

# Plasma Concentration of Calprotectin, but Not Serum Concentration, Is a Predictive Biomarker for Clinical Remission in Ulcerative Colitis

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## Keywords

Plasma calprotectin · Ulcerative colitis · Crohn's disease · Inflammatory bowel disease · Biomarker

## Abstract

**Introduction:** Fecal calprotectin is a validated biomarker for assessing disease activity in patients with inflammatory bowel disease (IBD). Blood calprotectin concentrations are correlated with disease activity in numerous immune-mediated inflammatory diseases. The aim of this study was to prospectively assess the diagnostic accuracy of plasma calprotectin as a potential biomarker of remission in IBD patients. **Methods:** This prospective observational study enrolled 131 patients at the time of infliximab administration alongside clinical assessment and blood analyses on the same day. The primary endpoint was to assess the diagnostic accuracy of plasma calprotectin for predicting remission in patients with IBD. **Results:** Plasma calprotectin concentration  $\leq 10.5$  ng/mL

had a sensitivity of 98.6%, specificity of 100%, positive predictive value of 100%, negative predictive value of 96.3%, and an area under the receiver operating characteristic (AUROC) curve of 0.999 for diagnosing remission in patients with ulcerative colitis (UC). Plasma calprotectin had poor diagnostic accuracy for diagnosing remission in Crohn's disease. In UC, plasma calprotectin had significantly greater diagnostic accuracy than C-reactive protein for diagnosing remission (absolute difference between AUROCs, 0.06; 95% CI: 0.008 to 0.113;  $p = 0.03$ ). Plasma calprotectin concentrations were not correlated with those measured in serum samples. The median serum-to-plasma calprotectin concentration ratio was 12-fold. **Conclusion:** Plasma calprotectin is a promising biomarker for predicting remission in UC patients treated with infliximab.

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## Introduction

Biomarkers are important for measuring the inflammatory burden of the gastrointestinal tract. Inflammatory bowel disease (IBD) is a chronic, immune-mediated disease, and the two most widely adopted biomarkers for measuring disease activity are fecal calprotectin and C-reactive protein (CRP).

Fecal calprotectin has been evaluated in numerous diagnostic studies in both adult and pediatric populations over the past 20 years. Fecal calprotectin has a high sensitivity for detecting gut inflammation and is reliably used to monitor disease activity in patients with IBD, in which levels are strongly correlated with the degree of clinical and endoscopic activity [1, 2]. However, there are several disadvantages, including low specificity for discriminating between different intestinal inflammatory etiologies and marked intraindividual variation [3]. Patient compliance has been shown to be poor, with only one-third of patients performing the test for reasons that include difficulty collecting stool samples, refusal to handle feces, and forgetfulness [4]. Other stool-based tests, such as those used for colorectal cancer screening, have also shown low compliance rates [5–7].

CRP is a validated and established clinical biomarker that is commonly used to distinguish IBD from irritable bowel syndrome and other functional gut disorders that have significant symptom overlap [3, 8]. CRP has a role in disease monitoring and assessing treatment response, but it is less sensitive and specific than fecal calprotectin at discerning mucosal disease activity in both ulcerative colitis (UC) and Crohn's disease (CD) patients [3].

There is an unmet need for a reliable, novel blood-based biomarker for IBD to allow disease monitoring with increased patient acceptability. High extracellular concentrations of calprotectin have been found in the blood and at inflammatory sites across a range of immune-mediated inflammatory conditions. There is a growing body of evidence indicating that for rheumatic diseases, such as rheumatoid arthritis and spondyloarthritis, blood calprotectin is significantly correlated with inflammatory markers and disease activity scores, and it acts as a biomarker of treatment response [9]. Regarding IBD, the evidence for calprotectin is less clear; only a few studies have been conducted, and the data are conflicting. Regarding serum calprotectin, some promising studies have shown that it can discriminate between active and inactive IBD and predict outcomes, but these findings have not been replicated in other studies [10, 11]. For plasma calprotectin, data regarding its utility as a biomarker in IBD are scarce; only one study showing superiority over serum calprotectin as a

biomarker of disease activity has been published, but this study included patients with UC only and was limited by its small sample size [12]. Thus, the aim of the present study was to prospectively assess the diagnostic accuracy of serum and plasma calprotectin in a comparatively large cohort of patients with both CD and UC. This study assessed the utility of using blood calprotectin as a potential biomarker of remission in patients with IBD.

## Materials and Methods

### *Study Design*

This was a prospective observational study that enrolled patients from the Nancy IBD cohort between December 1, 2010, and March 1, 2012, in the ambulatory care facility of the Department of Gastroenterology of the University Hospital of Nancy at the time of their infliximab schedule. Patients were not included in the study if they presented with signs of infection. The diagnosis of infection was based on clinical evaluation performed at the discretion of the investigator. This assessment could include patient-reported symptoms, physical examination findings suggestive of infection, and biological parameters such as leukocytosis (white blood cell count  $>12,000/\text{mm}^3$ ), elevated CRP (CRP  $>20 \text{ mg/L}$ ), or elevated procalcitonin levels ( $>0.5 \text{ ng/mL}$ ).

### *Primary and Secondary Objectives*

The primary objective of this study was to assess the diagnostic accuracy of plasma calprotectin for predicting remission in patients with IBD. The secondary objectives were as follows: (1) to compare the diagnostic accuracy of calprotectin and high-sensitivity C-reactive protein (hs-CRP) for predicting clinical remission in patients with IBD; (2) to assess the correlation between plasma calprotectin and hs-CRP among UC and CD patients and to compare correlation coefficients between IBD subtypes; (3) to compare the blood concentration of calprotectin in paired serum and plasma samples; and (4) to compare two calprotectin assays to assess the equality of measurements from two different analytical methods as part of quantitative bioanalytical method validation.

### *Measured Outcomes*

Clinical and biological disease activity markers were measured at the time of the patient visit. The simple clinical colitis activity index (SCCAI) [13] and the Crohn's disease activity index (CDAI) [14] were used for assessing clinical activity in patients with UC and CD, respectively. In patients with UC, clinical remission was

defined as an SCCAI <2 [15]. In patients with CD, clinical remission was defined as a CDAI <150 [16]. For each patient, biological data were collected prospectively in an electronic database and extracted for the purposes of the study using the General Laboratory Information Management System (GLIMS, version 8.11.6, MIPS France S.a.r.l., Paris, France). The following data were available in the electronic database: patient identification number; patient age at the time of blood collection; date and time of blood sampling; hemoglobin (g/dL); hematocrit (%); mean corpuscular volume (fL); mean corpuscular hemoglobin concentration (g/dL); red blood cells ( $\times 10^9$ /L); leukocytes ( $\times 10^9$ /L); neutrophils ( $\times 10^9$ /L); lymphocytes ( $\times 10^9$ /L); monocytes ( $\times 10^9$ /L); eosinophils ( $\times 10^9$ /L); basophils ( $\times 10^9$ /L); platelets ( $\times 10^9$ /L); albumin (g/L); hs-CRP (mg/L); and procalcitonin (ng/mL).

#### *Plasma Calprotectin Assays*

Plasma calprotectin was measured using the following two methods according to the manufacturer's recommendations: (1) Calprest<sup>TM</sup> and Eurospital ELISA kits for calprotectin quantification (Mast Diagnostic, Amiens, France), which is a manual technique, and (2) Calprotectin Fluorenszyme-Immunoassay (EliA<sup>TM</sup>, Thermo Fisher Scientific, Saint-Quentin-en-Yvelines, France), which is an automated technique. Blood samples for the calprotectin assay were collected on the same day as the patient's clinical evaluation. Blood samples were collected in EDTA tubes and stored at  $-20^{\circ}\text{C}$  until use in the Laboratory of Biochemistry Molecular Biology and Nutrition of the University Hospital of Nancy. The readers of the calprotectin assay were blinded to the clinical diagnosis and the inflammatory activity of the patients. The data are expressed in ng/mL.

#### *Statistical Analyses*

All quantitative variables are described as medians and percentiles (interquartile ranges [IQRs], 25th–75th percentiles). All proportions are expressed as percentages with 95% confidence intervals (95% CIs). The diagnostic accuracy of plasma calprotectin was evaluated using receiver operating characteristic (ROC) analysis according to DeLong et al. [17]. In each ROC analysis, patients with the classification criterion were opposed to patients without the classification criterion. For each ROC analysis, the diagnostic accuracy results were as follows: sensitivity, specificity, positive predictive value (PPV), negative predictive value, and area under the receiver operating characteristic (AUROC) curve with the associated *p* value. For each ROC analysis, the optimal calprotectin threshold was defined using the Youden index (*J*). The bias-corrected and

accelerated bootstrap interval after 10,000 iterations for the Youden index and its associated criterion value was calculated [18]. The comparison of AUROC values was performed using the procedure proposed by DeLong et al. [17]. Correlations were studied using Spearman's nonparametric correlation coefficients. The comparison of serum calprotectin concentrations according to disease activity subgroup was performed using the Kruskal-Wallis test. The comparison of the calprotectin concentration between paired plasma and serum samples was performed using the Wilcoxon test. Quantitative bioanalytical method validation for assessing the agreement between the two calprotectin assays was performed using Passing and Bablok regression [19]. Systematic differences, proportional differences, and random differences between the two methods were calculated. All the statistical analyses were conducted with MedCalc<sup>®</sup> (version 22.021, MedCalc Software Ltd., Ostend, Belgium) based on a two-sided type I error with an alpha level of 0.05.

## **Results**

#### *Description of the Studied Population*

Between December 1, 2010, and March 1, 2012, 308 visits (UC = 99 and CD = 209) were recorded for 131 IBD patients (UC = 32 and CD = 99) and were included in the study. The median age of patients at inclusion was 35 years (IQR, 28 to 49), and males represented 55% (72/131) of the total cohort. Among UC patients, the median SCCAI was 0, and clinical remission was noted in 74% (73/99) of patients. Among patients with CD, the median CDAI was 46, and remission was noted in 86% (179/209) of patients (Table 1). The hematological and biochemical parameters of the UC and CD groups were comparable, except for hs-CRP, which was significantly higher in the CD group (10.4 mg/L vs. 1.2 mg/L; *p* < 0.0001) (Table 1).

#### *Primary Objective*

##### *Ulcerative Colitis*

Among the UC patients, the median plasma calprotectin concentrations were 6.3 ng/mL and 5.4 ng/mL according to the EliA<sup>TM</sup> and Calprest<sup>TM</sup> assays, respectively (Table 2). Plasma calprotectin had good diagnostic accuracy for diagnosing remission among the UC patients. Using the EliA<sup>TM</sup> assay, a calprotectin concentration of 10.5 ng/mL had a sensitivity of 98.6%, a specificity of 100%, a PPV of 100%, a negative predictive value of 96.3%, and an AUROC of 0.999 for diagnosing remission (Table 3). In patients in clinical remission, the median plasma calprotectin concentration was 4.7 ng/mL (IQR, 3.6 to 6.8) compared to 11.1 ng/mL (IQR, 13.9 to 28.3) in

**Table 1.** Biochemical and hematological findings of UC and CD patients included in the study

|                                     | All patients (n = 131) |        |             | CD patient visits (n = 209) |        |             | UC patient visits (n = 99) |        |             |
|-------------------------------------|------------------------|--------|-------------|-----------------------------|--------|-------------|----------------------------|--------|-------------|
|                                     | n                      | median | IQR (25–75) | n                           | median | IQR (25–75) | n                          | median | IQR (25–75) |
| <b>Biochemical findings</b>         |                        |        |             |                             |        |             |                            |        |             |
| hs-CRP (mg/L)                       | 131                    | 7.9    | 1.3–15.9    | 209                         | 10.4   | 6.4–19.3    | 99                         | 1.2    | 0.6–7.2     |
| Albumin (g/L)                       | 131                    | 40.6   | 37.3–43.6   | 209                         | 40.2   | 37.0–43.0   | 99                         | 41.8   | 38.7–44.4   |
| Procalcitonin (ng/mL)               | 131                    | 0.09   | 0.07–0.12   | 209                         | 0.09   | 0.07–0.12   | 98                         | 0.09   | 0.08–0.12   |
| <b>Hematological findings</b>       |                        |        |             |                             |        |             |                            |        |             |
| Hemoglobin (g/dL)                   | 131                    | 13.9   | 13.0–14.9   | 178                         | 13.7   | 13.0–14.8   | 84                         | 14.2   | 13.3–15.0   |
| Hematocrit (%)                      | 131                    | 42.3   | 39.6–45.0   | 178                         | 42.0   | 39.5–45.0   | 84                         | 42.7   | 40.0–45.1   |
| MCV (fL)                            | 131                    | 88     | 85–92       | 159                         | 88     | 84–92       | 71                         | 89     | 87–92       |
| MCHC (g/dL)                         | 131                    | 32.9   | 32.1–33.3   | 159                         | 32.8   | 32.1–33.2   | 71                         | 33.0   | 32.1–33.8   |
| Red blood cells ( $\times 10^9$ /L) | 131                    | 4.83   | 4.43–5.16   | 159                         | 4.85   | 4.41–5.16   | 71                         | 4.79   | 4.49–5.17   |
| Leukocytes ( $\times 10^9$ /L)      | 131                    | 7.64   | 6.47–9.66   | 159                         | 7.96   | 6.42–10.04  | 71                         | 7.51   | 6.74–8.85   |
| Neutrophils ( $\times 10^9$ /L)     | 131                    | 5.08   | 3.96–6.75   | 159                         | 5.28   | 4.06–6.98   | 71                         | 4.50   | 3.21–5.61   |
| Lymphocytes ( $\times 10^9$ /L)     | 131                    | 1.99   | 1.55–2.47   | 159                         | 1.99   | 1.48–2.44   | 71                         | 2.00   | 1.74–2.78   |
| Monocytes ( $\times 10^9$ /L)       | 131                    | 0.49   | 0.38–0.61   | 159                         | 0.49   | 0.38–0.60   | 71                         | 0.51   | 0.36–0.65   |
| Eosinophils ( $\times 10^9$ /L)     | 131                    | 0.16   | 0.09–0.23   | 159                         | 0.15   | 0.09–0.22   | 71                         | 0.16   | 0.10–0.29   |
| Basophils ( $\times 10^9$ /L)       | 131                    | 0.04   | 0.03–0.06   | 159                         | 0.040  | 0.03–0.06   | 71                         | 0.04   | 0.03–0.06   |
| Platelets ( $\times 10^9$ /L)       | 131                    | 279    | 242–348     | 159                         | 287    | 247–366     | 71                         | 262    | 218–322     |

CD, Crohn's disease; CDAI, Crohn's disease activity index; CRP, C-reactive protein; SCCAI, simple clinical colitis activity index; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; IQR, interquartile range; UC, ulcerative colitis.

**Table 2.** Plasma calprotectin values in patients with UC and CD

|                                                       | Median | Range (min-max) | IQR (25–75) |
|-------------------------------------------------------|--------|-----------------|-------------|
| Plasma calprotectin, Calprest™ (manual method), ng/mL |        |                 |             |
| IBD                                                   | 9.1    | 0.0–199.3       | 3.9–16.4    |
| UC                                                    | 5.4    | 0.0–48.1        | 1.4–10.6    |
| CD                                                    | 10.8   | 0.0–199.3       | 5.6–17.1    |
| Plasma calprotectin, EliA™ (automated method), ng/mL  |        |                 |             |
| IBD                                                   | 9.9    | 1.7–231.1       | 5.1–17.6    |
| UC                                                    | 6.3    | 2.2–73.2        | 3.8–11.1    |
| CD                                                    | 11.7   | 1.7–231.1       | 6.3–20.2    |

Min, minimum; Max, maximum; IQR, interquartile range.

patients without clinical remission ( $p < 0.0001$ ) (Fig. 1a; online suppl. Table 1; for all online suppl. material, see <https://doi.org/10.1159/000545722>). There was no overlap in the plasma calprotectin concentration between these 2 patient subgroups (Fig. 1a).

#### Crohn's Disease

Among the CD patients, the median plasma calprotectin concentrations were 11.7 ng/mL and 10.8 ng/mL according to the EliA™ and Calprest™ assays, respectively (Table 2). Plasma calprotectin had poor diagnostic accuracy for diagnosing remission among the CD patients (Table 3).

#### Secondary Objectives

##### Comparison of the Diagnostic Accuracy of Plasma Calprotectin and hs-CRP

The ROC-defined optimal thresholds were used for calprotectin and hs-CRP. In the UC patients, plasma calprotectin (automated method EliA™) (AUROC = 0.993, standard error = 0.01, 95% CI: 0.951 to 1.000) had a significantly higher diagnostic accuracy than hs-CRP (AUROC = 0.933, standard error = 0.03, 95% CI: 0.864 to 0.973) for diagnosing remission, with an absolute difference between the AUROC values of 0.06 (95% CI: 0.008 to 0.113;  $p = 0.03$  for AUROC comparison) (Fig. 1b and online suppl. Table 2). In the CD visits, the diagnostic accuracy of plasma calprotectin using both techniques did not differ significantly from that of hs-CRP for diagnosing remission (online suppl. Table 3).

##### Analysis of Correlation Coefficients between hs-CRP and Plasma Calprotectin and Comparisons between UC Patients and CD Patients

In the IBD patients, plasma calprotectin was significantly correlated with hs-CRP (online suppl. Table 5). According to both calprotectin assay methods, the correlation between plasma calprotectin and hs-CRP was significantly higher in UC patients ( $\rho =$

**Table 3.** Diagnostic accuracy of plasma calprotectin and CRP for diagnosing remission in IBD patients

|                      | UC (n = 99)                            |                                         |             | CD (n = 209)                           |                                         |             |
|----------------------|----------------------------------------|-----------------------------------------|-------------|----------------------------------------|-----------------------------------------|-------------|
|                      | plasma calprotectin, EliA™ (automated) | plasma calprotectin, Calprest™ (manual) | hs-CRP      | plasma calprotectin, EliA™ (automated) | plasma calprotectin, Calprest™ (manual) | hs-CRP      |
| AUROC <sup>a</sup>   | 0.999 <sup>b</sup>                     | 0.986 <sup>b</sup>                      | 0.960       | 0.675 <sup>c</sup>                     | 0.662 <sup>c</sup>                      | 0.716       |
| SE                   | 0.001                                  | 0.01                                    | 0.02        | 0.05                                   | 0.05                                    | 0.05        |
| 95% CI               | 0.962–1.000                            | 0.939–0.999                             | 0.900–0.989 | 0.607–0.738                            | 0.594–0.726                             | 0.649–0.776 |
| Optimal threshold    | ≤10.5                                  | ≤8.1                                    | ≤5.9        | ≤10.2                                  | ≤13.3                                   | ≤11.8       |
| Sensitivity          | 98.63                                  | 87.67                                   | 90.41       | 48.04                                  | 67.6                                    | 62.01       |
| 95% CI               | 92.6–100.0                             | 77.9–94.2                               | 81.2–96.1   | 40.5–55.6                              | 60.2–74.4                               | 54.5–69.1   |
| Specificity          | 100                                    | 100                                     | 96.15       | 83.33                                  | 63.33                                   | 76.67       |
| 95% CI               | 86.8–100.0                             | 86.8–100.0                              | 80.4–99.9   | 65.3–94.4                              | 43.9–80.1                               | 57.7–90.1   |
| PPV                  | 100                                    | 100                                     | 98.5        | 94.5                                   | 91.7                                    | 94.1        |
| 95% CI               | 95.0–100.0                             | 94.4–100.0                              | 92.0–100.0  | 87.6–98.2                              | 85.6–95.8                               | 88.2–97.6   |
| NPV                  | 96.3                                   | 74.3                                    | 78.1        | 21.2                                   | 24.7                                    | 25.3        |
| 95% CI               | 81.0–99.9                              | 56.7–87.5                               | 60.0–90.7   | 14.2–29.7                              | 15.6–35.8                               | 16.7–35.5   |
| p value <sup>a</sup> | <0.0001                                | <0.0001                                 | <0.0001     | 0.0003                                 | 0.001                                   | <0.0001     |

CRP, C-reactive protein; 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value; SE, standard error. <sup>a</sup>Diagnostic accuracy calculated via ROC analysis according to DeLong et al. [17]. <sup>b</sup>Comparison of AUROC values for calprotectin accuracy between the two assays (EliA™ vs. Calprest™,  $p = 0.10$ ). <sup>c</sup>Comparison of the accuracy of the AUROC values for calprotectin between the two assays (EliA™ vs. Calprest™,  $p = 0.3$ ).

0.763) than in CD patients ( $\rho = 0.414$ ;  $p < 0.0001$  for correlation coefficient comparisons) (online suppl. Table 5; Fig. 2).

#### Comparison of Blood Concentrations of Calprotectin in Plasma and Serum

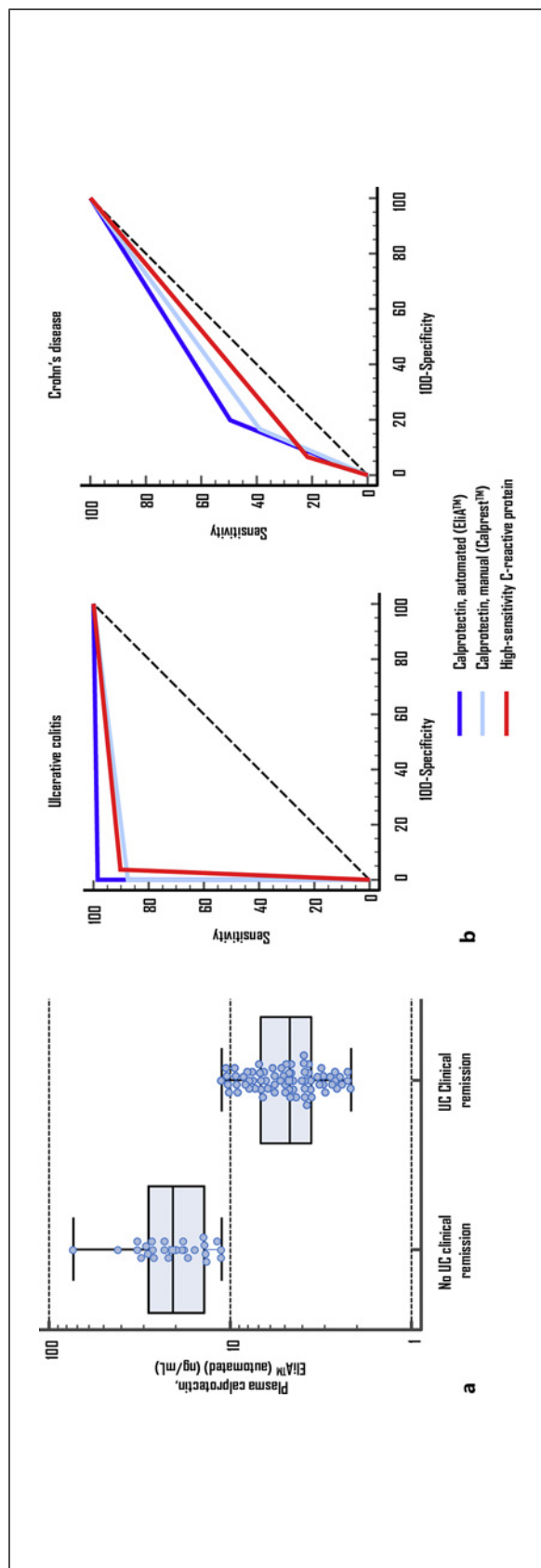
Plasma and serum calprotectin levels were measured in 19 patients with both UC and CD who had concomitant blood samples collected in dry tubes and EDTA tubes for serum and plasma preparation, respectively. These patients had a hs-CRP concentration less than 10 mg/L. The median concentration of calprotectin in the plasma samples was 7.0 ng/mL (IQR, 4.9 to 10.2; range: 2.3 to 24.8 ng/mL), whereas the median concentration of calprotectin in the serum samples was 72.5 ng/mL (IQR, 62.5 to 98.1; range: 12.2 to 254.9 ng/mL) ( $p < 0.0001$ , Wilcoxon test for paired samples). The Hodges-Lehmann median difference between serum and plasma calprotectin levels was 70.2 ng/mL (95% CI: 51.7–126.6;  $p < 0.0001$ ). Moreover, the plasma calprotectin concentrations were not correlated with those measured in serum samples ( $\rho = 0.181$ , 95% CI:  $-0.298$  to  $0.587$ ;  $p = 0.46$ ). The median serum-to-plasma calprotectin concentration ratio was 12-fold, ranging from 2- to 36-fold (Fig. 3).

#### Assessment of the Equality of Measurements from the Two Different Analytical Methods for Calprotectin Assays in Plasma Samples

Passing and Bablok regression was used to compare the two previously reported calprotectin quantification methods (Calprest™ and EliA™). The systematic difference between the two methods was 1.7 ng/mL (95% CI: 1.0 to 2.0), and the proportional difference had a slope of 1.05 (95% CI: 1.01 to 1.10). Regarding random differences, the residual standard deviation was 3.16 ( $\pm 1.96$  residual standard deviation interval:  $-6.19$  to  $6.19$ ) (online suppl. Table 4; Fig. 1A).

#### Discussion

There is an unmet need for novel blood-based biomarkers, as compliance with stool-based testing is poor [4]. Patient compliance with IBD monitoring is essential for timely detection of disease relapse and for monitoring treatment effectiveness to prevent disease progression and maximize patient quality of life. Given the strong associations seen with remission, this study showed that plasma calprotectin may represent a promising



**Fig. 1. a** Comparison of plasma calprotectin concentrations between UC patients with and without clinical remission. **b** Diagnostic accuracy of plasma calprotectin using both assays and hs-CRP for diagnosing clinical remission in patients with UC and CD (dark blue line: plasma calprotectin EliA™; light blue line: plasma calprotectin Calprest™; red line: hs-CRP).

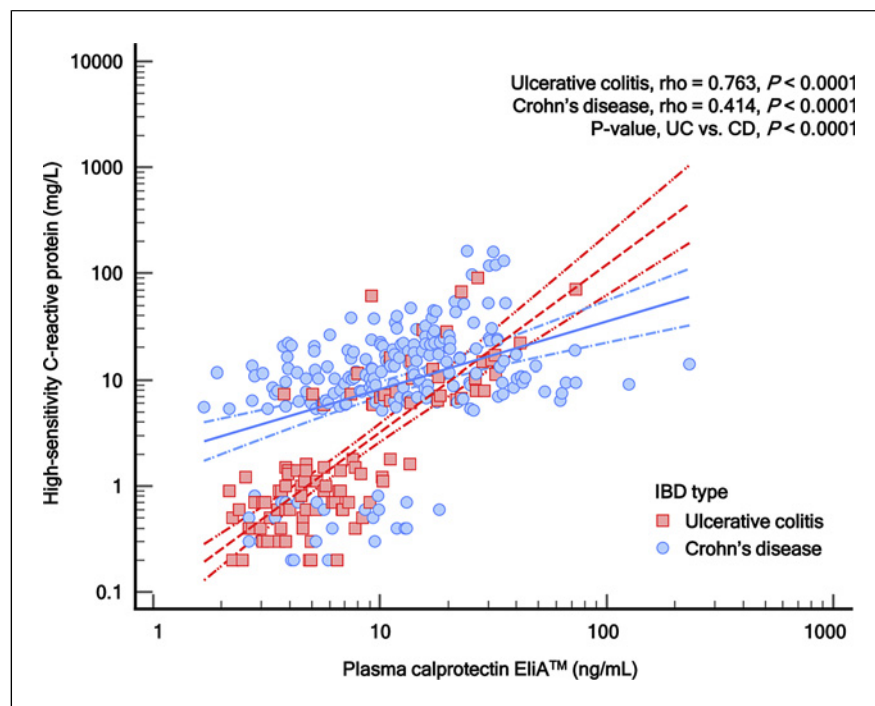
biomarker for predicting remission in patients with UC with a high diagnostic yield.

The utility of plasma and serum calprotectin in discriminating patients with active IBD from those with inactive IBD has previously been shown in a small number of studies. Only one study has reported the utility of plasma calprotectin. In a prospective study of 84 patients, Malham et al. [12] reported that plasma calprotectin levels distinguish patients with active UC from those in remission and that these levels are positively correlated with UC disease extent ( $\rho = 0.53$ ,  $p < 0.0001$ ), symptoms ( $\rho = 0.54$ ,  $p = 0.002$ ), endoscopy ( $\rho = 0.39$ ,  $p = 0.0003$ ), and histological outcomes ( $\rho = 0.28$ ,  $p = 0.01$ ). The present study showed that plasma calprotectin concentrations were not correlated with those measured in serum samples. However, Malham et al. [12] reported that plasma calprotectin is more strongly correlated with all outcome variables compared to serum calprotectin.

Data regarding the role of serum calprotectin as a biomarker are more conflicting, but several studies have shown promising results. Kalla et al. [20] prospectively derived a multi-biomarker model with both diagnostic and prognostic utility in a cohort of 177 patients. These researchers reported that serum calprotectin is strongly correlated with fecal calprotectin, and it is the strongest individual predictor of IBD compared to other blood parameters [20]. A prognostic model that combines CRP and albumin can predict treatment escalation in 65% of IBD patients (95% CI: 43–79%) and 80% of CD patients (95% CI: 31–94%) [20]. In the context of acute severe UC in a small prospective study, Hare et al. [21] showed that serum calprotectin significantly correlates with CRP ( $R^2 = 0.46$ ,  $p < 0.0001$ ) and albumin ( $R^2 = 0.12$ ,  $p = 0.023$ ), and serum calprotectin is comparable to CRP when predicting outcome. Similarly, Meuwis et al. [22] reported that serum calprotectin is significantly correlated with serum hs-CRP, and both are correlated similarly to the CDAI and significantly decreased after IFX treatment in patients with active disease. The correlation between serum and fecal calprotectin levels is weak but significant; however, while fecal calprotectin levels are correlated with endoscopic severity, serum calprotectin levels are not. Of note, other studies have not replicated these findings with serum calprotectin [10, 11].

In contrast to CD, intestinal inflammation in UC is characteristically restricted to the mucosal layer, and inflammatory infiltrates consist primarily of granulocytes during acute flare-ups and accumulate in crypt abscesses [1]. The movement of neutrophils into the colonic mucosa in UC is induced by chemokines, including IL-8 and leukotriene B4 [23]. In UC, neutrophil accumulation

**Fig. 2.** Correlation between plasma calprotectin and hs-CRP in patients with UC and CD. The line of equality (light brown) and LOESS (local regression smoothing) trendlines for UC (dotted brown line) and CD (blue line) patients are shown. The degree of smoothing is controlled by the span (%), which is the proportion (expressed as a percentage) of the total number of points contributing to each local fitted value. Larger values result in smoother trendlines.

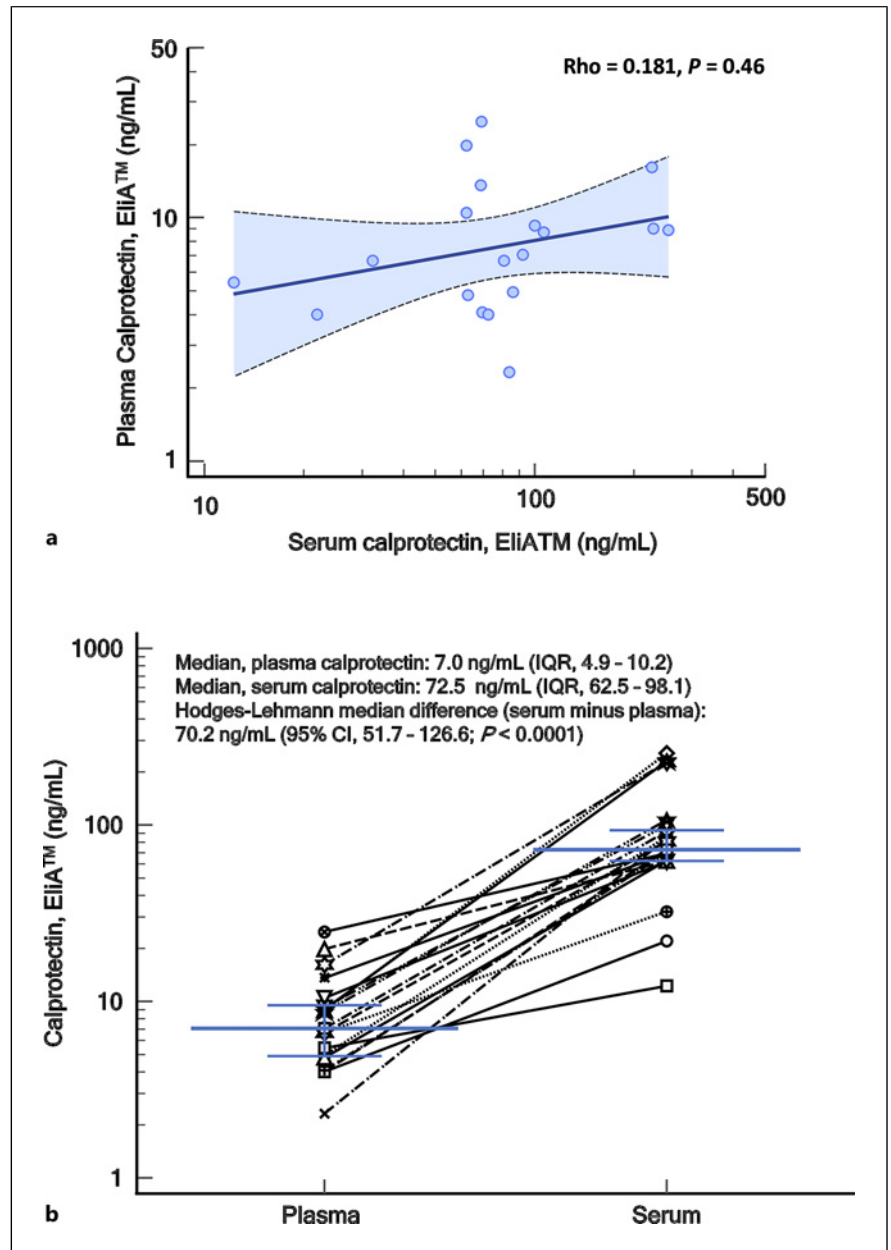


within epithelial crypts and in the intestinal mucosa directly correlates with clinical disease activity and epithelial injury [24]. In the present study, the correlation between hs-CRP and plasma calprotectin was significantly greater in UC patients than in CD patients, which may explain granulocytes being primarily involved in the pathogenesis of UC but not in that of CD [1]. Consistently, a recent meta-analysis of 13 studies has shown that fecal calprotectin is a reliable marker for assessing IBD activity and has a greater ability to evaluate disease activity in UC patients than in CD patients [25]. Furthermore, a previous study of 79 IBD patients followed for 12 months has shown that fecal calprotectin is an even stronger predictor of clinical relapse in UC patients than in CD patients [26].

During blood coagulation, neutrophils are activated and release the contents of their granules, including calprotectin, elastase, and lactoferrin [27]. In the present study, the median serum-to-plasma calprotectin concentration ratio was approximately 12-fold (range 2- to 36-fold). These differences were observed in paired samples collected simultaneously from the same patients, thereby minimizing the influence of clinical confounders such as medication use, disease duration, or sampling variability. We interpret this consistent discrepancy as primarily attributable to a pre-analytical artifact related to the biological processes involved in serum preparation. Specifically, neutrophil

degranulation during clotting has been shown to release intracellular calprotectin into the serum, leading to artificial elevation of its measured concentration. Recent studies have demonstrated that plasma calprotectin more accurately reflects in vivo levels of inflammation and avoids this clotting-induced bias. For example, in patients with UC, plasma calprotectin has been shown to better correlate with intestinal inflammation than serum calprotectin, highlighting its superior analytical validity [12]. Additional data further support that plasma calprotectin reflects true circulating levels, as intracellular stores within leukocytes are released into the serum during coagulation, significantly inflating serum concentrations [28, 29].

The measurement of plasma calprotectin may represent a potential biomarker for therapeutic monitoring of patients with UC. Plasma, rather than serum, should be used for calprotectin quantification to avoid underestimation due to neutrophil degranulation during clot formation. Plasma calprotectin offers several potential advantages in the routine monitoring of patients with IBD, including simplified sample handling, improved standardization, and better patient adherence compared to fecal calprotectin. Additionally, unlike fecal sampling, plasma collection is less invasive, more acceptable to patients, and more compatible with centralized testing workflows. IBD monitoring, particularly during drug development, requires an accurate, reproducible, and valid biomarker. In IBD, disease activity



**Fig. 3. a** Correlation between plasma and serum calprotectin concentrations in paired samples collected concomitantly from patients with IBD. **b** Comparison of calprotectin concentrations between plasma and serum samples collected concomitantly from patients with IBD.

biomarkers consist of a wide variety of substances found in blood, stool, or urine [3]. These biomarkers correlate with intestinal inflammation and act as surrogates for clinical endpoints. The main advantages of using biomarkers are reproducibility, ease of statistical handling as a continuous variable, and consistent measurement of treatment response.

There are other biomarkers in IBD that appear promising for monitoring therapeutic response. Increased levels of leucine-rich alpha-2 glycoprotein (LRG), which is predominantly derived from leucocytes, intestinal epithelial cells, and hepatocytes, have been found in

IBD patients with clinically and endoscopically active disease [3, 30–32]. While for UC, LRG was not able to outperform fecal calprotectin, there are some data showing superiority over CRP in CD [3, 30–33]. Similarly, anti-integrin  $\alpha\beta6$  autoantibodies may also serve as novel blood-based biomarkers for the diagnosis and monitoring of disease activity in IBD and could play a role in future decision-making [34–37].

The present study had several limitations. First, the results were obtained from a single center, indicating the need for verification in independent cohorts. Second, the absence

of direct correlation data with endoscopic activity or fecal calprotectin – both recognized as validated surrogates for mucosal healing – represents a key limitation of our study. Although objective evaluation was performed, endoscopy was not performed in patients at the time of their inclusion. In patients with UC, however, the SCCAI does have a specificity of 96% and a PPV of 94% for detecting endoscopically active disease [38]. The lack of fecal calprotectin also hindered comparison to serum and plasma calprotectin. Third, this study only recruited IBD patients receiving anti-TNF therapy; however, the primary aim of this study was to assess the diagnostic accuracy of plasma calprotectin as a potential biomarker of remission, a highly desirable goal in patients treated with biologics. Fourth, clinical remission was used as a gold standard in this study, which arguably should be definable without biomarker assistance for face-to-face assessments. Biomarkers aimed at identifying clinical remission may have limited utility outside remote monitoring by healthcare professionals, which is not feasible solely through blood tests. Finally, as we did not systematically capture the Montreal classification (for UC and CD) at enrolment, we were unable to perform a sensitivity analysis stratified by disease location, which is important as disease distribution may influence biomarker performance.

## Conclusion

Plasma calprotectin may represent a simple and noninvasive biomarker for monitoring disease activity in UC patients receiving infliximab. The data derived from this study are promising and warrant further detailed exploration and validation in large multicenter cohorts.

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## Statement of Ethics

The Nancy IBD cohort has been reported to the French National Commission for Data Protection and Liberties (CNIL No. 1404720), which supervises the protection of individuals regarding the processing of personal data. The present study was approved by the University Hospital of Nancy Ethics Committee (protocol ID, Version No. 1 of 08/02/2010). Written informed consent was provided for study enrolment, and the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

## Conflict of Interest Statement

S.H. has served as a speaker, a consultant, and/or an advisory board member for Pfizer, Janssen, AbbVie, Lilly, Banook, and Takeda, and has received travel grants from Ferring and Pharmacosmos. L.P.-B. reports consulting fees from AbbVie, Abivax, Adacyte, Alimentiv, Amgen, Applied Molecular Transport, Arena, Banook, Biogen, BMS, Celltrion, Connect Biopharma, Cytoki Pharma, Entera, Ferring, Fresenius Kabi, Galapagos, Genentech, Gilead, Gossamer Bio, GSK, IAC Image Analysis, Index Pharmaceuticals, Inotrem, Janssen, Lilly, Medac, Mopac, Morphic, MSD, Nordic Pharma, Novartis, Oncodesign Precision Medicine, ONO Pharma, OSE Immunotherapeutics, Pandion Therapeutics, Pfizer, Prometheus, Protagonist, Roche, Samsung, Sandoz, Sanofi, Takeda, Telavant, Theravance, Thermo Fisher, TiGenix, Tillotts, Viatrix, VectivBio, Ventyx, and YSOPIA; grant support from Celltrion, Fresenius Kabi, Medac, MSD, and Takeda; lecture fees from AbbVie, Amgen, Arena, Biogen, Celltrion, Ferring, Galapagos, Genentech, Gilead, Janssen, Lilly, Medac, MSD, Nordic Pharma, Pfizer, Sandoz, Takeda, Tillotts, and Viatrix; and travel support from AbbVie, Amgen, Celltrion, Connect Biopharma, Ferring, Galapagos, Genentech, Gilead, Gossamer Bio, Janssen, Lilly, Medac, Morphic, MSD, Pfizer, Sandoz, Takeda, Thermo Fisher, and Tillotts. All other authors declare no competing interests.

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## Author Contributions

S.H. interpreted the data and drafted the manuscript; A.O., I.A.-G., and L.P.-B. developed the study concept and design, acquired the data, interpreted the data, drafted the manuscript, critically revised the manuscript for important intellectual content, and approved the final draft; A.O. performed the statistical analyses; S.S.: acquired the data, critically revised the manuscript for important intellectual content, and approved the final draft; J.F. and J.P. performed the biochemical assays, critically revised the manuscript for important intellectual content, and approved the final draft; L.P.-B.: critically revised the manuscript for important intellectual content and approved the final draft; L.P.-B. and A.O. enrolled patients in the study.

## Data Availability Statement

Data are available on reasonable request. The data that support the findings of this study are not publicly available due to information that could compromise the privacy of research participants but are available from the corresponding author S.H. upon reasonable request (sailish.honap@kcl.ac.uk).

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