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Research Article

Fecal Neopterin Versus Fecal Calprotectin for Assessment of Disease Activity in Patients with Inflammatory Bowel Disease

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Abstract

Introduction: Diagnosis of inflammatory bowel disease (IBD) is a complicated issue combining patient's history, examination together with laboratory, endoscopic, histologic, and radiographic findings. Ileo-colonoscopy is the preferred method for diagnosis, assessment of disease extent, activity and follow up after therapy but repeated endoscopy is neither practical nor feasible, being invasive, time consuming, and not well tolerated.

Therefore, employment of non-invasive biomarkers is needed. Unfortunately, no single marker is ideal. Many studies focus on fecal calprotectin (FC) in IBD and confirm its value in diagnosis, disease activity evaluation, and relapse monitoring.

Neopterin, is a metabolite of cyclic guanosine monophosphate that is released by activated T lymphocytes and macrophages after induction by interferon γ. Neopterin release from activated macrophages may provide, at least theoretically by its intrinsic mechanism of release, an advantage over calprotectin which is not secreted and represents a neutrophil-derived protein.

Objectives: To investigate the relation between fecal neopterin (fNeo) excretion and IBD clinical and endoscopic activity indices and to compare its ability to discriminate active versus inactive disease with the routine usage of FC

Methods: 60 patients were included: 30 patients with ulcerative colitis (UC) (15 clinically in remission, 15 active) and 30 patients with Crohn's disease (CD) (15 clinically in remission, 15 active) and 20 healthy control subjects.

FC and fNeo were detected in stool samples by enzyme-linked immunosorbent assay (ELISA). The following indices were calculated at enrollment: for Crohn's disease the Crohn's disease activity index (CDAI) and simple endoscopic score for Crohn's disease (SES-CD); for ulcerative colitis, Simple Clinical Colitis Activity Index (SCCAI) and ulcerative colitis endoscopic index of severity (UCEIS).

Results: Among UC patients, fNeo was higher in those with either clinically active or inactive disease than in control subjects ($P = 0.001$, $P = 0.040$; for active and inactive disease vs. controls respectively) but there was no significant difference between both UC groups ($P = 0.225$). For CD patients, fNeo concentration was higher in those with active disease than in those with inactive disease ($P < 0.001$) or healthy controls ($P = 0.001$). Non-significant trends toward greater fecal neopterin concentration were observed with increased colonic disease involvement). fNeo was significantly correlated with CDAI ($r = 0.604$, $P < 0.001$), SES-CD ($r = 0.600$, $P < 0.001$) in CD patients but not with SCCAI, UCEIS in UC patients. fNeo was found to have comparable sensitivity and overall accuracy to FC in predicting endoscopic disease activity in CD patients but less in UC and combing both stool tests together increases the sensitivity and specificity of either alone.

Conclusion: Stool neopterin could be used to assess disease activity in CD but not in UC patients as it correlates positively with disease activity indices in CD but not in UC. Thus, its measurement represents a novel reliable biomarker useful to detect and monitor the severity of mucosal affection in CD than UC.

Keywords: *Inflammatory Bowel Disease; Fecal Neopterin*

Introduction

Inflammatory bowel disease (IBD) comprises Crohn's disease (CD) and ulcerative colitis (UC) characterized by intestinal chronic inflammation of unknown etiology. It has been postulated that it is a multifactorial disease involving interplay among aberrant immune response, environmental factors, and multiple genes [1,2].

The incidence of IBD is now rising in developing countries, it is being increasingly considered an emerging global disease. Developing nations have reported a lower prevalence of IBD, but the incidence is currently rising in many of these countries as they become more industrialized in Latin America and Asia [1,3,4].

Employment of non-invasive biomarkers is needed. Non-invasive biomarkers have the potential to avoid invasive diagnostic tests and inhibit potential complications. Laboratory tests mostly used are acute-phase proteins (C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)) [5].

White blood cells (WBCs) increases during the acute phase response and is also influenced by drugs utilized in IBD, such as glucocorticoids (increased) or azathioprine and 6-mercaptopurine (decreased). Albumin is a negative acute phase marker that decreases during inflammation especially in case of protein loosing entero-colitis or in malnutrition [5].

Stool markers are non-invasive, rapid, simple and low in cost. Fecal markers include a biologically heterogeneous group of substances that either leak from or are actively released by the inflamed mucosa [6]. Calprotectin exerts several effects, suggesting that it may have a more direct pathogenic role in IBD. Calprotectin-induced stimulation of monocytes/macrophages, acting through Toll-like receppror-4, activates nuclear factor-κB and other transcription factors, leading to the increased production of metalloproteinases and proinflammatory cytokines, in addition to stimulating interleukin-17-producing T cells, which have been implicated in IBD [7].

Neopterin, a pyrazino-[2, 3-d]-pyrimidine compound, is a metabolite of cyclic guanosine monophosphate that is released by activated T lymphocytes and macrophages after induction by interferon γ (IFN γ) [8].

It has been suggested that it is an excellent marker for the activation of the monocyte/macrophage axis in some clinical situations. Increased amounts of neopterin in body fluids are associated with a variety of diseases in which activation of the cellular immune mechanism is involved, such as certain malignancies, allograft rejection, autoimmune diseases and viral infections [9,10].

The release of neopterin, an immunologic marker that reflects the degree of cell-mediated immune response, may be detected earlier in the damage process in IBD when compared with other inflammatory markers such as calprotectin. This may give to fecal neopterin (fNeo) an advantage over FC because fNeo may be more accurate to differentiate early patients with endoscopically active from inactive disease. fNeo and FC are correlated to mucosal lesions severity distinguishing between clinically and endoscopically active and inactive IBD [9].

fNeo concentration could reliably distinguish between clinically active and inactive UC, it did not distinguish between clinically active and inactive intestinal CD but in colonic CD fecal neopterin was found greater in active disease. In UC it is also increased as more of the colon is affected [8].

Aim of the Work

The aim of the work was to

1- Assess the role of fecal neopterin concentration as a marker of disease activity in patients with IBD and to compare it to fecal calprotectin.

2- Evaluate the role of fecal neopterin concentration as an early marker of IBD diagnosis.

Subjects and methods

The study included eighty subjects divided as follow:

1. **Group I** included thirty patients with ulcerative colitis divided into 2 sub-groups:
   a. Group Ia includes fifteen patients with active UC.
   b. Group Ib includes fifteen UC patients in remission.

Disease activity was assessed clinically by Simple clinical colitis activity index (SCCAI).

2. **Group II** included thirty patients with Crohn's disease divided into 2 subgroups:
   a. Group IIa includes fifteen patients with active CD.
   b. Group IIb includes fifteen CD patients in remission.

Disease activity was assessed clinically by Crohn's disease activity index (CDAI).

3. **Group III** is a control group and included twenty subjects who have been scheduled for colonoscopy and were found to have normal findings.

A Written informed consent was taken from all subjects included in the study.

Inclusion criteria

Patients from both genders, adults, who were able to give consent either by themselves or by their guardians, were included in the study.

Exclusion criteria

Gastrointestinal Malignancy, Indeterminate colitis, Recent surgical intervention of the intestine within the last 6 months. Infectious diarrhea including bacterial, viral and parasitic. Colonic Polyposis and Diverticulosis. Non-steroidal anti-inflammatory drugs use within a month before colonoscopy. Autoimmune diseases as Rheumatoid arthritis. Pregnancy was also excluded from the study.

Methods

All patients were subjected to the following:

1. Through history taking and physical examination including abdominal examination with stress on signs of gastrointestinal disease such as tenderness, palpable organs or masses, or peri-anal fistula and EIM of IBD.

2. Routine lab investigations including Complete blood picture (CBC), ESR, CRP, Serum albumin.

3. Quantitative assessment of fecal calprotectin by ELISA (Calprest NG, Eurospital SpA, Italy) and Quantitative assessment of fecal neopterin by ELISA. IBL International, Germany) according to the manufacturer's instructions.

4. Imaging: CT enterocolonography.

Ileocolonoscopy was done for all subjects, endoscopic lesions were reported, endoscopic indices of activity were assessed and tissue specimens for histopathology confirmation of diagnosis and assessment of activity of disease were taken.

5. Assessment of disease activity

In ulcerative colitis patients clinically by SCCAI (Simple clinical colitis activity index) and Ulcerative colitis endoscopic index of severity

In Crohn's disease patients clinically by CDAI (Crohn's disease activity index), Crohn's disease patients endoscopically by SES-CD.

Results

Distribution of the studied cases in group I according to SCCAI

Group I included 30 UC patients divided into 2 groups. Group IA included 15 patients (50%) of active UC according to SCCAI. Group IB included 15 patients (50%) of UC patients in remission. This is shown in table 1.

SCCAI	No.	%
Inactive	15	50.0
Active	15	50.0

Table 1: *Distribution of the studied cases according to SCCAI in UC group (n = 30).*

Distribution of the studied cases in group II according to CDAI

Group II included 30 CD patients divided into 2 groups. Group IIA included 15 patients (50%) of active CD according to CDAI of which 6 patients (20%) were found to have Mild to moderate disease and 9 patients (20%) of moderate to severe disease and no patients (0%) were found to have severe to fulminating disease. Group IIB included 15 patients (50%) of CD patients in remission. This is shown in table 2.

CDAI	No.	%
Remission	15	50.0
Mild to Moderate	6	20.0
Moderate to severe	9	30.0

Table 2: *Distribution of the studied cases according to CDAI in CD group (n = 30).*

ESR: In group IA, ESR ranged from 40 to 100 mm/hr with a mean of 68.0 ± 19.61 mm/hr, in group IB, ESR ranged from 16.0 to 110.0 mm/hr with a mean of 44.65 ± 27.98 mm/hr, in group IIA, ESR ranged from 27.0 to 140.0 mm/hr with a mean of 85.87 ± 32.61 mm/hr, in group IIB, ESR ranged from 7.0 to 75.0 mm/hr with a mean of 39.53 ± 22.79 mm/hr. ESR was statistically significant higher in group IA than in group IB ($p_1 = 0.016$). Also, it was statistically significant higher in group IIA than in group IIB ($p_1 < 0.001$). However, there were no statistically significant differences in between group IA and IIA ($p_2 = 0.284$) and between group IB and IIB ($p_2 = 0.703$).

CRP: In group IA, CRP ranged from 5.60 to 87.0 mg/L with a mean of 18.77 ± 21.52 mg/L, in group IB, CRP ranged from 0.5 to 36.0 mg/L with a mean of 7.5 ± 8.78 mg/L, in group IIA, CRP ranged from 1.14 to 313 mg/L with a mean of 75.0 ± 97.05 mg/L, in group IIB, CRP ranged from 0.2 to 25.0 mg/L with a mean of 5.91 ± 6.55 mg/L. Patients in group IA have statistically significant higher CRP than patients in group IB ($p_1 = 0.014$). Also, patients in group IIA have statistically significant higher CRP than patients in group IIB ($p_1 = 0.001$) However, there was no statistically significant difference between group IA and IIA, and group IB and IIB ($p_2 = 0.627$, $p_2 = 0.627$).

Albumin: In group IA, Albumin ranged from 1.5 to 3.8 g/dl with a mean of 2.69 ± 0.56 g/dl, in group IB, albumin ranged from 3.2 to 4.0 g/dl with a mean of 3.75 ± 0.27 g/dl, in group IIA, Albumin ranged from 1.9 to 4.30 g/dl with a mean of 3.01 ± 0.72 g/dl, in group IIB, albumin ranged from 2.0 to 4.80 g/dl with a mean of 3.77 ± 0.74 g/dl. Patients in group IA have statistically significant lower albumin than patients in group IB ($p_1 < 0.001$). Also, patients in group IIA have statistically significant lower albumin than patients in group IIB ($p_1 = 0.006$). However, there was no statistically significant difference between group IA and IIA, and group IB and IIB ($p_2 = 0.453$, $p_2 = 0.999$).

Stool calprotectin level

In group IA calprotectin ranged between 258.0 and 2512.0 mg/kg with a mean of 837.87 ± 743.1 mg/kg, in group IB, it ranged between 22.0 and 450.0 mg/kg with a mean of 198.47 ± 164.0 mg/kg, in group IIA, it ranged between 31.0 and 2810.0 mg/kg with a mean of 413.87 ± 716.1 mg/kg, in group IIB, it ranged between 13.0 and 176.0 mg/kg with a mean of 41.80 ± 40.79 mg/kg. In the control group stool calprotectin ranged between 15.0 and 61.0 mg/kg with a mean of 33.20 ± 12.39 mg/kg.

Regarding the stool calprotectin, it was statistically significant higher in group IA, IB, IIA than the control group ($p_{control} = < 0.001$, $p_{control} = 0.003$, $p_{control} < 0.001$) respectively. While there was no statistically significant differences between group IIB and the control group ($p_{control} = 0.973$)

Comparing group IA and IB there was statistically significant higher stool calprotectin level ($p_1 = 0.008$) in group IA than in group IB, as well as between group IIA and group IIB ($p_1 = 0.001$). There was a statistically significant difference between group IA and group IIA ($p_2 = 0.036$) as patients in group IA has significantly higher calprotectin than patients in group IIA. Similarly, patients in group IB has significantly higher calprotectin ($p_2 = 0.005$) than patients in group IIB.

	UC		CD		Test of sig.	P
	IA (n = 15)	IB (n = 15)	IIA (n = 15)	IIB (n = 15)		
ESR						
Range	40.0 - 100.0	16.0 - 110.0	27.0 - 140.0	7.0 - 75.0	H = 21.041*	< 0.001*
Mean ± SD.	68.0 ± 19.61	44.65 ± 27.98	85.87 ± 32.61	39.53 ± 22.79		
Median	76.0	31.70	90.0	39.0		
p_1	0.016*		< 0.001*			
p_2			0.284	0.703		
CRP						
Range	5.60 - 87.0	0.50 - 36.0	1.14 - 313.0	0.20 - 25.0	H = 17.816*	< 0.001*
Mean ± SD.	18.77 ± 21.52	7.50 ± 8.78	75.0 ± 97.05	5.91 ± 6.55		
Median	12.0	5.0	53.0	4.0		
p_1	0.014*		0.001*			
p_2			0.627	0.627		
Albumin						
Range	1.50 - 3.80	3.20 - 4.0	1.90 - 4.30	2.0 - 4.80	F = 12.150*	< 0.001*
Mean ± SD.	2.69 ± 0.56	3.75 ± 0.27	3.01 ± 0.72	3.77 ± 0.74		
Median	2.80	3.80	2.90	3.90		
p_1	< 0.001*		0.006*			
p_2			0.453	0.999		

Table 3: *Comparison between the different studied groups according to ESR, CRP and Albumin.*

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey).

H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test).

p: p value for comparing between the different studied groups.

p_1: p value for comparing between A and B.

p_2: p value for comparing between UC and CD.

**: Statistically significant at p ≤ 0.05.*

	UC		CD		Control (n = 20)	H	P
	IA (n = 15)	IB (n = 15)	IIA (n = 15)	IIB (n = 15)			
Calprotectin (mg/kg)							
Range	258.0 - 2512.0	22.0 - 450.0	31.0 - 2810.0	13.0 - 176.0	15.0 - 61.0	47.341*	< 0.001*
Mean ± SD.	837.87 ± 743.1	198.47 ± 164.0	413.87 ± 716.1	41.80 ± 40.79	33.20 ± 12.39		
Median	596.0	136.0	148.0	28.0	32.0		
$p_{Control}$	< 0.001*	0.003*	< 0.001*	0.973			
p_1	0.008*		0.001*				
p_2			0.036*	0.005*			

Table 4: *Comparison between active UC, active CD, inactive UC, inactive CD according to stool calprotectin level.*

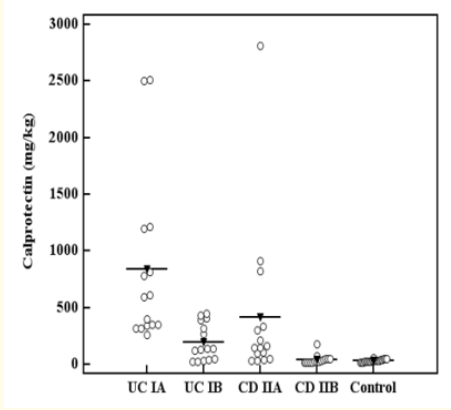


Figure 1: *Clustered box plots (log-scale) of fecal calprotectin concentrations by disease groups. Significant differences exist for Active and inactive UC relative to control for stool calprotectin concentration. (Significant differences also exist for Active CD relative to control but not for inactive CD).*

Neopterin level

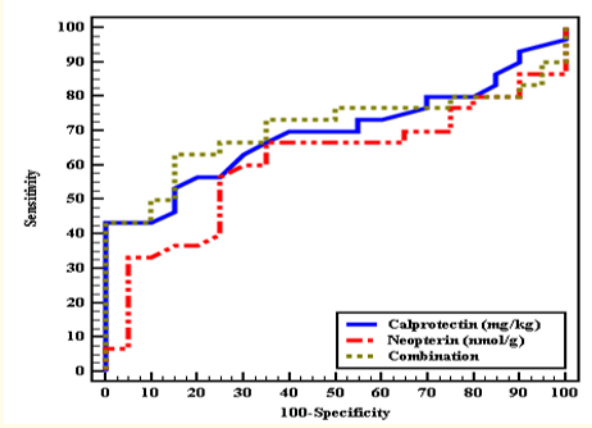
In group IA neopterin ranged between 293.0 and 2656.0 nmol/g with a mean of 935.7 ± 793.76 nmol/g, in group IB, it ranged between 10.0 and 2121.0 nmol/g with a mean of 602.67 ± 551.1 nmol/g, in group IIA, it ranged between 242.0 and 2323.0 nmol/g with a mean of 780.0 ± 532.0 nmol/g, in group IIB, it ranged between 10.0 and 576.0 nmol/g with a mean of 158.1 ± 175.44 nmol/g. In the control group stool neopterin ranged between 20.0 and 1444.0 nmol/g with a mean of 283.35 ± 317.1 nmol/g.

Like stool calprotectin, there was statistically significant higher stool neopterin level between group IA, IB, IIA than the control group ($p_{control}$ = 0.001, $p_{control}$ = 0.040, $p_{control}$ = 0.001) respectively. While there was no statistically significant differences between group IIB and the control group ($p_{control}$ = 0.245).

Comparing group IA and IB there was no statistically significant difference in stool neopterin level (p_1 = 0.225), but there was a statistically significant higher stool neopterin level in group IIA than group IIB (p_1 < 0.001). Stool neopterin level showed no statistically significant difference between group IA and group IIA (p_2 = 0.922). In contrast, patients in group IB has significantly (p_2 = 0.003) higher neopterin than patients in group IIB.

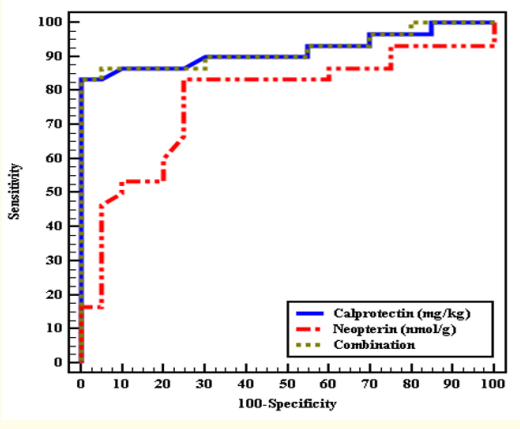
	UC		CD		Control (n = 20)	F	P
	IA (n = 15)	IB (n = 15)	IIA (n = 15)	IIB (n = 15)			
Neopterin (nmol/g)							
Range	293.0 - 2656.0	10.0 - 2121.0	242.0 - 2323.0	10.0 - 576.0	20.0 - 1444.0	30.840*	< 0.001*
Mean ± SD.	935.7 ± 793.76	602.67 ± 551.1	780.0 ± 532.03	158.1 ± 175.44	283.35 ± 317.1		
Median	551.0	576.0	727.0	111.0	217.0		
$p_{Control}$	0.001*	0.040*	0.001*	0.245			
p_1	0.225		< 0.001*				
p_2			0.922	0.003*			

Table 5: *Comparison between active UC, active CD, inactive UC, inactive CD according to stool neopterin level.*
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey).
p: p value for comparing between the different studied groups.



	AUC	P	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
			LL	UL						
Calprotectin (mg/kg)	0.695*	0.021*	0.550	0.840	> 36	63.33	70.00	76.0	56.0	66.0
Neopterin (nmol/g)	0.609	0.049*	0.451	0.768	> 232	66.67	65.00	74.1	56.5	66.0
Calprotectin (mg/kg)+ Neopterin (nmol/g)	0.707*	0.014*	0.560	0.853	> 36+ > 232	70.0	65.0	75.0	59.09	68.0

Figure 2: *ROC curve for Calprotectin, Neopterin and Calprotectin, Neopterin combined to diagnose CD from control.*



	AUC	P	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
			LL	UL						
Calprotectin (mg/kg)	0.918*	< 0.001*	0.837	1.000	> 52	83.33	95.00	96.2	79.2	88.0
Neopterin (nmol/g)	0.773*	0.001*	0.637	0.908	>313	76.67	75.00	82.1	68.2	76.0
Calprotectin (mg/kg)+ Neopterin (nmol/g)	0.920*	< 0.001*	0.840	1.000	> 52+ > 313	83.33	100.0	100.0	80.0	90.0

Figure 3: *ROC curve for Calprotectin, Neopterin and Calprotectin, Neopterin combined to diagnose UC from control.*

Regarding CD, the Roc curve of stool calprotectin level, stool neopterin level and combined both stool tests in patients with CD shows that stool calprotectin can significantly discriminate between CD patients and healthy controls at a cut off level > 36 mg/kg with a sensitivity of 63.33%, specificity of 70% and a positive predictive value of 76% with overall accuracy of 66%.

Regarding fecal neopterin, it can significantly discriminate between CD patients and healthy controls at a cut off level > 232 nmol/g with a sensitivity of 66.67%, specificity of 65% and a positive predictive value of 74% with overall accuracy of 66%. When using both stool tests combined at the same cut off values previously mentioned they can significantly discriminate between CD patients and healthy controls with a sensitivity of 70.0%, specificity of 65% and positive predictive value of 75% with overall accuracy of 68%.

	No.	Neopterin (nmol/g)	
		r_s	P
SCCAI	30	0.160	0.398
CDAI	30	0.604*	< 0.001

Table 6: *Correlation between Neopterin with clinical disease activity indices.*

	No.	Neopterin (nmol/g)			Test of sig.	P
		Min. - Max.	Mean ± SD.	Median		
CT CD findings						
Normal	14	10.0 - 1566.0	302.21±419.85	186.50	H = 7.362*	0.025*
Inflammatory	11	10.0 - 934.0	511.91 ± 286.11	525.0		
Structuring	1#		30.0			
Fistulizing	4	393.0 - 2323.0	1045.0 ± 866.87	732.0		
Colonic	5	71.0 - 934.0	467.60± 389.16	434.0	H = 0.535	0.765
Ileocolonic	15	10.0 - 2323.0	552.0 ± 642.46	394.0		
Ileal	10	10.0 - 768.0	345.40 ± 269.48	252.50		

Table 7: *Relation between Neopterin and CT CD findings.*

H: H for Kruskal Wallis test; U: Mann Whitney test.

p: p value for comparing between the different studied groups.

#: Excluded from the comparison due to small number of case (n = 1).

**: Statistically significant at p ≤ 0.05.*

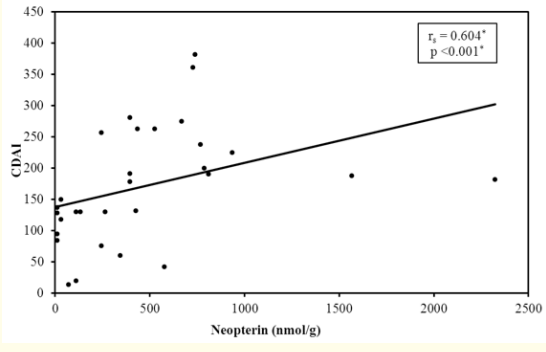


Figure 4: *Positive statistically significant correlation between fNeo and CDAI.*

Discussion

The major inflammatory bowel diseases, Crohn's disease and ulcerative colitis, are both debilitating disorders of the gastrointestinal tract, characterized by a dysregulated immune response to unknown environmental triggers. Both disorders have an important and overlapping genetic component. Although CD and UC share a number of clinical features, there are important distinctions in incidence patterns, disease localization, histopathology and endoscopic features [11].

In our study, ESR and CRP levels were found to be statistically significantly higher in active IBD group than in the inactive one. Another finding is the higher CRP level in CD active patients than in UC active but this was not statistically significant. One explanation suggested in previous studies is due to differences in disease behaviour between UC and CD, as CD is a transmural disease while UC is confined to the mucosa. Many studies [12,13] observed a statistically significant positive correlation between ESR, CRP and CDAI in CD patients ($r = 0.303$, $p = 0.033$) ($r = 0.402$, $p = 0.004$) respectively, and between ESR and UCEIS in UC patients($r = 0.334$, $p = 0.018$) but not statistically significant between CRP and UCEIS ($r = 0.243$, $p = 0.09$).

Albumin is a negative acute phase reactant and can be used as a marker of disease activity. In our patients: serum albumin levels were statistically significantly lower in the active IBD group (IA and IIA) compared to the inactive one (IB and IIB) ($p < 0.001$ for active UC Vs inactive UC, $p = 0.006$ for active CD Vs inactive CD). This coincides with previous studies [14] who concludes that albumin correlates with CDAI in CD patients thus it is a useful biomarker for identifying CD activity. Previous reports showed significantly lower levels of serum albumin in severely active UC as compared to moderate UC, mild UC, UC in remission [15].

Regarding the FC in the current study, there were statistically significant differences between groups of active and inactive UC and active CD over the control group ($p_{control} = < 0.001$, $p_{control} = 0.003$, $p_{control} < 0.001$) respectively. There was a statistically significant difference between UC and CD groups being higher in UC than in CD (Active groups $p_2 = 0.036$, inactive groups $p_2 = 0.005$).

Stool calprotectin can significantly discriminate between UC patients and healthy controls at a cut off level> 52 mg/kg with a sensitivity of 83.33%, specificity of 95% with overall accuracy of 88% and it can discriminate between CD patients and healthy controls at a cut off level > 36 mg/kg with a sensitivity of 63.33%, specificity of 70% with overall accuracy of 66%.

In a meta-analysis done by Gisbert and McNicholl in 2009 [16] concluded that fecal calprotectin is significantly higher in IBD patients over asymptomatic controls and IBS patients. Against our results, they found slightly higher accuracy for CD (sensitivity 83%, specificity 85%) than for UC (sensitivity 72%, specificity 74%). The finding of higher levels of FC in CD than UC in their studies could explain these differences.

There were statistically significant differences in stool Neopterin level between group IA (active UC), IB (inactive UC), IIA (Active CD) and the control group ($p_{control} = 0.001$, $p_{control} = 0.040$, $p_{control} = 0.001$) respectively. While there were no statistically significant differences between group IIB (inactive CD) and the control group ($p_{control} = 0.245$).

Nancey., *et al.* [9] in 2013 were the first to analyze the role of Neopterin as a fecal marker and evaluate its accuracy for predicting endoscopic disease activity in 133 consecutive IBD patients (78 CD and 55 UC) undergoing a colonoscopy.

Our data were opposed by the results of Husain., *et al.* [8] who founds that fecal Neopterin concentrations were higher in patients with active or inactive CD than in control subjects. Given these significant results of higher Neopterin in the IBD patients over controls, it suggests that this biomarker may have utility in the differentiation of symptoms from IBS and those from IBD although a special study designed to compare these 2 diseases should be carried out later.

There were no statistically significant differences in stool Neopterin levels between active and inactive UC ($p_1 = 0.225$), but there was a statistically significant difference between active and inactive CD ($p_1 < 0.001$). These results are against the results of Nancy., *et al.* [9] who found that fNeo level was significantly higher in patients with clinically active CD and UC when compared to inactive UC and CD, and also against the results of Husain., *et al.* [8] who confirmed a statistical significant higher fecal neopterin level in clinically active UC over the inactive and control.

The non-significant trend we observed for high Neopterin in inactive UC could be explained by an ongoing subclinical colonic inflammation that was not evident clinically and leads to elevation of fecal Neopterin.

Stool Neopterin levels showed no statistically significant differences between active UC and CD ($p_2 = 0.922$). In contrast, there were a statistically significant higher Neopterin levels in inactive UC over inactive CD ($p_2 = 0.003$).

The results of the present study showed that stool Neopterin performs less in predicting the diagnosis of UC over the controls in comparison to the usage of calprotectin with less both sensitivity and specificity and overall accuracy. Using both stool tests combined can increase the specificity and the positive predictive value to 100%.

In the present study the mean fNeo ± SD was significantly higher in endoscopically active CD according to SES-CD over the inactive ($p = 0.002$). Therefore, Neopterin was found to be positively correlated with SES-CD ($r = 0.600$, $p < 0.001$) This is exactly similar to the findings of Nancy., *et al.* [9] ($r = 0.46$. $p < 0.001$) and against the findings of Husain., *et al.* [8] who failed to detect any correlation between Neopterin and endoscopic scores.

In the present study the mean fNeo ± SD was not significantly higher in endoscopically active UC according to the UCEIS over the inactive ($p = 0.299$), therefore, Neopterin was not statistically significantly correlated with UCEIS ($r = 0.157$, $p = 0.408$). This matches the results of Husain., *et al.* [8] who failed to detect any correlation between Neopterin and endoscopic scores and against the results of Nancy., *et al.* [9] who found that fNeo differed significantly in UC patients without mucosal activity versus active endoscopic lesions according to Rachmilewitz index, Neopterin is correlated with the endoscopic severity scoring system. One explanation of this could be different endoscopic indices of activity used.

In CD patients, There was statistically significant difference in the stool Neopterin level according to the CT findings being higher in the fistulizing type followed by the inflammatory followed by the patients with normal CT findings ($p = 0.025$). This relation was not previously studied in the 2 above mentioned studies.

In CD patients, although stool Neopterin was found to be higher in the colonic CD than in the ileocolonic and ileal, this was not statistically significant ($p = 0.765$). In UC, Neopterin was found to be higher in pancolitis and left sided colitis than in proctitis but this was not statistically significant ($p = 0.087$). Therefore, we cannot definitively conclude whether Neopterin level is dependent on the location and or extent of the disease, given the too small sample size in each group.

Conclusion

In conclusion, we suggest that Neopterin measurement represents a novel reliable biomarker useful to detect and monitor the severity of mucosal affection in CD patients more than UC. More established results are in need to be confirmed by studies over a larger population of IBD patients with different disease behavior (especially in CD) and different ethnic groups and it will offer a new valuable and cheap marker of disease activity. The value of fecal Neopterin to predict further relapse and to assess the response to treatment needs to be investigated in further studies.

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