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Towards novel biomarkers and rational nutritional interventions in Inflammatory Bowel Disease

von Martels, Julius Zweder Hubertus

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5

A COMBINED SET OF FOUR SERUM INFLAMMATORY BIOMARKERS RELIABLY PREDICTS ENDOSCOPIC DISEASE ACTIVITY IN INFLAMMATORY BOWEL DISEASE

Submitted

Arno R. Bourgonje*, Julius Z. H. von Martels*, Ruben Y. Gabriëls*, Tjasso Blokzijl, Manon Buist-Homan, Janette Heegsma, B. H. Dianne Jansen, Hendrik M. van Dullemen, Eleonora A. M. Festen, Rinze W. F. ter Steege, Marijn C. Visschedijk, Rinse K. Weersma, Paul de Vos, Klaas Nico Faber#, Gerard Dijkstra#

*Authors contributed equally

Shared last authors

ABSTRACT

BACKGROUND

Mucosal healing is the ultimate treatment goal in inflammatory bowel disease (IBD). Disease activity in IBD is routinely measured with blood C-reactive protein (CRP) and fecal calprotectin levels, but this often does not reflect the degree of inflammation in the intestine as measured by endoscopy. Therefore, novel predictive biomarker(s) are urgently needed.

AIM

The aim of this study was to identify a combination of serum inflammatory biomarkers that are predictive for endoscopic disease activity.

METHODS

Serum concentrations of 10 inflammatory biomarkers were analyzed in 118 IBD patients (64 Crohn's disease (CD), 54 ulcerative colitis (UC)) and 20 healthy controls. Nonparametric ROC estimation with bootstrap inference was used to establish the best combination of inflammatory biomarkers predicting endoscopic disease activity.

RESULTS

Six (6) inflammatory biomarkers (serum amyloid A (SAA), Eotaxin-1, IL-6, IL-8, IL-17A and TNF-α) showed better prediction of IBD disease activity than routine measures (CRP, fecal calprotectin and HBI/SCCAI scores). The best combination of predictive inflammatory biomarkers consisted of serum SAA, IL-6, IL-8 and Eotaxin-1, showing an optimism-adjusted area under the ROC curve of 0.84 (95% CI: 0.73 – 0.94, P < 0.0001), which predicted significantly better (P = 0.002) than serum CRP levels with an AuROC of 0.57 (95% CI: 0.43 – 0.72, P = 0.32).

CONCLUSIONS

The combination of SAA, IL-6, IL-8 and Eotaxin-1 is superior over routine measures in predicting endoscopic disease activity in IBD and might be valuable for monitoring disease activity and management of the disease.

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are chronic idiopathic inflammatory bowel diseases (IBD), characterized by an inappropriate and uncontrolled immune response, stimulated by the gut microbiome in a genetically susceptible host.[1,2] In IBD, the extent of gut mucosal damage is clinically established by endoscopic disease scores, commonly represented by validated measures, such as the Mayo endoscopic subscore for UC and the Simple Endoscopic Score for CD (SES-CD).[3,4] Mucosal healing is the ultimate goal in IBD therapy.[5] Mucosal healing in IBD decreases surgical interventions and hospitalization and improves quality of life and economic participation.[6-9] Until now, the most reliable approach for diagnosing mucosal healing and monitoring IBD disease activity is invasive endoscopic investigation.[10] This procedure, however, has several disadvantages, such as a high patient burden, but also risks of serious complications, like bowel perforation or bleeding. In addition, it is costly and time-consuming. Alternatives for endoscopy are therefore urgently needed.

Non-endoscopic disease indices, such as the Crohn's Disease Activity Index (CDAI) or Harvey Bradshaw Index (HBI) for CD and the Simple Clinical Colitis Activity Index (SCCAI) for UC, fail to correlate well with endoscopically-proven intestinal inflammation.[11-14] Biomarkers for endoscopic disease activity have also been explored and are becoming increasingly important to predict the level of mucosal inflammation in IBD. Fecal calprotectin (FC) and serum C-reactive protein (CRP) levels are now widely used and considered predictive markers for the degree of inflammation, but also show inconsistent correlation with mucosal inflammation when compared to endoscopy.[15-17] This illustrates the need for better diagnostic measures for IBD exacerbations that preferably can be applied to patients with subclinical disease activity.[18]

Cytokines play a pivotal role in the pathogenesis of IBD, controlling intestinal inflammation and disease activity and might be better predictive markers for disease activity than FC and CRP.[19-21] In many diseases, combinations of inflammatory cytokines have been shown to be predictive for inflammatory state and are therewith adequate biomarkers for non-invasive disease activity monitoring.[22] Recently, we showed that for CD a positive correlation exists between multiple Th1- and Th17-associated serum cytokines and fecal calprotectin levels.[23] Although no endoscopic results were available for that patient cohort, it demonstrated the proof of principle and value of Th1- and Th17-associated serum cytokines for measuring inflammation in IBD.

In the present study, we compare several inflammatory biomarkers involved in IBD in qui-

escent and active state of the disease and compared results with outcome of endoscopic evaluation. Correlations between individual biomarkers and endoscopic disease activity were analyzed and used to compose an accurate prediction of endoscopic disease activity, based on a subset of these biomarkers. Finally, we compared the predictive accuracy of this panel of biomarkers with commonly applied measures of disease activity, such as clinical indices (HBI/SCCAI), serum CRP and FC levels.

5

MATERIALS AND METHODS

STUDY POPULATION

This cohort study included patients from the database of the IBD center of the University Medical Center Groningen (UMCG). Serum samples from 118 IBD patients, either CD (*n* = 64) or UC (*n* = 54) were collected. At the moment serum samples were obtained, all patients had an indication for starting new biological therapy. Inclusion criteria for this study were: age ≥ 18 years and an established diagnosis of IBD existing for at least 1 year. Diagnosis was based on clinical, endoscopic and histological criteria.[24] Clinically relevant data were retrieved from the patients' medical records: age, gender, body-mass index (BMI), smoking status, Montreal classification, maintenance medication (mesalamine, thiopurines, methotrexate, TNF-antagonists), previous anti-TNF therapy and surgical history. Clinical disease activity was recorded by scoring the Harvey Bradshaw Index (HBI) for CD and the Simple Clinical Colitis Activity Index (SCCAI) for UC.[25,26]

LABORATORY MEASUREMENTS

Serum samples for measurement of routine diagnostic laboratory parameters, including hemoglobin levels, C-reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR), white blood cell count (WBC) and thrombocyte counts, were obtained simultaneously with the serum samples collected for measurements of detected inflammatory biomarkers. At the same time, fecal calprotectin levels were quantified by enzyme-linked immunosorbent assays (ELISA) (BÜHLMANN Laboratories AG, Switzerland).

ETHICAL CONSIDERATIONS

Serum samples were obtained after patients gave written informed consent (study approved by the Institutional Review Board (IRB) of the UMCG registered as no. 08/338). Serum samples of 20 healthy individuals were included for comparison, which were retrieved from a UMCG biobank (PSI-UMCG [IRB no. 08/279]).

ENDOSCOPIC DISEASE ACTIVITY

Baseline endoscopy investigation was performed in a subset of 71 IBD patients (CD, *n* = 36; UC, *n* = 35) within 3 months prior to serum sample collection. Endoscopic disease activity was graded by certified, independent gastroenterologists from our university hospital, according to the validated Simplified Endoscopic Score for CD (SES-CD) and Mayo endoscopic subscore for UC.[3,4] To calculate the SES-CD, 5 different bowel segments were scored and defined as follows: ileum (excluding the ileocecal valve or ileocolonic anastomosis), ascending colon (including ileocecal valve, cecum and ascending colon until the hepatic flexure), transverse colon (between hepatic and splenic flexures), des-

5

cending colon (from splenic flexure to rectosigmoid junction) and rectum. All 5 segments were evaluated for 4 different endoscopic variables scored from 0 to 3: size of ulcers (none, aphthous ulcers (0.1 to 0.5 cm), large ulcers (0.5 to 2 cm) or very large ulcers (> 2 cm)), ulcerated surface (none, < 10%, 10-30% or > 30%), affected surface (unaffected, < 50%, 50-75% or > 75%) and presence of narrowings (none, single narrowing, multiple narrowings that can be passed or multiple narrowings that cannot be passed). Thus, for each of the 4 variables, the SES-CD score theoretically ranges from 0 to 15, except for the presence of narrowings (when an impervious stenosis is encountered, a '3' is scored, which makes further investigation impossible), that could range from 0 to 11. Therefore, the SES-CD score ranges from 0 to 56 in total. Ultimately, SES-CD scores were defined as previously described: endoscopic remission 0 – 3 points (category 0), mild disease activity 4 – 10 points (category 1), moderate disease activity 11 – 19 points (category 2) and severe disease activity ≥ 20 points (category 3).[27] For UC, the Mayo endoscopic subscore for endoscopic disease activity was obtained from endoscopy reports written by certified gastroenterologists. Here, Mayo 0 was defined as endoscopic remission (normal muco-sa), Mayo 1 as mild disease activity (erythema, decreased vascular pattern, mild friability), Mayo 2 as moderate disease activity (marked erythema, lack of vascular pattern, friability, erosions) and Mayo 3 as severe disease activity (spontaneous bleeding and ulceration). For the purpose of analysis, categories from both endoscopy scores of CD (SES-CD) and UC (Mayo endoscopic subscore) were merged on categorical level of mucosal damage (0 – 3) to finally create an IBD composite endoscopy score. Using this composite score, SES-CD scores < 2 for CD and Mayo endoscopic subscores ≤ 1 for UC were considered diagnostic for mucosal healing.[28]

MEASUREMENT OF INFLAMMATORY BIOMARKERS

A selection of 10 inflammatory biomarkers were measured based on a previously performed study and available literature.[23] In short, serum samples from all subjects were collected and stored in 1 mL aliquots at -80°C. After thawing and prior to analysis, samples were cen-trifuged for 3 minutes at 2,000 g to remove remaining debris. Measurement of serum levels of C-reactive protein (CRP), serum amyloid A (SAA), IFN-γ, TNF-α, IL-6, IL-8, IL-10, IL-17A, Eotaxin-1 and Eotaxin-3 was implemented using a customized electrochemiluminescence (ECL) multiplex assay (Meso Scale Discovery (MSD®), Meso Scale Diagnostics, Rockville, MD). ECL signals were fitted to a 4-parameter logistic model with $1/y^2$ weighting, ensuring a broad and dynamic range of molecule detection. Serum concentrations of all detected molecules were determined by using calibration curves to which the ECL signals were back-fitted. Final concentrations were calculated using the MSD Discovery Workbench ana-lysis software®. Of all detected biomarker concentrations, 94.0 % of values were within the detection range and remaining values (6.0%) were excluded from further analysis.

STATISTICAL ANALYSIS

Baseline demographic and clinical characteristics were presented as means ± standards errors (SEM) or proportions with corresponding percentages (n, %). Serum concentrations of inflammatory biomarkers were presented as median ± interquartile ranges (IQR). Assessment of normality of continuous variables was performed using normal Q-Q plots. Continuous variables were compared using Student's t-tests or Mann-Whitney U-tests according to normality. Categorical variables were compared using chi-square tests or Fisher's exact test, as appropriate. All consecutive analyses were performed in the subset of 71 IBD patients with available endoscopic results within 3 months prior to serum analysis. Simple correlations between inflammatory biomarkers and measures of disease activity were established using the nonparametric Spearman's correlation coefficient (ρ). To evaluate predictive performance of all detected inflammatory biomarkers regarding composite IBD endoscopic disease activity, receiver operating characteristics (ROC) curves were established with associated areas under the ROC curve (AuROCs) as overall measure of fit. ROC curves and associated AuROCs were established using the non-parametric, tie-corrected trapezoidal approximation method. Two correlated areas under the ROC curve were compared with each other using a non-parametric approach based on properties from generalized U-statistics to estimate a covariance matrix.[29] Optimal thresholds for the most promising serum inflammatory biomarkers (serum amyloid A (SAA), Eotaxin-1, IL-6, IL-8, IL-17A and TNF-α) were determined by equally maximizing sensitivity and specificity to compute the Youden's index (J statistic). Optimal thresholds or cut-off points (c) were established by selecting the highest Youden's index, defined as $J = \max_c$ {sensitivity(c) + specificity(c) - 1}. Combinations of classifiers were empirically tested for their predictive performance using a nonparametric ROC estimation of combined predicted probabilities (derived from multivariable logistic regression) with bootstrap inference. Data were analyzed using SPSS Statistics 23.0 software package (SPSS Inc., Chicago, ILL, USA) and STATA software (version 15.0, Stata Corp, College Station, Texas, USA; commands used: 'roctab', 'roccomp' and 'rocreg') and visualized using GraphPad Prism version 6.0 (La Jolla, CA, USA). In case of multiple testing, Bonferroni corrections were applied. Two-tailed P-values ≤ 0.05 were considered as statistically significant.

INTERNAL VALIDATION

Because all biomarker performances were tested on the same dataset, AuROCs and Youden's indices as overall measures of predictive performance could potentially be overestimated due to the correlated nature of the data. To adjust for this potential bias, a bootstrap resampling procedure using 20,000 replicates was performed as internal validation and to obtain standard errors (SE) and confidence intervals (CI) for the AuROCs of best biomarker combinations.

RESULTS

(1) STUDY COHORT CHARACTERISTICS

Baseline demographic and clinical characteristics of the total study population (n = 138) are presented in **Table 1**. The IBD study cohort consisted of 118 patients, of which 64 patients with CD and 54 patients with UC. For comparison, 20 healthy individuals (healthy controls, HC) were included in the study. IBD patients had a significantly lower mean age (CD: 43.8 ± 1.8 years; UC: 47.0 ± 2.0 years) as compared to healthy controls (56.1 ± 2.2 years), while no significant gender differences were observed (CD: 39 females (60.9%); UC: 26 females (48.1%); HC: 12 females (60.0%)). Further differences between CD and UC patients were largely related to disease-specific characteristics (**Table 1**).

For all IBD patients, different measures of disease activity were recorded and compared between CD and UC patients (**Table 2**). As clinical disease activity index, the Harvey Bradshaw Index (HBI) was calculated for CD patients, whereas the Simple Clinical Colitis Activity Index (SCCAI) was recorded for UC patients. Median HBI score was 8 points (IQR: 6 – 11) and median SCCAI score was 6 points (IQR: 4 – 8). Serum CRP (mg/l) levels and ESR (mm/h) were significantly higher in CD patients as compared to UC patients, whereas the latter group showed significantly higher levels of fecal calprotectin (FC, µg/g). Considering endoscopic disease activity, more CD patients fell into either remission (0 – 3 points) or mild (4 – 10 points) disease categories (CD: 47.2%; UC: 20.0%), whereas the majority of UC patients belonged to moderate (11 – 19 points) and severe (≥ 20 points) disease categories (UC: 80.0%; CD: 52.8%).

TABLE 1. Baseline demographic and clinical characteristics of the study population (n = 138) with comparisons drawn between CD patients (n = 64), UC patients (n = 54) and healthy controls (HC) (n = 20). Data are presented as numbers (proportions, n (%)) or mean ± SEM.

Variable	CD	UC	HC	*P*-value
	n = 64	n = 54	n = 20	
Age (years)	43.8 ± 1.8	47.0 ± 2.0	56.1 ± 2.2	**0.004**'
Female sex	39 (60.9)	26 (48.1)	12 (60.0)	0.348
BMI (kg/m²)	24.0 ± 0.6	26.1 ± 0.8	-	**0.034**
Active smoking	19 (32.8)	3 (6.7)	-	**0.005**
Medication	35 (54.7)	25 (46.3)	-	0.461
Mesalamine	4 (6.3)	15 (27.8)	-	
Thiopurines/MTX	29 (45.3)	7 (13.0)	-	
Combination therapy	2 (3.1)	5 (9.3)	-	
Prior anti-TNF (no.)	0.9 ± 0.1	0.8 ± 0.1	-	0.364
Prior IBD surgery (yes)	32 (50.0)	4 (7.4)	-	**< 0.001**
Disease location CD (Montreal)				
L1 (ileal)	15 (24.2)	-	-	

TABLE 1 continued.

Variable	CD	UC	HC	P-value
	n = 64	*n = 54*	*n = 20*	
Disease location CD (Montreal)				
L2 (colonic)	7 (11.3)	-	-	
L3 (ileocolonic)	40 (64.5)	-	-	
Disease location UC (Montreal)				
L1 (proctitis)	-	1 (1.9)	-	
L2 (left-sided colitis)	-	18 (34.6)	-	
L3 (pancolitis)	-	33 (63.5)	-	
Disease behavior CD (Montreal)				
B1 (non-penetrating, non-stricturing)	22 (36.1)	-	-	
B2 (stricturing)	22 (36.1)	-	-	
B3 (penetrating)	17 (27.9)	-	-	
Disease severity UC (Montreal)				
S1 (mild)	-	6 (11.5)	-	
S2 (moderate)	-	28 (53.8)	-	
S3 (severe)	-	18 (34.6)	-	

Differences between two groups were tested using Student's *t*-tests or Mann-Whitney *U*-tests (depending on normality) in case of continuous variables and Fisher's exact tests for categorical variables, as appropriate. ˈOverall *P*-value of one-way analysis of variance (ANOVA) test. *P*-values < 0.05 were considered statistically significant.

TABLE 2. Baseline clinical, biochemical and endoscopic disease activity measures for patients with either CD or UC. Data are presented as numbers (proportions, *n* (%)) and median (IQR), as appropriate.

Variable	CD	UC	P-value
Clinical			
HBI or SCCAI	8 (6 – 11)	6 (4 – 8)	
Biochemical			
Hemoglobin (mmol/l)	7.6 ± 0.1	7.6 ± 0.2	0.768
CRP (mg/l)	11.6 ± 1.8	4.6 ± 0.7	**0.001**
ESR (mm/h)	30.7 ± 3.3	20.5 ± 2.7	**0.021**
WBC (x10^9/l)	8.1 ± 0.5	7.9 ± 0.5	0.822
Thrombocytes (x10^9/l)	326 ± 14	283 ± 9	0.114
Fecal calprotectin (µg/g)	904 ± 140	1,824 ± 239	**0.004**
Endoscopic			
	SES-CD (*n* = 36)	Mayo (*n* = 35)	
0 (Remission)	4 (11.1)	0 (0.0)	
1 (Mild disease)	13 (36.1)	7 (20.0)	
2 (Moderate disease)	13 (36.1)	9 (25.7)	
3 (Severe disease)	6 (16.7)	19 (54.3)	
Composite IBD endoscopy score (*n* = 71)			
0 (Remission)	4 (5.6)		
1 (Mild disease)	20 (28.2)		
2 (Moderate disease)	22 (31.0)		
3 (Severe disease)	25 (35.2)		

Differences between groups were tested using Mann-Whitney U-tests in case of continuous variables and Fisher's exact tests for categorical variables. *P*-values < 0.05 were considered statistically significant.

(2) ANALYSIS OF 10 INFLAMMATORY BIOMARKERS IN 118 IBD PATIENTS AND 20 HEALTHY CONTROLS

Serum concentrations of 10 selected serum inflammatory biomarkers in IBD patients and healthy controls are presented in **Table 3**. In CD patients, four (4) out of 10 inflammatory biomarkers (CRP, SAA, IL-6 and IL-17A) showed significantly increased concentrations as compared to healthy controls (HC). Also, four (4) out of 10 biomarkers were significantly increased in UC compared to HC (SAA, IL-8, IL-10 and IL-17A), where SAA and IL-17A overlapped with CD. In addition, the levels of 6 inflammatory biomarkers were significantly different between CD and UC patients: levels of CRP, IFN-γ and IL-6 were significantly higher in CD, while IL-8, IL-10 and Eotaxin-1 levels were significantly higher in UC (**Figure 1**). No significant differences were observed for serum levels of TNF-α and Eotaxin-3 between CD, UC and HC.

TABLE 3. Median (IQR) of baseline serum concentrations of all detected molecules in CD (n = 64) and UC (n = 54) patients as compared to healthy controls (HC) (n = 20). Data are presented as median (IQR).

Detected molecules	CD	UC	HC	*P*-value
CRP (mg/l)	8.17 (2.42 – 17.3)	3.37 (0.86 – 9.48)	1.11 (0.71 – 3.08)	**< 0.001**
SAA (mg/l)	6.53 (3.31 – 14.5)	8.75 (2.85 – 40.9)	3.41 (1.67 – 5.12)	**0.005**
IFN-γ (pg/ml)	8.68 (5.03 – 16.1)	5.29 (3.67 – 8.04)	6.23 (5.03 – 8.40)	**0.007**
TNF-α (pg/ml)	2.15 (1.71 – 2.84)	2.29 (1.42 – 3.39)	2.12 (1.81 – 2.47)	0.578
IL-6 (pg/ml)	0.91 (0.69 – 1.92)	0.72 (0.40 – 1.46)	0.49 (0.38 – 0.62)	**< 0.001**
IL-8 (pg/ml)	6.16 (4.62 – 9.36)	8.42 (5.51 – 13.0)	5.47 (4.61 – 6.50)	**0.005**
IL-10 (pg/ml)	0.41 (0.28 – 0.51)	0.61 (0.34 – 1.58)	0.31 (0.22 – 0.41)	**0.004**
IL-17A (pg/ml)	2.30 (1.24 – 3.26)	2.76 (1.94 – 5.07)	1.04 (0.94 – 1.36)	**< 0.001**
Eotaxin-1 (ng/ml)	0.20 (0.16 – 0.29)	0.28 (0.20 – 0.36)	0.28 (0.23 – 0.33)	**0.018**
Eotaxin-3 (pg/ml)	17.0 (12.4 – 23.6)	19.2 (14.5 – 22.6)	19.6 (13.3 – 29.6)	0.454

Differences between groups were tested using Kruskal-Wallis tests. *P*-values < 0.05 were considered statistically significant (Bonferroni-adjusted).
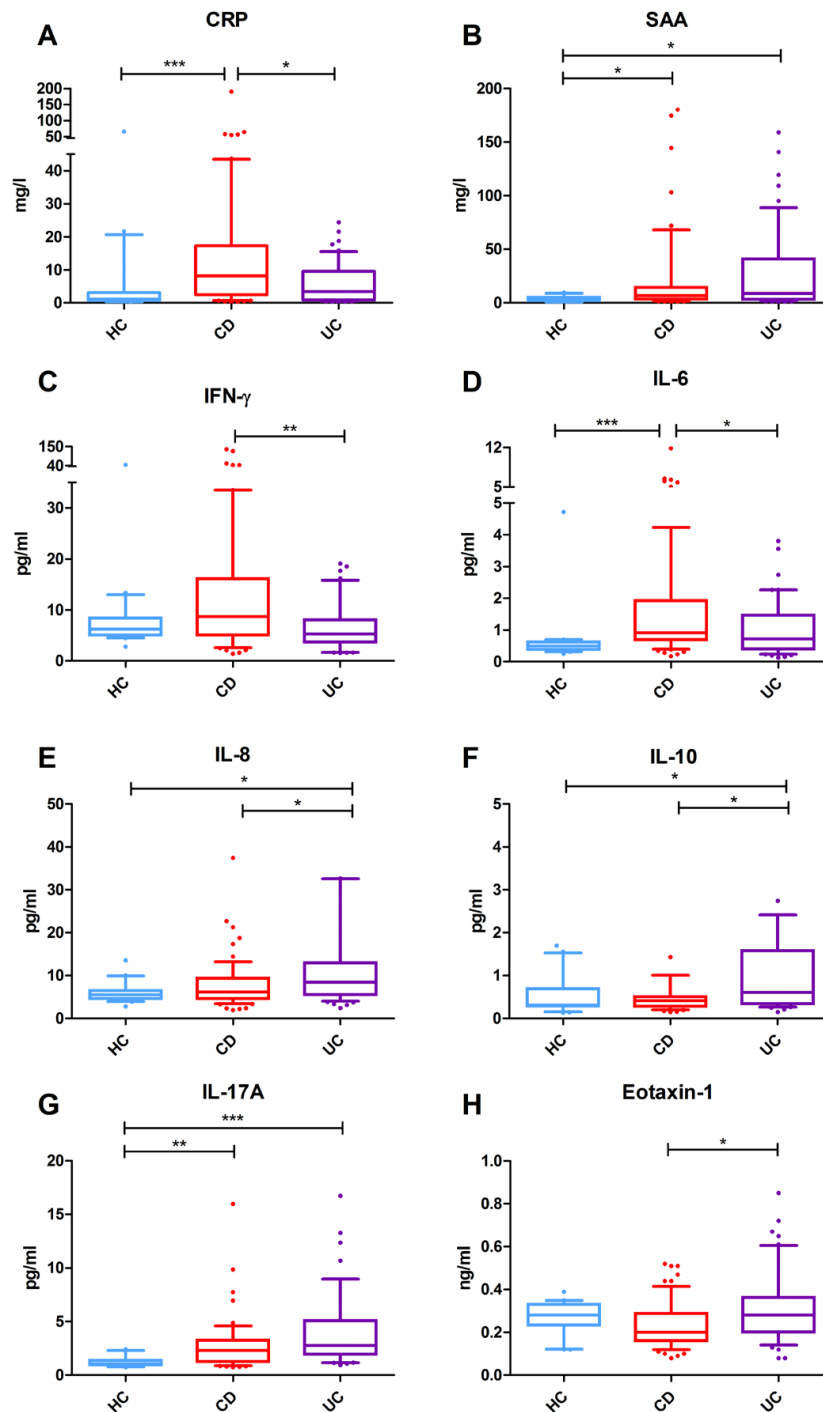
FIGURE 1 (A-H). Serum levels of selected inflammatory biomarkers in Crohn's disease (CD) (*n* = 64) and ulcerative colitis (UC) (*n* = 54) patients and healthy controls (HC) (*n* = 20). **(A)** Serum CRP levels (mg/l) are significantly increased in CD as compared to UC and healthy controls. **(B)** Serum SAA levels (mg/l) are significantly increased in IBD as compared to healthy controls. **(C)** Serum IFN-γ levels (pg/ml) are significantly more elevated in CD as in UC. **(D)** Serum IL-6 levels (pg/ml) are significantly increased in CD as compared to UC and healthy controls. **(E)** Serum IL-8 levels (pg/ml) are specifically more elevated in UC as compared to CD and HC. **(F)** Serum IL-10 levels (pg/ml) are also significantly increased in UC as compared to CD or HC. **(G)** Serum IL-17A levels (pg/ml) are strongly

significantly increased in both CD and UC as compared to HC. **(H)** Serum Eotaxin-1 levels (ng/ml) are significantly elevated in UC as compared to CD, but comparable with that of HC. $^{*}P$-values < 0.05 were considered statistically significant. $^{**}P$ < 0.01. $^{***}P$ < 0.001.

(3) CORRELATIONS OF INFLAMMATORY BIOMARKERS WITH ENDOSCOPIC DISEASE ACTIVITY IN IBD

Endoscopic examination of 71 (CD: n = 36 and UC: n = 35) of the 118 IBD patients was available and this subgroup was used to analyze correlations between the individual serum biomarkers and clinical (HBI/SCCAI), biochemical (CRP, fecal calprotectin) and endoscopic (CD: SES-CD score, UC: Mayo score, IBD: composite endoscopy score) measures of disease activity using Spearman's rank correlation coefficients (ρ). The data are presented in a correlation matrix (**Table 4**). The SES-CD score positively correlated with serum amyloid A (SAA) (ρ = 0.410, P < 0.05), closely followed by IFN-γ (ρ = 0.383, P < 0.05), IL-8 (ρ = 0.359, P < 0.05) and IL-17A (ρ = 0.352, P < 0.05), while the Mayo endoscopic subscore (for UC) correlated only significantly with serum levels of IL-6 (ρ = 0.356, P < 0.05). An IBD composite endoscopy score was created by merging both endoscopy scores of CD (SES-CD) and UC (Mayo) on categorical level of disease activity (0, 1, 2 or 3). Using this composite IBD endoscopy score (see **Table 2**; n = 71), significant correlations were observed for Eotaxin-1 (ρ = 0.316, P < 0.01), IL-8 (ρ = 0.295, P < 0.05) and SAA (ρ = 0.288, P < 0.05) (**Figure 2**). Furthermore, routinely-measured CRP levels (mg/l) correlated significantly with multiple biomarkers analyzed by the ECL multiplex assay (CRP, SAA, IL-6, IFN-γ and TNF-α). In contrast, fecal calprotectin (FC) levels did not show significant correlations with any of the detected inflammatory biomarkers. Similarly, clinical disease indices only showed a significant correlation with serum IL-6 levels (ρ = 0.349, P < 0.01), whereas the remaining inflammatory biomarkers did not correlate with either HBI or SCCAI scores.

TABLE 4. Correlations between serum levels of individual biomarkers with endoscopic (SES-CD, Mayo score and composite IBD endoscopy score), biochemical (CRP and fecal calprotectin, FC) and clinical (HBI or SCCAI) measures of disease activity.

	SES-CD	Mayo	Composite	HBI/SCCAI	CRP	FC
CRP (mg/l)	0.155	-0.053	-0.067	0.101	**0.871****	0.058
SAA (mg/l)	**0.410***	0.208	**0.288***	0.006	**0.605****	0.111
IFN-γ (pg/ml)	**0.383***	0.119	0.048	0.034	**0.325****	-0.129
TNF-α (pg/ml)	0.021	0.183	0.175	-0.048	**0.298****	-0.079
IL-6 (pg/ml)	0.164	**0.356***	0.129	**0.349****	**0.450****	0.104
IL-8 (pg/ml)	**0.359***	0.118	**0.295***	-0.076	0.002	0.021

TABLE 4 continued.

	SES-CD	Mayo	Composite	HBI/SCCAI	CRP	FC
IL-10 (pg/ml)	0.097	-0.023	0.127	0.172	-0.020	0.310
IL-17A (pg/ml)	**0.352**[*]	-0.073	0.202	-0.125	0.185	0.065
Eotaxin-1 (ng/ml)	0.212	0.144	**0.316**[**]	0.060	-0.121	0.129
Eotaxin-3 (pg/ml)	-0.205	-0.217	-0,110	-0.059	-0.098	0.076

Correlation matrix showing Spearman's rank correlation coefficients (ρ) for associations between all detected molecules and clinical, biochemical and endoscopic measures of disease activity. [*]*P*-values < 0.05 were considered statistically significant. [**]*P* < 0.01.

(4) PREDICTING ENDOSCOPIC DISEASE ACTIVITY USING INFLAMMATORY BIO-MARKERS

To test the predictive performances of selected inflammatory biomarkers, distributions of serum concentrations of all biomarkers were compared between IBD patients with binary categorized, composite IBD endoscopic disease activity: remission (0) or mild (1) endoscopic disease activity vs. moderate (2) or severe (3) endoscopic disease activity (**Table 5**). Subsequently, subgroup analyses were performed for CD and UC patients separately, which can be found in the supporting information (**Supplementary Table S1** and **Supplementary Figures S1-S4**).

TABLE 5. Distributions of serum concentrations of all detected molecules among binary categorized endoscopic disease activity (remission or mild disease (0-1) vs. moderate or severe disease (2-3)) using a composite IBD endoscopy score (CD: SES-CD, UC: Mayo score). Data are presented as median (IQR).

Detected molecules	Remission or mild disease (0-1)	Moderate or severe disease (2-3)	*P*-value
Composite Endoscopy Score	*n* = 24	*n* = 47	
CRP (mg/l)	3.95 (0.64 – 11.5)	5.82 (1.57 – 16.5)	0.189
SAA (mg/l)	3.93 (2.28 – 9.52)	13.6 (4.73 – 52.1)	**0.002**
IFN-γ (pg/ml)	6.33 (2.89 – 8.99)	8.29 (4.41 – 14.5)	0.118
TNF-α (pg/ml)	2.00 (1.24 – 2.62)	2.30 (1.87 – 3.05)	**0.039**
IL-6 (pg/ml)	0.67 (0.30 – 1.41)	0.96 (0.65 – 2.16)	**0.025**
IL-8 (pg/ml)	5.39 (3.58 – 8.06)	8.47 (5.46 – 11.7)	**0.005**
IL-10 (pg/ml)	0.40 (0.25 – 0.54)	0.48 (0.32 – 1.36)	0.103
IL-17A (pg/ml)	1.46 (1.05 – 2.75)	2.90 (1.69 – 4.34)	**0.005**
Eotaxin-1 (ng/ml)	0.18 (0.14 – 0.24)	0.27 (0.20 – 0.36)	**0.001**
Eotaxin-3 (pg/ml)	19.2 (14.1 – 26.9)	18.4 (11.4 – 22.8)	0.313

Differences between groups were tested using Mann-Whitney U-tests. *P*-values < 0.05 were considered statistically significant (Bonferroni-adjusted).
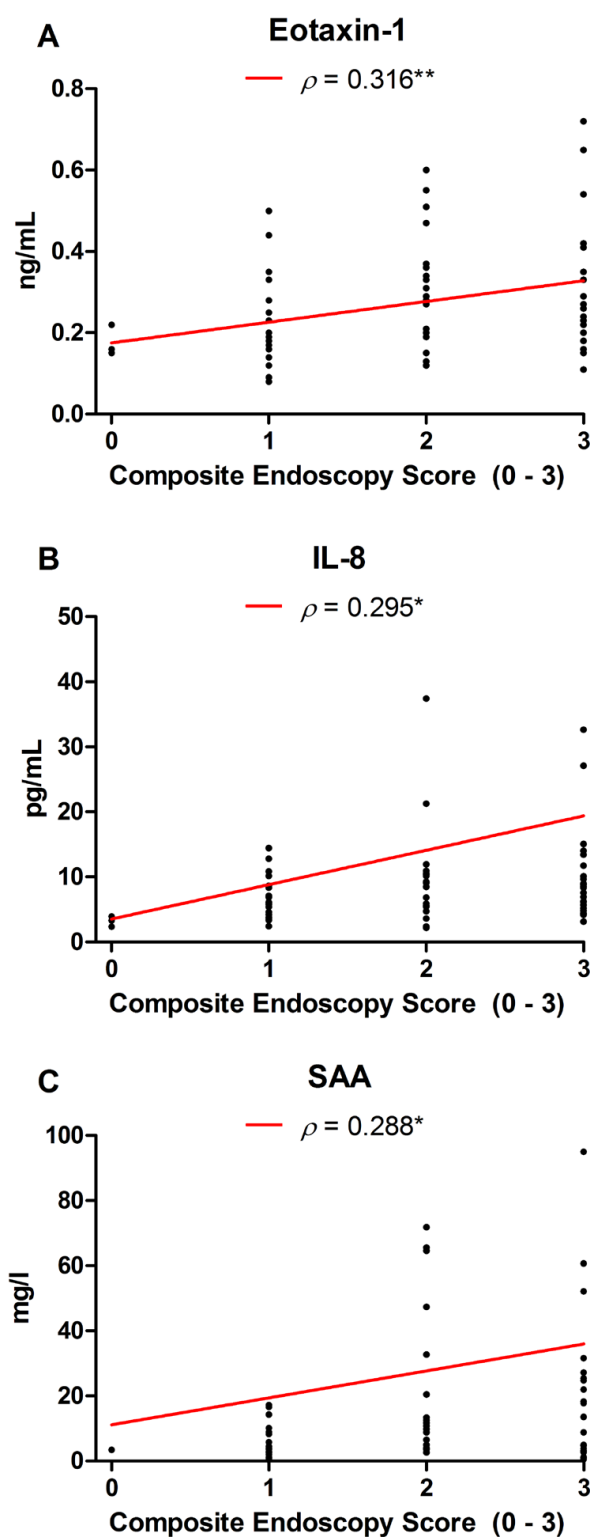
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FIGURE 2 (A-C). Serum levels of cytokines **(A)** Eotaxin-1, **(B)** IL-8 and **(C)** the acute-phase protein serum amyloid A (SAA) significantly correlate with the endoscopic disease activity as represented by the composite IBD endoscopy score (categories ranging from 0-3 are shown on x-axis). Correlations were established using Spearman's rank correlation coefficient (ρ). $^{*}P < 0.05$. $^{**}P < 0.01$.

Using the composite IBD endoscopy score, patients with high endoscopic disease activity (either moderate (2) or severe (3)) demonstrated significantly elevated serum concentrations of Eotaxin-1, SAA, TNF-α, IL-6, IL-8 and IL-17A as compared to patients with low endoscopic disease activity (either remission (0) or mild (1)) (**Figure 3**). In the CD subgroup, using the binary ordered SES-CD, significantly increased concentrations of SAA, IFN-γ, IL-6 and IL-17A were observed in patients with high endoscopic disease activity (**Supplementary Table S1; Supplementary Figure S1**). In UC, using the binary Mayo endoscopic subscore categories, serum concentrations of IL-6, TNF-α and Eotaxin-1 were significantly increased in moderate-to-severe disease activity as compared to remission or mild disease activity (**Supplementary Table S1; Supplementary Figure S3**).

To evaluate their predictive accuracies with respect to endoscopically active disease, receiver operating characteristics (ROC) curves were established (**Figure 4**). In the ROC analysis, serum levels of Eotaxin-1 (pg/ml) and SAA (mg/l) presented the best discriminative capacity regarding binary ordered, composite IBD endoscopic disease activity (area under the receiver operating characteristics curve (AuROC) 0.75 (SE: 0.06, 95% CI: 0.62 – 0.87, $P < 0.001$) for both serum Eotaxin-1 and SAA levels) (**Table 6**). Serum levels of IL-17A, IL-8, IL-6 and TNF-α were of subordinate, but still reasonable discriminative value.

TABLE 6. ROC analysis showing discriminative power of individual inflammatory biomarkers that are significantly increased in IBD patients with moderate (2) or severe (3) endoscopic disease activity as compared to patients with remission (0) or mild (1) disease activity, as determined by the binary categorized, composite IBD endoscopy score (CD: SES-CD, UC: Mayo endoscopic subscore).

	AuROC (95% CI)	Sensitivity / Specificity	Cut-off value	Youden's *J* statistic
Inflammatory biomarkers				
Eotaxin-1 (ng/ml)	0.75 (0.62 – 0.87)	74.5% / 66.7%	> 0.21 ng/ml	0.41***
SAA (mg/l)	0.75 (0.62 – 0.87)	48.8% / 95.2%	> 17.5 mg/l	0.44**
TNF-α (pg/ml)	0.65 (0.52 – 0.78)	38.3% / 87.5%	> 2.88 pg/ml	0.26*
IL-6 (pg/ml)	0.67 (0.53 – 0.81)	55.3% / 72.7%	> 0.91 pg/ml	0.28*
IL-8 (pg/ml)	0.70 (0.58 – 0.83)	68.1% / 66.7%	> 6.12 pg/ml	0.35**
IL-17A (pg/ml)	0.72 (0.57 – 0.86)	66.7% / 68.2%	> 2.40 pg/ml	0.35**
Routine measures				
CRP (mg/l)	0.57 (0.43 – 0.72)	51.1% / 66.7%	> 5.73 mg/l	0.18
FC (µg/g)	0.64 (0.44 – 0.85)	77.3% / 54.6%	> 735 µg/g	0.32
HBI/SCCAI	0.66 (0.49 – 0.83)	62.9% / 64.3%	> 6.5 points	0.27

*P-values were calculated for the difference between the area under the ROC curve and the no-discrimination line (AuROC = 0.50). *P-values < 0.05 were considered statistically significant. **P < 0.01. ***P < 0.001.
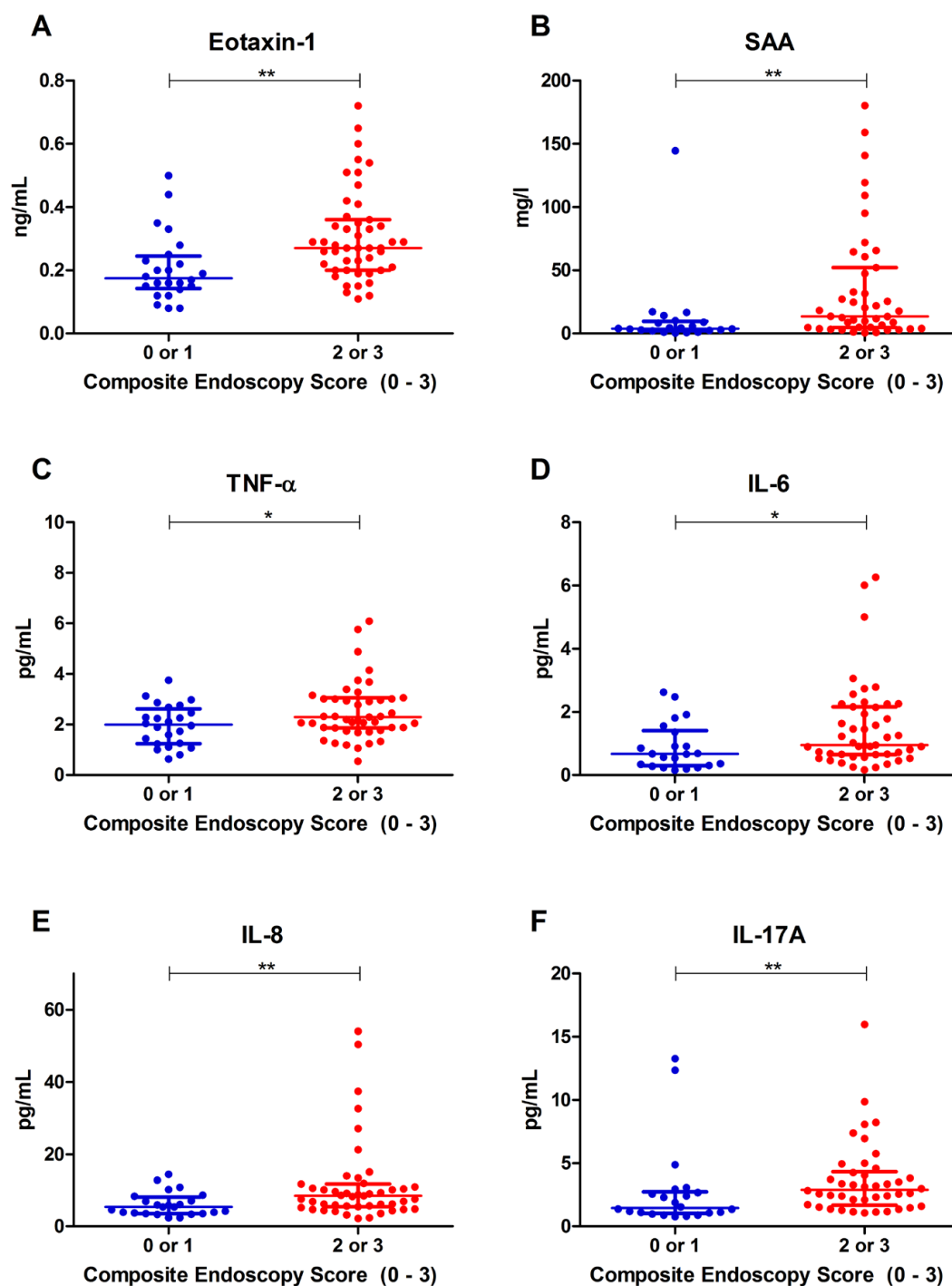
FIGURE 3 (A-F). Distributions of serum concentrations of **(A)** Eotaxin-1, **(B)** serum amyloid A (SAA), **(C)** tumor necrosis factor alpha (TNF-α), **(D)** interleukin-6 (IL-6), **(E)** interleukin-8 (IL-8) and **(F)** interleukin-17A (IL-17A), that were significantly different among binary ordered endoscopic disease activity, using a composite IBD endoscopy score (0 or 1 indicating remission or mild disease and 2 or 3 indicating moderate or severe disease, respectively). *$P < 0.05$. **$P < 0.01$.
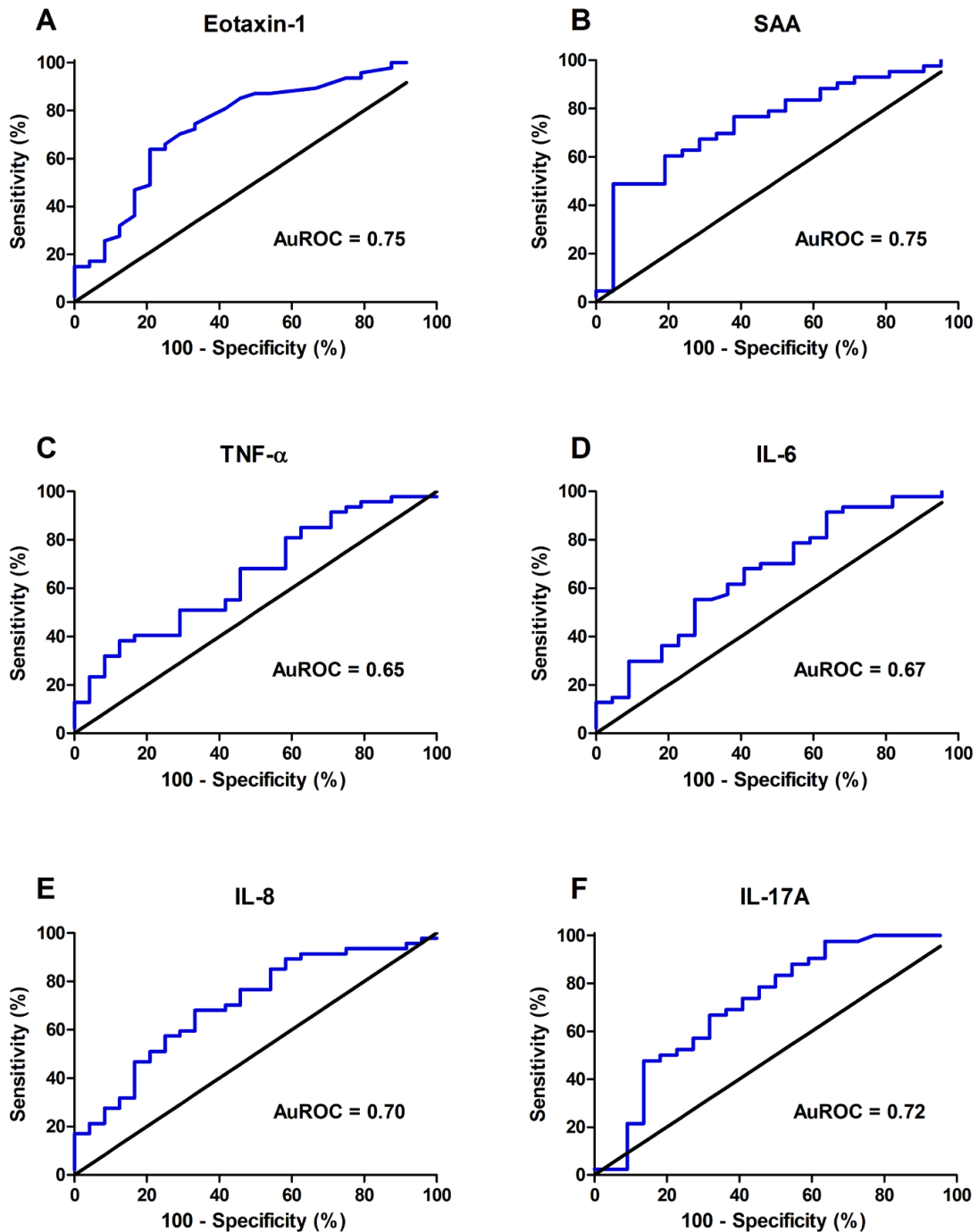
FIGURE 4 (A-F). Discriminative capacity of serum concentrations of **(A)** Eotaxin-1, **(B)** serum amyloid A (SAA), **(C)** tumor necrosis factor alpha (TNF-α), **(D)** interleukin-6 (IL-6), **(E)** interleukin-8 (IL-8), **(F)** interleukin-17A (IL-17A) regarding binary ordered endoscopic disease activity (remission (0) or mild (1) disease vs. moderate (2) or severe (3) disease), as represented by the area under the receiver operating characteristics curve (AuROC). Of all individual molecules shown, Eotaxin-1 and SAA display the best discriminative capacity regarding binary ordered endoscopic disease activity.

(5) BEST COMBINATIONS OF INFLAMMATORY BIOMARKERS TO PREDICT ENDO-SCOPIC DISEASE ACTIVITY

To achieve the best discrimination between remission (0) or mild (1) vs. moderate (2) or severe (3) endoscopic disease activity, multiple combinations of detected inflammatory biomarkers were empirically investigated for their predictive accuracy. Ultimately, for the composite IBD endoscopy score, the best predictive combination of inflammatory biomarkers was represented by the assembly of serum levels of SAA, IL-6, IL-8 and Eotaxin-1, showing an AuROC of 0.84 (SE: 0.05, 95% CI: 0.73 − 0.94, P < 0.0001, n = 64) (**Figure 5A**). In this combination, SAA could be replaced by serum CRP levels without losing predictive accuracy (correlation between CRP and SAA: ρ = 0.663, P < 0.01) (**Supplementary Figure S5**). Applying the algorithm for comparison of correlated ROC curves, the AuROC for this combination of biomarkers was significantly better as compared to that of serum CRP levels (P = 0.002), whereas no statistical significance emerged when compared to fecal calprotectin levels or the clinical disease scores (HBI/SCCAI) (P = 0.313 and P = 0.073, respectively). Serum CRP levels had an AuROC of 0.57 (SE: 0.07, 95% CI: 0.43 − 0.72, P = 0.32), fecal calprotectin (FC) levels 0.64 (SE: 0.10, 95% CI: 0.44 − 0.85, P = 0.18) and HBI or SCCAI scores 0.66 (SE: 0.09, 95% CI: 0.49 − 0.83, P = 0.08) (**Figure 5B-D**). The resulting combined calculated probability had a maximum sensitivity of 90.7% and specificity of 68.4% in correctly discriminating IBD patients with low or high endoscopic disease activity (Youden's J statistic = 0.58).

In the CD subgroup, regarding the predictive value for SES-CD scored endoscopic disease activity, serum levels of SAA presented the best discriminative capacity as represented by an AuROC of 0.79 (SE: 0.09, 95% CI: 0.61 − 0.96 , P < 0.01) (**Supplementary Figure S6**). In the UC subgroup, the combination of IL-6 and Eotaxin-1 demonstrated the best predictive performance (AuROC 0.97, SE: 0.03, 95% CI: 0.92 − 1.02, P < 0.001) (**Supplementary Figure S7**). Detailed subgroup analyses for both CD and UC cohorts are described in the supplementary data (**Supplementary Figure S6 and S7**).
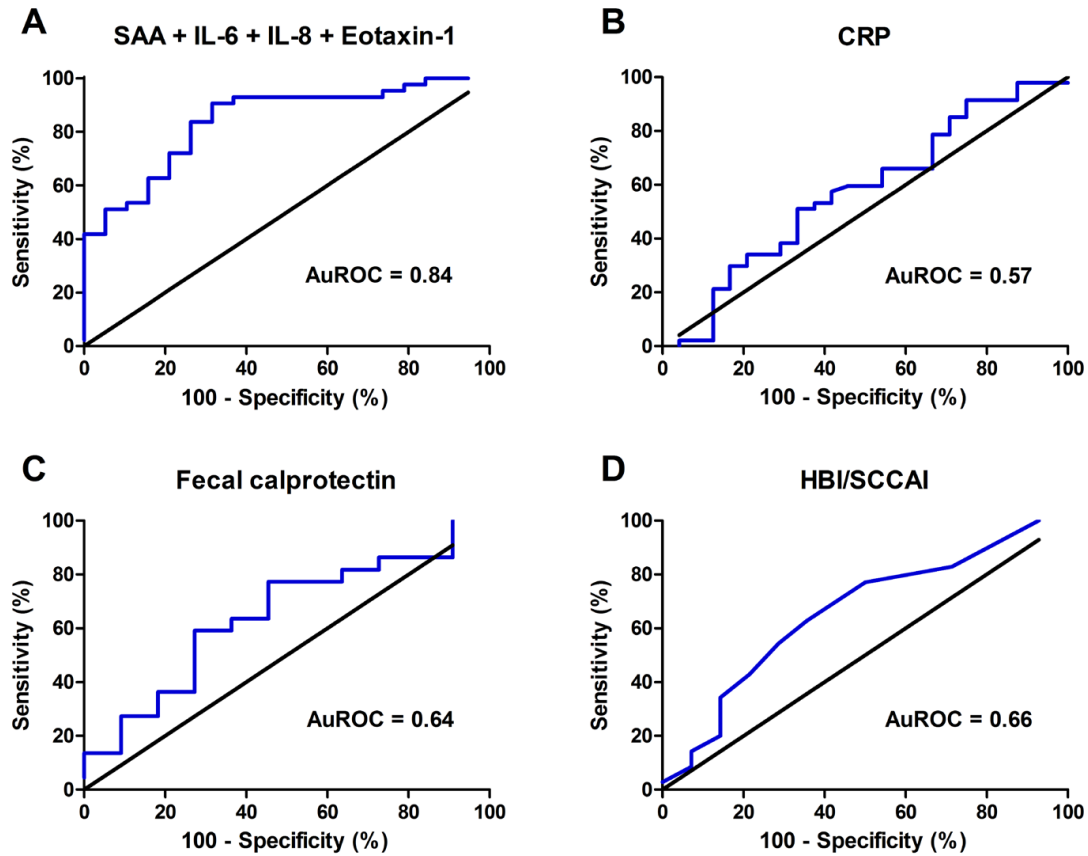
FIGURE 5 (A-D). Areas under the receiver operating characteristics curve (AuROC) for **(A)** the best predictive combination of biomarkers (serum amyloid A (SAA), interleukin-6 (IL-6), interleukin-8 (IL-8) and Eotaxin-1) (n = 64), **(B)** serum C-reactive protein (CRP) levels (mg/l), **(C)** fecal calprotectin (FC) levels (µg/g) and **(D)** Harvey Bradshaw Index (HBI) or Simple Clinical Colitis Activity Index (SCCAI).

DISCUSSION

In this study, we demonstrate that serum Eotaxin-1, SAA, IL-6, IL-8, IL-17A and TNF-α are better predictors of endoscopic disease activity in IBD than the routinely applied serum CRP, fecal calprotectin levels and HBI or SCCAI scores. A combined panel of Eotaxin-1, SAA, IL-6 and IL-8 showed the best prediction of the actual mucosal status in IBD with a sensitivity of 90.7% and specificity of 68.4%. Furthermore, only a few patients were misclassified as having high endoscopic disease activity, yielding a positive predictive value of 86.7%. The combination of these four inflammatory biomarkers demonstrated superiority in predicting endoscopic disease activity in IBD, compared to routinely applied measures of disease activity (i.e. serum CRP, fecal calprotectin levels and clinical disease indices (HBI/SCCAI)).

All biomarkers that were found to be predictive for endoscopically confirmed disease activity are involved in the pathogenesis of IBD. Eotaxin-1 (CCL11) is a selective chemoattractant and important in the activation and recruitment of eosinophils to the lamina propria of the gut.[30] Eotaxin-1 levels have been shown to be elevated in the serum of patients with (active) IBD.[31-34] In our study, however, we found higher serum Eotaxin-1 concentrations in UC as compared to CD. Remarkably, serum levels were generally reduced in CD as compared to healthy controls, though there was a large variation in Eotaxin-1 levels in this patient group (**Figure 1**). Despite this, we observed a clear positive correlation between inflammatory activity in the composite IBD endoscopy score and serum levels of Eotaxin-1 (**Figure 2**). Moreover, serum Eotaxin-1 showed discriminative value for differentiating IBD patients having either remissive or mild disease from patients with moderate or severe endoscopic disease activity (**Figure 4**). These findings of correlations between serum Eotaxin-1 levels and disease activity corroborate previous observations in human IBD and experimental colitis models that suggested that the eosinophil-selective chemokine Eotaxin-1 associates with disease pathogenesis.[35,36] Eotaxin-1 is produced by intestinal epithelial cells, endothelial cells and macrophages under the influence of several other cytokines that are involved in IBD disease activity, such as IL-17A.[37-40]

Serum amyloid A (SAA) was also predictive for IBD disease activity. SAA is an apolipoprotein of high-density lipoproteins (HDL) and belongs to the family of acute-phase reactants. It is produced by the liver upon enhanced serum levels of pro-inflammatory cytokines, such as TNF-α and IL-6, and is enhanced in several chronic inflammatory diseases.[41,42] Previously, it was demonstrated that circulating IL-6 and SAA are useful indicators of disease activity in IBD.[43] In contrast to the pro-inflammatory nature of most of the studied cytokines, it is unknown whether SAA contributes to inflammation. The positive correla-

tion with disease activity suggests a pro-inflammatory function, but recently it was also shown that SAA may protect the epithelial barrier by stimulating protective and anti-inflammatory IL-22-producing neutrophils.[44] Irrespective of its role in disease development, SAA has been shown to be the most sensitive acute-phase protein in IBD (when compared to other acute phase proteins, such as alpha-1-antichymotrypsin (alpha-1-ACT) and alpha 1-acid glycoprotein (alfa 1-AGP), or even CRP).[45] Therefore, SAA may be of added value as inflammatory biomarker in monitoring the acute-phase reaction, besides CRP.[46]

IL-6 was also part of the selected combination of predictive inflammatory biomarkers. IL-6 is one of the most ubiquitously present and pleiotropic cytokines that is involved in most (chronic) inflammatory diseases, including IBD.[47] IL-6 can change the balance of effector CD4^{+} T-cell subsets. It is produced by innate immune cells, such as macrophages, neutrophils and mast cells, and forms a bridge between the innate and the adaptive immune system. Upon acute inflammatory events, IL-6 is recognized as important stimulator of acute-phase reactant production in the liver, including CRP. In IBD, the importance of IL-6 is highlighted by the fact that serum concentrations rise concurrently with increasing inflammatory disease activity, as well as elevated soluble receptor complexes (sIL-6R/IL-6) that can bind to and activate IL-6R-lacking immune cells (trans signaling), contributing to chronic mucosal inflammation.[48] Pro-inflammatory actions of IL-6 have been demonstrated to predominantly occur via trans signaling, which is strongly associated with the development of and sustained intestinal inflammation in IBD.[49-51] Here, IL-6 levels fairly accurately differentiated between high and low endoscopic disease activity. As a result, serum IL-6 levels made a substantial contribution to the predictive power of the final biomarker combination. IL-8 is known as an important neutrophil chemoattractant, modulating recruitment and degranulation of neutrophils located in the intestinal mucosa.[52] Previously, it was demonstrated that serum IL-8 levels are elevated in active IBD, most prominently in UC, as compared to healthy subjects.[53] In line, we found significantly elevated serum concentrations of IL-8 in UC compared to CD and healthy controls. Therefore, IL-8 is suggested to be a key factor in the process of neutrophil-mediated intestinal inflammation in active UC. Previously, it was shown that mucosal IL-8 levels can predict future disease relapse in patients with quiescent UC.[54] Moreover, serum IL-8 levels present high accuracy in differentiating IBD from irritable bowel syndrome (IBS) patients.[55] In this respect, IL-8 might be particularly helpful in identifying an acute disease exacerbation, irrespective of the often non-specific clinical presentation.

Currently, disease activity in IBD is clinically assessed by evaluating a combination of symptoms (quantified with clinical risk scoring methods), biochemical measures such as serum CRP and fecal calprotectin, and ultimately endoscopic evaluation. However, the clinical scoring methods, such as the Harvey Bradshaw Index (HBI) or the Simple Clinical

Colitis Activity Index (SCCAI) correlate poorly with endoscopic disease activity.[25,26,56-58] Our results are in line with these studies, since only serum IL-6 levels correlated significantly with the clinical disease indices in our cohort. Moreover, associations between serum CRP and fecal calprotectin and endoscopic disease activity in IBD appear inconsistent.[59] Despite this, these parameters are the most frequently-used non-invasive biomarkers analyzed to monitor disease activity in IBD.[15,24,60] However, several studies have shown that one single biomarker is unlikely to accurately predict the mucosal status in IBD, given its complex immunological pathogenesis.[11,12,15,18,61] Mucosal healing is the ultimate goal and measure of therapeutic efficacy in IBD. Additional non-invasive markers are needed to be able to accurately represent mucosal healing.[62,63] Previous studies have developed disease activity indices reflecting mucosal status, based on clinical characteristics and standard laboratory measurements, but did not yet include inflammatory biomarkers as investigated in our study.[64] Incorporation of such inflammatory biomarkers in existing prediction models or disease indices may contribute to establishing an immunology-based prediction model for endoscopic mucosal status in IBD.

An important strength of the present study is the comprehensive analysis of a selected panel of serum inflammatory biomarkers using an electrochemiluminescence (ECL) assay. Using this highly-sensitive, validated detection method of serum inflammatory biomarkers, we were able to establish serum biomarker concentrations with a broad dynamic range of detection. However, biomarker concentrations were not within the detection range in a small number of samples (6.0%) and were excluded from the analyses. In order to determine whether this may skew the interpretation of our results, we performed a full statistical analysis on a dataset where missing values were replaced by the lower limit of detection (LLoD) or higher limit of detection (HLoD) as indicated by the signals obtained in the ECL assay. Importantly, these analyses further confirmed the final prediction model.

In an earlier study, we found correlations between several serum inflammatory cytokines in CD and fecal calprotectin levels, where we observed positive correlations for Th1- and Th17- associated serum cytokines (including CRP, SAA and IL-6) and fecal calprotectin levels.[23] However, that study was limited by a relatively small cohort of CD patients and the absence of endoscopic results, which prevented us from establishing correlations with IBD disease activity. Likewise, the current study has also some limitations. For instance, a larger cohort would have allowed us to predict endoscopic disease activity using the pre-defined categories as outcome parameter with values ranging from 0 to 3. Similarly, a greater sample size would have resulted in more reliable subgroup analyses for CD and UC. Moreover, this would have provided us with the ability to adjust for confounding variables (e.g. medication use, co-morbidity or acute inflammatory events).

Our results demonstrate that a combination of serum inflammatory biomarkers has the potential to identify patients with active IBD and differentiate them from patients with re-mission/quiescent disease in a minimally invasive manner. The panel of four biomarkers described in this study has a high accuracy, and it is important now to externally validate this combined array of biomarkers in another IBD cohort. Moreover, since cytokines play a pivotal role in the immunopathogenesis of IBD, it is interesting to analyze the effect of induction therapy on serum inflammatory status in relation to mucosal healing in IBD. Future studies are warranted that focus on the diagnostic potential of this distinct inflam-matory biomarker profile in predicting response to (biological) therapy in IBD.

In conclusion, the panel of four serum inflammatory biomarkers identified in this study shows a predictive value of endoscopic disease activity in IBD that is much better than current rou-tine laboratory tests. SAA, Eotaxin-1, IL-6, IL-8, IL-17A and TNF-α all individually showed bet-ter predictive performances compared to CRP, fecal calprotectin and HBI/SCCAI scores. The best prediction of luminal disease activity was observed when SAA, IL-6, IL-8 and Eotaxin-1 were combined, which, as a relatively small panel of biomarkers, harbors great potential to improve monitoring of intestinal inflammatory activity and therapeutic efficacy in IBD.

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5

SUPPORTING INFORMATION

## PREDICTING ENDOSCOPIC DISEASE ACTIVITY USING INFLAMMATORY BIOMARK-ERS

CD cohort

In the subgroup analysis of the CD cohort, using the binary ordered Simple Endoscopic Score for CD (SES-CD), patients with high endoscopic disease activity (i.e. moderate or severe) showed significantly increased concentrations of SAA, IFN-γ, IL-6 and IL-17A (**Supplementary Table S1; Supplementary Figure S1**).

SUPPLEMENTARY TABLE S1. Distributions of serum concentrations of all detected molecules among binary categorized endoscopic disease activity (remission or mild disease vs. moderate or severe disease) using the SES-CD for CD and Mayo endoscopic subscore for UC. Data are presented as median (IQR).

Detected molecules	Remission or mild disease (0-1)	Moderate or severe disease (2-3)	*P*-value
SES-CD	*n* = 17	*n* = 19	
CRP (mg/l)	4.04 (1.11 – 13.1)	14.8 (4.86 – 39.3)	0.064
SAA (mg/l)	4.17 (2.28 – 11.2)	17.8 (9.76 – 32.7)	**0.009**
IFN-γ (pg/ml)	7.44 (4.09 – 10.9)	12.0 (8.68 – 23.1)	**0.020**
TNF-α (pg/ml)	2.11 (1.42 – 2.61)	2.15 (1.68 – 2.93)	0.623
IL-6 (pg/ml)	0.69 (0.44 – 1.46)	1.58 (0.81 – 2.79)	**0.034**
IL-8 (pg/ml)	5.38 (3.72 – 6.60)	6.23 (4.72 – 10.4)	0.084
IL-10 (pg/ml)	0.39 (0.24 – 0.44)	0.41 (0.25 – 1.20)	0.525
IL-17A (pg/ml)	1.37 (0.92 – 2.65)	3.06 (1.67 – 3.95)	**0.005**
Eotaxin-1 (ng/ml)	0.18 (0.15 – 0.24)	0.23 (0.18 – 0.29)	0.159
Eotaxin-3 (pg/ml)	16.7 (14.0 – 26.0)	13.8 (9.45 – 23.5)	0.154
Mayo Score	*n* = 7	*n* = 28	
CRP (mg/l)	2.81 (0.54 – 9.65)	3.16 (0.92 – 7.64)	0.680
SAA (mg/l)	2.85 (2.07 – 8.88)	13.1 (3.81 – 58.5)	**0.053**
IFN-γ (pg/ml)	2.27 (1.62 – 6.24)	5.09 (3.79 – 9.81)	0.088
TNF-α (pg/ml)	1.44 (1.07 – 2.68)	2.61 (1.88 – 3.60)	**0.035**
IL-6 (pg/ml)	0.23 (0.18 – 1.09)	0.91 (0.54 – 1.70)	**0.024**
IL-8 (pg/ml)	6.90 (3.33 – 8.66)	9.05 (5.51 – 13.9)	0.070
IL-10 (pg/ml)	0.48 (0.26 – 0.76)	0.52 (0.32 – 1.38)	0.440
IL-17A (pg/ml)	2.15 (1.16 – 12.6)	2.71 (1.73 – 4.78)	0.678
Eotaxin-1 (ng/ml)	0.16 (0.08 – 0.33)	0.30 (0.25 – 0.40)	**0.035**
Eotaxin-3 (pg/ml)	21.3 (14.7 – 36.4)	19.0 (12.6 – 22.6)	0.455

Differences between groups were tested using Mann-Whitney U-tests. *P*-values < 0.05 were considered statistically significant.
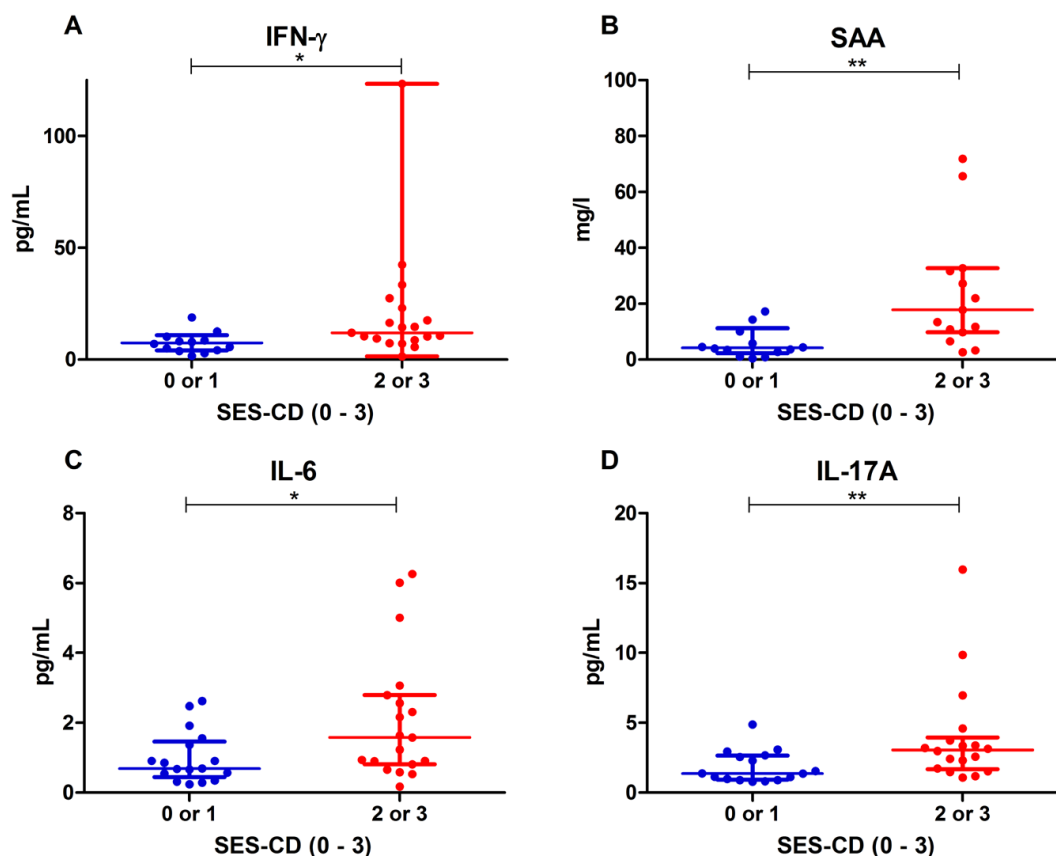
SUPPLEMENTARY FIGURE S1 (A-D). Distributions of serum concentrations of **(A)** interferon-gamma (IFN-γ), **(B)** serum amyloid A (SAA), **(C)** interleukin-6 (IL-6) and **(D)** interleukin-17A (IL-17A), that were significantly different among binary categorized endoscopic disease activity (remission (0) or mild (1) disease vs. moderate (2) or severe (3) disease), using the Simple Endoscopic Score for Crohn's disease (SES-CD). *P < 0.05. **P < 0.01.

Regarding their predictive value for SES-CD scored endoscopic disease activity, serum levels of SAA presented the best discriminative capacity as represented by an AuROC of 0.79 (SE: 0.09, 95% CI: 0.61 – 0.96 , P < 0.01). All other significantly elevated inflammatory biomarkers discriminated well (IFN-γ: AuROC 0.74 (SE: 0.09, 95% CI: 0.56 – 0.92), P < 0.05; IL-6: AuROC 0.71 (SE: 0.09, 95% CI: 0.54 – 0.88), P < 0.05; IL-17A: AuROC 0.78 (SE: 0.08, 95% CI: 0.63 – 0.94), P < 0.01), at least as compared to the standard measures of disease activity (CRP, fecal calprotectin levels and the HBI score) (**Supplementary Figure S2**).

UC cohort

In UC patients, using the Mayo endoscopic subscore as predicted outcome of binary categorized endoscopic disease activity, serum levels of IL-6, TNF-α and Eotaxin-1 were

significantly increased in moderate-to-severe disease activity as compared to remission or mild disease activity (**Supplementary Table S1; Supplementary Figure S3**).

In ROC analysis, IL-6 demonstrated the highest discriminative ability in predicting binary endoscopic disease activity with an AuROC of 0.82 (SE: 0.14, 95% CI: 0.55 – 1.10, $P < 0.05$). Predictive performances for TNF-α and Eotaxin-1 were 0.76 (SE: 0.10, 95% CI: 0.57 – 0.95), $P < 0.05$) and 0.77 (SE: 0.12, 95% CI: 0.53 – 1.00, $P < 0.05$), respectively. Lastly, SAA showed near-to-significance and had an AuROC of 0.74 (SE: 0.09, 95% CI: 0.56 – 0.92, $P = 0.052$) (**Supplementary Figure S4**).
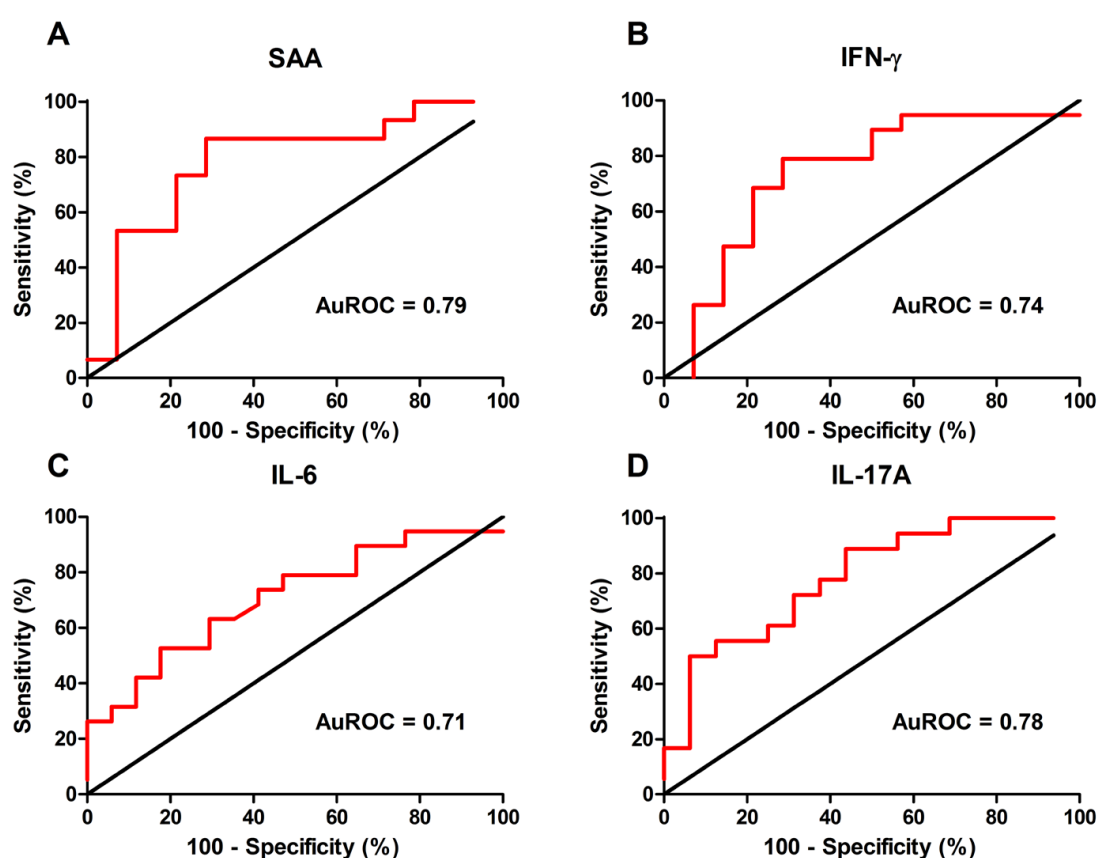
SUPPLEMENTARY FIGURE S2 (A-D). Discriminative capacity of **(A)** serum amyloid A (SAA), **(B)** interferon-gamma (IFN-γ), **(C)** interleukin-6 (IL-6) and **(D)** interleukin-17A (IL-17A) regarding binary categorized endoscopic disease activity in CD (remission (0) or mild (1) vs. moderate (2) or severe (3) disease) as represented by the area under the receiver operating characteristics curve (AuROC). Of all individual molecules shown, SAA displayed the best discriminative capacity regarding binary ordered SES-CD endoscopic disease activity.
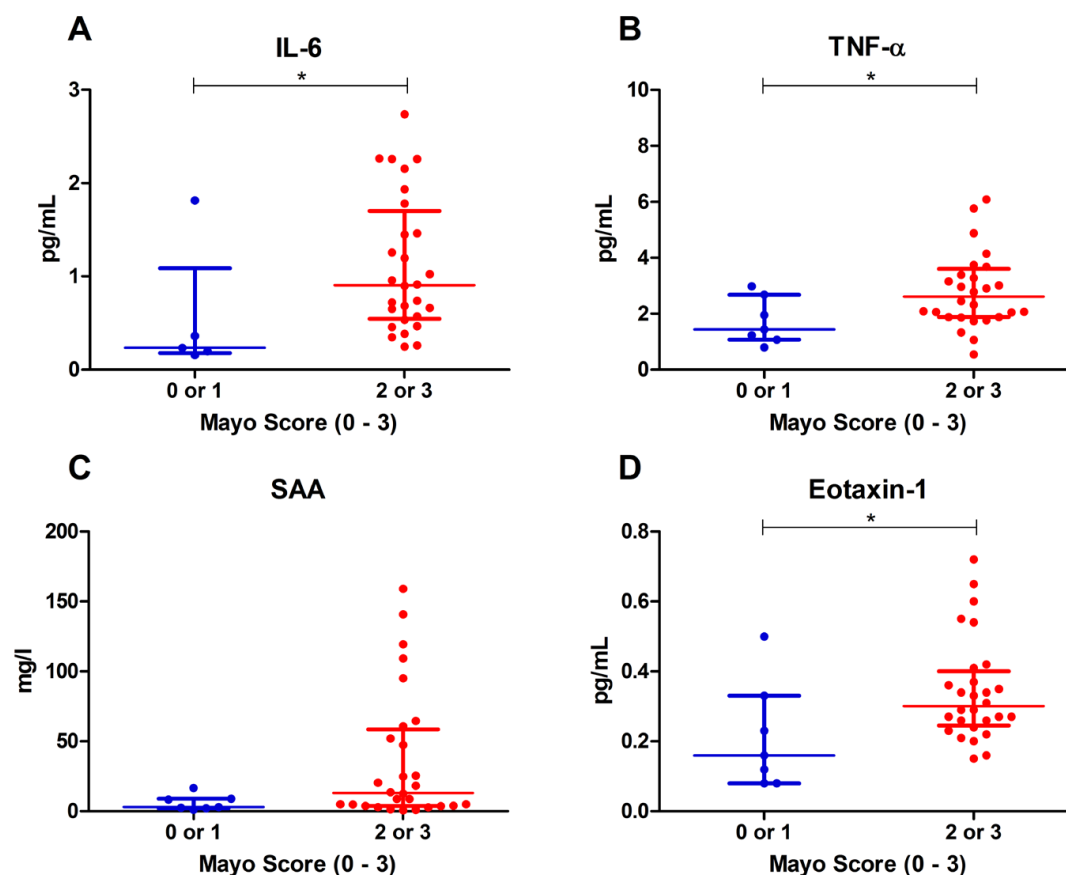
SUPPLEMENTARY FIGURE S3 (A-D). Distributions of serum concentrations of **(A)** interleukin-6 (IL-6), **(B)** tumor necrosis factor alpha (TNF-α), **(C)** serum amyloid A (SAA) and **(D)** Eotaxin-1, that were (almost) significantly different among binary categorized endoscopic disease activity (remission (0) or mild (1) disease vs. moderate (2) or severe (3) disease) using the Mayo endoscopic subcore for ulcerative colitis. *$P < 0.05$.

BEST PREDICTIVE COMBINATIONS OF INFLAMMATORY BIOMARKERS
Alternative best predictive combination by replacing SAA with CRP
Interestingly, regarding the final best combination of inflammatory biomarkers as repre-sented by the combination of SAA, IL-6, IL-8 and Eotaxin-1 (**Figure 5A**), SAA could be replaced by CRP levels without losing overall classification performance (**Supplementary Figure S5**) (AuROC 0.84, SE: 0.05, 95% CI: 0.73 − 0.94, $P < 0.0001$, $n = 69$).

Best predictive combinations for CD and UC cohorts
In the CD cohort, no combination of inflammatory biomarkers showed better predictive performance than serum levels of SAA with an AuROC of 0.79 (SE: 0.09, 95% CI: 0.61 − 0.96, $P < 0.01$), which showed higher discriminative capacity as compared to the standard measures of disease activity (CRP, fecal calprotectin levels and HBI score) (**Supplemen-**

tary Figure S6). Serum SAA levels showed a maximum sensitivity of 86.7% and specificity of 71.4% in correctly classifying CD patients into either low or high endoscopic disease activity (Youden's index = 0.57). In the UC cohort, the combination of IL-6 and Eotaxin-1 demonstrated significantly improved predictive performance (AuROC 0.97, SE: 0.03, 95% CI: 0.92 – 1.02, *P* < 0.001), definitely as compared to standard measures of disease activity (CRP, fecal calprotectin levels and SCCAI score) (**Supplementary Figure S7**). The combination of IL-6 and Eotaxin-1 presented a maximum sensitivity of 92.9% and specificity of 100% in correctly classifying UC patients into either low or high endoscopic disease activity (Youden's index = 0.93).
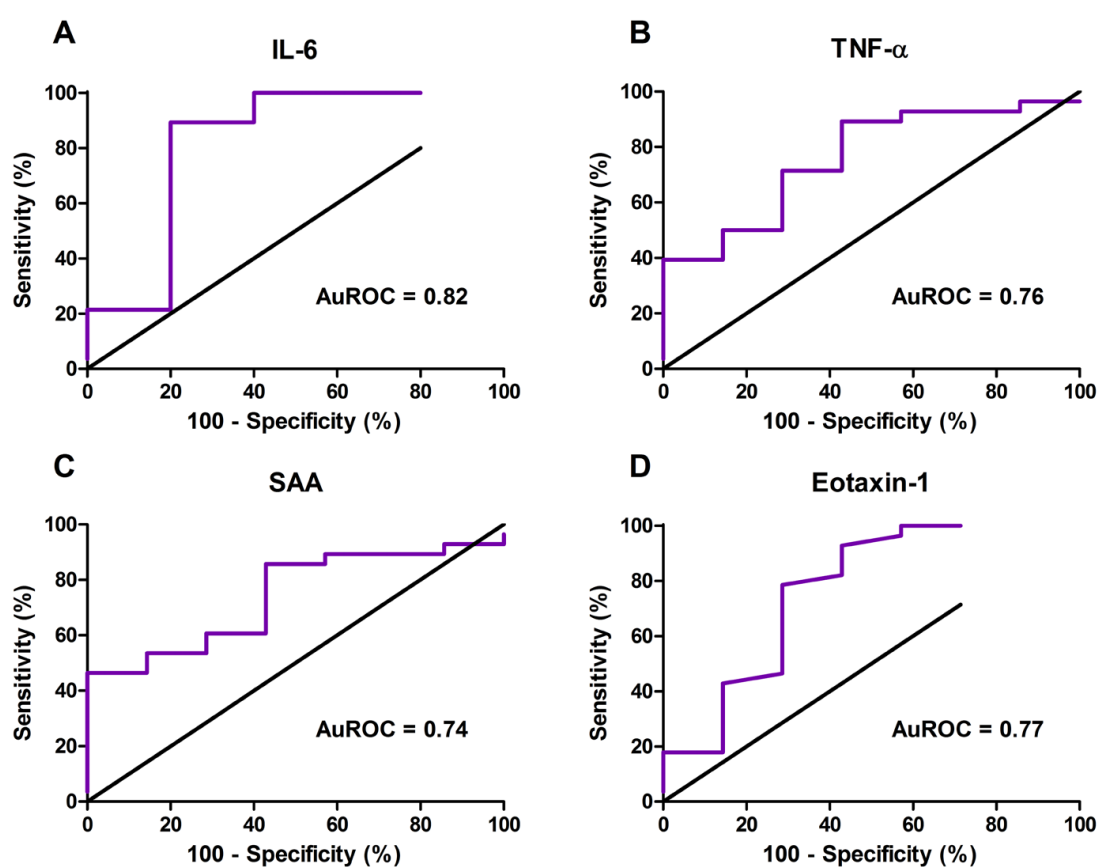

SUPPLEMENTARY FIGURE S4 (A-D). Discriminative capacity of **(A)** interleukin-6 (IL-6), **(B)** tumor necrosis factor alpha (TNF-α), **(C)** serum amyloid A (SAA) and **(D)** Eotaxin-1 regarding binary categorized endoscopic disease activity (remission (0) or mild (1) vs. moderate (2) or severe (3) disease) as represented by areas under the receiver operating characteristics curve (AuROC). Of all individual molecules shown, IL-6 displayed the best discriminative capacity regarding binary ordered Mayo endoscopic disease activity.
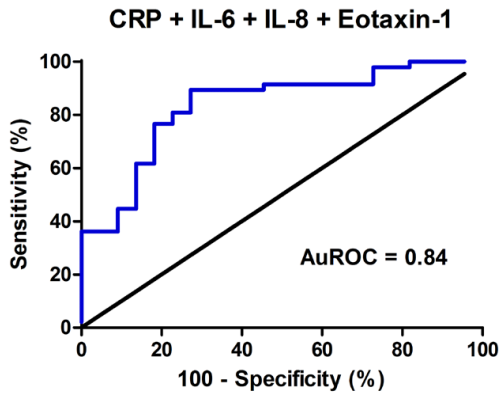
SUPPLEMENTARY FIGURE S5. The combination of C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-8 (IL-8) and Eotaxin-1 showed similar discriminative capacity as compared to the model presented in the main article (**Figure 5A**), but was not primarily presented since solely serum CRP showed bad predictive performance in our study cohort.
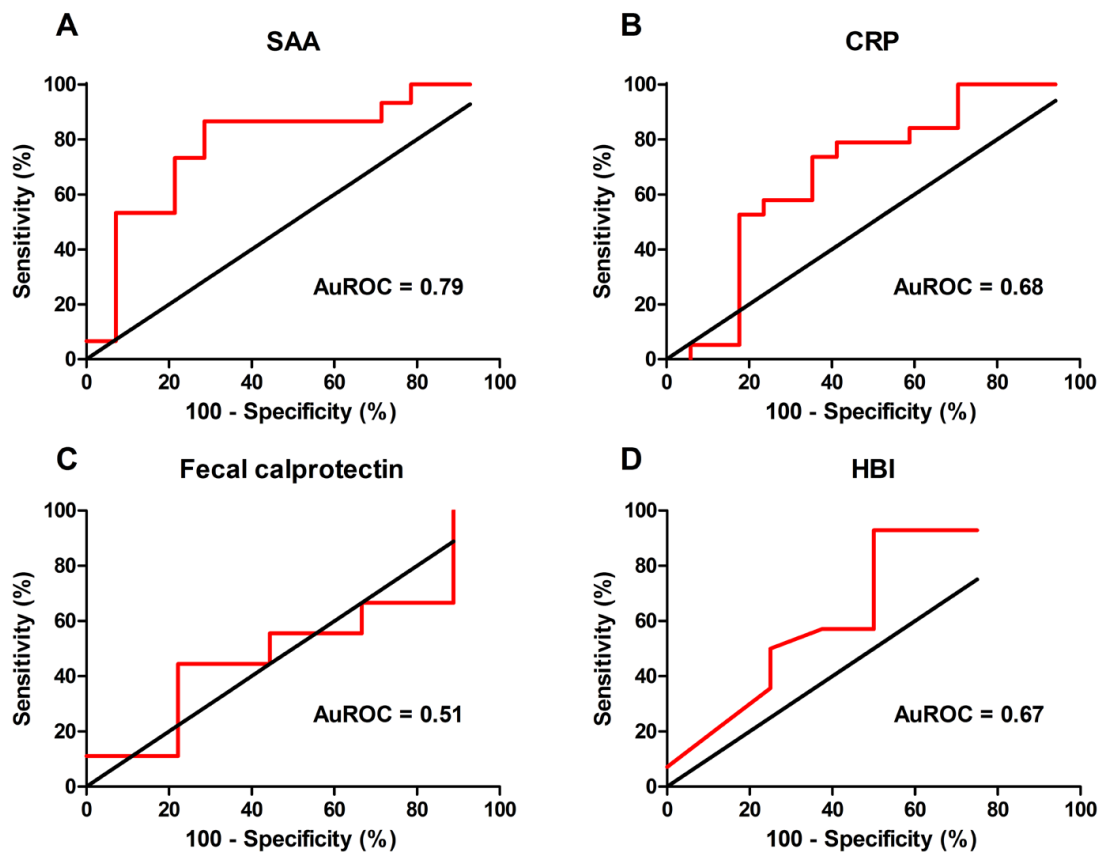
SUPPLEMENTARY FIGURE S6 (A-D). Areas under the receiver operating characteristics curve (AuROC) for **(A)** the best predictive performance as represented by serum amyloid A (SAA) levels (mg/l), **(B)** serum C-reactive protein (CRP) levels (mg/l), **(C)** fecal calprotectin (FC) levels (μg/g) and **(D)** Harvey Bradshaw Index (HBI). The best discriminative performance to predict binary ordered endoscopic disease activity using the SES-CD, is demonstrated by solely serum SAA levels (mg/l).
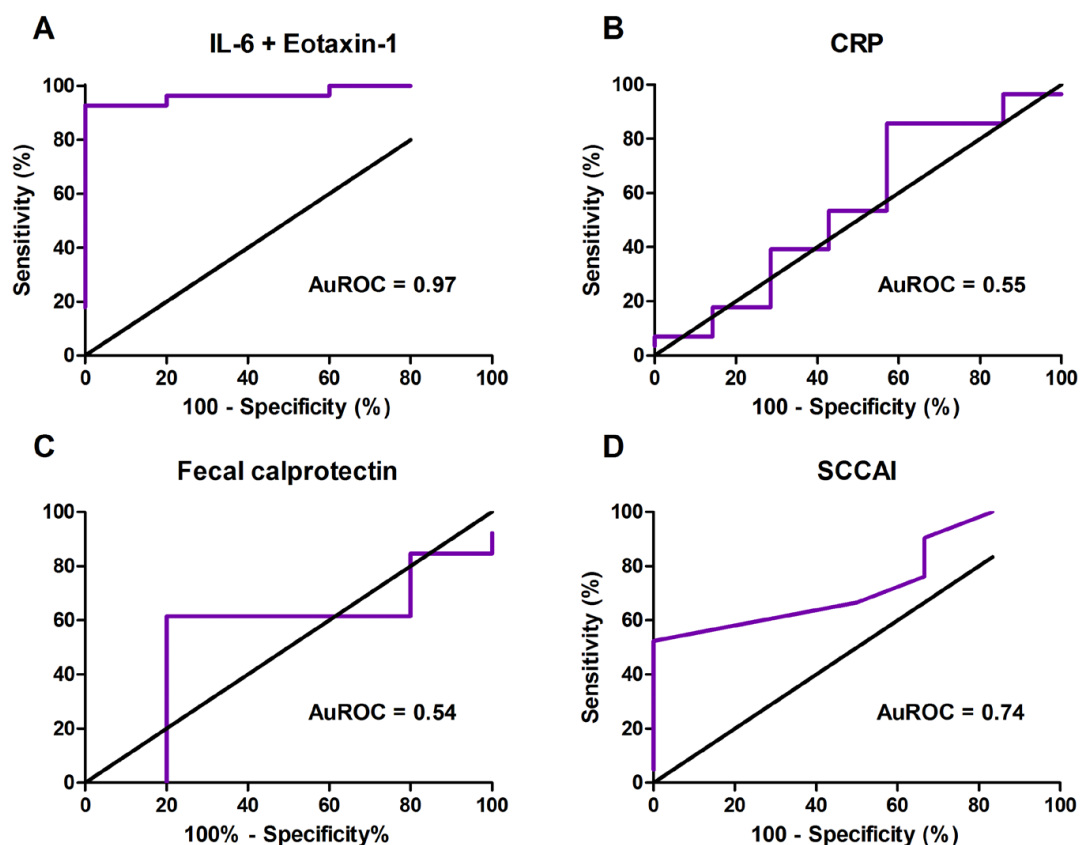
SUPPLEMENTARY FIGURE S7 (A-D). Areas under the receiver operating characteristics curve (Au-ROC) for **(A)** the best predictive performance as represented by the combination of interleukin-6 (IL-6) and Eotaxin-1 levels (pg/ml), **(B)** serum CRP levels (mg/l), **(C)** fecal calprotectin (FC) levels (µg/g) and **(D)** Simple Clinical Colitis Activity Index (SCCAI). The best discriminative performance to predict binary ordered endoscopic disease activity using the Mayo endoscopic subscore, is demonstrated by the combined serum IL-6 and Eotaxin-1 levels (pg/ml).

5