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Assessing the correlation between fecal calprotectin, blood markers and disease activity in pediatric inflammatory bowel disease

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Abstract

Background Crohn's disease (CD) and ulcerative colitis (UC) are the 2 main types of inflammatory bowel disease (IBD), a chronic inflammatory condition of the gastrointestinal tract. Management of IBD necessitates frequent clinical monitoring, including blood tests and occasionally endoscopy. Fecal calprotectin (FC) is a non-invasive measurement of luminal inflammatory activity, and can therefore be used as a useful monitoring tool. This study aimed to assess the relationship between FC, IBD activity indices and the commonly used blood markers in pediatric IBD.

Methods Electronic patient records were accessed to retrospectively collect patient data from a tertiary pediatric hospital from 2015-2021. CD and UC disease activity was quantified using the Pediatric CD Activity Index (PCDAI) and Pediatric UC Activity Index (PUCAI), respectively. The Paris classification was used for phenotype identification.

Results A total of 208 patients were included in the study, 115 with CD (18% <10 years and 82% 10-17 years) and 93 with UC (32% <10 years and 68% 10-17 years). There was a positive correlation between FC and PCDAI (r_s=0.546, P<0.001) and between FC and PUCAI (r_s=0.485, P<0.001). FC and activity indices were correlated positively with inflammatory markers/platelets and negatively with albumin and hemoglobin. FC correlated positively with PCDAI in all CD phenotypes, including isolated ileal disease.

Conclusion In pediatric IBD, FC shows a positive correlation with the clinical picture and blood markers in all disease phenotypes, and can provide an accurate non-invasive measure of disease activity.

Keywords Inflammatory bowel disease, fecal calprotectin, Pediatric Crohn's Disease Activity Index, Pediatric Ulcerative Colitis Activity Index

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Conflict of Interest: None

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Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal system, characterized by changes in bowel habit, abdominal pain, bloody stools, and extra-intestinal symptoms [1]. IBD comprises Crohn's disease (CD), ulcerative colitis (UC), and unclassified cases (IBDU). CD tends to involve the terminal ileum and colon, though it may affect the entire gastrointestinal tract, whereas UC is characterized by inflammation of the colon exclusively; IBDU has no characteristic features of either CD or UC.

Endoscopy is essential in both the initial diagnosis and the ongoing management of IBD. However, it is rather invasive and can be physically and emotionally challenging for pediatric patients and their families, particularly given the importance of bowel preparation for an effective colonoscopy [2-4]. The development of non-invasive monitoring methods for IBD

may minimize the frequency of colonoscopies in pediatric patients.

Fecal calprotectin (FC) is an antimicrobial protein found in abundance in neutrophil cytoplasm, and is released in the faeces in amounts proportional to the degree of neutrophil influx to the gut [5,6]. Consequently, levels of FC can be a useful measure of current inflammation of the gut; while this is informative for IBD-related investigations, high FC is not specific to IBD [7]. Nevertheless, FC serves as a useful investigatory tool with a high sensitivity and moderate specificity for IBD [8], but one that must be interpreted with care in younger patients, as healthy infants and young children have been shown to have elevated FC when compared to older children and adults [9-11].

Clinically, FC is a useful diagnostic marker for pediatric IBD, showing a positive correlation with both clinical disease activity and endoscopic scoring systems for both CD and UC, although the reported strength of this correlation varies in the literature [6-8,12,13]. Discrepancy also exists surrounding the normal values for pediatric FC [7,14]. Further studies are required, both to ascertain the efficacy of FC as a monitoring tool for pediatric IBD, and to determine what level of FC can be considered as "normal" for patient populations, improving IBD disease monitoring for pediatric patients.

This study aimed to quantify how strongly FC measurements correlated with disease activity and endoscopy findings in pediatric IBD. The project was registered with our institution clinical governance board as an audit to review clinical practice and a full ethical review was not required.

Materials and methods

Electronic patient records of pediatric patients (<18 years old) with a diagnosis of IBD between 2015 and 2021 at the Evelina London Children's Hospital in London were accessed and reviewed. Patient characteristics, FC measurements, blood test results, IBD activity indices and endoscopy results were retrieved and input to a central database.

Because of variation among the laboratories performing the FC tests, some measurements were represented as being above a certain concentration. For the purposes of data analysis, in these circumstances the lower limit of the range was taken (e.g., >1800 μg/mg was interpreted as 1800 μg/mg). All FC results included in this cohort were obtained from laboratories using enzyme-linked immunosorbent assays (ELISA) to measure FC. In our region, all primary care samples were sent to hospital laboratories for analysis.

To quantify clinical IBD disease activity, the Pediatric CD Activity Index (PCDAI) [15] and the Pediatric UC Activity Index (PUCAI) [16] were used for CD and UC, respectively. The phenotype of each patient's IBD disease was classified using the Paris classification [17]. Because of difficulties in accessing some patients' long-term records, the "Growth" factor for the CD Paris classification was not included in this study. We excluded children with incomplete medical records and children with a diagnosis of IBDU, in view of the absence of a phenotype-specific clinical and endoscopic scoring system.

Statistical analysis

Each FC value was matched with the relevant activity index calculated at the closest point in time possible, and the data were only included in the analysis if FC was measured within 14 days of the assessment. Spearman's rank correlations were used to assess the relationship between calprotectin values and activity indices. The significance level was set at $P<0.05$. Data analysis was carried out using Microsoft Excel and IBM SPSS Statistics 27.

Results

A total of 208 patients were included in the study, 115 with a CD diagnosis (35% females, mean age at diagnosis 14±3.8 years) and 93 (46% females, mean age at diagnosis 12±3.6 years) with a UC diagnosis. Children were treated according to standard medical therapy. Of the CD children, all were on azathioprine, 15 (13%) on infliximab and 15 (13%) on adalimumab, 4 (3.5%) on vedolizumab, 1 was on Ustekinumab, and 1 was on tofacitinib. Of the UC children, all were on 5-aminosalicylate, 41 (44%) on azathioprine, 15 (16%) on infliximab, and 15 (16%) on adalimumab. No child in this cohort had luminal surgery. Demographic profiles and details of the Paris classifications based on the IBD diagnosis can be seen in Tables 1 and 2, respectively.

The full Paris classification was not designated for 23 of the CD patients and 29 of the UC patients, either because no colonoscopy report was available, or because insufficient detail

Table 1 Paris classification of patients with Crohn's disease included in this study

Age at diagnosis	
A1a (0 to <10 years)	20 (17.7%)
A1b (10 to <17 years)	93 (82.3%)
A2 (17 to 40 years)	0 (0%)
Disease location	
L1 (distal 1/3 ileum ± limited caecal disease)	21 (24.7%)
L2 (colonic)	18 (21.2%)
L3 (ileocolonic)	46 (54.2%)
L4a (upper disease proximal to ligament of Treitz)	73 (97.3%)
L4b (upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum)	1 (1.3%)
L4ab (upper disease both proximal and distal to ligament of Treitz)	1 (1.3%)
Disease behaviour	
B1 (non-stricturing, non-penetrating)	85 (97.7%)
B2 (stricturing)	2 (2.3%)
B3 (penetrating)	0 (0%)
B2B3 (stricturing and penetrating)	0 (0%)
p (perianal disease modifier)	33 (36.7%)

Percentage values are relative to the number of patients with a value for that category. Because of the partially completed classifications, this number may differ between categories

was provided. For similar reasons, the full Paris classification was only partially completed for 24 CD patients and 1 UC patient. This resulted in 68 CD patients and 63 UC patients with complete Paris classifications.

Of the 92 CD patients who had a fully or partially designated Paris classification, 28 had multiple available colonoscopy reports. Of these, 14 patients had a different designated Paris classification on a subsequent colonoscopy relative to their original classification. Seven patients moved from L3 to L2, 5 from L3 to L1, a single patient moved from L1 to L3, and a single patient moved from L2 to L3.

FC values were collected for each CD and UC patient, including both results recorded directly by the Evelina London Children's Hospital, and those received via external correspondence from primary care and other healthcare professionals. For 14 CD and 23 UC patients, no FC value was included in their electronic patient records; 16 CD and 12 UC patients had only 1 recorded FC value, while 44 CD and 32 UC patients had 5 or more recorded FC values.

Table 2 Age at diagnosis and Paris classification of patients with ulcerative colitis (UC) included in this study

Age at diagnosis	
0 to <10 years	29 (31.5%)
10 to <17 years	63 (68.5%)
Disease extent	
E1 (ulcerative proctitis)	4 (6.3%)
E2 (left-sided UC distal to splenic flexure)	9 (14.1%)
E3 (extensive, hepatic flexure distally)	2 (3.1%)
E4 (pancolitis, proximal to hepatic flexure)	49 (76.6%)
Disease severity	
S0 (never severe)	60 (95.2%)
S1 (severe)	3 (4.8%)

S1 is classified as having at least 1 Pediatric UC Activity Index score ≥65 [16]. Percentage values are relative to the number of patients with a value for that category. Because of the partially completed classifications, this number may differ between categories. One UC patient had an unknown age at diagnosis

The correlation between FC and IBD activity index was assessed by matching each FC with the closest calculated relevant activity index score, and the data were only included in this study if FC measurements were obtained within 14 days of the assessment. For children with CD, the median PCDAI was 10 (interquartile range [IQR] 17.5) and the median FC was 445 (IQR 936). For UC, the median PUCAI was 15 (IQR 30) and the median FC was 393 (IQR 1022). Fig. 1 depicts the relationship between PCDAI and FC for CD patients. Using Spearman's rank correlation, a positive correlation was seen between PCDAI and FC (r_s=0.497, P<0.001, n=398). This correlation was stronger when only values that were measured within 14 days of each other were included (r_s=0.546, P<0.001, n=306).

The correlation between PCDAI and FC was strongest for patients designated L2 in the Paris classification (r_s=0.697, P<0.001, n=63), followed by L3 (r_s=0.540, P<0.001, n=144) and finally L1 (r_s=0.467, P<0.001, n=66).

Similarly, Fig. 2 demonstrates a positive relationship between PUCAI and FC for UC patients (r_s=0.454, P<0.001, n=250). This correlation was also stronger when only PUCAI and FC values measured within 14 days of one another were included (r_s=0.485, P<0.001, n=199).

Using Spearman's rank correlation, FC values and IBD activity index were also compared to various blood markers, where available (Tables 3 and 4). Generally, FC and IBD activity index correlated positively with erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cell and platelet counts, and correlated negatively with hematocrit, albumin, and hemoglobin (Hb) levels.

Discussion

In this cohort, there was a moderate correlation between both PCDAI and PUCAI and FC measured within 14 days of each other (r_s=0.546 and r_s=0.485 for CD and UC patients,

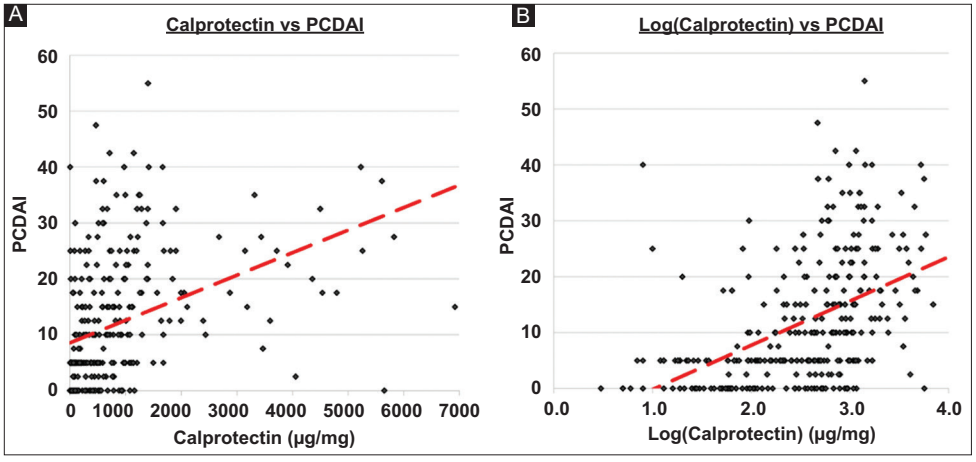

Figure 1 The relationship between fecal calprotectin (FC) and Pediatric Crohn's Disease Activity Index (PCDAI). Matched FC and PCDAI values that were measured within 14 days of each other were included. A linear trendline (red) is included for both graphs. (A) FC plotted against PCDAI. (B) Log_{10}(FC) plotted against PCDAI. r_s=0.546, P<0.001, n=306

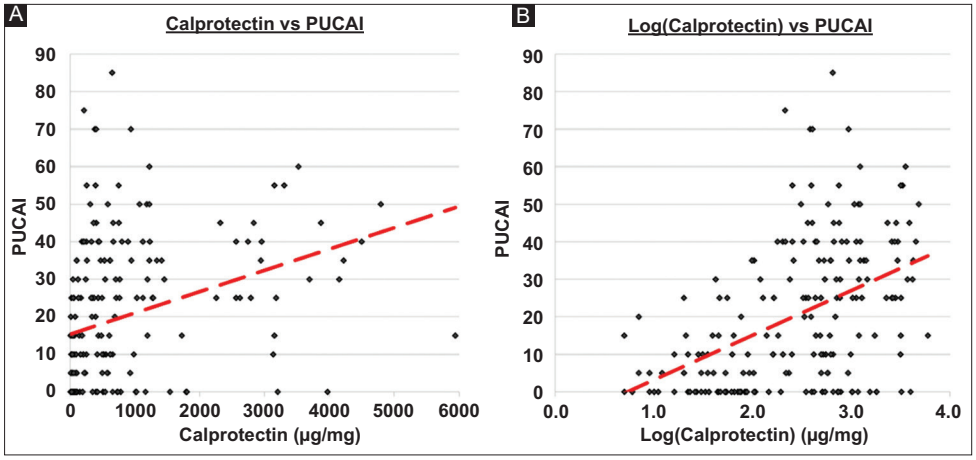

Figure 2 The relationship between fecal calprotectin (FC) and Pediatric Ulcerative Colitis Activity Index (PUCAI). Matched FC and PUCAI values that were measured within 14 days of each other were included. A linear trendline (red) is included for both graphs. (A) FC plotted against PUCAI. (B) Log_{10}(FC) plotted against PUCAI. r_s=0.485, P<0.001, n=199

Table 3 Values of FC and PCDAI vs. blood markers in patients with CD

PCDAI vs.	r_s	P-value	N
Hematocrit	-0.314	<0.001	303
ESR	0.485	<0.001	274
Albumin	-0.402	<0.001	297
CRP	0.54	<0.001	300
Hb	-0.353	<0.001	303
WBC	0.299	<0.001	303
Lymphocytes	-0.032	0.58	303
Platelets	0.433	<0.001	303
FC vs.	r_s	P-value	N
Hematocrit	-0.283	<0.001	246
ESR	0.482	<0.001	223
Albumin	-0.419	<0.001	242
CRP	0.584	<0.001	246
Hb	-0.354	<0.001	247
WBC	0.308	<0.001	247
Lymphocytes	-0.085	0.182	247
Platelets	0.518	<0.001	248

FC values were only correlated with blood markers if measured within 14 days of each other

FC, fecal calprotectin; CD, Crohn's disease; PCDAI, Pediatric CD Activity Index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; Hb, hemoglobin; WBC, white blood cells

Table 4 Values of FC and PUCAI vs. blood markers in patients with UC

PUCAI vs.	r_s	P-value	N
Hematocrit	-0.214	0.002	208
ESR	0.245	<0.001	179
Albumin	-0.271	<0.001	207
CRP	0.262	<0.001	205
Hb	-0.194	0.005	209
WBC	0.14	0.46	205
Lymphocytes	0.015	0.832	209
Platelets	0.352	<0.001	202
FC vs.	r_s	P-value	N
Hematocrit	-0.299	<0.001	162
ESR	0.355	<0.001	138
Albumin	-0.405	<0.001	161
CRP	0.307	<0.001	159
Hb	-0.306	<0.001	163
WBC	0.116	0.145	159
Lymphocytes	-0.081	0.307	162
Platelets	0.304	<0.001	156

FC values were only correlated with blood markers if measured within 14 days of each other

FC, fecal calprotectin; UC, ulcerative colitis; PUCAI, Pediatric UC Activity Index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; Hb, hemoglobin; WBC, white blood cells

respectively). The distribution of our patient cohort across the Paris classification categories is relatively similar to that seen in the cohort studied by Hoekman *et al* [13].

This study supports recent works in the literature that suggest FC as a useful biomarker for disease activity in pediatric IBD. Both Krzesiek and Hoekman *et al* found a positive correlation between FC and PCDAI and PUCAI [13,18], while Komraus *et al* reported an association between FC and PCDAI, in addition to alternative scoring systems for UC [19]. El-Matary *et al* found a strong correlation between FC and clinical activity indices, with positive FC measurements resulting in greater levels of treatment escalation compared to negative measurements [20]. FC has also been reported to correlate well with both the Simple Endoscopic Score for CD (SES-CD) and the Matts score for UC in a pediatric population [6], suggesting that FC is a useful tool for the care of pediatric IBD patients,

and can help to facilitate decision-making in conjunction with other clinical parameters.

Our results showed a stronger correlation when FC was measured within 2 weeks of a calculated activity index, suggesting a good association between luminal inflammation and clinical symptoms. Interestingly, FC was also correlated with activity indices even when they were measured up to 4 weeks apart, FC can thus be used as an accurate additional non-invasive and objective test to allow a comprehensive IBD assessment.

In children with CD, our findings revealed a stronger correlation between FC and the activity indices in children with colonic disease (L2), reflecting a high degree of fecal neutrophil shedding when the inflammation is confined to the colon, but isolated ileal inflammation (+/- limited cecal inflammation), as seen in L1, also appeared to elicit a significantly positive correlation between FC and PCDAI; however, the number of children and parameters measured within these cohorts was relatively small.

In this cohort, platelet count correlated strongly with both FC and the activity index. Thrombocytosis in response to inflammation is a recognised explanation for the increase in platelet counts in IBD patients, as has been noted in several studies [21,22]. Although a combination of platelet count, albumin, CRP, ESR and FC as holding diagnostic value in pediatric IBD has previously been discussed [23-25], we have reported on a detailed correlation between individual blood markers (Hb, ESR, CRP, albumin, hematocrit, platelets, white blood cell and lymphocyte counts) and FC and activity indices for both CD and UC, as detailed in Tables 3 and 4. In children with CD, PCDAI was strongly positively correlated with CRP and ESR (r_s=0.54, P<0.001 and r_s=0.49, P<0.001), respectively, followed by platelets (r_s=0.43, P<0.001), and was negatively correlated with albumin (r_s=0.40, P<0.001) and Hb (r_s=-0.35, P<0.001). FC was strongly positively correlated with CRP and ESR (r_s=0.58, P<0.001 and r_s=0.48, P<0.001, respectively), followed by platelets (r_s=0.52, P<0.001), and was negatively correlated with albumin (r_s=-0.42, P<0.001) and Hb (r_s=-0.35, P<0.001). Interestingly, in children with UC, the association between PUCAI and the blood markers was less pronounced, with only platelets showing a strong correlation (r_s=0.35, P<0.001), while FC had a positive correlation with ESR (r_s=0.36, P<0.001), CRP (r_s=0.31, P<0.001) and platelets (r_s=0.30, P<0.001), and was negatively correlated with albumin (r_s=-0.41, P<0.001) and Hb (r_s=-0.31, P<0.001).

This study had some limitations: a retrospective design based on a number of clinical databases may affect the consistency of reported data, but the authors reviewed all individual patient data to ensure consistency and validity. Unfortunately, attempts to quantify endoscopy findings using the SES-CD [26] and the Mayo Score for UC [27] were generally unsuccessful, because of insufficient information on endoscopy reports, but the association between endoscopic scoring and FC was reported previously [18,19]. Finally, full Paris classifications were not available for the whole cohort, so that only the included values were analyzed.

To conclude, this study assessed the relationship between serial FC measurements and IBD disease activity for a pediatric IBD cohort. A positive association between FC and both PCDAI and PUCAI was demonstrated for CD and UC patients. The correlation was stronger when FC was measured within 2 weeks of the activity indices. This study also found significant correlations between FC, activity indices and various blood markers. FC can be used alongside select blood markers and validated activity indices to provide a non-invasive comprehensive assessment of children with IBD.

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Summary Box

What is already known:

- Fecal calprotectin (FC) can be used to identify gastrointestinal luminal inflammation in children with inflammatory bowel disease (IBD)
- FC can be useful to monitor luminal inflammation in IBD

What the new findings are:

- FC was strongly correlated with activity indices in all pediatric Crohn's disease and ulcerative colitis phenotypes
- FC was strongly correlated with albumin, C-reactive protein, erythrocyte sedimentation rate, and platelet count
- FC can accurately be used for longitudinal monitoring of children with IBD

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