



Original article

Plasma calprotectin as a biomarker of inflammatory activity in ulcerative colitis



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ABSTRACT

Background: An ideal test to evaluate the inflammatory burden in ulcerative colitis is still an unmet need. Fecal calprotectin (FCP) and C-reactive protein (CRP) have significant limitations. Plasma calprotectin (PC) seems to be promising in inflammatory diseases, but its value in IBD is still to be determined. Our aim was to assess whether PC correlates with inflammatory activity in UC.

Methods: Prospective single center cohort study. Consecutive patients previously diagnosed with UC undergoing endoscopy were included (June 2021–September 2022). Demographic, clinical, analytical (CRP, PC and FCP), endoscopic and histologic data was collected at the time of colonoscopy. PC was assessed with Gentian Calprotectin Immunoassay and, in a subgroup of patients, also with QUANTA Flash Circulating Calprotectin from INOVA.

Results: Inclusion of 98 patients (60.2% male) with a median age 49 (38–61) years. The extent of colitis was distal in 12 (12.2%), left-sided in 49 (50%), and extensive in 37 (37.8%). Mesalazine was taken by 65 (66.3%) patients, with biologic monotherapy used in 24 (24.5%) and combination therapy in 6 (6.1%). Clinical, endoscopic and histological remission were detected, in 56 (57.1%), 48 (49%) and in 55 (56.1%) patients, respectively.

Comparing MES 0/1 vs MES 2/3, a statistically significant difference was found with PC, CRP and FCP. Concerning endoscopic (MES = 1) and histological (GS < 2) remission, FCP was the only biomarker able to detect these outcomes. PC (Gentian) and PCi (INOVA) were highly correlated with CRP.

Conclusion: PC has low value in distinguishing patients in remission from patients with endoscopic or histologic activity in UC. This essential role must continue to be played by FCP.

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La calprotectina plasmática como biomarcador de actividad inflamatoria en la colitis ulcerosa

RESUMEN

Palabras clave:

Calprotectina plasmática

Calprotectina fecal

Colitis ulcerosa

Enfermedad inflamatoria intestinal

Introducción: Existe la necesidad de disponer de un examen ideal para evaluar la carga inflamatoria en la colitis ulcerosa (CU). La calprotectina fecal (FCP) y la proteína C reactiva (PCR) presentan limitaciones significativas. La calprotectina plasmática (PC) parece prometedora en enfermedades inflamatorias, pero su valor en la enfermedad inflamatoria intestinal aún está por determinarse. Nuestro objetivo fue valorar si la PC se correlaciona con la actividad inflamatoria en la CU.

Métodos: Estudio de cohortes prospectivo en un solo centro. Se incluyeron pacientes consecutivos previamente diagnosticados de CU mediante endoscopia (junio 2021–septiembre 2022). Se recopilaron datos demográficos, clínicos, analíticos (PCR, PC y FCP), endoscópicos e histológicos en el momento de la colonoscopia. Se evaluó la PC con el inmunoensayo de calprotectina Gentian y, en un subgrupo de pacientes, también con QUANTA Flash Circulating Calprotectin de INOVA.

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Resultados: Un total de 98 pacientes fueron incluidos (60,2% hombres), con una edad media de 49 (38–61) años. La extensión de la colitis fue distal en 12 (12,2%), izquierda en 49 (50%) y extensa en 37 (37,8%). Mesalazina fue tomada por 65 (66,3%) pacientes, con monoterapia biológica en 24 (24,5%) y terapia combinada en 6 (6,1%). Se detectó remisión clínica, endoscópica e histológica, respectivamente, en 56 (57,1%), 48 (49%) y 55 (56,1%) pacientes.

Al comparar MES 0/1 vs. MES 2/3, se encontró una diferencia estadísticamente significativa con PC, PCR y FCP. En cuanto a la remisión endoscópica (MES = 1) e histológica (GS ≤ 2), la FCP fue el único biomarcador capaz de detectar estos resultados. PC (Gentian) y PC (INOVA) mostraron una alta correlación con la PCR. **Conclusión:** La PC tiene poco valor para distinguir a los pacientes en remisión de los pacientes con actividad endoscópica o histológica en la CU. Este papel esencial debe seguir siendo desempeñado por la FCP.

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Introduction

Inflammatory bowel disease (IBD) is a chronic condition with a remitting pattern. Although the incidence of IBD has reached a plateau in developed countries, the prevalence of both ulcerative colitis (UC) and Crohn's disease (CD) is increasing, being thought to surpass 0.3% globally.¹ In spite of this, both the diagnosis and the monitoring of relapses lack a non-invasive gold standard.² Evaluation of inflammatory activity in IBD is performed with a combination of clinical, biochemical, endoscopic, imagological and histologic parameters.² Regarding monitoring of activity, endoscopic evaluation is still considered the point of reference. Nevertheless, colonoscopy is invasive, not extensively available, expensive and painful, requiring sedation in a wide proportion of patients. As far as biochemical biomarkers are concerned, inflammatory markers as C-reactive protein (CRP) and albumin may be raised or diminished, respectively, but in the majority of mild to moderate relapses, these biomarker's levels are within normal ranges. Additionally, albumin and CRP are not specific of intestinal inflammation and many concomitant conditions may lead to their alteration.³

However, likely due to the transmural inflammation, CRP has demonstrated better accuracy CD patients compared to those with ulcerative colitis (UC). There is a lack of a reliable biomarker for the non-invasive assessment of UC activity.⁴

Fecal calprotectin (FCP) is the biomarker with the higher sensitivity regarding the detection of ileocolonic inflammation. Moreover, FCP has revealed a good correlation with endoscopic disease activity as demonstrated in two recent meta-analysis.^{5,6} However, this biomarker has some limitations such as intra and interindividual variability and may be influenced by some medications like non-steroidal anti-inflammatory drugs and proton-pump inhibitors.⁷ Additionally, the sampling process is difficult and uncomfortable to many patients which can lead to a suboptimal disease monitoring. To overcome these limitations serum and plasma calprotectin have been studied. Calprotectin is a calcium-binding protein mostly found in neutrophils. The elevation of this protein functions as a signal of inflammatory activity mainly induced by neutrophils.⁸ Systemic calprotectin has been claimed as a good biomarker in inflammatory diseases such as rheumatoid arthritis both in the diagnosis and in monitoring disease activity.^{9–11} However, the value of these protein in IBD is still to be determined. Various works have tried to evaluate both serum and plasma calprotectin in IBD, with conflicting results.^{12–17} As a matter of fact, plasma calprotectin (PC) seems more accurate in the assessment of inflammatory activity in systemic conditions such as rheumatoid arthritis.¹⁸ This results from the fact that calprotectin is released in the serum during the coagulation cascade when creating serum, leading to increased variability in the evaluations and possibly to overestimation of levels. Additionally, plasma levels of calprotectin are more stable during the storage processing than serum ones.¹⁹

Moreover, a recent study has revealed that PC seems to be more accurate predicting intestinal inflammatory activity than the serum protein.²⁰ We thus aim to assess whether PC correlates with inflammatory activity in ulcerative colitis (UC).

Methods

Study design

We conducted a prospective single center cohort study in a tertiary hospital. Consecutive patients with UC undergoing endoscopic evaluation between June 2021 and September 2022 were included. All the patients had a previous diagnosis of UC. Patients with consumption of non-steroidal anti-inflammatory drugs in the previous 6 months or with concomitant other immune-mediated diseases were excluded from the study. Baseline information collected included: gender, age, duration and extension of the disease, smoking habits and current and past therapies for IBD. Moreover, clinical, analytical (CRP, PC and FCP), endoscopic (Mayo endoscopic subscore (MES) and Ulcerative Colitis Endoscopic Index of Severity (UCEIS)) and histologic (Geboes score (GS)) data was prospectively collected at the time of colonoscopy.

Clinical remission was defined according the Adapted Mayo score: stool frequency ≤ 1 and rectal bleeding = 0.

To address potential sources of bias, consecutive patients referred to colonoscopy were included, managing to have patients both in active stages and in remission in the study.

Laboratory assessment of biomarkers

FC was performed by Fluorimetric Enzyme-Lined Immunoassay (FEIA) using ImmunoCap 250® from Thermo Fisher Scientific®.

PC was assessed with Gentian Calprotectin Reagent Kit (GCAL) from Gentian® (PCg) using Optilite system from Binding Site® and, in a subgroup of patients, also with QUANTA® Flash Circulating Calprotectin from INOVA (PCi).

Endoscopic evaluation of activity

Endoscopic grading of activity was performed with Mayo endoscopic subscore (MES) following the recommended in ECCO guidelines.²¹ Disease extent was categorized in accordance with the Montreal classification.²²

Endoscopic remission was defined as a MES = 0 and a separate evaluation was also performed to assess differences between MES 0–1 and 2–3 (MES 0: normal or cicatricial mucosa; MES 1: presence of erythema, mild friability and/or decreased vascular pattern; MES 2: presence of marked erythema, moderate to severe friability, absence or vascular pattern and/or erosions; MES: spontaneous bleeding and/or ulcerations).²³

Histological evaluation of activity

Geboes score was used to assess histologic activity, as, despite not being fully validated, it is one of the most used scores.²⁴ Histologic remission was defined as Geboes score <2. Definitions were in accordance with a recent position statement of ECCO.²⁴

Ethical considerations

The project was subjected to the standards of good clinical practice and always complied with the ethical precepts of the Helsinki's Declaration. All the data was completely anonymized before analysis. Written informed consent was obtained previous to the inclusion in the study. The study protocol was reviewed and approved by our institution's Ethics Committee (OB.SF.171/2021).

Statistical analysis

Data was analyzed with Stata (StataCorp LP®) (version 16.0). Descriptive statistics were used in the description of clinical and analytical data. Continuous variables were described with median and interquartile range (IQR) and categorical variables with frequencies. Correlation between PCi and PCg was evaluated with Spearman's correlation. Univariate analysis of the factors associated with levels of CRP, FCP, PCi and PCg was performed with Mann–Whitney test (binomial variables) or Kruskal–Wallis test (categorical variables) and Spearman's correlation with Bonferroni correction (continuous variables). Multivariable analysis of factors associated with each biomarker was performed with median regression. For multivariable analysis, the factors used were those with $p < 0.2$ in the univariate analysis and those that were clinically relevant.

Receiver operating characteristic (ROC) curves were introduced to calculate the area under the curve (AUC) values. Relevance of a biomarker was considered in the case the AUC was ≥ 0.7 . Comparison between AUC of different biomarkers was also computed. A p -value of < 0.05 was considered statistically significant.

Results

Patient characteristics

A total of 98 patients were included. The majority (60.2%) were from male sex. Demographic and clinical characteristics are detailed in Table 1. The majority of patients (56 (57.1%)) were in clinical remission.

Median levels of CRP, FCP and PC are detailed in Table 2. PCg was measured in all the study participants and additionally PCi was

Table 1

Demographic and clinical characteristics of study population ($N = 98$ patients) with previously diagnosed ulcerative colitis undergoing endoscopic evaluation.

Characteristic	N (%)
Age (years) median (IQR)	49 (38–61)
Age at the diagnosis (years) median (IQR)	34 (23–42)
Gender (male)	59 (60.2%)
Active smoking	11 (12.2%)
Family history of IBD	13 (14.6%)
Extension of the disease	
Proctitis	12 (12.2%)
Left colitis	49 (50%)
Extensive colitis	37 (37.8%)
Current medication	
Mesalazine	65 (66.3%)
Biologics	24 (24.5%)
Concomitant azathioprine	6 (6.1%)
Topical mesalazine	14 (14.3%)
Monotherapy	2 (2%)

IQR: interquartile range.

measured in a subgroup of 74 patients, because this measurement was only available in part of the course of the study. A very high degree of correlation was found between PCi and PCg ($\rho = 0.76$).

As far as endoscopic evaluation is concerned, 48 (49%) patients were in remission, 28 (28.5%) had MES = 1; 14 (14.3%) patients had MES = 2 and 8 (8.2%) had MES = 3. Regarding histology, remission was present in 55 (56.1%).

Plasma calprotectin and demographics and disease characteristics

The results of the univariate analysis assessing the associations between CRP, FCP, PCi and PCg and clinical parameters are detailed in Table 3.

Plasma calprotectin and clinical activity

As far as the evaluation of symptomology is concerned, the patients in clinical remission had statistically significant lower values of CRP, FCP and PCi ($p = 0.042$, $p = 0.004$ and $p = 0.014$, respectively), but not with PCg ($p = 0.156$).

Plasma calprotectin and endoscopic activity

Concerning PC, a statistically significant association was found between MES and Gentian's biomarkers ($p = 0.02$), but not with INOVA's kit ($p = 0.24$).

There were no statistically significant differences in the values of CRP by subgroups of patients with different MES ($p = 0.086$).

Table 2

Median levels (interquartile range (IQR)) of C-reactive protein, fecal calprotectin and both kits of plasma calprotectin (Gentian and INOVA diagnostics) in predicting endoscopic and histological outcomes. Comparison between AUCs. Statistical significance with $p < 0.05$.

	CRP	FCP	PCg	PCi
Median (IQR)	0.27 (0.09–0.57)	152 (47–588)	0.23 (0.13–0.48)	0.525 (0.38–0.82)
MES				
0/1	0.2 (0.08–0.49)	75 (36–488)	0.21 (0.11–0.39)	0.5 (0.36–0.72)
2/3	0.58 (0.16–1.36)	824 (259–1529)	0.46 (0.17–0.8)	0.76 (0.51–1)
Endoscopic remission				
MES 0	0.19 (0.09–0.51)	55 (23–245)	0.22 (0.13–0.39)	0.48 (0.33–0.77)
MES ≥ 1	0.33 (0.11–1)	470 (113–1206)	0.28 (0.13–0.5)	0.59 (0.41–0.85)
Histologic remission				
GS <2	0.2 (0.09–0.5)	63 (36–257)	0.21 (0.13–0.4)	0.51 (0.36–0.71)
GS ≥ 2	0.39 (0.11–1.08)	484 (83–1356)	0.3 (0.12–0.54)	0.66 (0.41–0.95)

CRP: C-reactive protein; FCP: fecal calprotectin; GS: Geboes score; MES: Mayo endoscopic subscore; PCg: plasma calprotectin assessed with Gentian immunoassay; PCi: plasma calprotectin assessed with INOVA immunoassay.

Table 3

Univariate analysis of the association between inflammatory biomarkers and clinical characteristics study population ($N=98$ patients). (Mann–Whitney test (binomial variables) or Kruskal–Wallis test (categorical variables)); statistical significance with $p < 0.05$.

Characteristics	CRP	FCP	PCg	PCi
Gender	0.96	0.56	0.007	0.296
Age	0.133	0.671	0.01	0.024
Extension of UC	0.174	0.117	0.267	0.086
Time from diagnosis	0.134	0.677	0.647	0.771
Family history of IBD	0.813	0.98	0.816	0.961
Smoking habits	0.199	0.897	0.497	0.935
Smoking load	0.607	0.591	0.087	0.003
Biological therapy	0.25	0.624	0.331	0.83
Topical mesalazine	0.746	0.142	0.631	0.935

CRP: C-reactive protein; FCP: fecal calprotectin; IBD: inflammatory bowel disease; PCg: plasma calprotectin assessed with Gentian immunoassay; PCi: plasma calprotectin assessed with INOVA immunoassay; UC: ulcerative colitis.

In bold, values <0.05 .

Table 4

Areas under the ROC curve (AUCs) of C-reactive protein, fecal calprotectin and both kits of plasma calprotectin (Gentian and INOVA diagnostics) in predicting endoscopic and histological outcomes. Comparison between AUCs. Statistical significance with $p < 0.05$.

	CRP	FCP	PCg	PCi	p-Value
MES 0/1 vs MES 2/3	68.1%	79.6%	71.5%	67.1%	0.522
Endoscopic remission	57.7%	76.1%	57.2%	58.9%	0.128
Histologic remission	57.5%	71.4%	58%	60%	0.172

CRP: C-reactive protein; FCP: fecal calprotectin; MES: Mayo endoscopic subscore; PCg: plasma calprotectin assessed with Gentian immunoassay; PCi: plasma calprotectin assessed with INOVA immunoassay.

Regarding values of FCP, a statistically significant association was found with MES ($p < 0.001$).

Median levels of CRP, FCP and PC according to endoscopic remission and differences between MES 0/1 and MES 2/3 are detailed in [Table 2](#).

Comparing MES=0 with MES>0, the only biomarker that was able to achieve a statistically significant difference between endoscopic remission and activity was FCP ($p < 0.001$). However, when differences between MES 0/1 and MES 2/3 were evaluated, all the biomarkers had statistically significance (CRP: $p = 0.011$; FCP: $p < 0.001$; PCg: $p = 0.002$; PCi: $p = 0.04$).

Plasma calprotectin and histologic activity

Histological grades, according to the Geboes score, were only associated, with statistical significance, with FCP ($p < 0.001$). No other biomarkers had significant differences regarding histology (CRP: $p = 0.192$; PCg: $p = 0.573$; PCi: $p = 0.265$). Median levels of CRP, FCP and PC regarding histologic remission are described in [Table 2](#).

Concerning histologic remission, the only biomarker with a statistically significant difference between Geboes score <2 and ≥ 2 was FCP ($p < 0.001$). Lower levels of CRP, PCg and PCi were not associated with absence of inflammatory infiltrate in the epithelium ($p = 0.221$, $p = 0.18$ and $p = 0.15$, respectively).

The areas under the ROC curve (AUCs) of biomarkers in predicting endoscopic remission and response and histologic remission are described in [Table 4](#). The relevance of PC was shown in the assessment of differences between MES 0/1 and MES 2/3.

Plasma calprotectin and other biomarkers

A statistically significant association between values of both PCg and PCi and values of CRP was found ($p < 0.001$). No association was revealed between PCg and PCi and FCP ($p = 0.333$ and $p = 0.253$, respectively).

Discussion

An ideal non-invasive biomarker of IBD's activity is still yet to be discovered. FCP has shown good reliability in predicting endoscopic activity, but the sampling process leads to discomfort of many patients. Regarding serum biomarkers currently used, they are not adequate for monitoring disease activity as they are not specific of intestinal inflammation and only show a good sensitivity in case of severe relapses.² In fact, the evaluation of UC' activity lacks a non-invasive biomarker to monitor mild to moderate relapses.² Actually, probably due to the transmural inflammation of CD, CRP is more useful in this condition than in UC.⁴ Taken all the above into consideration, the authors considered that PC should firstly be tested in UC patients.

The major findings of our work were that PC, despite being able to distinguish between higher and lower levels of inflammatory activity in UC, is not capable of detecting clinical useful outcomes as endoscopic and histological remission. In fact, the Gentian's biomarker, but not the INOVA's, revealed to be able to assess differences between MES 0/1 and MES 2/3. Nevertheless, the only biomarker that was able to differentiate between endoscopic remission and activity was FCP. This is not in accordance with previous studies of Malham et al. and Ferrer et al. in which PC was capable of distinguishing endoscopic remission and activity.^{16,20}

Concerning histology, the only biomarker with a statistically significant difference between remission and activity was again FCP. Previous works reached different conclusions, having concluded that PC correlated with grades of histological inflammation.²⁰

Moreover, our work showed that PC was highly correlated with CRP, in line with previous works.^{12–14,17,25} This makes the authors infer that PC may be similar to CRP in predicting inflammatory activity as they are both acute phase reactants. These findings must make us suggest that the potential role of PC is in predicting systemic inflammatory activity and not specifically intestinal inflammation. As a matter of fact, the authors consider that PC may be more advantageous in CD than in UC, in line with CRP.

Additionally, despite a visible tendency towards significance in the values of CRP between subgroups of MES, this difference was not statistically significant in our study. As a matter of fact, this limitation had already been pointed out in the literature.³ In fact, FCP has been considered as the best surrogate marker of endoscopic and histological activity in IBD.^{2,3} In our work, the values of this biomarker were associated with endoscopic and histological activity, which is in accordance with previous studies.^{5,6,26}

The major strength of our study is the evaluation of PC in a large cohort of patients and the assessment of its correlation, not only with other biomarkers and endoscopic findings, but also with histologic activity. To the best of our knowledge, this is the largest cohort of adult patients in whom the value of PC was assessed.

Nevertheless, our study has some limitations. The patients are from a single center and there was no comparison of values of PC from IBD patients with healthy controls. Moreover, a large proportion of patients were in remission, which may have influenced the results. Additionally, the majority of the evaluations were performed in patients in histologic remission.

PC has low value in distinguishing patients in remission from patients with endoscopic or histologic activity in UC. This essential role must continue to be played by FCP.

Ethical considerations

The project was subjected to the standards of good clinical practice and always complied with the ethical precepts of the Helsinki's Declaration. All the data was completely anonymized before analysis. Written informed consent was obtained prior to the inclusion

in the study. The study protocol was reviewed and approved by our institution's Ethics Committee (OB.SF.171/2021).

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Conflict of interest

Francisco Portela received speaker fees from Abbvie, Falk, Ferring, Janssen, Pfizer, Pharmakern, Takeda and Tillotts.

The other authors of this manuscript report no conflict of interest.

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