Comparison of CRP, Clinical Activity Indices and Fecal Lactoferrin with Disease Status in Ileocolonoscopy of Patients with IBD and IBS

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INTRODUCTION

Determining the presence of intestinal inflammation is the main criteria for the differentiation of irritable bowel syndrome (IBS) from inflammatory bowel disease (IBD) and for tailoring medical therapy. Clinical indices have proven too complex and time-consuming for daily routine practice leading to an assessment of clinical symptoms and use of independent lab parameters. Serological parameters for systemic inflammation such as C-reactive protein (CRP) and sedimentation rate are utilized often in the clinical assessment of IBD but are limited by low sensitivity and specificity for intestinal inflammation. Lactoferrin, a neutrophil-derived protein, has been shown to be a sensitive and specific indicator of intestinal inflammation in IBD. Recent studies have shown the correlation of elevated fecal lactoferrin ($\geq$7 ug/mL) to disease activity in IBD and the increase in levels as an indicator of relapse. Fecal lactoferrin is baseline in healthy subjects and in IBS. The aim of our study was to assess the correlation between levels of lactoferrin, serum CRP and disease activity indices to grades of intestinal inflammation as determined by endoscopic and histopathologic examinations of subjects suspected of IBD and IBS.

METHODS

Subject Population: A total of 63 adult patients, 22 Crohn's disease (CD), 21 ulcerative colitis (UC) and 20 IBS were enrolled following informed consent at an adult IBD clinic over an 8 month period. A total of 35 patients was scored as active IBD by endoscopy. The mean age was 42 years and the male:female ratio was 1:2.5.

Lab Parameters: Fecal lactoferrin was determined using ELISA (TECHLAB® IBD-SCAN®) with a cut-off for elevated levels of $\geq$7 ug/mL. Serum CRP was determined using an ELISA (CRPLX, Tina-quant, Roche/Hitachi) with a positive cut-off of $\geq$ 1 mg/dL.

Activity Indices: A Colitis Activity Index (CAI) was used to assess subjects with UC using a cut-off of > 5 calculated score for indicating active disease. The Crohn's Disease Activity index (CDAI) was calculated for CD and considered positive at > 150 calculated score. In the analysis for correlation, the CAI and CDAI indices were combined.

Endoscopic Score: Endoscopically obtained histopathology specimens in addition to macroscopic colonoscopy results were used as the standard reference. Each endoscopy was scored regarding inflammation: 0 for "no acute inflammation", 1 for "mild acute inflammation", 2 for "moderate acute inflammation" and 3 for "high acute inflammation". "No inflammation" was defined as the appearance of a healthy mucosa with no ulcerations. "Mild inflammation" was defined by erythema, decreased or absent vascular pattern, friability of mucosa and single aphthous lesions. "Moderate inflammation" was defined as additional multiple aphthous lesions and small ulcers. "High inflammation" was characterized by additional presence of spontaneous bleeding, large ulcerous lesions, nodules and/or narrowing.

Histopathology: Tissue biopsies were retrieved during the endoscopy exam from areas of disease involvement as determined visually. Slides were prepared using conventional hematoxylin-eosin (HE) stain and the magnification ranged from 50x to 400x. Each stain was graded as follows: "No inflammation" featured misshaped and asymmetric crypt architecture. "Mild inflammation" showed single crypt abscesses. "Moderate inflammation" was indicative of frequent crypt abscesses. "High inflammation" was defined as increased infiltration of inflammatory cells (neutrophils) into the lamina propria.

RESULTS

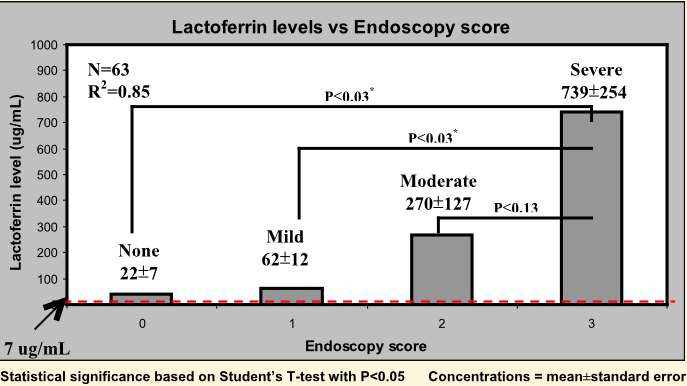### Figure 1. Correlation of Lactoferrin Levels to Endoscopy Score for Intestinal Inflammation

Lactoferrin levels vs Endoscopy score

N=63, R^2=0.85; None 22±7; Mild 62±12; Moderate 270±127; Severe 739±254; P<0.03*; P<0.03*; P<0.13; 7 ug/mL

*Statistical significance based on Student's T-test with P<0.05   Concentrations = mean±standard error

Figure 2. Correlation of CRP Levels to Endoscopy Score for Intestinal Inflammation

CRP levels vs Endoscopy score

N=63, R^2=0.73; None 0.5±0.1; Mild 1.1±0.3; Moderate 1.3±0.4; Severe 5.2±1.9; P<0.04*; P<0.06; P<0.07; 1 mg/dl

*Statistical significance based on Student's T-test with P<0.05   Concentrations = mean±standard error
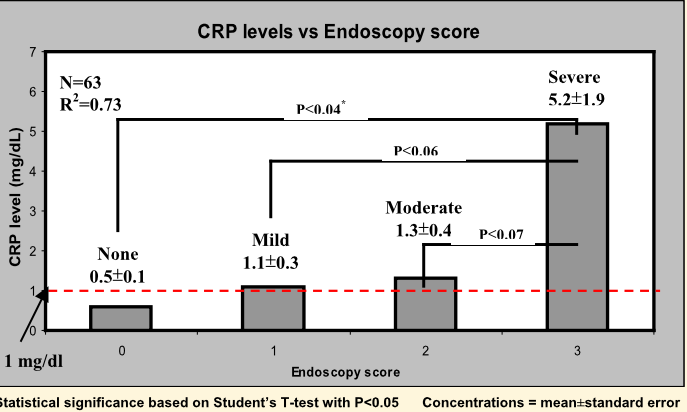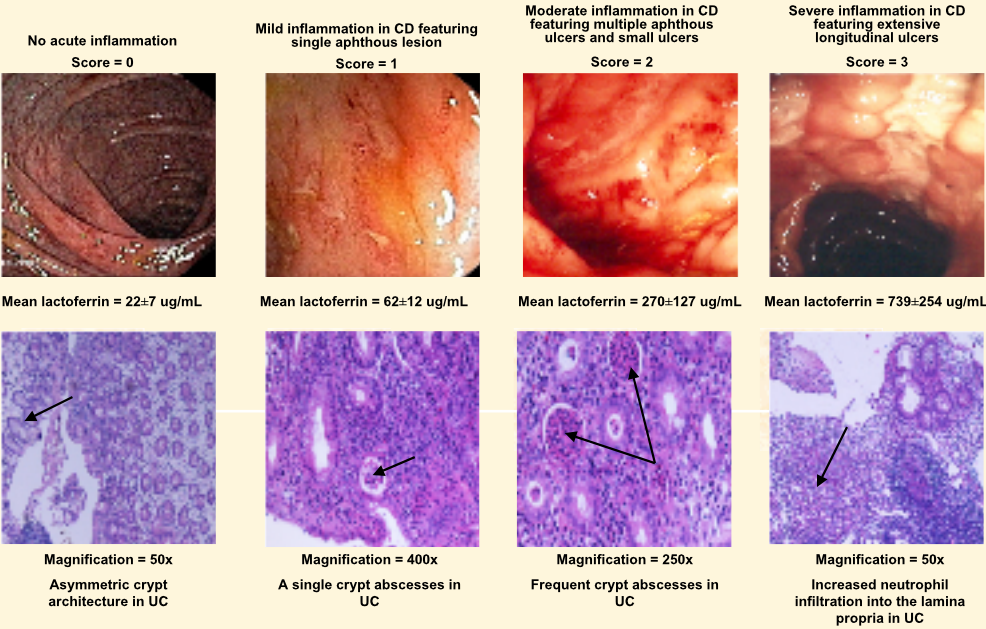
Table 1. Correlation Between Lactoferrin, CRP and Activity Index to Endoscopy

Parameter	Fecal Lactoferrin Level	Serum CRP Level	Activity Index (CAI/CDAI)
Correlation to Endoscopy	R= 0.73	R= 0.68	R = 0.58
Confidence intervals (95%)	0.59 to 0.80	0.52 to 0.79	0.19 to 0.67
Sensitivity	81%	51%	46%

RESULTS

Table 2. Endoscopic Pictures for CD and Histologic Slides for UC

No acute inflammation
Score = 0
Mean lactoferrin = 22±7 ug/mL

Mild inflammation in CD featuring single aphthous lesion
Score = 1
Mean lactoferrin = 62±12 ug/mL

Moderate inflammation in CD featuring multiple aphthous ulcers and small ulcers
Score = 2
Mean lactoferrin = 270±127 ug/mL

Severe inflammation in CD featuring extensive longitudinal ulcers
Score = 3
Mean lactoferrin = 739±254 ug/mL

Magnification = 50x
Asymmetric crypt architecture in UC

Magnification = 400x
A single crypt abscesses in UC

Magnification = 250x
Frequent crypt abscesses in UC

Magnification = 50x
Increased neutrophil infiltration into the lamina propria in UC

CONCLUSIONS

• Lactoferrin levels showed the highest sensitivity and linear correlation (R^2) to endoscopy score for grading intestinal inflammation in patients being assessed for IBD and IBS.

• A significant difference (p<0.05) was observed with lactoferrin between levels of mild and severe intestinal inflammation, and with no inflammation. CRP only showed a significant difference between severe and no intestinal inflammation.

• Lactoferrin and serum CRP showed a similar overall correlation to endoscopy and histopathology results.

• The CDAI and CAI Indices showed a poor correlation to endoscopic and histologic results.

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