LABORATORY MARKERS IN IBD: USEFUL, MAGIC, OR UNNECESSARY TOYS?

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SUMMARY

Laboratory markers have been investigated in inflammatory bowel disease (IBD) for diagnostic and differential diagnostic purposes, for assessment of disease activity and risk of complications, for prediction of relapse, and for monitoring the effect of therapy. The introduction of biological therapies in IBD has renewed interest in inflammatory markers (especially C reactive protein (CRP)), given their potential to select responders to these treatments.

Of all the laboratory markers, CRP is the most studied and has been shown to have the best overall performance. CRP is an objective marker of inflammation and correlates well with disease activity in Crohn's disease (CD). Increased CRP levels are associated with better response rates and normal CRP levels predict high placebo response rates in clinical trials with biologicals. However, despite the advantages of CRP over other markers, it is still far from ideal. Furthermore, CRP correlates less well with disease activity in patients with ulcerative colitis (UC) as compared with CD.

Other laboratory markers, including erythrocyte sedimentation rate (ESR), leucocyte and platelet count, albumin, and α_1 acid glycoprotein (orosomucoid), have been studied either less extensively in IBD or have proven to be less useful than CRP.

Faecal markers seem promising and may be more specific in detecting gut inflammation in patients with established IBD. Promising results have been reported with the use of faecal calprotectin in CD as well as in UC. Recent data however suggest that the performance of the faecal calprotectin test is superior for UC than for CD.

Taken together, laboratory markers are useful and should be part of the global management of our IBD patients. They are however not magic and until more data become available, the use of CRP and other laboratory markers should be seen as an additive tool to clinical observation and physical examination rather than a replacement.

INTRODUCTION: IS THERE A NEED FOR LABORATORY MARKERS IN IBD?

Many aspects of the IBDs, CD and UC, still present challenges for physicians treating this disorder: diagnosis, prognosis, assessment of disease activity and severity, as well as outcome of therapy. For each of these aspects, there is no single ''gold standard'' test or examination. Instead, physicians apply a combination of symptoms, clinical examination, laboratory indices, radiology, and endoscopy with histology to make the diagnosis, to assess severity, and to predict the outcome of disease.

There are several reasons why laboratory markers have been studied in IBD in the past decades: firstly, to gain an objective measurement of disease activity as symptoms are often subjective; and secondly, to avoid invasive (endoscopic) procedures which are often a burden to the patient.

An ideal marker should have many qualities (table 1). It should be easy and rapid to perform, cheap, and reproducible between patients and laboratories. The ideal laboratory marker should furthermore be able to identify individuals at risk for the disease and should be disease specific; it should be able to detect disease activity and monitor the effect of treatment; and finally it should have a prognostic value towards relapse or recurrence of the disease.

If the ideal marker exists for IBD, it would greatly facilitate the work of the gastroenterologist or surgeon treating these patients. Unfortunately, no single marker has proven to possess all the above listed qualities (table 1) although some interesting markers have been identified. In this overview, we will first briefly discuss markers of inflammation studied in IBD, with special reference to CRP, ESR, and faecal calprotectin. Thereafter, the use of laboratory markers in IBD in various clinical situations will be discussed.

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Table 1 Performance and qualities of an ideal marker

Performance	Qualities
► Simple	Be disease specific: identify individuals at risk for IBD and differentiate IBD from non-IBD
► Easy to perform	Able to objectively measure disease activity
► Not or minimally invasive	Able to predict the disease course (relapse or recurrence)
► Cheap	Able to monitor the effect of treatment
► Rapid	Have a prognostic value in assessing morbidity/mortality
► Reproducible between labs and individuals	

IBD, inflammatory bowel disease.

THE ACUTE PHASE RESPONSE AND IBD

During the acute phase response to infection, inflammation, necrosis, neoplasia, trauma, severe stress, and childbirth, the human organism will react by up- or downregulation of a number of acute phase proteins (table 2).[1] On resolution of the event which triggered the production of these proteins, their concentrations will return to normal levels but not all with the same speed.

The presence of active gut inflammation in patients with IBD is associated with an acute phase reaction and migration of leucocytes to the gut, and this is translated into the production of several proteins, which may be detected in serum or stools.[2–5]

C REACTIVE PROTEIN

CRP is a pentameric protein consisting of five monomers and is one of the most important acute phase proteins in humans.[6] Under normal circumstances CRP is produced by hepatocytes in low quantities (<1 mg/l). However, following an acute phase stimulus such as inflammation, hepatocytes rapidly increase production of CRP under the influence of interleukin (IL)-6, tumour necrosis factor α (TNF-α), and IL-1β, and may reach peak levels of 350–400 mg/l. Generally, CRP levels of 10–40 mg/l are found in cases of mild inflammation or viral infections. Severe active inflammation or bacterial infection will typically generate CRP levels of 50–200 mg/l, and very high levels of >200–250 mg/l are only found in severe conditions and burns.[7–10]

CRP has a short half life (19 hours) compared with other acute phase proteins and will therefore rise early after the onset of inflammation and rapidly decrease after resolution of the inflammation.

The function is CRP in vivo is still incompletely understood. CRP binds to phosphocholine containing microorganisms or particles which in turn lead to C1q and classical complement activation. CRP also plays a role in the opsonisation of infectious agents and damaged cells.[9–12]

Although CRP is upregulated in most inflammatory diseases, including IBD, there is remarkable heterogeneity in the CRP response between CD and UC. Whereas CD is associated with a strong CRP response, UC has only a modest to absent CRP response.[4 13] This is an important feature to keep in mind when using CRP in clinical practice. There is no good explanation for this heterogeneity given that in UC increased amounts of IL-6, IL-1β, or TNF-α are also detected. However, in the study of Gross *et al*, serum IL-6 concentrations were significantly increased in patients with CD compared with UC and healthy controls and 68.5% of CD patients had serum IL-6 concentrations of ⩾4 U/ml compared with only 21.7% of UC and 0% of healthy controls.[14] Another explanation may lie in the fact that in UC the inflammation is confined to the mucosa whereas in CD it is transmural. However, this is unlikely to explain all of the differences. Recent studies have suggested that polymorphisms in the CRP gene, located on the long arm of chromosome 1 (1q23–24), account for interindividual differences in baseline CRP production in humans.[15–17] Results are however conflicting and one recent study investigating CRP polymorphisms in IBD patients showed no clear association with serum CRP levels.[18]

ERYTHROCYTE SEDIMENTATION RATE

ESR is the rate at which erythrocytes migrate through the plasma. Inevitably, ESR will depend on the plasma concentration and on the number and size of the erythrocytes. Conditions such as anaemia, polycytemia, and thalassemia affect ESR.[19] 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. Increases in ESR with age have been described.[1]

OTHER LABORATORY MARKERS

More generally used laboratory markers include white blood cell count, platelets, and albumin. White blood cell count will increase as part of the acute phase response. Increased

Table 2 Acute phase proteins

	Increased	Decreased
Proteinase inhibitors	α_1 Antitrypsin, α_1 antichymotrypsin, α_2 macroglobulin*	
Coagulation and fibrinolytic proteins	Fibrinogen, prothrombin, factor VIIII, plasminogen, tissue plasminogen activator antithrombin	Factor XII
Complement system	C1s, C2, B, C3, C4, C5, C1INHibitor, C9	Albumin, transferrin
Transport proteins	Haptoglobin, haemopexin, caeruloplasmin	Insulin-like growth factor, α-fetoprotein, cholinesterase
Other	C-reactive protein, serum amyloid A, ferritin Fibronectin, orosomucoid (α1-acid glycoprotein)	

*α_2 macroglobulin behaves differently in animals compared with humans (positive and negative acute phase protein, respectively).

leucocytosis is therefore not a specific feature of IBD and may be seen in other inflammatory conditions and stressful events. White blood cell count is also influenced by some treatments used in IBD, such as glucocorticoids (increased) or azathioprine and 6-mercaptopurine (decreased). Platelet count will also increase and is therefore an indication of, without being a specific marker of, inflammation. Given the wide range of normal values for platelet count, it has been less useful. Albumin is a typical example of a negative acute phase reactant and decreased levels may be found during inflammation. However, other conditions such as malnutrition and malabsorption also cause low albumin levels.

Other acute phase reactants include sialic acid, α_1 acid glycoprotein or orosomucoid, fibrinogen, lactoferrin, β_2 microglobulin, serum amyloid A, α_2 globulin, and α_1 antitrypsin. Most of these markers have not been studied widely in IBD and many have shown conflicting results. Furthermore, their use in IBD has not proved superior to CRP in general, mainly due to the longer half life of these proteins.

β_2 Microglobulin is a low molecular weight protein and is released by activated T and B lymphocytes on activation. The estimated half life is two hours.[20] β_2 Microglobulin is filtered through the glomeruli and levels increase with age and also with decreasing kidney function.

Orosomucoid has been shown to correlate well with disease activity but its half life of five days makes this a less useful marker in clinical practice.[21 22]

FAECAL CALPROTECTIN AND OTHER FAECAL MARKERS

An obvious reason to search for faecal markers is that stools are easy accessible in IBD patients. Furthermore, serum markers may be increased by various conditions other than gut inflammation and therefore faecal markers would have a higher specificity for IBD in the absence of gastrointestinal infection. Also, if faecal markers are representative of mucosal inflammation in the bowel in IBD patients, endoscopic examinations could potentially be avoided.

A number of neutrophil derived proteins present in stools have been studied, including faecal lactoferrin, lysozyme, elastase, myeloperoxidase, and calprotectin.[5 23] Calprotectin, a 36 kDa calcium and zinc binding protein, is probably the most promising marker for various reasons. In contrast with other neutrophil markers, calprotectin represents 60% of cytosolic proteins in granulocytes. The presence of calprotectin in faeces can therefore be seen as directly proportional to neutrophil migration to the gastrointestinal tract. Although calprotectin is a very sensitive marker for detection of inflammation in the gastrointestinal tract, it is not a specific marker and increased levels are also found in neoplasia, IBD, infections, and polyps.[5] Faecal calprotectin is a very stable marker (stable for more than one week at room temperature) and is resistant to degradation, which makes it attractive. Early studies using faecal calprotectin in IBD have shown a good correlation with ^{111}In labelled leucocyte excretion and intestinal permeability.[24] Increased faecal calprotectin levels have been reported after the use of non-steroidal anti-inflammatory drugs as well as with increasing age.[25]

USE OF LABORATORY MARKERS IN INFLAMMATORY BOWEL DISEASE

Laboratory markers have been investigated in IBD for various purposes—diagnosis, differential diagnosis, monitoring of disease activity, response to therapy, and prediction of relapse. In the second part of this overview, the role of laboratory markers in each of these indications will be discussed.

Use of laboratory markers in the diagnosis and differential diagnosis of IBD

Only a few studies have investigated the value of laboratory markers in identifying individuals at risk for IBD and furthermore not all studies used the same markers.

An early study from St Mark's Hospital, London, UK, investigated 82 adults referred with abdominal symptoms.[26] In all patients, clinical examination as well as a rectal biopsy were performed and ESR, CRP, and α_1 glycoprotein were determined. Of these markers, CRP was increased in all patients who subsequently were diagnosed with CD (n = 19), in 50% of patients diagnosed with UC (n = 22), but in none of the 41 patients with functional bowel symptoms. A paediatric study by Beattie *et al* undertook a similar approach and studied 91 children (mean age 11 years) referred for symptoms of abdominal pain, diarrhoea, rectal bleeding, weight loss, or mouth ulceration.[27] All children underwent extensive blood analysis (including haemoglobin, leucocyte count, platelet count, ESR, albumin, and CRP), ileocolonoscopy, and small bowel follow through. Twenty six children were finally diagnosed with CD, 13 with UC, eight with polyps, two with tuberculosis, three with indeterminate colitis, two with lymphoid nodular hyperplasia, and 37 had a normal investigation. The best laboratory marker in differentiating IBD from normals was CRP. Similar to the study of Shine and colleagues,[26] 100% of CD patients but only 60% of UC patients had increased CRP compared with none of the children with polyps and none of the children with a normal investigation. ESR proved to be the second best marker, with 85% of CD and 23% of UC patients positive compared with none of the children with a normal investigation. Finally, a larger study on 203 individuals referred for symptoms suggestive of lower bowel disease also showed that CRP was a good marker in differentiating IBD from irritable bowel syndrome (IBS).[28]

Taken together, these studies seem to suggest that CRP is the most sensitive marker in detecting IBD but values range between 50% and 60% for UC and between 70% and 100% for CD, and also depend on the cut off value used. Some authors have suggested using more sensitive cut off values which would allow an increase in sensitivity to 100%.[28] In this respect, high sensitivity CRP assays have been studied in other inflammatory conditions, such as atherosclerotic heart disease, but no studies investigating the role of hs-CRP in the detection of IBD have been published.

Faecal calprotectin has been shown to enable diagnosis of IBD. In this respect, a cut off of 30 μg/g had 100% sensitivity in discriminating active CD from IBS in the study of Tibble and colleagues.[5] In the paediatric study by Fagerberg and colleagues,[29] 36 children with symptoms and suspected inflammation of the colon were subjected to stool analysis for faecal calprotectin and an ileocolonoscopy. Twenty two patients showed inflammation on endoscopy (of whom 20 were later diagnosed with IBD), and calprotectin levels were much higher in these patients than in children without inflammation on endoscopy. The authors concluded that faecal calprotectin is helpful in the detection of colonic inflammation in children with gastrointestinal symptoms suggestive of IBD and that a positive test may prioritise

endoscopy. Interestingly, increased faecal calprotectin has been described in healthy first degree relatives of patients with CD.[30] Follow up of these individuals will determine if faecal calprotectin may identify relatives at risk of developing IBD.

Use of laboratory markers to monitor disease activity in IBD

In general, patients with severe disease more often have abnormal inflammatory markers, compared with patients without or with only low grade inflammation. This has been shown in a prospective study by Tromm and colleagues[31] who investigated laboratory markers ESR, serum albumin, α_1 proteinase inhibitor, cholinesterase, CRP, and haematocrit, and correlated these markers with endoscopic activity.

One of the early studies in IBD showed a good correlation between ESR and clinical activity.[32] The correlation was however dependent on disease location, and ESR correlated less well with UC restricted to the rectum and with CD restricted to the upper small bowel.[32 33] The study by Fagan *et al* showed that both CRP and ESR correlated well with disease activity but the correlation was better for CRP.[34] This was also the conclusion from various other studies where CRP was either the best marker or the only marker which correlated significantly with clinical activity status.[21] However, a wide range of CRP values was observed and overlap existed between mild to moderate (10–50 mg/l), moderate to severe (50–80 mg/l), and severe disease (>80 mg/l). What is undoubtedly more important than a particular cut off value for CRP is the comparison of the CRP value with previous values in a given patient.

With respect to CD and UC, the correlation of laboratory markers with disease activity has been shown to be much stronger for CD than for UC.[34] Apart from clinical activity, data from the Mayo Clinic have also shown good correlation between CRP and endoscopic and histological activity in CD. For UC, again, this correlation was less strong.[35]

Faecal calprotectin also correlates well with endoscopic and histological activity in patients with UC[36] and in CD,[37] and increased calprotectin levels normalise once the inflammation is resolved.

Finally, some authors showed good correlation between β_2 microglobulin and disease activity.[38–40] However, data were conflicting and not all authors were able to confirm these findings.[41]

Use of laboratory markers to predict the disease course

IBD follows a variable disease course and both CD and UC are characterised by periods of remission altered with flares. Disease flares occur in a random way and are often unpredictable. However, if a relapse could be reliably predicted, one could try and avoid them or treat with early and more aggressive therapies.

CRP has been shown to be a good marker for predicting disease course and outcome in a number of diseases. Most well known is its association with cardiovascular disease and poor outcome after myocardial infarction.[42–44] Also, in multiple myeloma, serum CRP is a highly significant prognostic factor and high CRP and high β_2 microglobulin levels are associated with worse survival.[45]

A number of studies in CD have investigated a panel of laboratory markers in predicting clinical relapse. A prospective study by Brignola *et al* analysed 41 CD patients with clinically inactive disease (CD activity index <150) for six months for a panel of inflammatory markers (ESR, white blood cells, haemoglobin, albumin, α_2 globulin, serum iron, CRP, α_1 glycoprotein, and α_2 antitrypsin).[46] All patients were followed up until relapse. A total of 17/41 patients relapsed. ESR, α_2 globulin, and α_1 glycoprotein were best at distinguishing relapsers from non-relapsers. Based on these markers, a prognostic index (PI) was calculated and the threshold of discriminant power was 0.35. Using this threshold, all patients with a PI >0.35 relapsed over a period of 18 months, compared with 5/29 patients with a PI <0.35. Therefore, although normal values did not guarantee remission in all patients, high values predicted relapse in the following 1–2 years. A few years later, Boirivant *et al* prospectively followed 101 outpatients with CD.[47] Half of the patients had a raised CRP and this correlated well with clinical activity. Approximately one third of CD patients presented with active disease despite normal CRP and one third had raised CRP but clinically inactive disease. The likelihood of relapse after two years was higher the patients with an increased CRP compared with patients with normal CRP.

More recently, the GETAID group prospectively followed 71 CD patients with medically induced remission and measured laboratory markers (full blood count, CRP, ESR, α_1 antitrypsin, orosomucoid) every six weeks.[48] In total, 38 patients relapsed (defined as a CD activity index >150 with an increase of >100 points from baseline) after a median of 31 weeks. Only two laboratory markers were predictive of relapse: CRP (>20 mg/l) and ESR (>15 mm). Patients with both markers positive had an eightfold increased risk for relapse with a negative predictive value of 97%, suggesting that normal CRP and ESR could almost certainly rule out relapse in the next six weeks.

It is clear that we still cannot rely on CRP alone to predict clinical relapse in CD. One of the crucial questions is how early or late CRP and other inflammatory markers start increasing and what the ideal time would be to measure them.

There are much less data on the value of laboratory markers in assessing disease course and outcome in UC. A prospective study from Oxford evaluated 49 severe UC patients treated with hydrocortisone and/or ciclosporin (n = 49). On day 3, a frequency of >8 stools/day or 3–8 stools/day together with an increased CRP (>45 mg/l) predicted with 85% certainty the need for colectomy.[47]

More recently, faecal calprotectin was shown to predict relapse of CD.[50–52] In the study by Tibble *et al*, calprotectin levels of 50 μg/g or more predicted a 13-fold increased risk for relapse.[50] Costa *et al* included 38 CD and 41 UC patients in remission for a mean of five months.[51] A baseline level of calprotectin of 150 μg/g or more was predictive for a relapse in the next year. Although sensitivity was high for both CD (87%) and UC (89%), specificity was much lower in the case of CD (43%) compared with UC (82%). In this study, ESR or CRP was not predictive of relapse.

Also, in a more recent study, faecal calprotectin predicted clinical relapse but here the test performed better in patients with UC than in those with CD.[52] It is however difficult to conclude from cut off values from these studies.

Role of laboratory markers for monitoring the effect of treatment

A change in CRP following therapy is a good parameter to assess the effect of the drug on the underlying inflammation.

Table 3 Pros and cons of including C reactive protein (CRP) in future clinical trials in inflammatory bowel disease

In favour of including CRP in clinical trials	Against including CRP in clinical trials
(1) Allows objective selection of patients with active disease	(1) Carries risk of restricting drugs to patient with high CRP
(2) Higher likelihood of response and lower placebo response rates	(2) Risk of more restrictive FDA label
(3) Objective marker for follow up effect of treatment	(3) Yet unclear which CRP cut off value is best

A decrease in CRP in response to therapy is objective evidence that the drug has a beneficial effect on gut inflammation and this even in patients with little change in symptoms. On the other hand, persistently raised CRP indicates failure of the therapy to control mucosal inflammation. Introduction of biological therapies in IBD has led to a major improvement in treatment options. Anti-TNF-α antibodies are very efficacious in patients with CD. Nevertheless, anti-TNF treatment fails in approximately 25% of patients. In a Belgian study, in 153 patients treated with infliximab, a baseline CRP >5 mg/l before the start of therapy was associated with a higher response (76%) compared with patients with CRP <5 mg/l (46%) ($p = 0.004$).[53] Very similar results have been demonstrated for the humanised anti-TNF molecules CDP-571 and CDP-870, and for the fully human anti-TNF antibodies adalimumab and antiadhesion molecule strategies.[54–56] Along the same lines, low or normal baseline CRP values have been associated with a high placebo response and remission rate in clinical trials.[57]

These findings raise the question of whether CRP should be included in selection of patients in future clinical trials? There are certainly pros and cons of such a strategy (table 3). On the one hand, including only patients with raised CRP will select patients with active disease who are more likely to respond and will reduce placebo response rates. This approach may therefore be beneficial in optimising treatment and reaching primary end points in clinical trials. However, including only patients with raised CRP carries the risk that a drug and its FDA label may be restricted only to certain patients. When considering results from the study by Louis *et al* on infliximab, 46% of CD patients with low or normal CRP still showed a response.[53] Restriction of certain treatments only to patients with increased CRP would deny them a good drug. Less important but nevertheless an issue which will need to be discussed is which cut off value to use when including CRP in clinical trials.

CONCLUSION

Although various laboratory markers have been investigated in IBD, none has been shown to be ideal or superior to our current diagnostic tools. Nevertheless, CRP is a useful marker and should be preferred in CD as it correlates well with disease activity. The situation in UC is however different and CRP correlates less well with disease activity compared with CD. Faecal calprotectin is a useful, non-invasive, and sensitive stool marker for gut inflammation in both CD and UC. Recent data suggest that the calprotectin assay performs better for UC than for CD. Whereas other acute phase reactants and markers of inflammation such as ESR also give reliable information on disease activity, their longer half life and interference with other factors make them less useful in clinical practice compared with CRP. In conclusion, laboratory markers are useful and should be integrated in the overall management of the IBD patient.

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