Salivary calprotectin: a potential biomarker in inflammatory bowel disease?

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Background: Biomarkers are used frequently for evaluation and monitoring of IBD. Fecal calprotectin (FCP) is an already established biomarker in IBD, nevertheless patients often do not feel comfortable with stool manipulation. A saliva sample collection is particularly interesting due to its noninvasiveness and readiness of collection. We aimed to assess if salivary calprotectin (SCP) could be used as a new biomarker to assess disease activity.

Methods: In this observational cohort study, saliva was collected through the passive drool technique from IBD patients. All patients were submitted to a previous oral examination performed by a dentist. Recent history of antibiotic treatment (within the preceding month), proton pump inhibitors or non-steroidal anti-inflammatory drugs were exclusion criteria. Determination of FCP and SCP levels was accomplished using Elia Stool Extraction Kit and Elia Calprotectin 2 test.

Results: 92 patients (56 with CD and 36 with UC) were included. Clinical characteristics are presented in Table 1. Patients had a median FCP of 45 mg/Kg and a median SCP of 206.5 mg/Kg. No significant correlations between SCP and FCP, CRP (C-reactive protein), ferritin and ESR (erythrocyte sedimentation rate) were found. However, there was a significant correlation between fecal calprotectin, CRP and ESR. Disease activity scores did not correlate significantly with SCP, although there was a significant correlation between FCP and Partial Mayo Score (table 2).

IBD patients were stratified based on a composite criterion for inactive disease (Harvey-Bradshaw index (HBI) score <5 for CD or a Partial Mayo Score ≤ 2 for UC, and FCP cutoff value of 150 μg/g[1,2]), with no significant difference in SCP between the two groups, despite the significant difference in FCP (table 3). No significant differences between different treatments groups or no treatment in salivary calprotectin concentration were found using Kruskal–Wallis H tests (figure 1). 77% of patients with IBD do not have periodontal health. Nonetheless, there was no significant difference in the median SCP value between patients with or without periodontal health. Although the median SCP value is higher in patients with UC without periodontal health, the difference is not statistically significant (table 4).

Conclusion: SCP does not correlate with FCP and do not represent a reliable biomarker to distinguish disease activity in patients with IBD. In our opinion, a larger number of patients with active disease and the inclusion of healthy controls is required, since the influence of oral health problems on the measurement of SCP might demonstrate the infeasibility of its use in everyday clinical practice.

Table 1. Demographics and clinical characteristics of patients					
		IBD (N=92)	**CD (N= 56)**	**UC (N=36)**	**p**
Age (y) [a]		45 (24)	42 (29)	49 (±15)	0.07
Gender (F/M)[b]		41/ 51	21/35	20/16	0.089
Disease duration (y) [a]		8.5 (10)	6 (8.5)	12 (9.75)	**0.018**
Smoking[c]**, n (%)**	Yes	9 (9.8)	8 (14.3)	1 (2.8)	0.084
	No	83 (90.2)	48 (85.7)	34 (97.2)	
Montreal Classification, n (%)	L1 (ileal)		36 (64.3)		
	L2 (colonic)		6 (10.7		
	L3 (ileocolonic)		14 (25.0		
	+ L4 (Upper GI)		2 (3.6)		
	+ p (perianal disease)		15 (26.8)		
	+ L4 & p		0 (0)		
	B1 (inflammatory)		42 (75.0)		
	B2 (stenosing)		3 (5.4)		
	B3 (penetrating)		11 (19.6)		
	E1 (proctitis)			5 (13.9)	
	E2 (left side)			14 (38.9)	
	E3 (extensive)			17 (47.2)	
Previous Surgery, n (%)	Yes	19 (33.9)	19 (33.9)	0 (0)	
	No	37 (66.1)	37 (66.1)	36 (100)	
Main previous therapy, N (%)	5-ASA	31 (33.7)	8 (14.3)	23 (63.9)	
	Immunomodulators	17 (18.5)	12 (21.4)	5 (13.9)	
	Immunomodulators + biologic	12 (13.0)	11 (19.6)	1 (2.8)	
	1 biologic	15 (16.3)	10 (17.9)	5 (13.9)	
	2 biologics	4 (4.3)	2 (3.6)	2 (5.6)	
	≥ 3 biologics	2 (2.2)	2 (3.6)	0 (0)	
	Corticosteroids	2 (2.2)	2 (3.6)	0 (0)	
	Without treatment	9 (9.8)	9 (16.1)	0(0)	
Current therapy, N (%)	5-ASA	21 (22.8)	5 (8.9)	16 (44.4)	
	Immunomodulators	9 (9.8)	7 (12.5)	2(5.6)	
	Immunomodulators + biologics	10 (10.9)	7 (12.5)	3 (8.3)	
	1 biologic	40 (43.5)	25 (44.6)	15 (41.7)	
	Without treatment	12 (13.0)	12 (21.4)	0 (0)	
HBI, N (%)	<5		47 (83.9)		
	≥ 5		9 (16.1)		
Partial Mayo score, N (%)	≤ 2			31 (86.1)	
	>2			5 (13.9)	
Fecal Cp (mg/Kg) [a]		45 (141.8)	37 (148.5)	49 (143.3)	0.645
N (%)	<150	70 (76.1)	42 (75)	27 (75)	
Salivary Cp (mg/Kg)[a]		206.5 (559.8)	179.5 (654.3)	243.0 (555.3)	0.911
CRP (mg/dL)[a]		0.115 (0.318)	0.115 (0.49)	0.11 (0.313)	0.763
Ferritin (ng/mL)[a]		75.5 (464)	76 (92.5)	75.5 (99)	0.99
ESR (mm/hr)[a]		2.5 (3)	2 (4)	3 (3)	0.697
Hemoglobin (g/dL) [a]		14.35 (2.1)	14.614 (±1.3611)	13.8 (2.3)	0.327

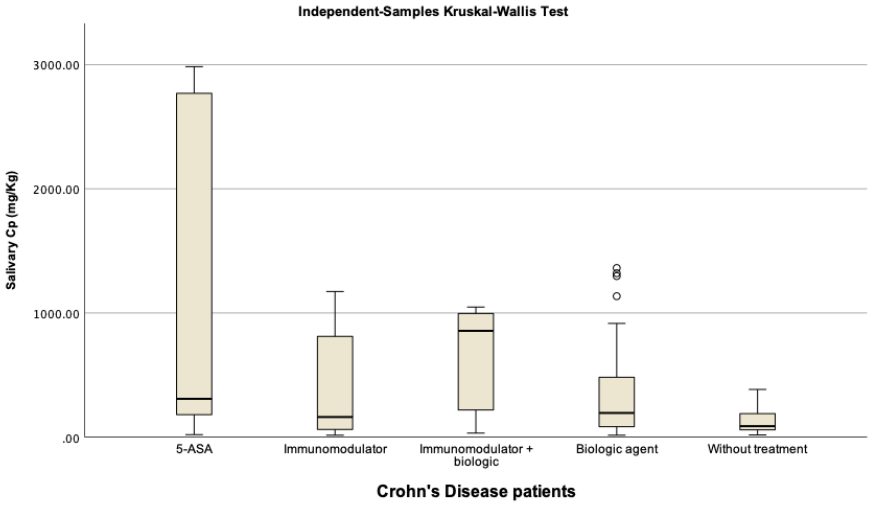
ESR: Erythrocyte sedimentation rate; CD: Crohn's disease; Cp: calprotectin; CRP: C-Reactive Protein; F: Female; HBI: Harvey-Bradshaw Index; IBD: inflammatory bowel disease; UC: ulcerative colitis; M: Male; Mayo PS: Mayo Partial Score; 5-ASA: 5-aminosalicylic acid; y: years.

Variables reported as: means with standard deviation for normally distributed data, median with interquartile range (IQR) for non-normally distributed data and numbers with percentages for categorical data; [a] Mann-Whitney U test for non-normally distributed variables; [b] Chi2 test, [c]Fisher exact test.

Table 2. Correlations between disease activity scores, calprotectin levels in feces and saliva, CRP, ferritin and ESR in patients			
	N	r statistic	p value
Salivary Cp and Fecal Cp[a]	92	0.116	0.271
Salivary Cp and CRP[a]	92	-0.017	0.873
Salivary Cp and ferritin[a]	92	0.027	0.798
Salivary Cp and ESR[a]	92	0.035	0.738
Salivary Cp and HBI[b]	56	-0.087	0.376
Salivary Cp and Mayo PS[b]	36	-0.107	0.416
Fecal Cp and and CRP[a]	92	0.532	**<0.001**
Fecal Cp and ferritin[a]	92	-0.108	0.305
Fecal Cp and ESR[a]	92	0.264	**0.011**
Fecal Cp and HBI[b]	56	0.057	0.565
Fecal Cp and Mayo PS[b]	36	0.368	**0.006**
[a]Spearman rho correlation; [b]Kendall's tau correlation			

Table 3. IBD patients and CD and UC patients stratified into two groups, "remission" and "active disease"			
	Remission (n =65)	**Active disease** (n=27)	**p**
Salivary Cp (mg/Kg)	194 (428)	226 (639)	0.787
Fecal Cp (mg/Kg)	28 (43.9)	440 (1300)	**<0.001**

	CD (n=56)		**p**	**UC** (n= 36)		**p**
	Remission (n =39)	Active disease (n=17)		Remission (n =26)	Active disease (n=10)	
Salivary Cp (mg/Kg)	172 (719)	181 (561.5)	0.587	226.5 (359.5)	253.5 (578.3)	0.306
Fecal Cp (mg/Kg)	28 (38)	440 (1254.5)	**<0.001**	19 (51.3)	687 (4977.5)	**<0.001**



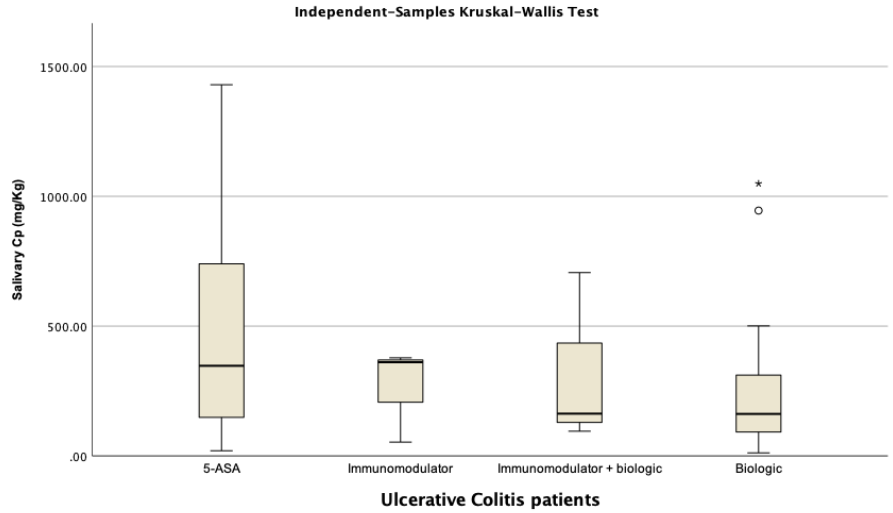


Figure 1 – Salivary calprotectin levels in CD and UC patients between different group treatments or no treatment (Kruskal–Wallis H tests: CD ($\chi2(4) = 6.211$, $p = 0.184$; UC group ($\chi2(3) = 1.201$, $p = 0.753$))

Table 4.

Periodontal health	**IBD** (n=92)	**CD** (n=56)	**CU** (n=36)	p value
Yes, n (%)	21 (22.8)	14 (25.0)	7 (19.4)	0.536
No, n (%)	71 (77.2)	42 (75.0)	29 (80.6)	
Chi2 test, CD vs UC				

	Periodontal health	**IBD** (n=92)	**p**	**CD** (n=56)	**p**	**CU** (n=36)	**p**
Salivary Cp (mg/Kg)	Yes, n (%)	214 (635.5)	0.170	213.5 (688.8)	0.233	236 (630)	0.484
	No, n (%)	181 (447)		145 (527.3)		250 (538)	
Mann-Whitney U test.							

References

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Annex

Periodontitis staging and grading, n (%)	**IBD** (n=92)	**CD** (n=56)	**CU** (n=36)
SI, GA	2 (2.2)	-	2 (5.6)
SI, GB	8 (8.7)	7 (12.5)	1 (2.8)
SII, GA	1 (1.1)	-	1 (2.8)
SII, GB	13 (14.1)	4 (7.1)	9 (25.0)
SIII, GB	23 (25.0)	14 (25)	9 (25.0)
SIV, GB	9 (9.8)	6 (10.7)	3 (8.3)
Gingivitis	11 (12.0)	9 (16.1)	2 (5.6)
Without teeth	4 (4.3)	2 (3.6)	2 (5.6)
Periodontal health	21 (22.8)	14 (25)	7 (19.4)