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Luisa Agnello, Caterina Maria Gambino, Fabio Del Ben, Roberta Vassallo, Francesco Cacciabaudo, Josephine Vastarella, Martina Tamburello, Anna Masucci, Ambrogio Orlando & Marcello Ciaccio

Evaluating the correlation between fecal and serum Calprotectin in Inflammatory Bowel Disease

Luisa Agnello[1,2#], Caterina Maria Gambino[1,2#], Fabio Del Ben[3], Roberta Vassallo[1], Francesco Cacciabaudo[1], Josephine Vastarella[4], Martina Tamburello[1], Anna Masucci[1], Ambrogio Orlando[4], Marcello Ciaccio[1,2*]

1. Department of Biomedicine, Neurosciences and Advanced Diagnostics, Institute of Clinical Biochemistry, Clinical Molecular Medicine, and Clinical Laboratory Medicine, University of Palermo, Palermo, Italy.
2. Department of Laboratory Medicine, University Hospital Paolo Giaccone, Palermo, Italy.
3. CRO Aviano National Cancer Institute IRCCS, Aviano, Italy
4. IBD Unit, 'Villa Sofia-Cervello' Hospital, Palermo, Italy.

*These Authors contributed equally.

*Corresponding Author: Prof. Marcello Ciaccio, marcello.ciaccio@unipa.it

Abstract

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), require accurate assessment of inflammatory activity to guide treatment and monitor disease course. While fecal calprotectin (FC) is an established non-invasive biomarker of intestinal inflammation, serum calprotectin (SC) has been proposed as a more convenient systemic marker, though its relationship to FC remains unclear. We conducted a prospective observational cohort study in 426 patients with IBD (277 CD, 149 UC). Both stool and blood samples were analyzed using the QUANTA Flash® Calprotectin chemiluminescent immunoassay. Overall, the correlation between fCal and sCal was weak (Spearman's $\rho = 0.21$), with substantial proportional bias and wide limits of agreement, indicating poor concordance between the two biomarkers. No significant association was observed at low or intermediate fCal concentrations, while a moderate correlation emerged only at fCal levels >250 µg/g, although systematic bias and heteroscedasticity persisted. fCal correlated more closely with symptom-based disease activity in ulcerative colitis, whereas sCal showed only weak associations with clinical indices, particularly in Crohn's disease. Both biomarkers demonstrated modest but significant correlations with C-reactive Protein, reflecting overlapping but distinct inflammatory pathways. In conclusion, serum calprotectin cannot be considered a surrogate for fecal calprotectin in assessing intestinal inflammation in IBD. However, it may provide complementary information on systemic inflammatory activity at higher disease burden, while fecal calprotectin remains the gold-standard noninvasive marker of mucosal inflammation.

Keywords

Inflammatory bowel disease, Crohn's disease, ulcerative colitis, fecal calprotectin, serum, calprotectin, biomarker

Introduction

Inflammatory bowel diseases (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing inflammatory disorders of the gastrointestinal tract characterized by periods of exacerbation and remission [1]. Accurate assessment of disease activity is essential for guiding treatment decisions, monitoring therapeutic response, and predicting relapse [2]. Traditionally, endoscopy remains the gold standard for evaluating mucosal inflammation, but its invasive nature, cost, and patient burden limit its use for routine monitoring [3].

Biomarkers have therefore gained increasing attention as non-invasive tools for assessing disease activity in IBD. Among these, calprotectin, a calcium- and zinc-binding protein derived primarily from neutrophils, has emerged as a reliable surrogate biomarker of intestinal inflammation. Fecal calprotectin (FC) is well established as a sensitive indicator of mucosal inflammation. It is widely used in clinical practice to differentiate IBD from functional disorders, monitor disease activity, and predict relapse [4-6]. Serum calprotectin (SC), on the other hand, has recently been investigated as a systemic biomarker of inflammation, with potential utility for assessing IBD activity through a simple blood test [7].

Although both fecal and serum calprotectin reflect neutrophil-driven inflammation, their relationship in IBD remains incompletely understood. While FC is more specific to intestinal inflammation, SC may capture both intestinal and extraintestinal inflammatory activity [8]. Establishing the degree of correlation between fecal and serum calprotectin levels could clarify whether SC may serve as a surrogate biomarker to FC, particularly in patients where stool sampling is difficult or adherence is limited.

In this study, we investigated the correlation between fecal and serum calprotectin concentrations in patients with IBD. Understanding this relationship may enhance the clinical applicability of calprotectin testing and optimize biomarker-based disease monitoring strategies.

Material and Methods

Study population

In this prospective observational cohort study, we considered all patients attending the IBD Unit, 'Villa Sofia-Cervello' Hospital, Palermo, Italy, from February 2025 to July 2025, with a clinically confirmed diagnosis of IBD. The diagnosis of UC or CD was made according to the current ECCO guidelines [9]. The exclusion criteria were: i) age <18 years; ii) lack of informed consent; iii) inadequate biological sample (stool or blood).

Clinical disease activity indices were collected for all participants. In patients with ulcerative colitis, disease activity was assessed using the partial Mayo score (PMS). In patients with Crohn's disease, clinical activity was evaluated using the Harvey–Bradshaw Index (HBI). Clinical indices were recorded at the time of serum and fecal calprotectin sampling to ensure temporal alignment between biomarker measurements and clinical assessment.

These indices were used to explore the relationship between clinical disease activity and fecal and serum calprotectin levels. Given the observational nature of the study, clinical indices were collected as part of routine clinical care using standardized definitions, and no intervention or treatment modification was mandated by the study protocol.

The study protocol was reviewed and approved by the Local Ethics Committee Palermo 1 (no. 01, 13 January 2025). The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion.

Laboratory analysis

At enrollment, each patient provided a stool sample and underwent venous blood collection in a dry tube. Serum obtained through centrifugation of the whole sample was stored at -80°C until analysis. Stool samples were extracted using a sterile sampling device designed to collect approximately 56 mg of stool, which was then transferred into a tube containing 2.8 mL of extraction buffer. The mixture was thoroughly vortexed and centrifuged at 3000×g for 10 minutes to obtain a clear supernatant. Samples with atypical consistency were handled

according to standardized procedures. Highly liquid stools were processed by pipetting 56 µL directly into the extraction buffer. For very hard stools, 100 µL of saline solution was added and the sample was incubated at room temperature for 60 minutes to soften prior to collection and extraction. Following extraction, all supernatants were stored at −80 °C until further measurement.

FC and SC concentrations were measured using the IVD QUANTA Flash® Calprotectin assay (Inova Diagnostics, San Diego, CA, USA). This chemiluminescent sandwich immunoassay was conducted at 37 °C using the automated BIO-FLASH Instrument (INOVA Diagnostics Inc.), providing quantitative determination of calprotectin levels with high analytical sensitivity and specificity. Specifically, anti-calprotectin antibodies coated on paramagnetic beads were mixed with the patient sample and assay buffer in a cuvette at 37 °C. Isoluminol-conjugated monoclonal anti-calprotectin antibodies were then added to the cuvette and incubated again at 37 °C. Following incubation, the beads were magnetized and washed repeatedly to remove unbound reagents. Subsequently, Trigger 1 (Fe(III) coproporphyrin in sodium hydroxide solution) and Trigger 2 (urea-hydrogen peroxide in sodium chloride solution) were added to initiate oxidation of the isoluminol conjugate. The resulting chemiluminescent reaction produced a flash of light, which was measured as Relative Light Units (RLU) by the BIO-FLASH® optical system, which directly correlated with both the amount of isoluminol conjugate bound and the quantity of calprotectin captured on the bead surface. To determine calprotectin concentrations, the instrument software used a master curve generated from three calibrators to convert RLUs into ng/mL. These values were then converted to µg/mL and mg/kg for serum and fecal samples, respectively, using a calculation that accounts for sample dilution.

For SC, the manufacturer’s recommended a serum cut-off value ≥ 2.00 µg/mL with a limit of detection (LoD) of 0.07 µg/mL. For FC, results were interpreted as negative < 50 mg/kg, borderline 50–120 mg/kg, positive > 120 mg/kg, with a LoD of 2.4 mg/kg.

All analyses were performed at the Institute of Clinical Biochemistry, Clinical Molecular Medicine, and Clinical Laboratory Medicine of the University Hospital of Palermo “Paolo Giaccone”.

For each patient, we collected data on C-reactive protein (CRP) and cell blood count (CBC), which were performed as part of the routine work-up.

Statistical analysis

All statistical analyses were performed using R (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria). Method comparison between fecal and serum calprotectin concentrations was carried out using the Passing–Bablok regression, implemented with the mcr package (version 1.3.3.1). Regression parameters (intercept and slope) and their 95% confidence intervals were estimated with the bootstrap (quantile) method. Correlation between the two measurements was assessed using Spearman's rank correlation coefficient (rho).

Agreement between the two methods was further evaluated using Bland–Altman analysis. Both absolute differences and ratios were analyzed, the latter with the help of log-transformation. For each Bland–Altman plot, the mean bias and the 95% limits of agreement (mean ± 1.96 standard deviations of the differences) were calculated.

Subgroup analyses were performed according to fCal thresholds commonly used in clinical practice: <50 µg/g, 50–250 µg/g, and >250 µg/g. Correlation between fCal and sCal was assessed using Spearman's rank correlation coefficient. In patients with fCal >250 µg/g, exploratory Passing–Bablok regression was performed to evaluate the presence of a consistent linear relationship between sCal and fCal at higher levels of intestinal inflammation. Bland–Altman analyses were additionally used for exploratory visualization of systematic bias and proportional disagreement across the measurement range. Given that sCal and fCal are measured in different biological matrices and expressed in different units, these analyses were not intended to assess method interchangeability, but rather to describe the nature and limitations of the relationship between the two biomarkers. A two-sided significance level of 0.05 was adopted throughout.

Results

We enrolled a total of 426 Caucasian patients with IBD, 277 CD and 149 UC (table 1). Median age was 52 years, 54% males, with a median disease duration of 156 months. Among patients with UC, disease extent according to the Montreal classification most patients were classified as E3 (44.1%) or E2 (40.5%), while a smaller proportion had proctitis (E1, 14.4%). In patients with CD, a wide distribution of Montreal phenotypes was observed, reflecting marked disease heterogeneity. Regarding age at diagnosis, most patients belonged to the A2 and A3 categories. Disease behavior was most frequently inflammatory (B1) or stricturing (B2), with a substantial proportion exhibiting penetrating features (B3) and/or perianal involvement. Ileal (L1) and ileocolonic (L3) disease locations were the most common, whereas isolated colonic disease (L2) and upper gastrointestinal involvement were less frequently observed. Complex phenotypes combining stricturing and/or penetrating behavior with ileal or ileocolonic location accounted for a relevant share of the cohort. Most patients (67.9%) had not undergone prior intestinal resections, while approximately one-third (31.8%) had a history of one intestinal resection. Only one patient had undergone two previous resections. All patients were under treatment, with 56% assuming biological drugs and 31% 5-aminosalicylates. Additionally, 2% undertook non-steroidal anti-inflammatory drugs and 27% proton pump inhibitors.

In the whole population, the median fCal concentration was 119.45 [186.2] µg/g. The median sCal concentration was 96.75 [48.33] ng/mL.

Passing–Bablok regression between fecal and serum calprotectin showed a weak association (Spearman’s rho = 0.212). The fitted regression equation was serum calprotectin = 76.74 + 0.13 × fecal calprotectin (n = 426). The slope close to zero indicates a poor proportional relationship between the two matrices, and the large intercept suggests a systematic offset (Fig. 1a).

To evaluate whether the observed relationship between fecal and serum calprotectin was influenced by clinical or therapeutic heterogeneity, we performed multivariable linear regression using log-transformed values. Given that 56% of the cohort was receiving biological therapy, we first tested for

interactions between treatment and fecal calprotectin; however, biological therapy did not significantly confound the relationship (p=0.48), and no significant interaction was observed (p=0.72). Similarly, disease location according to the Montreal classification did not significantly impact the association (p=0.38). In a comprehensive final model adjusting for diagnosis, biological therapy, disease location, age, and sex, log-fecal calprotectin remained the only significant independent predictor of log-serum calprotectin (estimate: 0.085; p<0.001). Despite this statistical significance, the overall model explained only a minimal proportion (6%) of the total variance in serum levels (adjusted R2=0.06).

In the residual plot of the Passing–Bablok regression, residuals were randomly distributed around zero up to fCal values of approximately 500 µg/L, indicating no evident systematic bias in the lower concentration range. However, at higher concentrations, the residuals showed a clear downward curvature, suggesting that serum values became progressively lower than those predicted by the model. This pattern indicates non-linearity of the relationship at elevated concentrations, with the Passing-Bablok model overestimating serum calprotectin relative to fecal levels in the upper range (Fig. 1b). The Bland–Altman analysis in absolute differences showed a mean bias of −103.7, with 95% limits of agreement ranging from −1010.2 to +802.8. This indicates that serum values tend to underestimate fecal concentrations on average, with considerable disagreement at the individual level (Fig. 2a). In the Bland–Altman analysis of ratios, the mean ratio was 1.15, with limits of agreement ranging from 0.09 to 14.31. This representation underscores the poor concordance, particularly at low fCal concentrations, where serum values span a wide range, thereby confirming the substantial variability between the two measurement methods (Fig. 2b).

When stratified by fCal thresholds, different patterns of association between sCal and fCal were observed. In patients with fCal <50 µg/g (Fig. 3A), no significant correlation was detected between sCal and fCal (Spearman’s ρ = 0.11, p = 0.30; n = 90), with wide dispersion of sCal values across low fCal concentrations. Similarly, no significant correlation was observed in patients

with fCal between 50 and 250 µg/g (Fig. 3B; Spearman's ρ = 0.11, p = 0.12; n = 188), indicating a lack of association in the intermediate "grey zone" range.

In contrast, a moderate but statistically significant positive correlation was identified in patients with fCal >250 µg/g (Fig. 3C; Spearman's ρ = 0.36, 95% CI 0.16–0.54, p < 0.001; n = 86), suggesting that sCal reflects increasing intestinal inflammatory burden only at higher levels of fCal.

Based on this finding, Passing–Bablok regression was performed exclusively in the fCal >250 µg/g subgroup. The regression analysis demonstrated a proportional relationship between the two biomarkers, with the fitted equation sCal = 73.57 + 0.06 × fCal and deviation from the line of identity, indicating systematic differences between the two measurement matrices. Residual analysis showed an acceptable distribution around zero, with increasing variability at higher fCal values, consistent with heteroscedasticity at higher inflammatory levels. Bland–Altman analysis further demonstrated a negative mean bias (−640.7 units), indicating that fCal values were substantially higher than sCal values at increased concentrations, with wide limits of agreement. The ratio-based Bland–Altman plot on a logarithmic scale showed increasing proportional disagreement with increasing biomarker levels, underscoring that sCal and fCal are not directly interchangeable but may provide complementary information, particularly in the setting of marked intestinal inflammation.

Passing–Bablok regression and Bland–Altman analysis are conventionally employed in method comparison studies to evaluate agreement between analytical procedures measuring the same measurand in the same matrix. In this study, fecal and serum calprotectin represent related but analytically distinct measurands derived from different biological matrices and expressed in different units. Accordingly, these analyses cannot be interpreted as evidence of analytical agreement or interchangeability.

We applied Passing–Bablok and Bland–Altman analyses in an exploratory, descriptive manner, restricted to samples with elevated fecal calprotectin concentrations, to characterize proportionality, systematic bias, and heteroscedasticity in the relationship between the two biomarkers at higher inflammatory burden [10]. The observed proportional bias and wide limits of

agreement indicate substantial analytical and biological variability, reinforcing that serum calprotectin cannot be considered a surrogate for fecal calprotectin. Instead, serum calprotectin may serve as a complementary biomarker reflecting overlapping but distinct pathophysiological processes. From a laboratory medicine perspective, these findings highlight the importance of defining clinical decision limits and outcome-based performance characteristics before serum calprotectin can be integrated into routine inflammatory bowel disease monitoring algorithms.

We explored the relationship between fecal and serum calprotectin levels and clinical disease activity indices. In patients with CD, fCal showed no significant correlation with the HBI (rho = −0.05, 95% CI −0.18 to 0.08; p = 0.41; n = 244). In contrast, serum calprotectin demonstrated a weak but statistically significant positive correlation with HBI (rho = 0.15, 95% CI 0.03 to 0.27; p = 0.0097; n = 283).

In patients with UC, fCal was moderately correlated with the PMS (rho = 0.28, 95% CI 0.10 to 0.44; p = 0.0024; n = 117). Serum calprotectin showed a weak, non-significant association with the partial Mayo score (rho = 0.15, 95% CI −0.02 to 0.31; p = 0.08; n = 136).

Overall, these findings indicate that fCal correlates more closely with symptom-based disease activity in UC, whereas sCal shows only weak associations with clinical indices, particularly in CD.

We assessed the relationship between CRP and both fecal and serum Cal. With respect to fCal, patients with elevated CRP had significantly higher fCal levels than those with normal CRP (median 167.4 vs 112.4 µg/g; p = 0.003). Furthermore, fCal demonstrated a weak but statistically significant positive correlation with CRP values measured within three months of calprotectin sampling (Spearman’s rho = 0.20, 95% CI 0.09–0.30; p < 0.001; n = 351). Similarly, sCal levels were significantly higher in patients with elevated CRP compared with those with normal CRP (median 113.95 vs 92.20 ng/mL; p < 0.001). sCal also showed a weak but significant correlation with CRP values over the same time frame (Spearman’s rho = 0.20, 95% CI 0.10–0.29; p < 0.001; n = 409). Overall, these findings indicate that both fecal and serum calprotectin

are associated with systemic inflammatory activity as reflected by CRP, although the strength of these correlations is modest. This is consistent with prior literature and supports the concept that CRP and calprotectin capture overlapping but biologically distinct aspects of inflammation.

Discussion

Calprotectin is a calcium-binding protein complex released by activated immune cells during inflammation. It forms stable heterodimers or heterotetramers that regulate inflammatory responses and inhibit microbial growth. Its levels rise sharply during inflammation, with fecal calprotectin showing very high accuracy in distinguishing Crohn's disease from irritable bowel syndrome. Fecal calprotectin is the preferred noninvasive biomarker for assessing intestinal inflammation in patients with IBD, as it correlates closely with endoscopic and histologic disease activity and is recommended by the American Gastroenterological Association for diagnosis, monitoring, and relapse prediction [11]. Although fecal calprotectin reliably distinguishes IBD from non-inflammatory conditions and guides clinical decision-making, it has some limitations, especially in IBD patients [12,13]. In cases of constipation, reduced stool frequency, or during remission when bowel habits normalize, timely sample collection may be particularly challenging. Conversely, in active disease with diarrhea, obtaining a properly handled and uncontaminated sample can also be problematic. A substantial proportion of patients report difficulty with sample collection, with common issues including aversion to handling stool, embarrassment, and forgetfulness. In a large multicenter survey, 37% of IBD patients found fecal calprotectin testing difficult, with sample collection being the most frequently cited challenge; younger age and shorter disease duration were associated with greater difficulty [14]. Compliance rates are low, with only about one-third of patients completing the test when prescribed, and the main reasons for non-compliance are forgetfulness, lack of perceived benefit, constipation, and refusal to handle feces [15].

Preanalytical factors further complicate interpretation. Indeed, fecal calprotectin levels exhibit significant intra-individual variability, both within a single stool and between bowel movements on the same day, which can affect reliability when only a single sample is used [16,17]. Additionally, improper storage (e.g., at room temperature for 24–72 hours or more) can degrade calprotectin and yield inaccurate results. In this context, we evaluated whether serum calprotectin could serve as a surrogate for fecal calprotectin in a large, well-characterized IBD cohort. Across the entire study population, the association between fecal and serum calprotectin was weak, indicating that an increase in fecal calprotectin is specific to intestinal inflammation and does not necessarily parallel systemic calprotectin concentrations. The Passing–Bablok regression revealed a slope close to zero and a large intercept, indicating that changes in fCal were only minimally reflected in serum concentrations and that a systematic offset exists between the two matrices. Residual and Bland–Altman analyses further highlighted marked inter-individual variability and increasing non-linearity at higher concentrations. Together, these findings clearly indicate that fecal and serum calprotectin are not interchangeable and should not be interpreted as measuring the same biological signal. Stratified analyses according to fecal calprotectin thresholds provided additional nuance. At low and intermediate fecal calprotectin concentrations, corresponding to remission or the so-called “grey zone,” serum calprotectin showed no meaningful association with fecal levels, with wide dispersion of serum values. This lack of correlation suggests that serum calprotectin is not sensitive enough to detect low-grade or localized intestinal inflammation and is therefore unlikely to be useful as a screening or rule-out tool in patients with suspected mucosal quiescence. In contrast, at fecal calprotectin levels above 250 µg/g, a moderate, statistically significant correlation emerged, indicating that serum calprotectin begins to reflect the intestinal inflammatory burden only with more pronounced inflammation. Even in this subgroup, however, proportional bias and heteroscedasticity persisted, reinforcing that serum calprotectin cannot substitute for fecal measurements but may provide complementary information at higher disease activity. Thus, serum calprotectin, while elevated in IBD and associated with systemic inflammation, is less specific for gut mucosal activity.

From a methodological standpoint, it is important to emphasize that fecal and serum calprotectin are analytically and biologically distinct measurands, derived from different compartments and expressed in different units. Although Passing–Bablok regression and Bland–Altman analyses are traditionally used to assess agreement between methods measuring the same analyte, we applied these tools in an exploratory manner to characterize proportionality, bias, and variability at higher inflammatory burden. The observed discrepancies likely reflect fundamental biological differences: fecal calprotectin predominantly mirrors neutrophil migration into the intestinal lumen and mucosal inflammation, whereas serum calprotectin reflects systemic release from activated neutrophils and monocytes, potentially influenced by extraintestinal inflammation, comorbidities, and pharmacological treatments. Our results are consistent with this biological compartmentalization and suggest that sCal and fCal provide complementary rather than interchangeable information.

Beyond its role as a biomarker of inflammatory activity, emerging evidence suggests that blood-based calprotectin may also serve as an indicator of disease progression and the development of complications in inflammatory bowel disease. Elevated circulating calprotectin levels have been associated with an increased risk of disease progression, including escalation of therapy and worsening disease course over time [18]. More recently, blood-based calprotectin has also been linked to the occurrence of disease-related complications, supporting its potential utility as a prognostic biomarker rather than solely a measure of current inflammatory burden [19]. These observations suggest that systemic calprotectin may reflect broader inflammatory and immune activation processes relevant to long-term disease outcomes. In this context, the limited agreement observed between serum and fecal calprotectin in the present study may indicate that these biomarkers capture distinct, albeit complementary, aspects of disease biology.

Strengths and limitations of this study should be mentioned. This is one of the largest prospective observational studies to date evaluating both fecal and serum calprotectin in a well-characterized cohort of IBD patients. The study included patients with both Crohn’s disease and ulcerative colitis, enhancing generalizability across the IBD spectrum. Additionally, calprotectin levels were

measured using a standardized, fully automated chemiluminescent immunoassay with high analytical sensitivity and reproducibility. On the other hand, the cross-sectional design did not allow for assessment of longitudinal changes or the predictive value of serum calprotectin in monitoring disease course. Although we observed statistically significant associations between calprotectin levels and clinical activity indices, these correlations were generally weak, particularly in Crohn's disease. Moreover, the absence of concomitant endoscopic or histologic assessment represents an important limitation, as it precludes any definitive conclusions regarding the relationship between serum calprotectin and mucosal inflammation. Therefore, our findings do not support the use of serum calprotectin as a surrogate marker of mucosal healing. Future studies incorporating endoscopic and/or histologic endpoints are warranted to clarify the potential role of serum calprotectin in reflecting intestinal inflammatory burden.

The primary objective of this study was to evaluate whether sCal could serve as a clinically meaningful surrogate to fCal. The weak correlation and poor agreement observed between sCal and fCal suggest that sCal cannot reliably substitute for fCal when the clinical aim is assessment of intestinal inflammation. Interpretation of our findings should consider the well-recognized biological variability of fecal calprotectin, including intra- and inter-individual fluctuations that may occur independently of disease activity [20,21]. Importantly, in the present study, fecal calprotectin was measured using a single standardized automated assay, thereby minimizing analytical variability. As a result, the assay-related differences widely reported in the literature do not represent a confounding factor in our analysis [21].

In summary, in this large prospective cohort of patients with IBD, serum calprotectin did not correlate significantly with fecal calprotectin levels. These findings indicate that elevations in serum calprotectin reflect systemic inflammatory activity but are not a reliable surrogate for intestinal inflammation as measured by fecal calprotectin. While serum calprotectin may offer complementary insights into extraintestinal inflammation and overall disease burden, fecal calprotectin remains the gold standard non-invasive biomarker for assessing mucosal intestinal inflammation in IBD in routine

clinical practice. Future studies should further investigate the potential role of serum calprotectin in prognostication and in combination with other biomarkers to refine personalized disease monitoring strategies.

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Competing interest: The authors declare no competing interests.

Data availability statement: The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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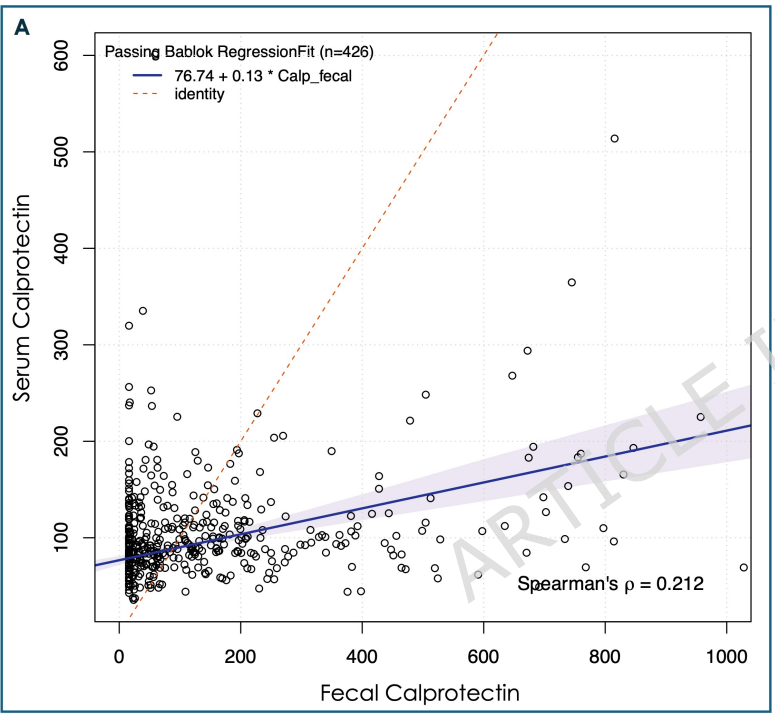
Figure legends

Figure 1. A. Passing–Bablok regression of serum versus fecal calprotectin concentrations. The blue solid line represents the fitted regression line, and the shaded area indicates the 95% confidence interval. The red dashed line corresponds to the line of identity (y = x). B. Residual plot of the Passing–Bablok regression. Optimized residuals (y-axis) are plotted against estimated fecal values (x-axis). The red dotted line marks the zero-residual reference.

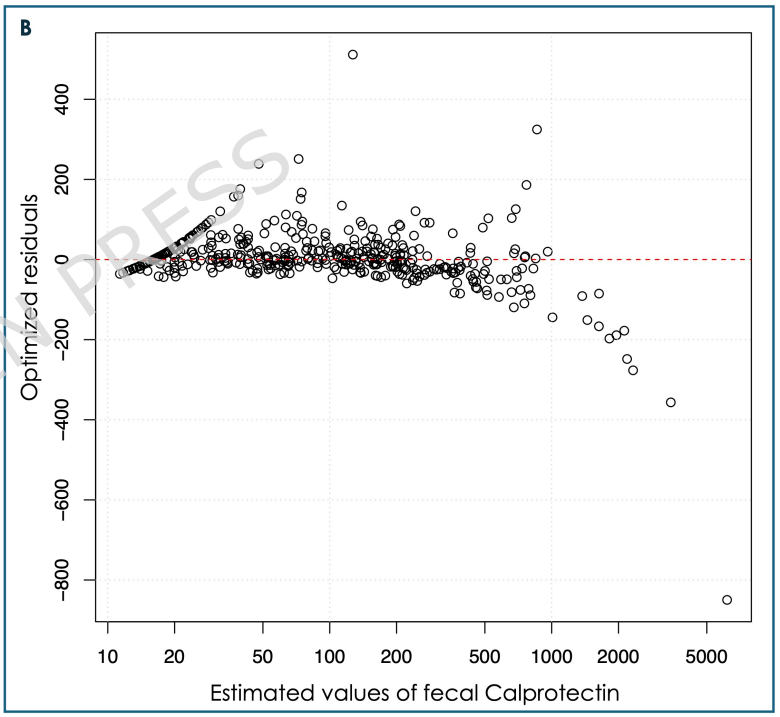
Figure 2. A. Bland–Altman plot comparing serum and fecal calprotectin concentrations. Each point represents the difference between serum and fecal values plotted against their mean. The red solid line indicates the mean difference, while the red dotted lines represent the upper and lower limits of agreement (mean ± 2 SD). B. Ratio Bland–Altman plot. The red solid line marks the mean log ratio of serum to fecal, with red dotted lines indicating the upper and lower limits of agreement (mean ± 2 SD).

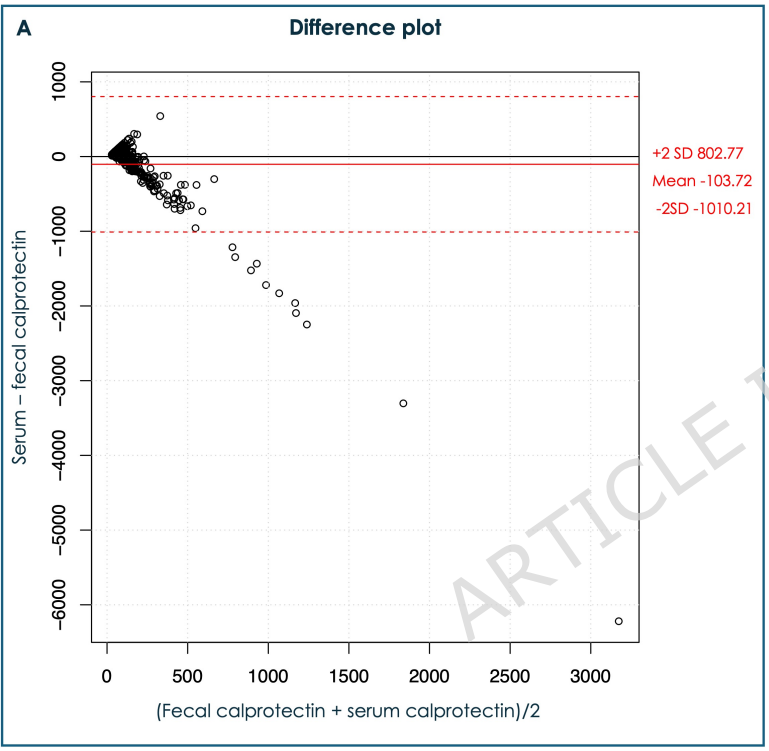
Figure 3. Correlation between fecal calprotectin (fCal) and serum calprotectin (sCal) according to clinically relevant fCal thresholds. Scatter plots show the relationship between fCal and sCal in patients with (A) fCal <50 µg/g, (B) fCal between 50 and 250 µg/g, and (C) fCal >250 µg/g. Blue lines represent linear regression fits with 95% confidence intervals (shaded areas). Spearman's correlation coefficient (ρ), p values, 95% confidence intervals, and the number of paired samples are reported for each subgroup. Marginal histograms display the distribution of fecal (top, green) and serum (right, orange) calprotectin values. A significant positive correlation was observed only in patients with fCal >250 µg/g.

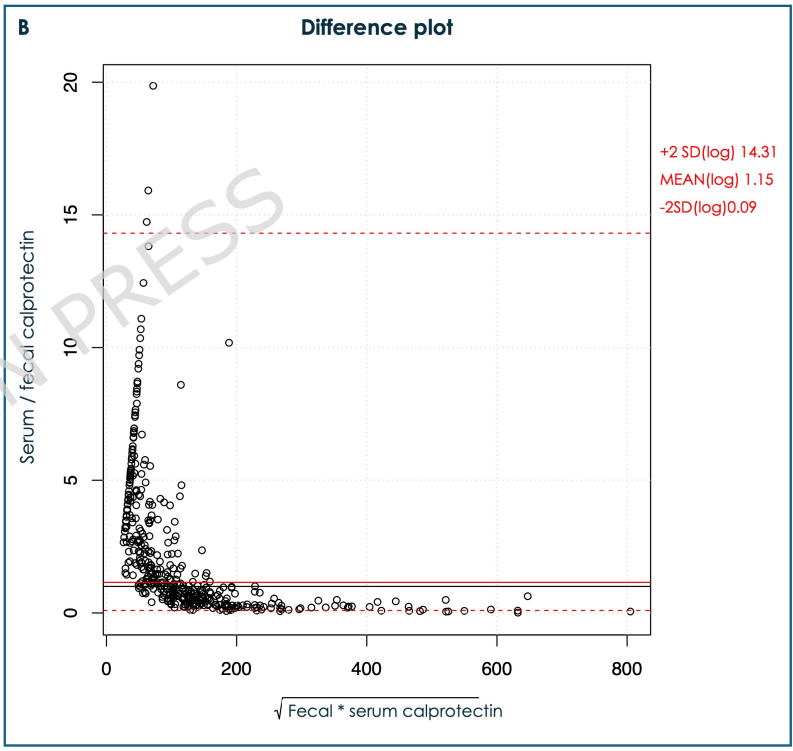
Passing Bablok Regression fit

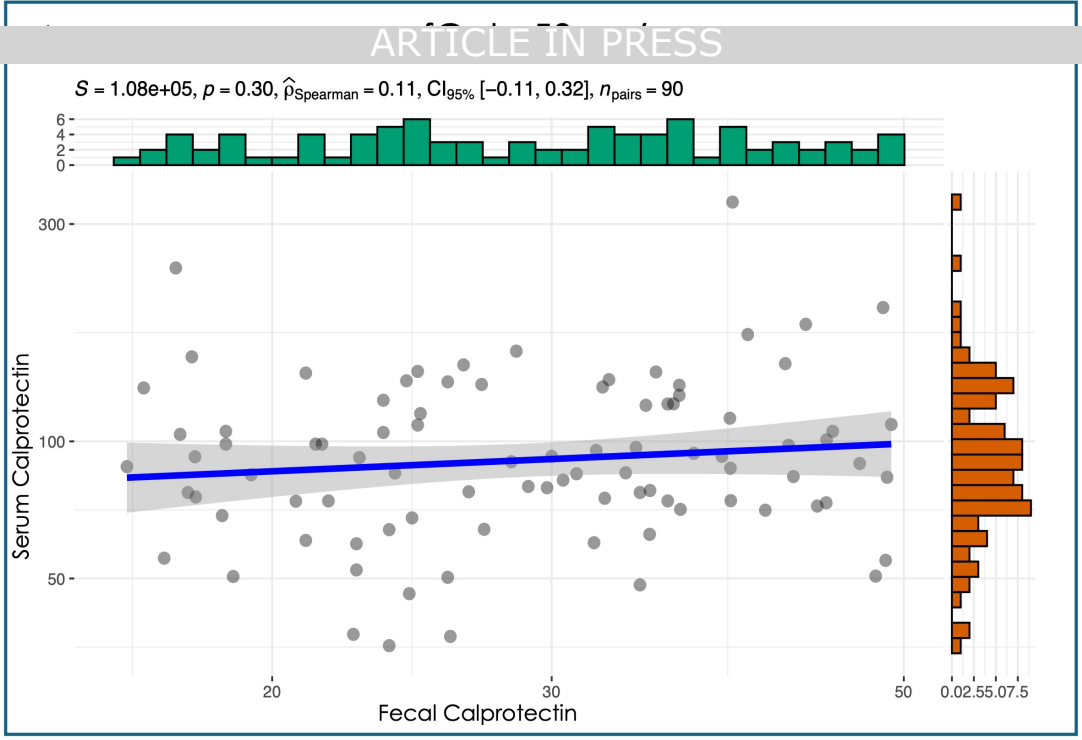


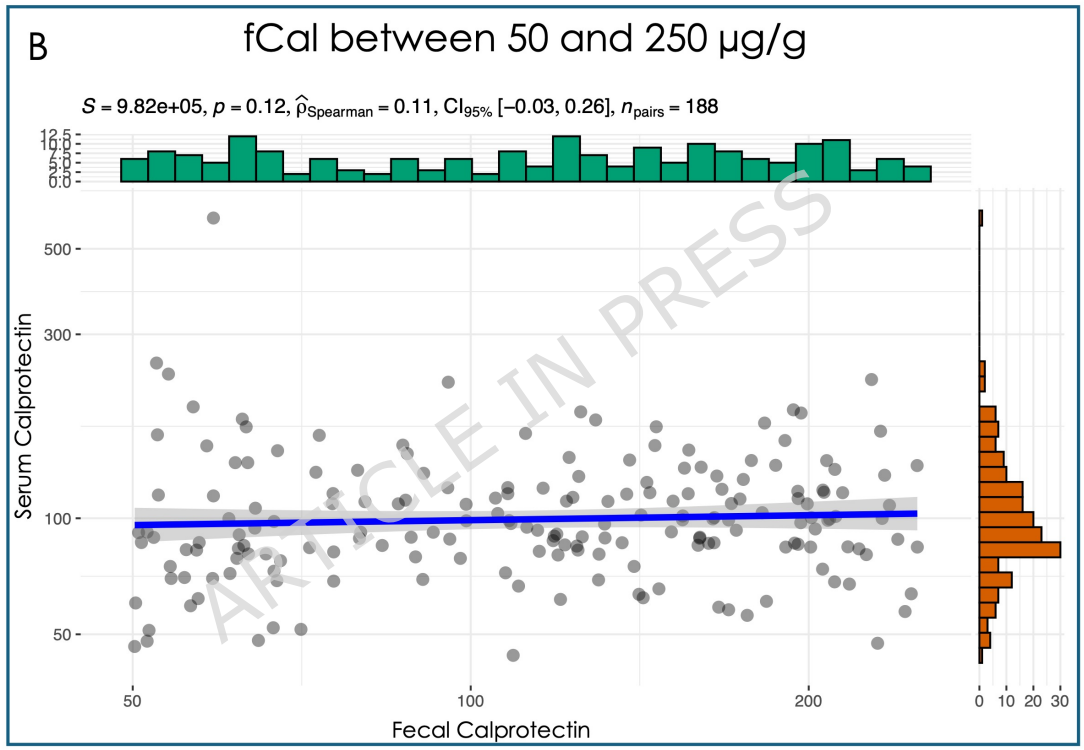
Residual plot for Passing Bablok Regression fit











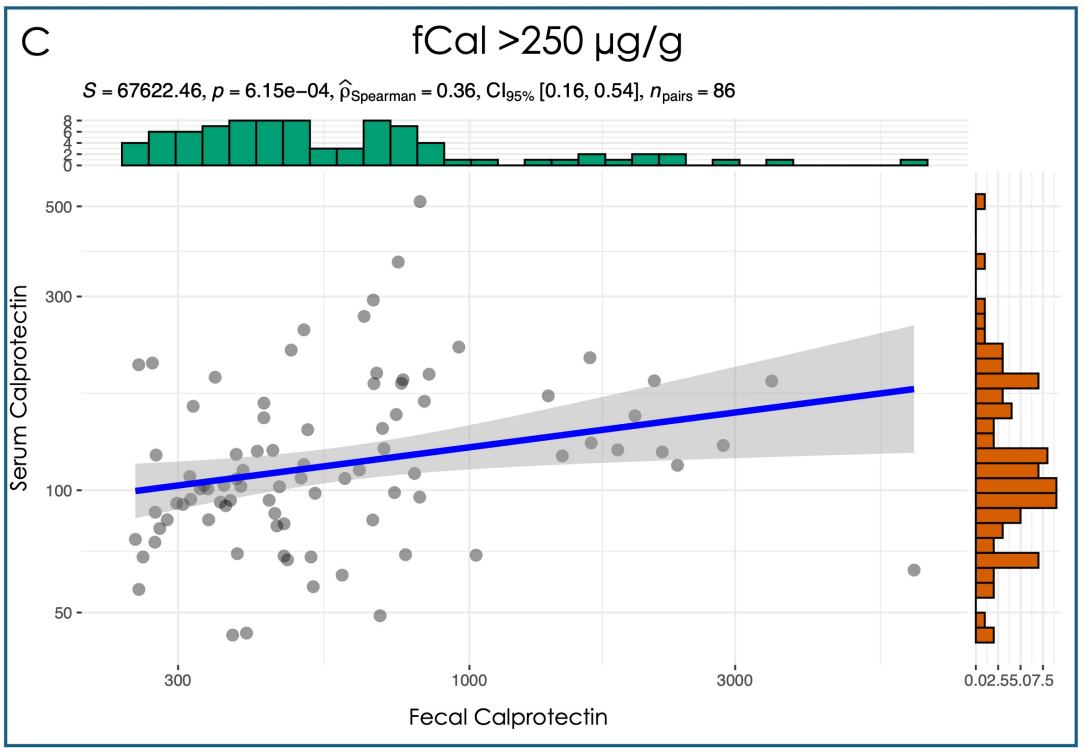


Table 1. Demographic and clinical features of the study population.

Nr. patients, n.	426
Age, years	52 years
Males	54%
BMI, kg/m²	24.5 [22.0–27.5]
IBD subtype *Crohn's disease* *Ulcerative colitis*	 66% 34%
Disease duration	177.6 ± 131.6 months
Age at diagnosis	420 months ± 32 months
Montreal classification *Crohn's disease:* *- A1* *- A2* *-A3* *- L1* *- L2* *- L4* *- B1* *- B2* *- B3* *Ulcerative colitis* *- E1* *- E2* *- E3*	 2.5% 43.2% 54.3% 40% 8% 52% 52% 43% 44% 22.5% 32.5% 45%
History of IBD-related surgery	30%
Concomitant treatments *5-ASA* *Corticosteroids* *Immunomodulators* *Biologic therapies*	 37% 9% 7% 47%
Fecal caprotectin, median *Crohn's disease* *Ulcerative colitis*	 118.42 ± 208.2 µg/g 122.03 ± 157.1 µg/g
Serum caprotectin, median	98.9 ± 54.1 µg/mL

Crohn's disease *Ulcerative colitis*	89.9 ± 40.1 µg/mL