PhiCal™ Test

"Helps to guide the patient in the right direction"

A very useful test for the distinction between organic and functional gastro-intestinal disorders. Consider it when you suspect inflammatory bowel disease or colorectal cancer.

IN THIS BOOKLET:

Introduction

The **PhiCal test**, or the assessment of faecal calprotectin, is a new principle for an objective determination of the inflammation in IBD (Inflammatory Bowel Disease), i.e. ulcerative colitis (UC) and Crohn´s disease (CD). Traditionally, the disease activity has been evaluated by clinically indices like the Crohn´s disease activity index (CDAI) or by the Harvey-Bradshaw "simple index" (SI). However, the use of more sophisticated methods, for instance the use of isotope labelled white cells, has shown that such indices tend to be inaccurate (1).
The quantitation of the neutrophil marker protein calprotectin in simple extracts of small (0.1g) stool samples is now available as an attractive alternative to the previous methods (2). This PhiCal test is non-invasive, objective and can be used repeatedly for the early detection of relapse in IBD, monitoring disease activity and document response to medical treatment. In children with chronic abdominal symptoms where IBD may be suspected, most physicians will hesitate to use isotope labelled leukocytes or endoscopy under general anaesthesia as diagnostic procedures. The PhiCal test has such a high negative predictive value that active IBD can be ruled out and invasive procedures avoided (3).

PhiCal test can be performed by Calprest kits produced by Eurospital, S.p.A.
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Calpro AS is responsible for worldwide marketing and sales.

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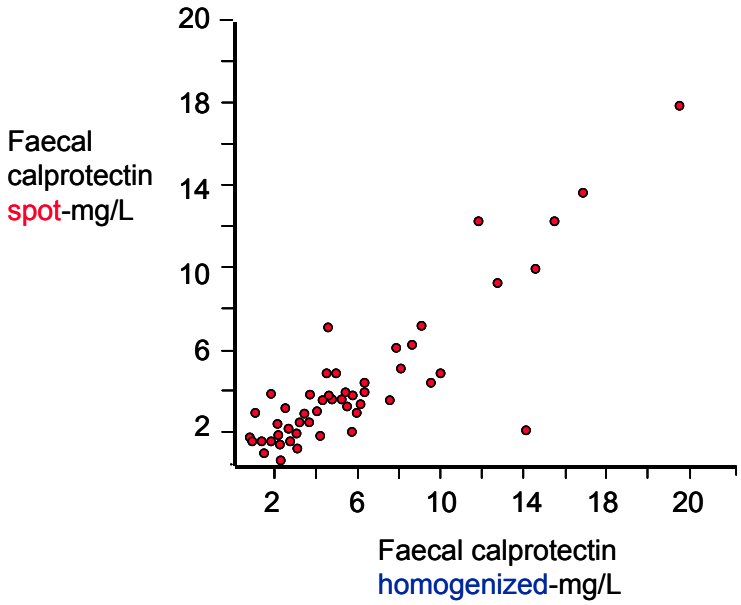
Sampling

Traditionally, biological markers in stools have been assessed in 24 hour stool collections, but it is rather inconvenient for out-patients to collect and send such materials to the laboratory. Fortunately, calprotectin is so evenly distributed in stools (fig 1) that only small stool samples (typically one teaspoon full) are necessary for analysis. Furthermore, this protein is so stable that stools can be sent by ordinary mail. In fact, samples can be stored at room temperature for up to seven days The stability of calprotectin is caused by its binding of calcium (4).

Fecal extracts:

A new hygienic extraction procedure has been developed where stool samples of only about 0.1 g are extracted with about five ml of a new buffer in a closed tube on a rotator for about 30 minutes. The supernatant after centrifugation is run on a simple ELISA (5).

Fig 1



There is a exellent correlation (r = 0.90) between calprotectin values determined in spot samples (5g) and in samples from homogenized 24 hr stool collections.

Scand J Gastroenterol 1992;27:793 - 98

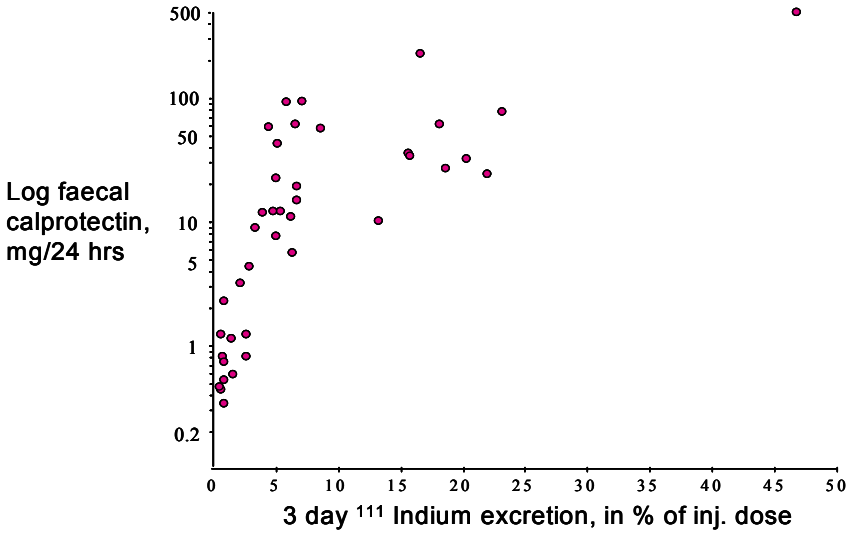
ELISA

Calprotectin is assessed by a simple ELISA, using polyclonal rabbit antibodies for capture and affinity purified, enzyme (alkaline phosphatase) labelled antibodies (against six different epitopes on calprotectin) for development. Using six epitopes makes the ELISA more robust and less likely to give falsely low values. In contrast, assays using monoclonal antibodies against a single epitope, may give low values due to masking or destruction of the epitope due to complex formation or enzymatic degradation in the bowel. The Phical ELISA has between and within assay variations of 14.8% and 1.9% respectively (2,3).
The new faecal extraction is much more efficient so that both in healthy controls and patients higher values are found. For this reason the new cut off value is now 50 mg/kg in contrast to the previously reported 10 mg/l (5).

The PhiCal test versus isotope labelled leukocytes:

Excretion of isotope labelled leukocytes has been regarded as the "gold standard" of disease activity in IBD. However, this requires three days of hospitalisation for the collection of stools and the determination of the isotope. Furthermore, it also exposes the patient to a potentially hazardous radiation, making this method unsuited for repeated use in children and fertile women.
We have compared this method with the PhiCal test in nine healthy controls, 10 patients with UC and 19 patients with CD. (5) Although these were all out patients with very low disease activity, a highly significant correlation was found between the three day excretion of 111-indium labelled granulocytes and that of faecal calprotectin (r=0.87, p<0.0001) (figure 2).

Fig 2



There is a good correlation between the excretion of calprotectin and 111-indium labelled granulocytes (r= 0.87, p<0,0001).

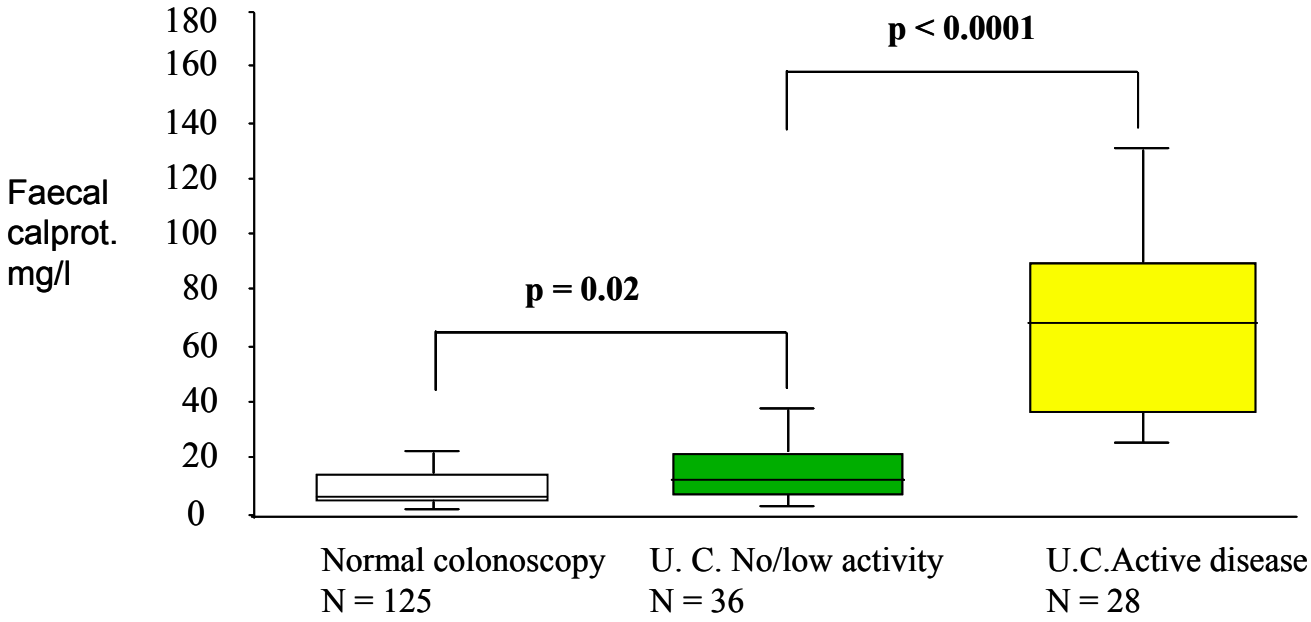
Scand J Gastroenterol 1999;34:50-54

In a similar study published lately, this close relationship was confirmed. (3)
This suggests that the PhiCal test can replace the use isotope method. Its simplicity and low cost makes it an attractive alternative allowing follow-up by repeated testing.

Calprotectin in patients with Ulcerative Colitis:

Data from a study of 62 outpatients referred for colonoscopy are shown in Fig.3. The disease activity was determined by endoscopy and histology using accepted criteria. There was a highly significant increased concentration of faecal calprotectin in the group of patients with active disease as determined by histological criteria when compared to patients with no/low disease activity and those with normal colonoscopy. Furthermore, calprotectin levels were better correlated to the degree of inflammation than the extension of the disease. (7)

Fig 3



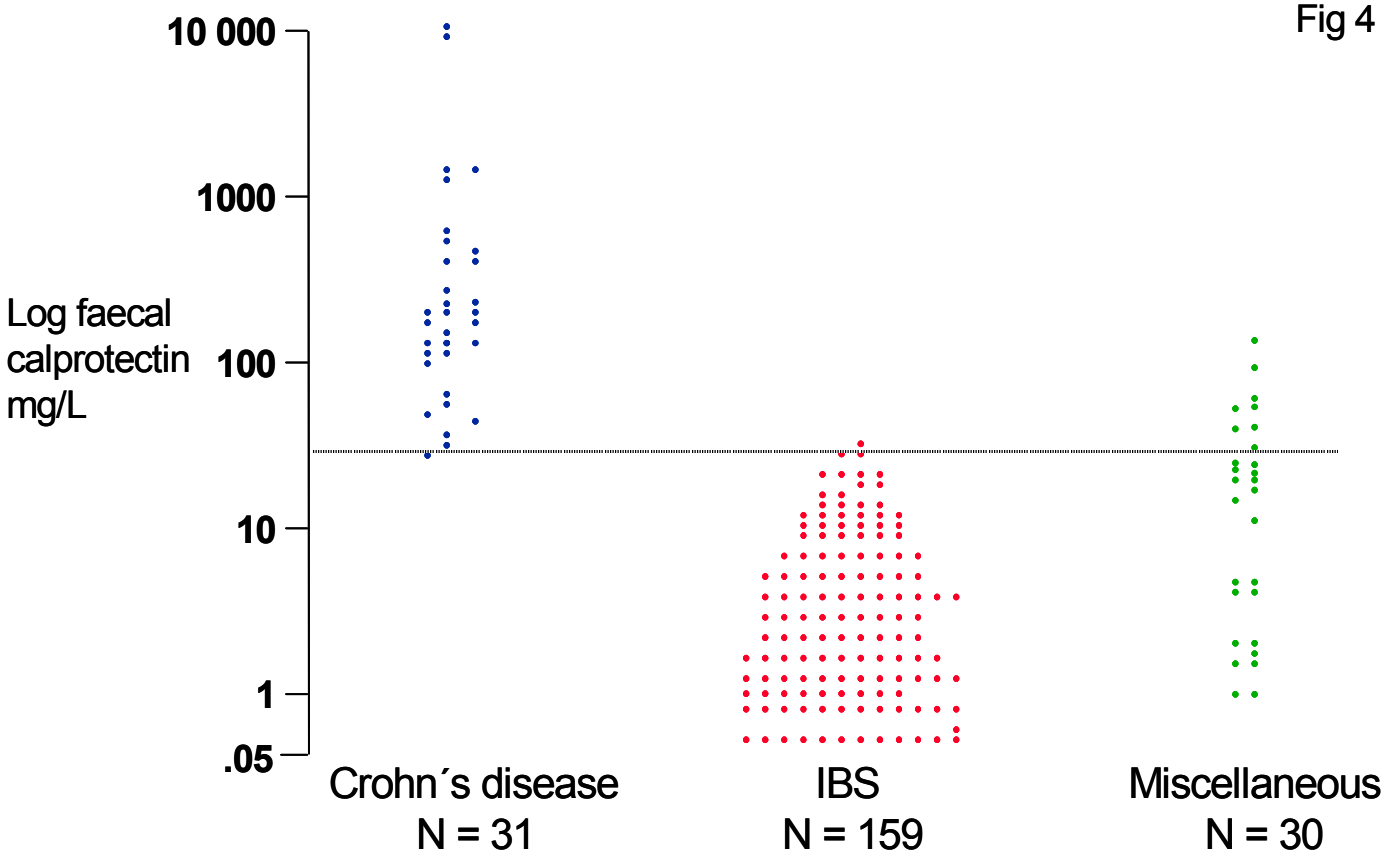
There is a good agreement between calprotectin levels and histologically determined disease activity. This is demontrated by the box-plots showing 10/90 percentiles and 25/50/75 percentiles.

Digestion 1997;58:176-80

PhiCal test in Crohn´s disease and irritable bowel syndrome:

It is often difficult to differentiate between patients with IBS and CD by clinical parameters. This results in many unnecessary colonoscopic examinations adding an substantial economical burden on the society.
Calprotectin was determined in 220 patients referred for colonoscopy at King´s College, London. (3) The patients were evaluated by the ROME criteria for possible IBS. The results showed that patients with CD had significantly higher faecal calprotectin levels than those with IBS ($p<0.0001$) (Fig 4).

Fig 4



Distribution of faecal calprotectin levels in patients with Crohn´s disease, irritabel bowel syndrome (IBS) and miscellaneous colonic diagnoses (see below)

Gut 2000;47:506-513

A **ROC plot analysis** revealed that a cut-off level of 30 mg/l (corresponding to 150 mg/kg in the new method) distinguished between CD and IBS with 100% sensitivity and 97% specificity (fig 5).
Among 30 patients with other disorders, elevated PhiCal tests were found among 2/2 colorectal cancer, 2/3 adenomas and 6/6 with microscopic colitis. Similar results were reported in a study at Mayo Clinic USA. (8)

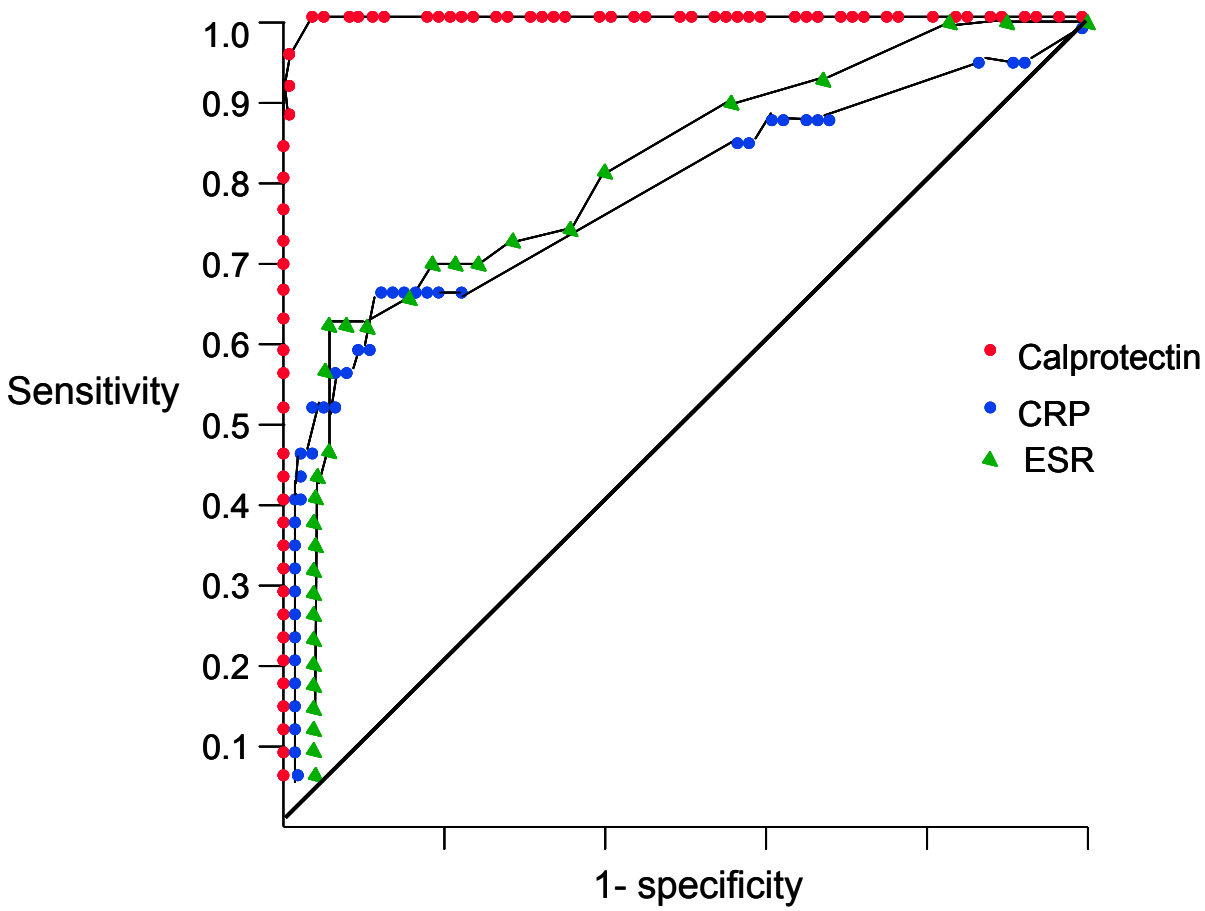


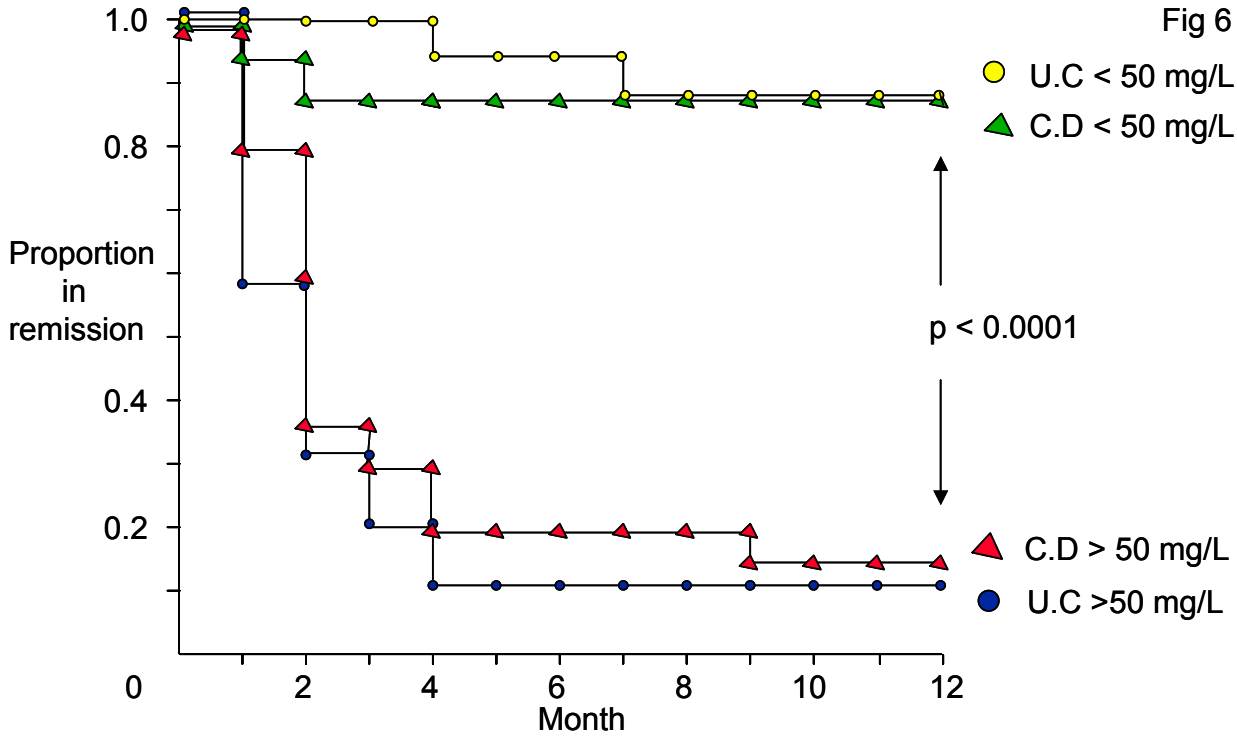
Fig 5

ROC analysis of the ability of calprotectin, CRP and ESR to discriminate between patients with IBS and IBD.

Gut 2000;47:506-513

The PhiCal test as a predictor of relapse of IBD:

Tibble et al reported in Gastroenterology last year a study of 43 patients with CD and 37 with UC, and concluded that the PhiCal test was useful to predict a future relapse of IBD (Fig 6). In each patient, faecal calprotectin was assessed in a single stool sample at inclusion, and changes in the disease activity were assessed by the Harvey Bradshaw "Simple Index".



Kaplan-Meier " time to relaps curves" for patients with CD and UC in relation to faecal calprotectin. Patients with elevated calprotectin levels (>50 mg/l) had a significantly increased (p<0.0001) risk for a relaps during the 12 month observation period.

Gastroenterology 2000;119:15-22

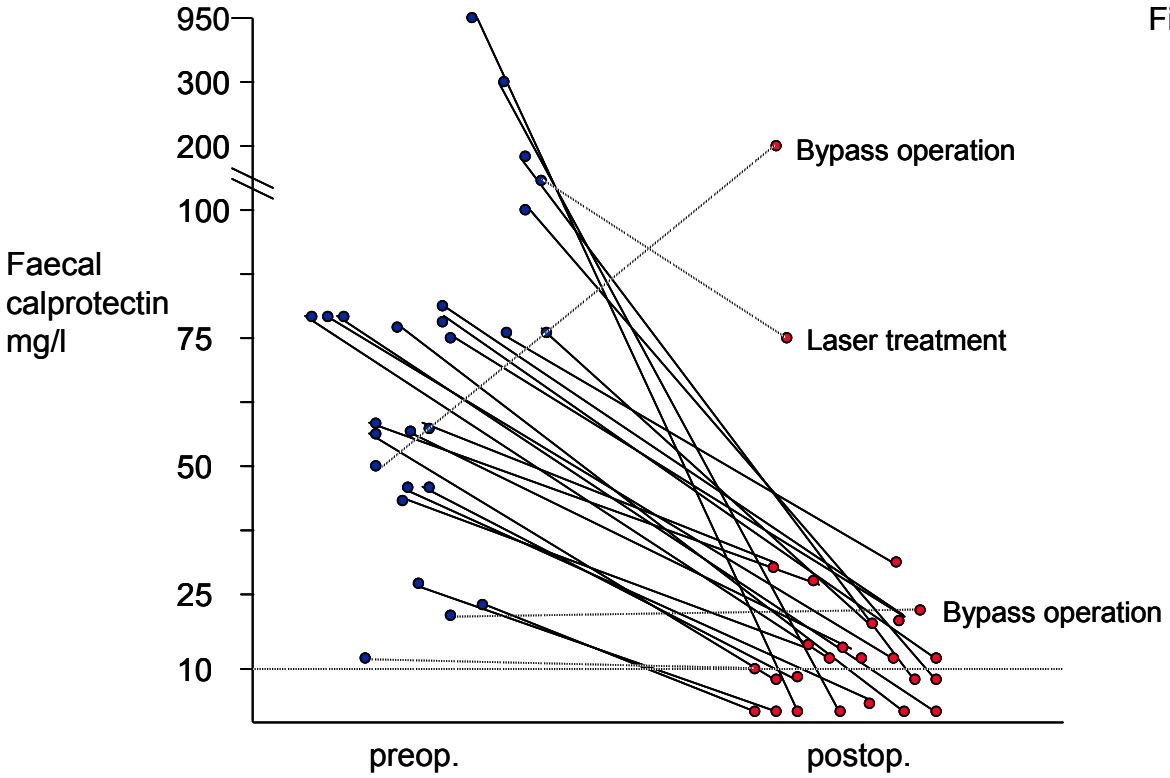
The **Kaplan-Meier curves** show that 58% of patients with CD and 51% of those with UC had relapsed during the one-year follow-up period. ROC analysis revealed that faecal calprotectin level of 50 mg/l (250mg/kg by the new method) predicted a relapse with 90% sensitivity and 83% specificity. These results may suggest that the PhiCal test can be helpful in adjusting the medical treatment of IBD and thereby reduce side effects and probably the development of secondary changes in the GI tract in IBD patients. (9)

The PhiCal test and colorectal neoplasia:

Several publications have reported increased levels of faecal calprotectin in patients with colorectal cancer and adenomas.

As shown in Fig.7 levels normalise or drop dramatically after surgery, indicating a close relationship between increased calprotectin and the presence of the tumour. Most publications agree that increased calprotectin levels are found in approximately 90% of symptomatic cancers and in 60-70% of adenomas (5,10,11) The mechanism behind is probably ulcerations and increased permeability of the mucosa so that increased numbers of neutrophils migrate into the gut lumen towards the high concentrations of bacterial chemotactic substances there.
A negative predictive value of 99.1% indicates that patients with a normal PhiCal test have a very low risk of having colorectal cancer. (5)

Fig 7



Faecal calprotectin levels in 26 patients with colorectal cancer, before and after surgery.

Dis Colon Rectum 1998;41:316-21

Conclusion:

Calprotectin is a non-specific marker of gastrointestinal disease of both inflammatory and neoplastic character. The high sensitivity and negative predictive values can be useful to select patients for colonoscopy.
Calprotectin is also an interesting marker of disease in various organs since elevated concentrations are found in plasma/serum, cerebrospinal fluid, synovial fluid or urine during infections or inflammatory conditions.

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