μg/ml; 0.2–88 μg/ml; $n=128$) as well as concomitant upper gastrointestinal disease (14.7 μg/ml; 1.2–48 μg/ml; $n=26$) than in those who had isolated ileal disease (1.2 μg/ml; 0.4–47 μg/ml; $n=15$; $P<0.0001$) or colonic disease (1.0 μg/ml; 0.1–576 μg/ml; $n=60$; $P<0.0001$; **Figure 1b**). Serum levels of GM-CSF Ab did not significantly differ with respect to stricturing and/or penetrating disease behavior of CD (data not shown).

Correlation of serum GM-CSF Ab with extent and severity of UC

Levels of GM-CSF Ab were significantly higher in patients with moderate/severe disease (0.67 μg/ml; 0.1–38 μg/ml; $n=241$; $P<0.0004$) and mild disease (0.70 μg/ml; 0.1–8 μg/ml; $n=145$; $P<0.05$) than in those patients with UC who had no signs of disease activity (0.49 μg/ml; 0.02–14 μg/ml; $n=228$). Furthermore, GM-CSF Abs were highest in patients with left-sided (distal) UC (0.64 μg/ml; 0.1–21 μg/ml; $n=295$) compared with those with ulcerative proctitis (0.54 μg/ml; 0.1–13 μg/ml; $n=113$; $P<0.004$) and those with pancolitis (0.58 μg/ml; 0.02–38 μg/ml; $n=206$; $P<0.05$; **Figure 1b**).

Association of serum GM-CSF Ab with disease activity indices and inflammatory markers

A significant correlation was found between GM-CSF Ab and fecal calprotectin (**Table 4**). GM-CSF Abs were not correlated with clinical disease activity scores, fecal S100A12, C-reactive protein, erythrocyte sedimentation rate, white blood cell counts, hemoglobin, erythrocyte counts, platelet count, or hematocrit (**Table 4**).

Accuracy of serum GM-CSF Ab in detecting clinical relapse

GM-CSF Ab concentrations of patients who had a relapse during follow-up were higher at inclusion into the study (1.7 μg/ml; 0.1–88 μg/ml; $n=81$) than in those who were continuously in remission (0.7 μg/ml; 0.2–61 μg/ml; $n=87$, $P<0.05$). Receiver operating characteristics curve analyses were performed to analyze the sensitivity and specificity of GM-CSF Ab in differentiating patients with IBD with relapse from those in remission at 2–6 months before the relapse becomes clinically apparent (**Figure 3a** and **b**). Thus, a GM-CSF Ab concentration of 1.7 μg/ml in CD (**Figure 3a**) and 0.5 μg/ml in UC (**Figure 3b**) gives the best accuracy in predicting clinical relapse. We performed parallel receiver operating characteristics curve analyses to analyze the sensitivity and specificity of fecal calprotectin in differentiating patients with relapse of IBD from those in remission at 2–6 months before the relapse becomes clinically apparent (**Figure 3c** and **d**). We found that the diagnostic accuracy of serum GM-CSF Ab for the early detection of IBD relapses is clearly superior to fecal calprotectin (**Figure 3**).

Accuracy of serum GM-CSF Ab in predicting clinical relapse

The percentage of patients with IBD relapsing out of a status of disease remission during follow-up (CD, $n=37$; UC, $n=53$) was higher in patients having high GM-CSF Ab concentrations at the time of study enrollment in disease remission compared with those with low levels of GM-CSF Ab. A baseline GM-CSF Ab level of >1.7 μg/ml was significantly associated with clinical relapse of CD within 18 months (**Figure 4a**), whereas the difference in the proportion of patients with UC who relapsed over a 1.5-year period was not statistically significant when GM-CSF Ab concentrations (< or >0.5 μg/ml) were considered (**Figure 4b**). We performed comparative Kaplan–Meier time-to-relapse analyses using fecal calprotectin and found that fecal calprotectin is like serum GM-CSF Ab a useful biomarker for the *a priori* risk prediction of disease relapse in patients with CD (but not with UC) in clinical remission. A baseline fecal calprotectin level of >29 mg/kg was significantly associated with clinical relapse of CD within 18 months (**Figure 4c**), whereas the difference in the proportion of patients with UC who relapsed over a 1.5-year period was not statistically significant when fecal

Table 4. Correlation between serum GM-CSF auto-antibodies, disease activity, and inflammatory markers

	n	*r*	95% Confidence interval	*P* value
Fecal S100A8/A9	306	0.203	0.089 to 0.311	<0.0005
Fecal S100A12	610	0.056	−0.026 to 0.137	0.168
CDAI	166	0.132	−0.025 to 0.283	0.090
PCDAI	67	0.081	−0.170 to 0.321	0.516
UCAI	604	0.024	−0.058 to 0.106	0.555
PUCAI	23	−0.258	−0.614 to 0.185	0.234
CRP	781	−0.005	−0.077 to 0.067	0.888
ESR	565	−0.024	−0.108 to 0.062	0.577
White blood cells	772	−0.059	−0.131 to 0.014	0.103
Platelets	772	0.072	−0.002 to 0.142	0.051
Hemoglobin	772	−0.011	−0.083 to 0.062	0.766
Red blood cells	226	−0.061	−0.193 to 0.074	0.365
Hematocrit	241	−0.018	−0.147 to 0.113	0.786

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GM-CSF, granulocyte macrophage colony-stimulating factor; n, number of pairs; (P)CDAI, (pediatric) Crohn's disease activity index; (P)UCAI, (pediatric) ulcerative colitis activity index; *r*, Spearman's correlation.

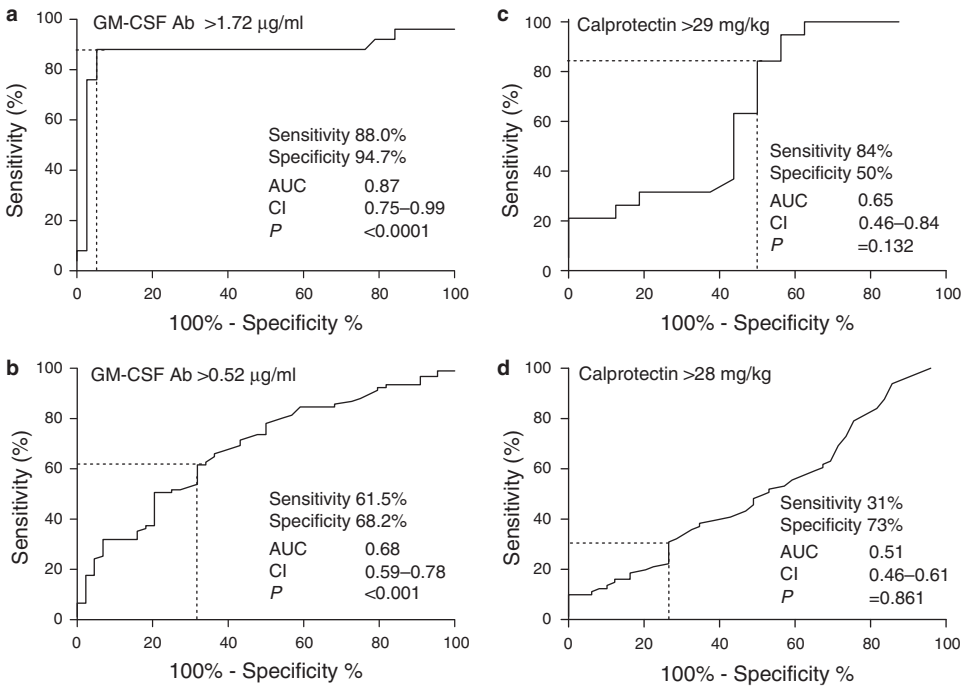

Figure 3. Detection of clinical relapse. Receiver operating characteristics (ROC) curve analyses were performed to analyze the sensitivity and specificity of granulocyte macrophage colony-stimulating factor (GM-CSF) auto-antibody (Ab) (**a**, **b**) and fecal calprotectin (**c**, **d**) in differentiating patients with Crohn's disease (CD) (**a**, **c**) and ulcerative colitis (UC) (**b**, **d**) with disease relapse from patients with CD (**a**, **c**) and UC (**b**, **d**) in remission during the visits 2–6 months before the relapse becomes clinically apparent. Shown are the area under the curve (AUC), 95% confidence interval (CI), and the *P* value. The GM-CSF Ab serum and fecal calprotectin concentration that gives the best accuracy is shown.

calprotectin concentrations (< or >28 mg/kg) were considered (**Figure 4d**). Combined predictive logistic modeling showed that the odds ratio (OR) of IBD relapse in patients with both elevated levels of GM-CSF Ab and fecal calprotectin at baseline is 3.8 (95% CI 1.4–10.3; $n=126$; $P<0.01$). The OR of elevated GM-CSF Ab levels at baseline was 2.6 (95% CI 1.4–4.8; $n=170$; $P<0.005$),

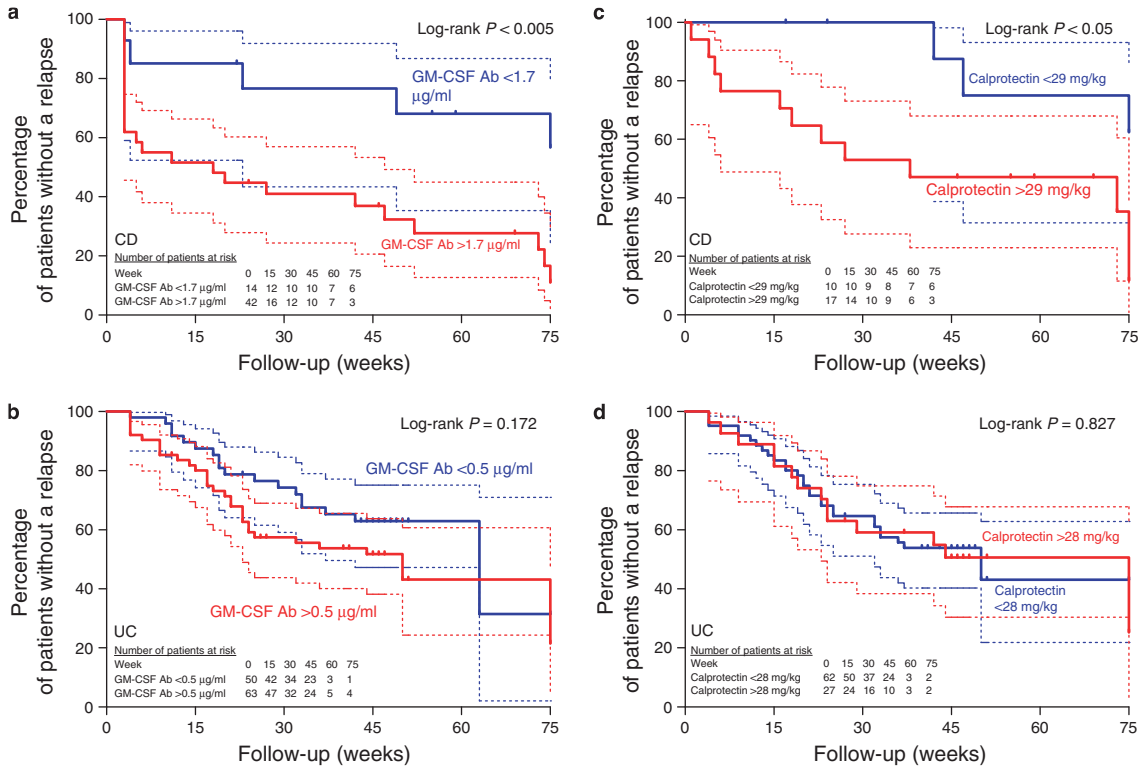

Figure 4. Prediction of clinical relapse. Differences in the proportion of patients who relapsed over a 1.5-year period depending on granulocyte macrophage colony-stimulating factor (GM-CSF) auto-antibody (Ab) (**a**, **b**) or fecal calprotectin (**c**, **d**) at the time of inclusion into the study were analyzed and shown are Kaplan–Meier time-to-relapse curves for patients with Crohn's disease (CD) (**a**, **c**) and ulcerative colitis (UC) (**b**, **d**). Dashed lines represent 95% confidence intervals.

whereas the OR of elevated calprotectin concentrations at baseline was 2.0 (95% CI 1.0–4.1; $n = 135$; $P = 0.050$).

DISCUSSION

The factors that promote clinical relapse in IBD are poorly understood. Endogenous cytokine Abs have a role in a number of autoimmune and chronic inflammatory disorders (14). Given the advent of therapeutic anti-cytokine antibodies use in IBD, a better understanding of the role of endogenous cytokine Abs in pathogenesis and treatment response is potentially of great importance (31). We have reported previously that elevated levels of GM-CSF Ab are associated with an increase in intestinal permeability, increased titers of several anti-microbial seroreactivities, and increased rates of stricturing behavior and surgery in adult and pediatric CD (19,20,22). 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. In recent decades, a number of fecal biomarkers have been evaluated for their ability to differentiate and monitor IBD disease activity (32,33) but an ideal fecal marker for IBD has yet to be identified (25,34–36).

In the present study, we examined for the first time whether serum GM-CSF Abs are a suitable marker for the confirmation of stable remission or the prediction of relapses of IBD during long-term follow-up. We found that serum GM-CSF Ab levels are significantly elevated in patients with active CD or active UC compared with those with inactive IBD and that serum GM-CSF Ab titers correlate significantly with concentrations of fecal calprotectin (S100A8/A9). We have recently shown that S100 proteins are reliable biomarkers of intestinal inflammation in IBD (25) and that higher serum GM-CSF Ab concentrations are associated with lower neutrophil bacterial killing (19). Thus, it could be speculated that in IBD fluctuating GM-CSF Ab titers have biologic effects on myeloid cell functions, which in turn increases the risk of disease relapses due to an impaired modulation of gut intestinal homeostasis and intestinal barrier function (21). Two previous cross-sectional studies on the role of GM-CSF Ab in IBD found no differences of serum GM-CSF Ab in subjects with inactive vs. active disease (19) or the authors did not assess the direct impact of GM-CSF Ab titers on disease activity (22). These studies did not examine the relationship between serum GM-CSF Ab and disease activity within subjects longitudinally. In the current study, we clearly show, using a longitudinal prospective design, that GM-CSF Ab levels increase starting up to 6 months before clinical relapse; peak during relapse; and decline steadily during 9 months after relapse to baseline levels. Furthermore, we found that serum GM-CSF Ab titers in patients with IBD in stable remission were

low and showed no relevant fluctuation as seen in patients with disease relapse.

We also examined the association of GM-CSF Ab levels and CD location and behavior and UC extent and severity. GM-CSF Ab titers were significantly higher in patients with ileo-colonic CD or concomitant upper gastrointestinal disease and in patients with left-sided (distal) UC. GM-CSF Ab levels did not differ as regards CD behavior or severity of active UC. Previous studies have shown that elevated GM-CSF level are significantly associated with ileal or ileo-colonic location of CD and stricturing or penetrating disease behavior (19,22). However, the proportion of patients with CD and stricturing/penetrating disease was significantly higher in these studies (35–58%) compared with our present study (16%) and the association was not observed in subjects with colon-only disease (19,22). In addition, different results might be explained by the fact that our study design did not include systematic endoscopic and/or radiologic (re-) evaluation of disease location and extent. It will be important in future longitudinal studies to correlate GM-CSF Ab levels with the location and extent of CD and UC based on follow-up radiologic, endoscopic, or surgical examination.

Similar to previous studies on the role of GM-CSF Ab in IBD, we confirm that GM-CSF Ab expression is elevated in CD when compared with UC (19,22). The reason for the different result for GM-CSF Ab expression in CD and UC is not clear. However, we have shown in a recent study that both free GM-CSF and GM-CSF Abs are produced by the affected tissue in ileal surgical specimens of patients with CD and GM-CSF Abs were increased markedly in lamina propria mononuclear cells isolated from the stricture, suggesting a specific local tissue response (21). These data are consistent with the hypothesis that prolonged exposure to the cognate cytokine, in this case GM-CSF, leads to loss of T-cell tolerance, and expansion of an existing pool of B cells producing GM-CSF Abs (14). It will be important in future studies to determine whether and to what extent *in vivo* production of GM-CSF Ab occurs also in UC.

Although it is unlikely that 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 patients with IBD. Fecal markers are a non-invasive way of objectively measuring intestinal inflammation and disease activity. A number of fecal markers have been evaluated for their ability to differentiate and monitor IBD disease activity (32,33). Two members of the S100 family of calcium-binding proteins (calprotectin and S100A12) are among the most promising disease-specific markers, which have the potential to advance diagnostic and disease monitoring practices (33). However, the capacity of fecal S100 proteins in predicting relapse of quiescent IBD is not ideal and shows a sensitivity of 70% (S100A12) to 78% (calprotectin) and a specificity of 73% (calprotectin) to 83% (S100A12) (25,34,37). Furthermore, evidence suggests that fecal S100 proteins are a stronger predictor of relapse in UC than in CD (25,38). In the present study, we examined for the first time whether GM-CSF Ab titers are a suitable marker for the confirmation of stable remission or the prediction of relapses of IBD during long-term follow-up. To investigate the accuracy of serum GM-CSF Ab in predicting relapse of IBD, we determined GM-CSF Ab levels in prospectively collected serum samples of patients with CD and UC. Time course analysis of GM-CSF Ab up to 9 months before and after relapse showed a clear increase of Ab titers up to 6 months before clinical relapse followed by a steady decrease, likely indicating the success of the intensified therapies. Thus, an elevated serum GM-CSF Ab titer in patients with IBD in clinical remission as defined by clinical disease activity indices may represent an early stage of increased intestinal permeability, bacterial translocation, neutrophil dysfunction, and reduced antimicrobial activity (19–21). It is conceivable that the potentially impaired intestinal homeostasis progresses to cause an eventual clinical relapse of the disease. Conversely, our results suggest that measuring GM-CSF Ab may serve as a tool for monitoring relapses and for measuring the effects of treatment. We would recommend monitoring GM-CSF Ab levels every 3–4 months in stable patients and more often in patients with recurrent/frequent relapses and/or refractory IBD. Future studies are needed to investigate whether or not GM-CSF Ab levels can predict the need for immunosuppressants, immunomodulators, or biologics.

A baseline GM-CSF level of $>1.7\,\mu g/ml$ was significantly associated with relapse of CD within 18 months. At $1.72\,\mu g/ml$ the sensitivity and specificity of GM-CSF Ab for predicting relapse of CD already 2–6 months earlier were 88% and 95%, respectively. Consistently, Han *et al.* have previously defined elevated GM-CSF Ab as a serum concentration $\geq 1.7\,\mu g/ml$. We found that, in contrast to fecal S100 proteins, GM-CSF Ab levels are a stronger predictor of relapse in CD than in UC. Thus, the combined determination of biomarkers, that is, the measurement of GM-CSF Ab levels and fecal S100 protein concentrations, could be useful to assess the risk of relapses in patients with IBD, both with CD and UC. Fecal calprotectin may tend to excel in colonic disease, and anti-GM-CSF in ileal disease. However, the inhomogeneity of our study population does not allow reliable subanalyses by age and disease type. Future larger study will be needed to adjust our findings for age and IBD subtypes. In addition, it would be interesting to know the GM-CSF Ab levels at the time of diagnosis and how serum titers change over time during the course of IBD. However, this issue is beyond the scope of our present study because we included patients with known IBD in remission (and not at the time of diagnosis of CD or UC). Thus, further studies are needed to address the question if GM-CSF Abs are an acquired phenomena or integral to disease pathogenesis.

The fact that GM-CSF Ab levels start to increase up to 6 months before the disease relapse becomes clinically apparent could explain the overall lack of correlation with clinical disease activity scores. We assume that GM-CSF Ab might rather reflect the preceding impairment of intestinal homeostasis at an early stage, which results in intestinal inflammation and disease relapse later on. Thus, GM-CSF Ab may already be elevated in the absence of significant intestinal inflammation and clinically detectable disease activity. This assumption is supported by the observed overlap of GM-CSF Ab levels in patients with active and inactive disease as

defined by clinical disease activity scores (**Figure 1a**). This might also underpin the fact that clinical disease activity scores cannot sufficiently reflect subclinical intestinal inflammation and are potentially hindered by inaccuracy as a result of subjective components. However, it is important to mention that GM-CSF Ab levels correlate with clinical disease activity score at the time of disease relapse. Furthermore, it has also been shown that C-reactive protein and erythrocyte sedimentation rate are not suitable for the prediction and early detection of disease relapses in IBD. We were therefore not surprised by the lack of correlation between these acute phase reactants and GM-CSF Ab in our longitudinal study, given the fact that serum levels of GM-CSF Ab start to increase up to 6 months before the clinical relapse of the disease. Although C-reactive protein and erythrocyte sedimentation rate reflect the presence and intensity of an inflammatory process, GM-CSF Ab might have an underlying mechanistic role in the pathogenesis of IBD relapse independent of inflammatory responses. Thus, GM-CSF Ab might rather reflect the preceding impairment of the intestinal homeostasis at an earlier stage. Specifically, elevated GM-CSF Ab levels might be an indicator of intestinal permeability, bacterial translocation, neutrophil dysfunction, and reduced antimicrobial activity. The breakdown of intestinal homeostatic functions may then lead to intestinal inflammation and disease relapse with a concomitant increase of acute phase reactants like C-reactive protein and erythrocyte sedimentation rate.

Interestingly, it has recently been shown that smokers with ileal CD have significantly lower GM-CSF Ab concentrations and that elevated GM-CSF Ab (≥5 μg/ml) were associated with significantly higher odds of having ileal disease location among non-smokers (22). However, in the present study we found no interaction of smoking status with CD location or GM-CSF Ab titers (data not shown). This may be caused by differences in the smoking status and the number of patients with ileal CD. The study of Gathungu *et al.* included 477 patients with CD, of whom 35% had ileal disease and 42% were current smokers or ex-smokers; whereas our present study included 61 patients with CD, of whom only 11% had ileal disease and only 6% were current smokers or ex-smokers. Furthermore, the effect of smoking on GM-CSF Ab expression in the study by Gathungu *et al.* did not reach significance in their second cohort and in a subset of CD patients with colon-only location. Nevertheless, it will be important in future studies to consider the effects of smoking on GM-CSF Ab levels in IBD.

In summary, our study shows for the first time that regular measurements of GM-CSF Ab levels reliably detect IBD relapse at an early stage, which makes the test a promising tool for monitoring and optimizing therapy, and may reduce the need for invasive investigations during disease follow-up. As GM-CSF is required for myeloid cell anti-microbial functions and homeostatic responses to tissue injury, serum GM-CSF Ab levels might reflect the degree of bowel permeability and bacterial translocation (20) and therefore identify IBD patients at risk of disease relapse. Our ongoing studies will determine whether neutrophil and monocyte function varies with longitudinal GM-CSF Ab levels, and whether these myeloid cell functions decrease before flares in association with increased GM-CSF Ab levels.

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CONFLICT OF INTEREST

Guarantor of the article: Jan Däbritz, MD.

Specific author contributions: Planned the study and led the clinical trial: Jan Däbritz and Dirk Foell; recruited and enrolled patients to the study: Jan Däbritz, Jost Langhorst, and Dirk Foell; performed the research: Jan Däbritz, Erin Bonkowski, Claudia Chalk, Bruce C. Trapnell, Jost Langhorst, Lee A. Denson, and Dirk Foell; interpreted and analyzed the data: Jan Däbritz, Lee A. Denson, and Dirk Foell; performed statistical analyses: Jan Däbritz; wrote the manuscript and created tables and figures: Jan Däbritz; critically reviewed the manuscript: Lee A. Denson and Dirk Foell; revised and submitted the manuscript: Jan Däbritz; read and approved the final version of the manuscript: Jan Däbritz, Erin Bonkowski, Claudia Chalk, Bruce C. Trapnell, Jost Langhorst, Lee A. Denson, and Dirk Foell.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Neutralization of granulocyte macrophage colony-stimulating factor (GM-CSF) increases intestinal permeability and bacterial translocation in mice.
- ✓ GM-CSF Ab production is enriched within the strictured ileum in Crohn's disease (CD), and high titers of GM-CSF Ab are associated with reduced GM-CSF bioactivity and neutrophil bacterial killing.
- ✓ Elevated neutralizing GM-CSF Abs are associated with an increase in bowel permeability in patients with CD.

WHAT IS NEW HERE

- ✓ We show for the first time a relationship between a cytokine auto-Ab and clinical relapse in inflammatory bowel disease (IBDs).
- ✓ Serum GM-CSF Ab levels correlated with disease activity, location, and extent; and baseline serum GM-CSF Ab levels are able to predict IBD relapse.
- ✓ Regular measurements of GM-CSF Ab levels reliably detect IBD relapse at a very early stage and may demonstrate a novel pathogenic mechanism of disease.

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S U P P L E M E N T

Granulocyte-macrophage colony stimulating factor auto-antibodies and disease relapse in inflammatory bowel disease

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SUPPLEMENTAL DATA

Supplementary Figure S1.

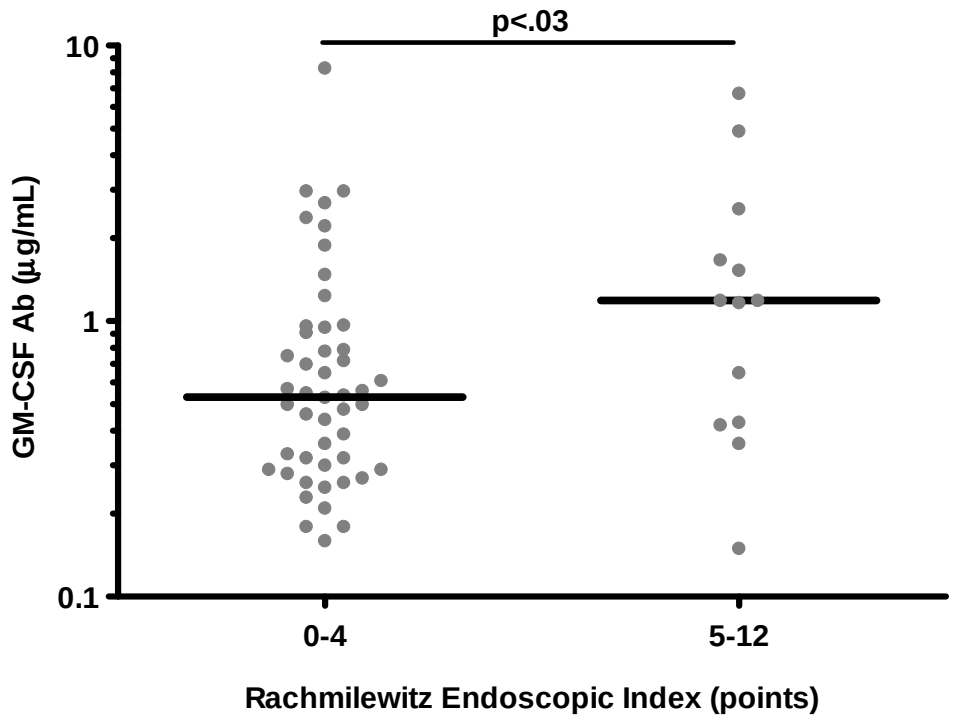

Figure S1. Correlation of the endoscopic activity index subgroups with serum GM-CSF Ab levels. Endoscopic data was available for 63 patients with ulcerative colitis, in whom serum GM-CSF Ab levels were measured at the time of colonoscopy. Experienced board-certified gastroenterologists with at least 5 years experience in colonoscopy performed the endoscopies and were unaware of clinical and laboratory results to avoid bias. Colonoscopy findings were graded by the gastroenterologists according to the endoscopic part of the Rachmilewitz Activity Index (1) consisting of 4 items: granulation scattering reflected light, vascular pattern, vulnerability of mucosa, and mucosal damage (mucus, fibrin, exudates, erosions, ulcer). Scores range from 0 to 12 points. Endoscopic remission was defined as an endoscopic index score of 0-4 points.

Supplementary Figure S2.

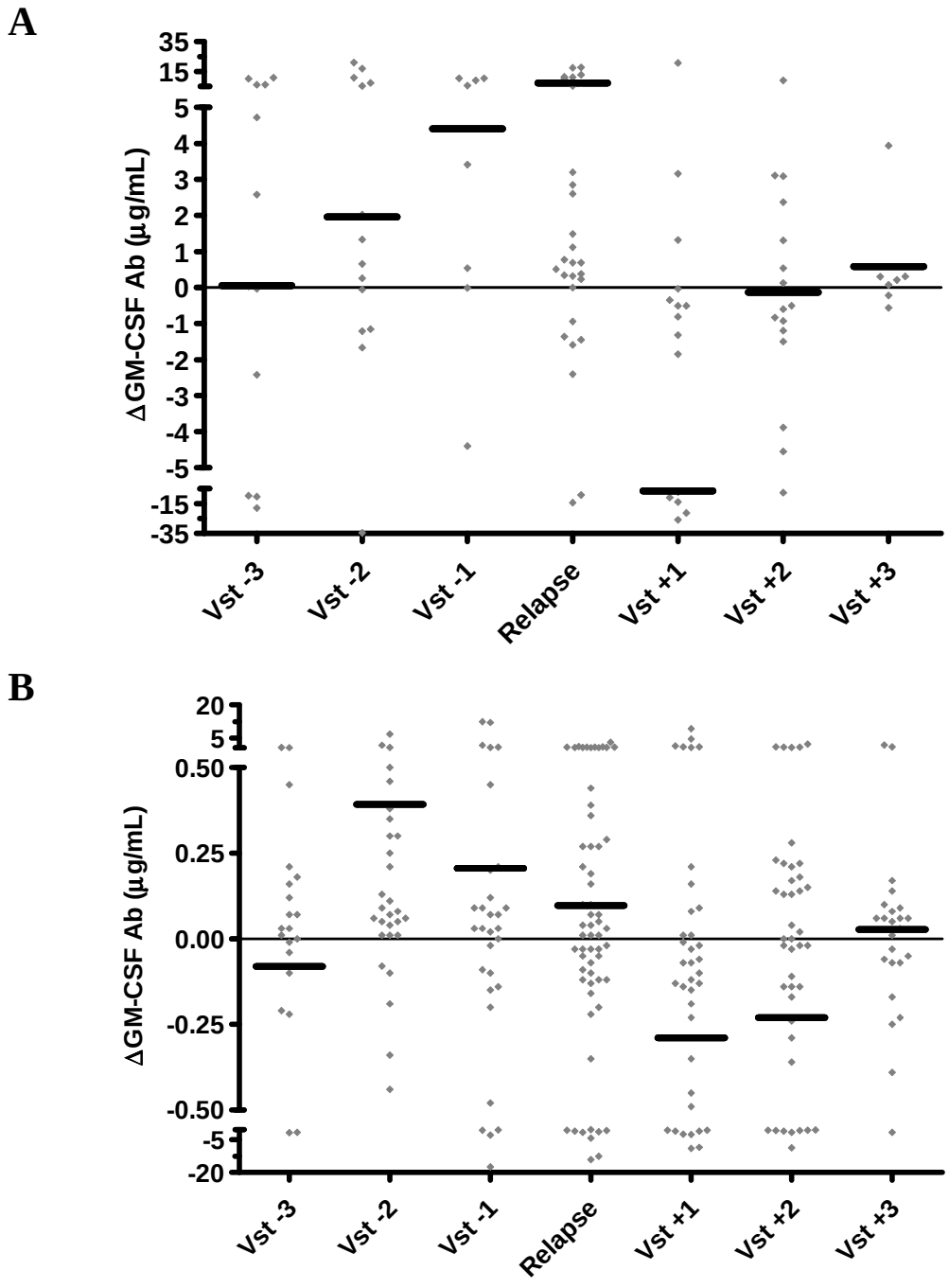

Figure S2. Serum GM-CSF Ab and monitoring of disease activity. Changes in serum levels (ΔGM-CSF Ab) of individual patients with CD **(A)** and UC **(B)** are shown for different time points before, during and after disease relapse in relation to GM-CSF Ab levels at the time of the previous study visit (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 to 3 month before/after relapse).

SUPPLEMENTAL REFERENCES

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