



## Faecal Lactoferrin- A novel test to differentiate between the irritable and inflamed bowel?

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**Faecal Lactoferrin- A novel test to differentiate between the irritable and inflamed bowel?**

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DSS designed the study. All authors contributed to the data collection. RS performed the

initial analysis and wrote the initial draft, all ten authors were involved in the subsequent revisions, critical analysis and final draft. David Sanders is the guarantor for this manuscript.

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Abbreviations used: Faecal lactoferrin (LF), Crohn's disease (CD), Ulcerative colitis (UC), Inflammatory bowel disease (IBD), Harvey Bradshaw Index (HBI) interquartile range (IQ)

**Key words:** faecal lactoferrin, irritable bowel syndrome, inflammatory bowel disease

**Abstract**

**Background:** Distinguishing between inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) can be challenging.

**Aims:** To investigate the utility of faecal lactoferrin (LF) as a marker of inflammation in patients with IBD, IBS and controls.

**Methods:** Disease activity in IBD patients were assessed using the modified Harvey-Bradshaw Activity Index. Stool samples were analysed using an ELISA assay.

**Results:** 137 patients with IBS, 126 ulcerative colitis (UC) and 104 Crohn’s disease (CD) and 98 healthy volunteers were recruited. The median  $\pm$  IQ LF concentration (ug/g faecal weight) was  $0 \pm 1.4$  for IBS patients,  $6.6 \pm 42$  for UC patients,  $4 \pm 12.7$  for CD patients and  $0.5 \pm 2$  for healthy controls. LF levels were significantly higher in IBD patients compared to IBS/healthy controls ( $p<0.001$ ). The median LF concentrations were significantly higher in active UC & CD patients compared to inactive patients (( $p<0.001$  and  $p=0.002$  respectively). The sensitivity, specificity, positive and negative predictive values of LF in distinguishing active IBD from IBS/healthy controls was 67% and 96%, 87% and 86.8% respectively.

**Conclusion:** LF is useful to differentiate between IBD and IBS and can be used as an adjunct to blood parameters to determine IBD patients who have ongoing inflammation.

## Introduction

Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are common entities in the Western population<sup>1-3</sup>. Both conditions may present with similar clinical features such as diarrhoea and abdominal pain. Patients with IBD oscillate between periods of active and inactive disease and may even present with concomitant functional IBS. The discrimination of IBS from active IBD can be resourcefully challenging for clinicians and may delay effective treatment. Some investigations may also be perceived as uncomfortable or invasive for the patient. Clinical criteria such as ROME II IBS have been devised to aid the diagnosis of IBS<sup>4,5</sup>. The determination of inflammatory activity is crucial for patients with IBD for the diagnosis, monitoring and step up of therapy. Clinical indices are widely used but are hampered by the subjective nature of symptom reporting and have been shown to be poorly correlated with mucosal activity<sup>6</sup>. Colonoscopy is the accepted gold standard for investigation of the colon but is invasive and associated with risks<sup>7</sup>. Whilst there is emerging evidence of activation of the mucosal innate defence system toward a pro-inflammatory response in IBS patients, the absence of endoscopic and histological inflammation remains an accepted approach to the diagnosis of IBS by the bedside<sup>8</sup>.

Lactoferrin (LF) is an iron binding glycoprotein secreted by most mucosal membranes and a major component of secondary granules of polymorphonuclear neutrophils, a component of the inflammatory response<sup>9,10</sup>. Elevated LF has been used as a marker of active IBD<sup>11-16</sup> and for monitoring patients for response to treatment<sup>17</sup>. Some studies report a high sensitivity of LF for active IBD in comparison to IBS. However the use of LF for the distinction of inactive IBD and IBS is less clear<sup>13,14,18,19</sup>. Table 1 tabulates the comparative

studies of patients with IBD and IBS using LF<sup>11, 13-16, 19</sup>.

The aim of this study was to investigate the clinical utility of LF as a marker of GI inflammation in patients with active and inactive IBD compared to patients with diarrhoea predominant IBS and healthy controls.

**Methods**

***Patients***

Consecutive patients were recruited from the outpatient clinic. Patients with inflammatory bowel disease were questioned about their general well being, the frequency of bowel habit, the presence/ absence of abdominal pain or blood in the stool. Patients with established IBD were given a Harvey Bradshaw Index (HBI) for Crohn's disease and a (previously validated) modified HBI for Ulcerative colitis (**Appendix 1**)<sup>15</sup>. Patients with HBI of  $\geq 4$  were considered to have active disease. All patients who had diarrhoea with the presence of abdominal discomfort and who fulfilled the Rome II criteria for diarrhoea predominant IBS were also recruited.<sup>4, 5</sup>

All patients were investigated and treated according to the British Society of Gastroenterology guidelines<sup>4</sup>. Colonoscopy was requested based on clinical need. Healthy controls were recruited after exclusion of disease with a questionnaire. All participants were requested to return a stool sample in a container provided. Ethical approval was obtained from the North Sheffield Ethics Committee.

***Stool analysis***

Analysis was performed blind to the clinical details of the patient. Stool samples were frozen at -20 degrees Celsius (C) immediately on receipt. Quantitative ELISA (*IBD SCAN*)

fecal lactoferrin test were performed on each thawed sample. The stool analysis kits were provided by ScheBo Biotech UK Limited and Techlab, USA (Blacksburg, VA, USA). A cut off level of  $>7.25\mu\text{g/g}$  was deemed positive, based on the manufacturers guide.

### ***Statistical analysis***

The data was analyzed using SPSS version 15. Non parametric tests (Mann Whitney U test) were used to compare lactoferrin concentrations between groups as the data was not normally distributed. Kendal tau correlations were performed to assess the relationship between lactoferrin concentration and disease activity (HBI). Assistance was also sought from the University of Sheffield statistics department.

### **Results**

Four hundred and sixty five patients were recruited between November 2006 and October 2008. The mean age in the IBS, UC and Crohn's group was 42 years, 58 years and 56 years respectively. The median LF levels were significantly higher in patients with IBD compared to patients with IBS ( $P<0.001$ ) and healthy controls ( $p<0.001$ ). Table 2 tabulates the mean and median LF values for each group whilst Figure 1 shows the distribution of LF values in all patients.

Among patients with IBD, there was a trend towards higher LF values in patients with UC compared to patients with CD ( $p=0.051$ ). As for stratification based on severity of symptoms/ disease activity, the median LF ( $\mu\text{g/g}$ ) levels were significantly higher in patients with active disease ( $\text{HBI} \geq 4$ ) compared to patients with inactive disease for both UC and CD ( $p<0.001$  and  $p=0.002$  respectively). Analysis of LF levels in IBD patients based on disease activity is tabulated in Table 3.



Comparisons were also made between patients with inactive IBD (HBI <4) and diarrhoea predominant IBS. Patients with inactive IBD had significantly higher LF levels compared to patients with IBS. The median LF (ug/g)  $\pm$  IQ for patients with inactive UC and inactive CD was 3.1ug/g (8.5) and 1ug/g (5.8) respectively compared to 0 ug/g (1.4) for patients with IBS ( $p<0.001$  and  $p=0.002$  respectively).

The correlation between LF values and the disease activity (Harvey Bradshaw Index) was fair. The correlation coefficient for patients with UC was 0.4, whilst it was 0.2 for patients with CD and 0.3 for any diagnosis of IBD.

The sensitivity and specificity of LF for active IBD versus IBS patients was 67% and 96% respectively with positive and negative predictive values of 92% and 80% respectively. Similar calculations for active UC and CD patients are tabulated in Table 4. ROC curves were calculated to illustrate the trade off between the sensitivity and specificity for each group as shown in Figure 2a-2c.

**Discussion**

This study, the largest to date on the use of LF (n=465), has shown that LF has a high sensitivity and specificity for the discrimination of patients with active IBD against patients with IBS and healthy controls. In addition, LF levels were significantly higher in patients with inactive IBD compared to patients with IBS, making it a valuable investigative tool in patients where the differentiation is difficult, based on clinical history alone. Whilst the poor correlation between symptom reporting and disease activity in IBD has been demonstrated before<sup>6</sup>, previous LF studies making similar comparisons have shown conflicting results with some studies failing to show a difference between inactive IBD and IBS<sup>13, 18, 19</sup>.

We have also demonstrated a significant difference in LF levels in patients with active IBD compared to patients with inactive IBD. This suggest that LF could be used in conjunction with other parameters (clinical and blood inflammatory markers) to determine the subset of patients who have active disease or who may require a step up of therapy. A paediatric study (n=5) showed that LF levels is potentially a useful as a biomarker for response to anti-tumour necrosis factor (anti-TNF) therapy<sup>20</sup>. It has also been suggested that the course of LF may be an early predictor of a relapse<sup>11, 20</sup>. In a recent study LF predicted post operative recurrence in Crohn's disease with greater accuracy than C-reactive protein, platelet count or endoscopic appearance<sup>17</sup>.

In our study, the median LF levels in IBD patients were comparatively lower compared to other studies in the published literature<sup>14, 15</sup>. This could be explained by the larger number of patients with inactive disease. The inclusion of patients into this study was based on recruitment from routine outpatient clinics from a single centre as opposed to specially selected patients with severe symptoms.

A perceived limitation of our study is the lack of correlation with endoscopic and histological grading. In our study, colonoscopy was only performed based on clinical need and represented less than 30% of the population group. In addition, the endoscopies which were performed as routine care were done by a number of endoscopists. Similarly biopsies from these patients were also analysed by a number of histopathologists making meaningful comparisons difficult. Previous investigators have demonstrated that LF has a good correlation with endoscopic grading<sup>14</sup>.

LF is an inexpensive and non invasive test that can provide the clinician with a marker to differentiate between IBD (particularly active disease) and IBS and stratify patients who

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require endoscopic investigations. In addition, LF can also be used as an adjunct to blood parameters and clinical symptoms to determine IBD patients who have ongoing inflammation.

For Peer Review

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Table 1: Studies investigating the utility of Faecal lactoferrin in comparison of Inflammatory bowel disease and Irritable bowel syndrome patients

Author & Year	Country	Study Comparative Groups	Total number of Patients (Irritable bowel syndrome/IBS)	Results
<b>Walker 2007</b>	USA	Faecal lactoferrin, blood parameters and clinical activity index	148 (IBS n=7)	Sensitivity 84% Specificity 97%
<b>Langhorst 2008</b>	Germany	Faecal markers, activity index, blood parameters, endoscopy	140 (IBS n=54)	Sensitivity 85% Specificity 77%
<b>Schoepfer 2008</b>	Switzerland	Faecal markers, activity index, blood parameters & endoscopy	136 (IBS n=30)	Sensitivity 87% Specificity 96%
<b>Schroder 2007</b>	Germany	Faecal markers & endoscopy	88 (IBS n=31)	Sensitivity 82% Specificity 100%
<b>Kane 2003</b>	USA	Faecal markers& activity index	271 (IBS n=31)	Sensitivity 90% Specificity 100%
<b>Dai 2007</b>	China	Faecal markers, activity index & endoscopy	177 (IBS n=25)	Sensitivity 92% Specificity 88%

Table 2: Mean and Median Faecal Lactoferrin concentration (ug/g) in all groups.

	Ulcerative Colitis	Crohn's Disease	Irritable Bowel Syndrome	Healthy Controls
No of Patients	126	104	137	98
Mean LF (ug/g) (±SD)	69.5 (168)	41.4 (139)	1.39 (3.4)	2.4 (7.2)
Median LF (ug/g) (±IQ)	6.6 (42)	4 (13)	0 (1.4)	0.5 (2)

Table 3: Comparison of Faecal Lactoferrin values in patients with Inflammatory bowel disease based on disease activity

	<b>Ulcerative Colitis (n=126)</b> <b>Median LF (IQ)</b>	<b>Crohn's disease (n=104)</b> <b>Median LF (IQ)</b>
<b>Active disease</b>	N=51 Median 26 (102)	N=51 Median 8.4 (32)
<b>Inactive disease</b>	N=75 Median 3 (8.5)	N=53 Median 1 (6)
<b>P values</b>	<0.001	<0.002



Table 4: Comparison of patients with active inflammatory bowel disease and patients with Irritable bowel syndrome.

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Comparison of Active UC against IBS	78%	96%	86%	92%
Comparison of Active CD against IBS	58%	96%	83%	86%

UC: Ulcerative Colitis  
CD: Crohn's disease  
IBS: Irritable bowel syndrome

Figure 1: Faecal lactoferrin concentrations in all patients.

Figure 2a: ROC curve for all patients, area under the curve 0.75

Figure 2b: ROC curve for patients with active Inflammatory bowel disease compared to patients with Irritable bowel syndrome (area under the curve 0.84)

Figure 2c: ROC Curve for Patients with Active versus Inactive Inflammatory Bowel Disease (area under the curve 0.81)

Table 1: Studies investigating the utility of Faecal lactoferrin in comparison of Inflammatory bowel disease and Irritable bowel syndrome patients

Table 2: Mean and Median Faecal Lactoferrin concentration (ug/g) in all groups.

Table 3: Comparison of Faecal Lactoferrin values in patients with Inflammatory bowel disease based on disease activity

Table 4: Comparison of patients with active inflammatory bowel disease and patients with Irritable bowel syndrome.

*U*CACT: Active Ulcerative colitis  
*U*CINACT: Inactive Ulcerative Colitis  
*C*DACT: Active Crohn's disease  
*C*DINACT: Inactive Crohn's disease  
*I*BS: Irritable bowel syndrome

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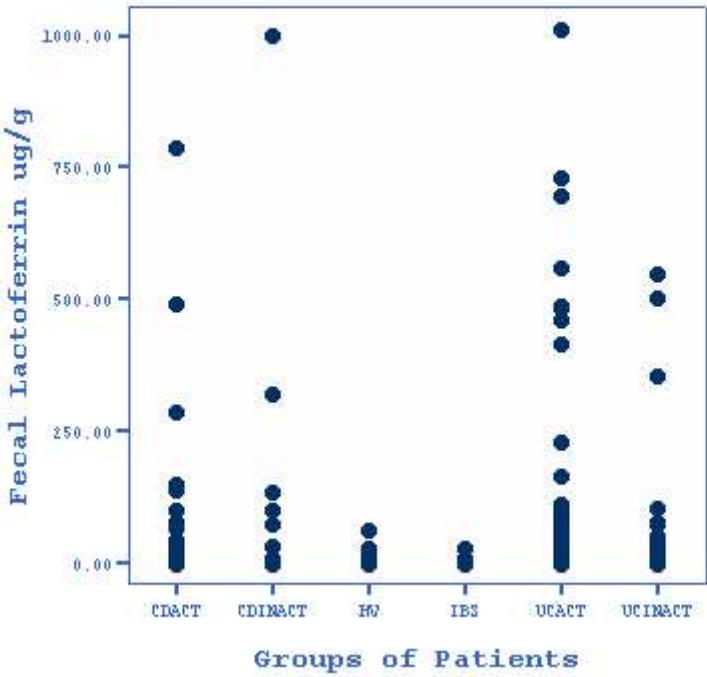


Figure 1  
110x105mm (96 x 96 DPI)

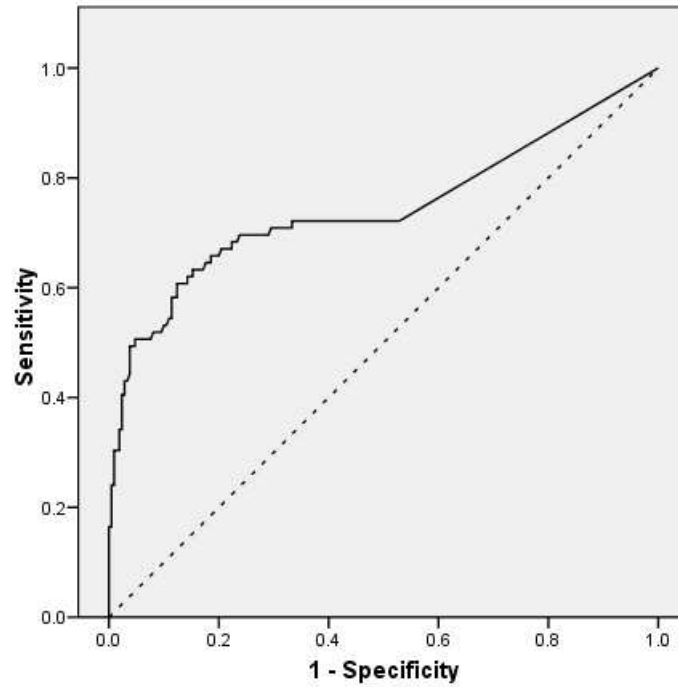


Figure 2a  
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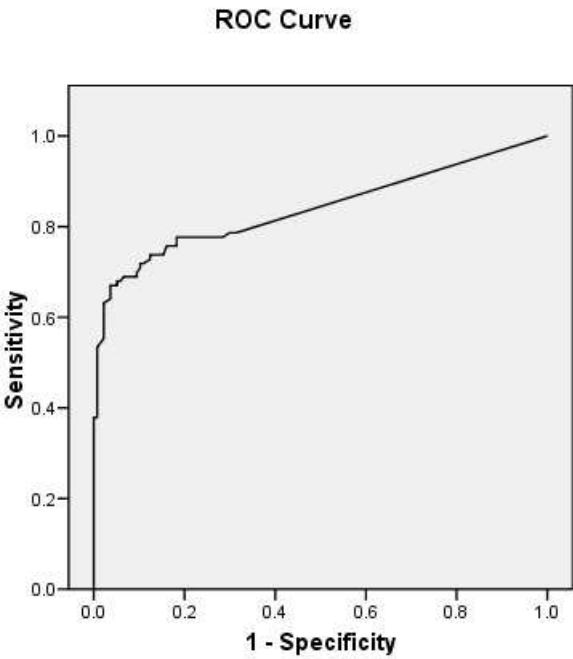


Figure 2b: ROC curve for patients with active Inflammatory bowel disease compared to patients with Irritable bowel syndrome (area under the curve 0.84)  
165x132mm (96 x 96 DPI)

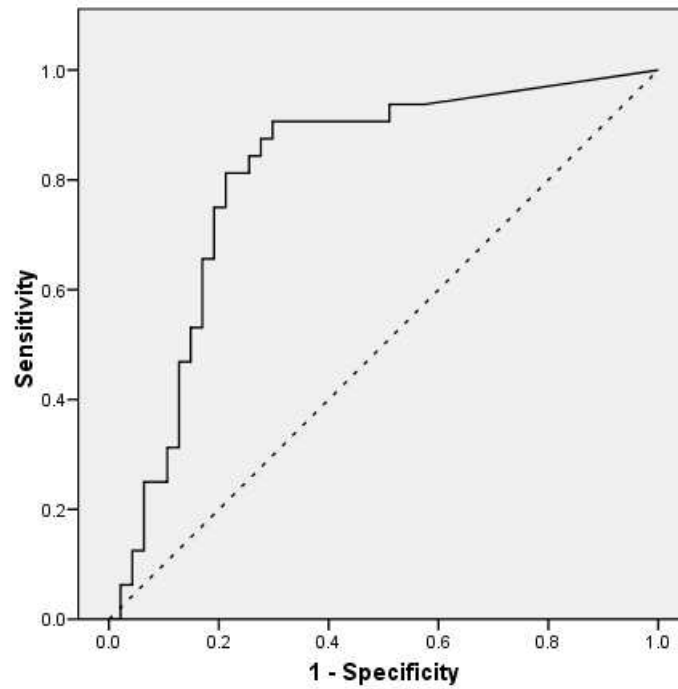


Figure 2c  
165x132mm (96 x 96 DPI)



**Appendix 1**

**Harvey Bradshaw Index for Crohn’s disease**

- 1) Number of liquid stools per day
- 2) Abdominal pain, sum of seven daily ratings:  
(0- none, 1-mild, 2-moderate, 3-severe)
- 3) General well being  
(0- very well, 1 –slightly below par, 2-poor, 3-very poor, 4-terrible)
- 4) Complications (score 1 point per item)
  - Arthritis/arthralgia
  - Skin/mouth lesions
  - Iritis/uveitis
  - Anal fissure, fistula/perianal abscess
- 5) Abdominal mass  
(0- none, 1-questionable, 2-definite, 3-definite & tender)

**Modified Harvey Bradshaw Index for Ulcerative Colitis**

- 1) Number of liquid stools per day
- 2) Abdominal pain, sum of seven daily ratings:  
(0- none, 1-mild, 2-moderate, 3-severe)
- 3) General well being  
(0- very well, 1 –slightly below par, 2-poor, 3-very poor, 4-terrible)
- 4) Complications (score 1 point per item)
  - Arthritis/arthralgia
  - Skin/mouth lesions
  - Iritis/uveitis
  - Anal fissure,fistula/perianal abscess
- 5) Bleeding per rectum  
(0-none, 1-slight, 2-moderate, 3-severe)

**STARD checklist for reporting of studies of diagnostic accuracy**  
(version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	1-2
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	4
METHODS			
<i>Participants</i>	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	5
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	5
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	5
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	5
<i>Test methods</i>	7	The reference standard and its rationale.	5
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	5
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	5
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	N/A
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	N/A
<i>Statistical methods</i>	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	6
	13	Methods for calculating test reproducibility, if done.	N/A
RESULTS			
<i>Participants</i>	14	When study was performed, including beginning and end dates of recruitment.	5
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	5
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	5
<i>Test results</i>	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	N/A
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	5
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	N/A
	20	Any adverse events from performing the index tests or the reference standard.	N/A
<i>Estimates</i>	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	6

	22	How indeterminate results, missing data and outliers of the index tests were handled.	N/A
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	6
	24	Estimates of test reproducibility, if done.	N/A
DISCUSSION	25	Discuss the clinical applicability of the study findings.	7-8

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