Original article

Stool lactoferrin as a biochemical marker in Crohn's disease; correlation with clinical, laboratory and endoscopic indices and its use as a surrogate marker for endoscopic healing

Ibrahim Mohamed Ibrahim Naguib[*1] , Hanan Abdel-Haleem[1] , Mahmoud Abdo[1] , Sherif Hamdy[1], Tarek Ramzy[2], Ahmed Moustafa[1], Hany Shehab[1], Hedy Ayman[1], AbdelAziz Gaber[1]*

1- Endemic medicine department, Faculty of medicine, Cairo university

2- Department of clinical and chemical pathology, Cairo university

A R T I C L E   I N F O

A B S T R A C T

Background: A significant limitation of mucosal healing as a treatment target in Crohn's disease is that Ileocolonoscopy remains the gold-standard for assessing disease activity. Identification of optimal biomarkers and their cut-off values is an unmet need in the context of tight monitoring strategy. **Aim of work:** To study the performance of Fecal lactoferrin in patients with Crohn's disease in comparison to endoscopic assessment, clinical indices and fecal calprotectin to address whether it correlates with endoscopic severity of inflammation and whether it could be used as reliable surrogate marker of endoscopically detected mucosal healing after therapy. **Methods:** In this prospective study, 35 patients with active Crohn's disease were recruited. All patients provided stool samples for lactoferrin and calprotectin at baseline and underwent colonoscopy. Twenty-six (26) of these patients were followed up after 6-9 months of therapy initiation or upgrade. Fecal lactoferrin was compared with the simple endoscopic score of Crohn's disease (SES-CD), Harvey-Bradshaw index (HBI) and fecal calprotectin. Data was then analyzed to identify cut-off levels to detect endoscopic response/remission. **Results:** Lactoferrin showed excellent performance in detecting remission (AUC 0.93) with a sensitivity of 85.7% and a specificity of 88.9% at a cut-off value of 21.5μg/g. A drop of more than 26.2% from baseline values is 100% sensitive and specific in detecting endoscopic response (≥50% reduction from baseline SES-CD). Lactoferrin showed a strong correlation with SES-CD (r=0.74) and calprotectin (r=0.91) **Conclusion:** Fecal lactoferrin is a reliable marker of response to therapy and mucosal healing, making endoscopic monitoring of treatment success less necessary. Whether lactoferrin is superior to calprotectin or not would require further investigation.

Introduction

Crohn's disease (CD) is a chronic inflammatory gastrointestinal disorder characterized by a pattern of relapses and remissions, with a tendency to progress over time. While it often manifests as ileo-colitis, it can affect any segment of the gastrointestinal tract. If left unaddressed, the illness is associated with a significant risk of complications and the potential for long-term disability [1]. Over the past two decades, there has been a growing acknowledgment of the significance

* *Corresponding author:* Ibrahim Mohamed Ibrahim Naguib
E-mail address: ibrahim.naguib@cu.edu.eg

of achieving mucosal healing as a crucial therapeutic goal in treating inflammatory bowel diseases and is linked to enhanced long-term clinical outcomes [2]. A significant drawback of targeting mucosal healing is that while ileocolonoscopy stands as the benchmark for evaluating disease activity, it is a relatively invasive procedure and frequent follow up is not always feasible, particularly within the framework of rigorous monitoring approaches [3]. Fecal calprotectin (FC) is the most commonly used biomarker in practice. However, there are still knowledge gaps regarding its use in the assessment of treatment response and diagnosis of mucosal healing [4]. The optimal threshold value for disease activity monitoring and the most suitable measurement interval remains unclear, particularly in CD compared to ulcerative colitis. Consequently, there is considerable variability in the reported data in the literature, leading to wide fluctuations in reported values with sensitivity ranging from 36% to 100% and specificity ranging from 25% to 100% [5].

Numerous studies have been conducted to address these limitations, yet no definitive conclusions have been reached. Assessing the effectiveness of alternative fecal biomarkers may, therefore, aid in determining under what circumstances they might outperform fecal calprotectin [5].

Fecal lactoferrin (FL) is another possible marker for evaluating therapy response; however, it has not been thoroughly explored [5].

Lactoferrin is an 80 kDa glycoprotein known for its ability to bind iron. As a component of innate immunity, lactoferrin is found in most exocrine fluids, exhibiting antimicrobial properties. It is also a major component of the secondary granules of neutrophils and is released during degranulation upon neutrophil activation. In the presence of intestinal inflammation, neutrophils migrate to the mucosa, resulting in an increased concentration of FL due to elevated neutrophil apoptosis and degradation [6-8].

Although an ideal biomarker has not been identified, enhancing our understanding of existing biomarkers and devising algorithms to direct management is crucial for tight monitoring strategies and better outcomes [4].

The purpose of this study was to assess the value of FL in Egyptian patients with CD to further evaluate its utility as a surrogate marker of endoscopically detected mucosal healing and its correlation with clinical, laboratory and clinical indices. No studies have assessed the role of FL in the follow up of CD in the Egyptian population.

Patients and Methods

This is a prospective longitudinal study in which thirty-five patients with active CD from the IBD clinic in the Integrated Clinical and Research Center for Intestinal Disorders (ICRID), Endemic medicine department, Cairo university, were included. Diagnosis was based on based on clinical, laboratory, radiological, endoscopic and histological assessment. After index colonoscopy, therapy was initiated in accordance with the European Crohn's and Colitis Organization (ECCO) guidelines [9] with conventional oral medications (systemic steroids and azathioprine) or initiated with/upgraded to Anti-TNF therapy. Out of the total number of patients, only 26 were included in the follow-up after treatment. The remaining nine patients either underwent colo-rectal surgery (4), declined to follow up (3), or were deceased (2). The cause of death in these two patients was not related to CD.

Endoscopic response was assessed 6-9 months after therapy upgrade or initiation. Colonoscopy findings were scored according to SES-CD. Scores of 0-2 suggesting inactive disease, 3-6 mild activity, 7-15 moderate activity and >16 suggesting severe disease[10]. Response to therapy was defined as >50% reduction from the baseline score and endoscopic remission was defined as a score of 0-2 in a patient with baseline evidence of endoscopic activity[11]. Harvey-Bradshaw index was used to assess clinical activity with scores >4 suggesting clinically active disease[12].

Blood tests and fecal markers

Thirty-five patients provided stool samples within two weeks of index colonoscopy, of whom only 26 provided a 2nd sample within two weeks of follow-up colonoscopy. Samples were stored at −30° C until analysis. Lactoferrin was measured by means of a quantitative ELISA (EA0063 Hu Human Lactoferrin ELISA kit, BT Labs, China). Values <7.25µg/g of stool are frequently quoted in literature as normal [13]. However, the manufacturer quotes <50µg/g of stool as normal. Fecal calprotectin was also measured by means of quantitative ELISA (QUANTA Lite, Inova diagnostics, USA). Patients provided blood samples during the same visits for complete blood count and C-reactive protein.

Statistical analysis

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 27. Data were explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. Comparisons of numeric variables between two groups were done by independent t test and Mann-Whitney test for non-normally distributed. Comparisons overtime between pre and post treatment were done by Wilcoxon signed rank test for not normally distributed variables and paired t test for normally distributed one. Comparisons between categorical variables were performed using the chi square test or fisher exact test as appropriate. Spearman's correlation tests were used for linear correlation between variables. (ROC) curve was constructed to permit selection of threshold values for test results. A p-value less than 0.05 was considered statistically significant. All tests were two tailed.

Ethics

The institutional review board, faculty of medicine, Cairo university, approved the study protocol and the written informed consent signed by the study participants. The study was conducted in accordance with the Helsinki Declaration (6th revision, 2008).

Results

Baseline parameters

Patient characteristics are shown in **table (1)**. The majority of patients (57.1%) had moderately active disease on their index colonoscopy. The ileum (n=26) and right colon (n=19) were the most commonly involved sites. Two participants were in clinical remission but had elevated FC and endoscopic evidence of inflammation. One patient who had isolated jejunal involvement, proven by imaging, enteroscopy and histopathology, had a baseline SES-CD of 0. All patients had FL levels >7.25μg/g but only 71% had values > 50μg/g. No significant differences in baseline FL levels were observed between responders and non-responders and between those who started conventional therapy and those who started anti-TNF or eventually underwent surgery. The only significant difference ($p=0.025$) between treatment groups was lower median SES-CD (6 vs 10) in the subgroup starting conventional treatment than the Anti-TNF group (10). The median pretreatment FL was higher in non-responders (74.5μg/g) than responders (55.7μg/g, p=0.06) and those who achieved remission (47.9μg/g, $p=0.001$)

Post treatment parameters

Lactoferrin and SES-CD

A significant reduction from baseline was seen in FL and SES-CD.

No statistically significant difference was seen in median SES-CD and FL values post treatment between both treatment groups .

On combining pre- and post-treatment data, FL showed a strong positive correlation with SES-CD (r=0.74, $p<0.001$).

The median pre-treatment SES-CD was 8 in responders and 11 in non-responders. Post treatment, the scores were 3 and 12, respectively.

Based on changes in SES-CD, 16 (61.5%) patients showed endoscopic response, of whom only 7 (26.9%) patients were in endoscopic remission (SES-CD<3). Median FL in responders declined from 55.7μg/g (18.9-94.1) to 21.5μg/g (7.9-35.8) compared to 74.5μg/g and 82.8μg/g in non-responders. A median change of –64% was seen in responders as opposed to +2.5% in non-responders.

Post treatment FL < 16.8μg/g gave a sensitivity of 71%, specificity of 100%, PPV of 100% and NPV of 90% in detecting endoscopic remission. Raising the value to < 21.5μg/g, the optimum cut-off chosen by the statistician, improves sensitivity to 85.7% with specificity of 88.9% **(Figure1)**. A drop of >26.2% from baseline FL values show an AUC of 1 with 100% sensitivity and specificity in detecting endoscopic response **(Figure 2)**. The same value showed an AUC of 0.7 with 100% sensitivity and 55.6% specificity in detecting remission **(Figure 3)**.

Lactoferrin and HBI

The median HBI dropped from 8 and 9 at baseline to 3 and 7 after treatment in responders and non-responders, respectively. HBI showed moderate linear correlation with FL (r=0.52, p<0.001). Likewise, HBI correlated moderately with SES-CD (r=0.53, p<0.001).

Lactoferrin and laboratory parameters

With pre- and post-treatment data combined, the highest correlation was seen with fecal Calprotectin (r=0.91, $p<0.001$). A weak linear correlation was found between FL and platelets (r=0.41, p=0.001). No linear correlation was shown between FL and Hemoglobin, Total leukocytic count or CRP. In the latter, a moderate correlation

was seen on analyzing post-treatment data separately (r=0.52, *p*=0.01).

Table 1. Study group characteristics.

		n(%)
Age	Mean ±SD	33.8±13.9
	Range	18-72
Gender	Male	21(60)
	Female	14(40)
Harvey-Bradshaw index		
	0-4	2(5.7)
	5-7	11(31.4)
	8-16	22(62.9)
	>16	1(2.9)
SES-CD	0-2	1(2.9)
	3-6	10(28.6)
	7-15	20(57.1)
	≥ 16	4(11.4)
Disease phenotype	Inflammatory	15(42.9)
	Stricturing	9(25.7)
	Penetrating	11(31.4)
Lab tests		**Median (range)**
	Hemoglobin	11±1.4(9-14.8)
	White blood cells	7±3.2(3.6-11.4)
	Platelets	382±125(181-628)
	CRP	18.7(1-118)
	Fecal calprotectin	488.5(96-1500)
	Stool lactoferrin	68.4(18.94-97.4)

Figure 1. AUC for post treatment values of FL for detecting endoscopic remission.
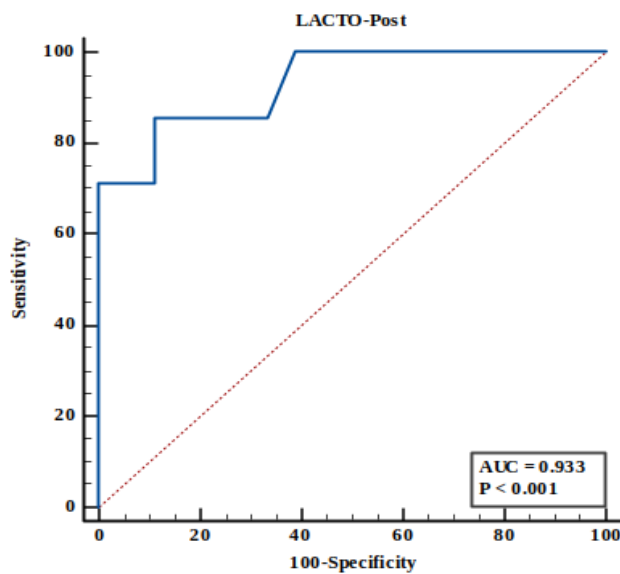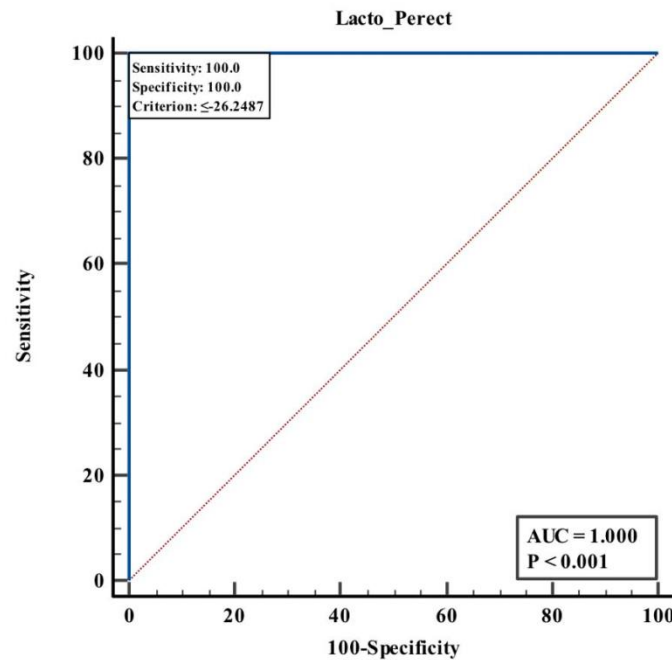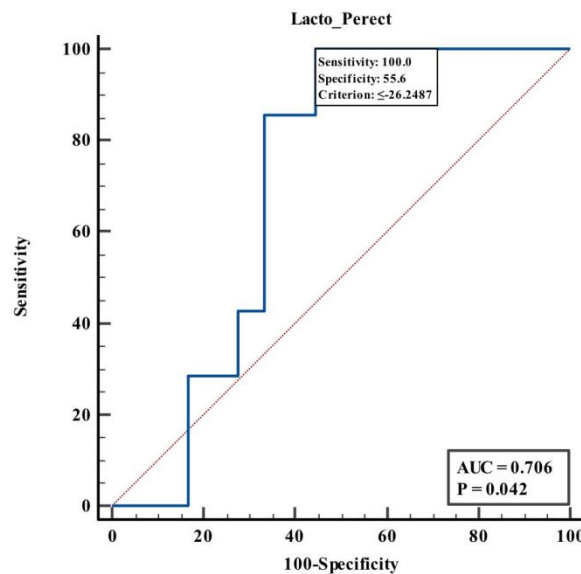
Figure 2. AUC for % drop of lactoferrin from baseline in detecting endoscopic response.

Figure 3. AUC for % drop of Lactoferrin from baseline in detecting endoscopic remission.

Discussion

In this prospective study, our objective was to assess the performance of fecal lactoferrin in Egyptian patients with CD. We aimed to compare FL with endoscopic and clinical indices to determine their correlation. Additionally, we investigated whether FL could serve as a dependable surrogate indicator for detecting mucosal healing after therapy.

Data on fecal markers in Egyptian patients with CD is scarce. **Makhlouf et al**.[14] Proposed FL cut-off levels of 37.5μg/g to differentiate between inflammatory bowel disease and irritable bowel syndrome and healthy subjects in this population. In his study, however, less than 10 CD patients were included, and the cut-off levels differed between

IBS and subjects without gastrointestinal symptoms. Our study is the first to assess the marker before and after therapy and its overall correlation with FC and clinical and endoscopic indices.

Globally, several studies have consistently reported higher FL levels in clinically active than in inactive disease when compared to clinical activity indices [5]. Our findings are consistent with most reports.

Four studies proposed cut-off levels [15-18] for FL for endoscopically detected healing with sensitivity ranging from 66 to 81% and specificity from 59 to 91% with pooled values of 75% and 80% sensitivity and specificity, respectively. 7.1μg/g, 10μg/g and 25μg/g were the proposed cut-offs in three of these studies. The fourth reported the values as optical density [15].

Sorrentino and Gray[19] demonstrated the sensitivity of FL to detect therapy-induced changes in activity in a timely manner and potential to guide management in cases of loss of response. In their study, FL levels normalized or partly decreased in patients who showed clinical response after therapy. **Sorrentino and colleagues** [20] have also demonstrated that the percentage drop in FL levels from baseline can serve as a criterion for remission with AUC of 1 (100% sensitive and specific) when a 42% or more reduction from the baseline after the second dose of biological therapy is seen. Both studies, however, didn't report endoscopic data and only relied on clinical scores. We report that a 26% drop from baseline is 100% sensitive and specific in detecting endoscopic response but much less specific in detecting remission (55%) with no other value showing better performance. In fact, measuring changes from baseline may be a better strategy to identify response while absolute cut-offs may serve as better markers for endoscopic remission since the correlation between the markers and the endoscopic indices is never perfect. Moreover, experience from clinical trials reveals the difficulty in attaining endoscopic healing in CD [21].

The cut-off level for endoscopic healing reported in our study (21.5μg/g) is on average higher than most cut-offs reported [5]. Additionally, the percentage drop in FL in responders was also lower. Whereas we have shown a median drop of 64% in responders, others have shown a drop of more than 90% on average[19, 20]. A possible explanation is that chronic subclinical inflammation from frequent

GI infections may result in impaired intestinal mucosal function and hence the elevated FL threshold and baseline [22]. Another possible explanation is the heterogeneity of studies, where most have relied on clinical rather than endoscopic assessment and in those studies where endoscopic assessment was done, the definition of remission differed from one to the other [5]. Last but not least, most of these studies have grouped UC and CD patients together rather than separately analyzing each disease.

As shown in our results, A moderate linear correlation (r=0.53) is seen between HBI and FL. It is well recognized that clinical remission is not synonymous to endoscopic remission and that mucosal inflammation may persist even when clinical remission is achieved in CD [23]. In literature, the correlation between FL and clinical indices is moderate at best [18][24, 25].

In our study, FL showed a strong correlation with FC. This is in keeping with a recent meta-analysis on fecal biomarkers in CD where FL demonstrated similar performance to FC [26]. Inspite of their comparable performance, FL has not been widely endorsed by medical practitioners in clinical practice[26,27].

The strength of our study is that it is amongst the very first studies addressing this issue in our population, however, it has some limitations; First of all, the sample size is relatively small which might have affected our analyses and conclusions. Regardless, our findings are largely consistent with the existing literature.

Another limitation is the heterogeneity of the medications used and their routes. This could be looked at as a strength rather than a limitation as it confirms the applicability of a stool marker based strategy irrespective of the type of therapy. Finally, consecutive measurements of the stool marker could have added valuable information and insight. Endorsing a strategy that requires frequent measurements may not be cost-effective, however, a study assessing the marker at different phases of therapy will help define the most informative timepoint.

Conclusion

Fecal lactoferrin is a reliable marker of response to therapy in CD with comparable performance to FC. Successful incorporation into management plans will make frequent endoscopic follow-ups less necessary.

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