Original Article

Non-Invasive Monitoring of Inflammatory Bowel Disease: Time To Use Newer Tools?

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Abstract

Introduction: In inflammatory bowel disease (IBD), commonly used biomarkers employed for non-invasive monitoring of disease activity are the C-reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR). Ulcerative colitis (UC) has a modest to absent CRP response despite active inflammation. Iron deficiency anaemia (IDA) is often a marker of active disease in IBD.

Methods: CRP, ESR, and Haemoglobin level taken within 7 days of a colonoscopy were analysed and compared with histopathological findings from colonic and ileal biopsies.

Results: Colonic biopsies from 95 colonoscopies in UC patients; and colonic and ileal biopsies from 98 colonoscopies in CD patients were analyzed. The Positive Predictive Values and Negative Predictive Values relating to ESR, CRP and iron deficiency anaemia in the two groups of patients were calculated.

Conclusion: UC has a similar CRP response to CD in active inflammation. Commonly used biomarkers have poor sensitivities in demonstrating active mucosal disease. IDA has little value when used as a marker of disease activity on its own but may be used as an adjunct to ESR and CRP. Faecal biomarkers and novel antibodies may help to increase the sensitivity and specificity in non-invasive monitoring of IBD.

Keywords

Colitis, Crohn's, CRP, Lactoferrin, Calprotectin

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Introduction

In inflammatory bowel disease (IBD), biomarkers are desirable tools that are often used to gain objective measurements of disease activity and severity, as well as to quantify responses to therapy. The ideal biomarker for IBD does not exist and more than one biomarker is usually employed. Biological markers that have found use in assessing IBD include acute-phase proteins, faecal markers, antibodies and novel genetic determinants.

The acute-phase proteins most used in clinical practice are the C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR). They are potential laboratory surrogate markers for disease activity and are associated with endoscopic inflammation and severely active histologic inflammation.[1] CRP and ESR are also the two main biomarkers used in gastroenterology out-patients clinics to measure disease activity in patients with ulcerative colitis and Crohn's disease. But how accurately do these tests measure IBD activity?

CRP is the most studied acute-phase protein and it has been shown to be an objective marker of inflammation. Solem et al showed that CRP elevation in IBD patients is associated with clinical disease activity, endoscopic inflammation, severely active histologic inflammation (only in Crohn's disease patients), and several other biomarkers of inflammation, but does not correlate with radiographic activity.[2] The authors observed that CRP had 54% sensitivity and 75% specificity for Crohn's disease in 105 patients. In a study of 43 patients with ulcerative colitis, 19 of 37 (51%) patients with active disease based on colonoscopic analysis had increased levels of CRP whereas 0 of 6 patients without endoscopic evidence of disease activity had increased levels of CRP.[2]

The production of CRP occurs mostly in the liver by the hepatocytes as part of the acute phase response. Hepatocytes synthesize CRP extremely rapidly, with a 500 to 1,000 fold higher increase than under basal circumstances occurring within 24 – 48 hours of the

onset of inflammation. The reduction in plasma CRP concentration as the acute phase response subsides may be similarly rapid. The biological half life of the circulating protein itself is short (19 hours) thus making CRP a valuable marker to detect and follow up disease activity in Crohn's disease (CD). In contrast, ulcerative colitis is believed to have only a modest to absent CRP response despite active inflammation and the reason for this is unknown.[3]

Erythrocyte Sedimentation Rate (ESR) analysis is commonly performed in IBD. ESR measures the distance that erythrocytes have fallen after one hour in a vertical column of anticoagulated blood under the influence of gravity.[4] ESR varies with plasma protein concentration and the haematocrit values and in IBD provides a crude and rapid assessment of the plasma protein alterations of the acute phase response. ESR tends to be influenced by multiple factors including increasing age, gender, pregnancy, anaemia, temperature, handling of the ESR tube, infection, malignancy, red blood cell abnormalities and technical factors.[5] Repeatedly, ESR determinations have been shown to be satisfactory monitors of acute-phase response to disease after the first 24 hours, while CRP tends to be a better indicator in the first 24 hours.[6] Compared with CRP, ESR will peak much less rapidly and may also take several days to decrease, even if the clinical condition of the patient or the inflammation is ameliorated.[7]

Iron deficiency anaemia (IDA) is also another marker of mucosal inflammation, though the time required for iron deficiency to develop is even longer and it usually takes a number of weeks after the onset of inflammation for the Mean Corpusclar Volume (MCV) and the Haemoglobin to drop. Iron deficiency anaemia occurs when the Haemoglobin is less than 14 g/dl in men and less than 12 g / dl in women in the presence of reduced iron stores (low serum ferritin <30 pg/L, serum iron < 10 pmol/L, transferring saturation <20% or total iron binding capacity > 45 pmol/L). Since ferritin is an inflammatory marker and may be raised in active inflammation, checking serum iron, transferrin saturation and total iron binding capacity may be necessary for the diagnosis of iron deficiency. While CRP is the fastest rising acute phase protein with ESR rising after the first 24 hours, iron deficiency develops over a number of weeks and therefore might represent a marker of longstanding disease activity.

Aim

The aim of this study was to assess the reliability of ESR and CRP in detecting active mucosal inflammation in inflammatory bowel disease. The reliability of IDA in IBD as a marker of recent ongoing inflammation was also analysed. We also studied the relationship between disease location and behaviour in Crohn's disease with the sensitivity, specificity and predictive values of CRP.

Methods

Patients with endoscopically and histologically confirmed ulcerative colitis and Crohn's disease were studied. Through the iSOFT® laboratory results database system, all colonic biopsies taken at Mater dei Hospital between May 2010 and May 2011 from these patients were analysed retrospectively. Using the same software system, any CRP, ESR, Haemoglobin level, serum ferritin and Mean Corpuscular Volume (MCV) taken within 7 days of the colonic and terminal ileal biopsies were collected. The data was stored in a spreadsheet (Microsoft Office Excel 2007®) and the biochemical data was compared with the histopathology reports. Any histological evidence of inflammation (including mild inflammation) was taken as evidence of disease activity. The sensitivity, specificity, positive and negative predictive values of the CRP, ESR, IDA and both inflammatory markers together were analysed. Sensitivity, specificity, positive and negative predictive values of the CRP in different Crohn's disease phenotypes (depending on Crohn's disease location and type, as classified by the Montreal classification) was also analysed. The Montreal classification describes Crohn's disease according to the following criteria:

- Age at Diagnosis:
  - A1: Diagnosed < 17 years
  - A2: Diagnosed at 17 – 40 years
  - A3: Diagnosed > 40 years
- Disease Location:
  - L1: Ileal disease
  - L2: Colonic disease
  - L3: Ileo-colonic disease
- Disease Type:
  - B1: non-stricturing, non-pentrating disease
  - B2: structuring disease
  - B3: penetrating disease

Results

Colonic biopsies from 95 colonoscopies done in 71 different patients with known ulcerative colitis were analysed. Table 1 describes the sensitivities, specificities, positive and negative predictive values of CRP, ESR and IDA in patients with ulcerative colitis. In patients with histological and endoscopic evidence of left sided active colitis, the sensitivity of CRP was 50% (true positive: 11, false negative: 11 cases). In patients with active proctitis, the sensitivity was 33.3% (true positive: 3, false negative: 6) while in patients

with pancolitis, the sensitivity was 42.3% (true positive; 11, false negative: 15 cases).

Marker	Sensitivity	Specificity	PPV	NPV
CRP	44.6%	94.1%	92.6%	50.7%
ESR	64.7%	89.3%	91.7%	58.1%
IDA	24.6%	100%	100%	39.5%
ESR & CRP	75.9%	90%	93.2%	67.5%
ESR, CRP & IDA	70%	85.3%	89.3%	61.7%

***Table 1**: Sensitivities, specificities and predictive values of inflammatory markers and iron deficiency anaemia in ulcerative colitis patients. (PPV – Positive Predictive Value, NPV – Negative Predictive Value)*

Marker	Sensitivity	Specificity	PPV	NPV
CRP	54.5%	71.0%	80%	42.3%
ESR	55.4%	89.7%	91.2%	50.9%
IDA	44.1%	87.5%	93.8%	42.4%
ESR & CRP	70.9%	70.0%	83.0%	53.8%
ESR, CRP & IDA	74.6%	68.7%	83.3%	56.4%

***Table 2:** Sensitivities, specificities and predictive values of inflammatory markers and iron deficiency anaemia in Crohn's disease patients. (PPV – Positive Predictive Value, NPV – Negative Predictive Value)*

CRP	L1	L2	L3
Sensitivity	80%	62.9%	46.8%
Specificity	50%	90.9%	62.5%
PPV	57.1%	94.4%	83.3%
NPV	75%	50%	22.7%

***Table 3:** Crohn's Disease Location and CRP. (L1 – ileal disease, L2 – colonic disease, L3 – ileocolonic disease, PPV – Positive Predictive Value, NPV – Negative Predictive Value*)

Colonic and terminal ileal biopsies from 98 colonoscopies in 62 different patients with known Crohn's disease were analysed. Table 2 describes the sensitivities, specificities positive and negative predictive values of CRP, ESR and IDA in Crohn's disease patients. Table 3 describes the sensitivities, specificities and predictive values of CRP with disease location as classified by the Montreal Classification while Table 4 shows the sensitivities, specificities and predictive values of CRP in predicting Crohn's disease behaviour as classified by the same classification. CRP in patients on biological therapy for Crohn's disease showed a sensitivity of 55%, a specificity of 54.5%, a positive predictive value of 81.5% and a negative predictive value of 25% (True Positive – 22, False Positive – 5, True Negative – 6, False Negative – 18).

CRP	B1	B2	B3
Sensitivity	55.5%	66.6%	0%
Specificity	100%	50%	50%
PPV	100%	70.6%	0%
NPV	47.8%	45.5%	50%

***Table 4:** Crohn's Disease behaviour and CRP. (B1 – non-stricturing non-penetrating disease, B2 – stricturing disease, B3 – penetrating disease, PPV – Positive Predictive Value, NPV – Negative Predictive Value).*

Discussion

CRP exhibits similar sensitivities, specificities and predictive values in UC and CD. We have shown that UC has a similar CRP response to CD in active inflammation. However, both the CRP and the ESR tend to have a poor sensitivity in identifying disease activity. Sensitivity tends to improve if both inflammatory markers are analysed together. Specificity also tends to be unacceptably low since a false positive result means that patients will need to undergo unnecessary invasive endoscopies or an increase in their treatment.

Disease location and behaviour in Crohn's disease may also affect the sensitivity and specificity of the C-Reactive Protein, with ileal and stricturing disease having the best sensitivities (see Tables 3 and 4). However, the sensitivities and specificities of different disease locations and behaviours still remain unacceptably low.

Limitations in this study may affect the value of the statistical measures described. One of the limitations is that blood tests taken up to one week before the endoscopy were included, thus potentially affecting sensitivity since the CRP with its short half-life might have improved in the interim. In fact, when the ESR and CRP were analysed together there was an improved sensitivity in detecting disease activity.

Another limitation is that even with serial colonic biopsies, areas of inflammation may be missed during endoscopic examination of the colon. This is even more evident in Crohn's disease affecting the small bowel where histological evidence of inflammation is usually very difficult to obtain. A normal colonoscopy does not exclude the presence of ongoing inflammation in the small bowel. In fact, specificity of the inflammatory markers in Crohn's disease was lower than in ulcerative colitis.

Mucosal inflammation may lead to a drop in haemoglobin (secondary to anaemia of chronic disease) or a rise in serum ferritin. While iron

deficiency is the commonest cause of anaemia in IBD, serum iron, transferrin saturation and total iron binding capacity levels may be needed to confirm the presence of iron deficiency during active inflammation.

Notwithstanding these limitations, the poor sensitivity and specificity of ESR and CRP is reflected in our every day practice. Patients frequently present with symptoms of ongoing active disease, like diarrhoea, bleeding per rectum, weight loss, and anaemia but with normal inflammatory markers.

Iron deficiency anaemia has good specificity but very poor sensitivity in both Crohn's disease and ulcerative colitis. While IDA may be used as an adjunct to ESR and CRP or other biomarkers, it has little value when used as a marker of disease activity on its own.

Therefore better biomarkers are needed for the non-invasive monitoring of ulcerative colitis and Crohn's disease. Fecal calprotectin and lactoferrin concentrations correlate better with colonic than ileal disease activity although extent of colonic disease does not appear to be important.[9-11] The sensitivities of tests for calprotectin to detect any mucosal disease range from 70% to 100% with a specificity range of 44% to 100%, depending on the cut off point used.[12-17] Sensitivities and specificities of tests for lactoferrin are similar.

In general, the correlation between CRP and endoscopic activity is lower than that observed between feacal markers and activity. Similarly, sensitivity and specificity for active mucosal inflammation is likely to be lower for CRP compared with fecal markers. In the study by Solem et al, 86% of patients (n=43) with any clinical symptoms of Crohn's disease and with increased levels of CRP had evidence of mucosal inflammation based on colonoscopic findings.[2] Some patients have persistently normal levels of CRP despite active disease.[18] For these patients, feacal biomarkers should be used preferentially to differentiate quiescent from active disease. Feacal calprotectin and lactoferrin also tend to have higher sensitivity and specificity in predicting mucosal healing.[19]

Siponnen et al found a 66-71% sensitivity and 83-92% specificity with fecal lactoferrin, 70-91% sensitivity and 44-92% specificity with calprotectin and 48% sensitivity and 91% specificity with CRP in Crohn's disease patients.[16] Schoepfer et al showed an 89% sensitivity and 58% specificity with fecal calprotectin versus 68% sensitivity and 58% specificity with CRP.[14]

As opposed to regular CRP, high sensitivity CRP (hsCRP) assays may allow detection of low grade inflammation in patients with IBD although the routine use of this test is not yet readily available.[20]

In IBD, biomarkers may play a useful role in distinguishing between Crohn's disease and ulcerative colitis. Antibodies against luminal antigens like antineutrophil cytoplasmic autoantibodies (ANCA), anti-Saccharomyces cerevisae antibodies (ASCA), OmpC 12 and CBir1 Flagellin are specifically associated with Crohn's disease. The contribution of serologic markers, specifically the anti-glycan antibodies, to IBD diagnosis may be in differentiating IBD from other gastrointestinal diseases, in differentiating Crohn's disease from ulcerative colitis, in better classifying indeterminate colitis and in decision-making prior to proctocolectomy in UC patients. The anti-glycan antibodies are specifically important in ASCA-negative Crohn's disease patients.[21]

Conclusion

In inflammatory bowel disease biomarkers may help in assessing disease activity and mucosal healing. Ulcerative colitis has a similar CRP response to Crohn's disease in active inflammation. IDA has little value when used as a marker of disease activity on its own but may be used as an adjunct to ESR and CRP or other biomarkers. No single test provides 100% sensitivity and specificity. Combinations of fecal and serological markers may be used to identify patients who should undergo earlier invasive testing or who require a step-up in treatment. Biomarkers such as calprotectin and lactoferrin provide better sensitivities and specificities and can be used to assess mucosal healing without the need for invasive testing or radiation. Antibodies against luminal antigens may prove useful in the future but are still too expensive for every day practice and require further research before they can be recommended.

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