

Measurement of calprotectin in faeces

Abstract

Background. Calprotectin is a calcium-binding protein found in neutrophils. Inflammatory damage of the intestinal mucosa causes an influx of neutrophils into the intestinal lumen, after which increased calprotectin levels can be measured in stools. This review is based on publications identified through a non-systematic search in Medline and our clinical experience from measuring calprotectin in faeces for more than 10 years.

Results. Calprotectin is first of all a very good marker of intestinal inflammation. The test is therefore a useful tool for investigation of abdominal discomfort when symptoms and clinical examination make it difficult to determine whether the condition is organic or functional. At the time of diagnosis, all patients with inflammatory bowel disease have clearly increased levels of faecal calprotectin; in patients with irritable bowel syndrome the levels are normal. Normalization of faecal calprotectin seems to be a strong indicator of healing of the intestinal mucosa.

Interpretation. Determination of calprotectin in faeces can therefore contribute to reducing the number of unnecessary colonoscopies, which is especially important in children. Faecal calprotectin is a simple test and an objective parameter of treatment response and disease course in patients with inflammatory bowel disease.

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Calprotectin is a small protein that can be detected in various body fluids and tissues by using ELISA methodology and immunohistochemistry. The protein originates mainly from neutrophils. In the 1990s, detection of calprotectin in faeces was marketed as a test for colorectal cancer. The specificity of the test was too low, which was one of the reasons for the test never being accepted.

Calprotectin has received a lot of attention during recent years, and only during the last year more than 50 articles have been published about calprotectin measurements in faeces or in other body fluids and tissues. The article discusses measurement of calprotectin in faeces in diagnostics and follow-up of patients with inflammatory bowel disease and irritable bowel syndrome.

Material and method

This review article is based on literature identified through a non-systematic search in Medline and own clinical experience with measuring calprotectin in faeces for more than 10 years.

Calprotectin in faeces

After granulocytes are released from bone marrow, they circulate freely in the blood. Thereafter they spend a few days in various peripheral tissues before they end their life in the gastrointestinal tract (1), where they release a number of antimicrobial substances (among them calprotectin). It is assumed that calprotectin has an important biological function in regulation of the intestinal microbiota.

The intestinal mucosa is continuously exposed to enormous amounts of microbes and their toxic products, among them are chemotactic substances such as endotoxins. This is probably the explanation for the concentration of calprotectin in normal intestinal environments being many times higher

than that in blood. Magne Fagerhol and collaborators first described calprotectin in 1980 (2). Every granulocyte contains about 20 pg of calprotectin which comprises approximately 5% of the total amount of protein, and as much as 60% of the protein in cytosol fractions in these cells. For comparison, there is about as much calprotectin in a granulocyte as there is haemoglobin in an erythrocyte.

Calprotectin binds both calcium and zinc and has a number of biological characteristics. As mentioned it has an antimicrobial function, it inhibits many metalloproteinases (4) and induces apoptosis in malignant and non-malignant cell cultures (5). The protein is released from granulocytes upon activation and the concentration in blood and faeces may rise over 100 times the normal value, e.g. in active rheumatoid arthritis (6), meningococcal sepsis (M. Fagerhol personal communication) and active inflammatory intestinal disease (7–9).

In the presence of calcium the protein is stable and resistant to enzymatic breakdown, and unchanged levels have been measured in faecal samples after storage for seven days at room temperature (7). This means that doctor's offices or patients may send samples directly to the laboratory with ordinary post. A number of investigations in Norway, England and the USA show that healthy individuals generally have calprotectin levels in faeces below 50 mg/kg, while patients with active inflammatory bowel disease (irrespective of location in the gastrointestinal tract) often have concentrations above 500–1 000 mg/kg (8–11). Patients with coeliac disease have calprotectin in faeces at the same levels as healthy individuals (12).

Calprotectin analyses are carried out at

Main message

- Calprotectin in faeces is a good marker for inflammation in the gastrointestinal tract
- Determination of calprotectin in faeces is of great value in the differential diagnosis between irritable bowel syndrome and inflammatory bowel disease
- The measurement can be used to assess disease course and treatment effect in inflammatory bowel disease

Box 1

The Rome III criteria for diagnosis of irritable bowel syndrome

The patient should have recurrent abdominal pain or discomfort for more than six months. In addition, the complaints should be present at least three days per month for three months. At the same time at least two of the following features have to be present:

- The complaints are alleviated in connection with defecation and/or
- change in stool frequency with symptom start
- and/or changes in the faecal appearance with symptom start

Fürst laboratory in Oslo and in several hospitals in Norway: among them Oslo University Hospital, Ullevaal; University Hospital Northern Norway, Haukeland University Hospital and Nordland Hospital Trust. A qualitative quick test has also been developed and is expected to be clinically available shortly (13).

Irritable bowel syndrome

Persisting abdominal pain is a common cause for consulting general practitioners. Irritable bowel syndrome is the most common cause and occurs in 10–20% of the adult population in the western world (14). It is estimated that about 40% of all referrals to colonoscopy are for irritable bowel syndrome (15). The disorder can present at all ages, also in children, but is most common at the age 20–40 years, with a slightly higher prevalence among women. The diagnosis is made by using the Rome III criteria (Box 1), but many consider it an exclusion diagnosis (made by excluding organic disease). Symptoms of irritable bowel syndrome can easily be mixed up with untreated coeliac disease, lactose intolerance and not least ulcerative colitis and Crohn's disease. Doctors with a special interest for the syndrome deny that it is necessary to do extensive examinations

to make the diagnosis (16). As the condition is so common, the costs for examinations such as lab tests, endoscopy and imaging are very large. Calculations from the USA and the UK show that this comprises as much as 0.5% of the total health budget. Indirect costs, such as absence from work and sick leave, come in addition (17). A simple and reliable test to discriminate organic disease from functional conditions will be important. It is especially important to avoid unnecessary invasive examinations in children.

Inflammatory bowel disease

Several studies have shown that calprotectin levels in faeces are highly sensitive for detection of active inflammatory bowel disease. Blood tests such as sedimentation rate (SR) and C-reactive protein (CRP) can often be normal even if there is extensive inflammatory activity in the intestinal mucosa (7, 9, 18–20). It is not uncommon that patients with inflammatory bowel disease also have symptoms of irritable bowel syndrome that can be very difficult or impossible to discriminate from symptoms caused by active inflammation in the intestine. In such situations, measurement of calprotectin in faeces may be very helpful and can replace colonoscopy in many cases.

SR and CRP are much used as objective parameters for disease activity in inflammatory bowel disease, but the usefulness seems to be relatively low (19, 20). Several clinical indices have been developed, but common for them all is that they are based on subjective symptom descriptions and have a low reproducibility for individual patients (21). Colonoscopy is often necessary and gives the possibility for direct inspection of the intestinal mucosa in addition to taking biopsies. On the negative side is discomfort for the patient and the invasive nature of the investigation. Sipponen and collaborators suggested in a recent publication that faecal markers (such as calprotectin and lactoferrin) should completely or partly replace colonoscopy in follow-up of Crohn's disease (20). The calprotectin value in faeces correlates very well with the excretion of isotope-labelled granulocytes ($r = 0.87$,

$p = 0.0001$) (22). There is also a very good correlation between calprotectin levels in faeces and inflammatory activity, as assessed by endoscopy and histology (8, 23, 24). At Oslo University Hospital, Aker patients routinely send faeces samples as part of the follow-up. Inflammatory bowel disease is a chronic condition with a variable level of disease activity. With clinical relapse, it may be important to quickly intensify treatment. In a prospective study, Tibble and collaborators showed that calprotectin can be used to foresee the risk of exacerbation of the disease. Among patients in clinical remission (with faecal calprotectin levels above 250 mg/kg), 85% had a relapse during the next 12 months, while for patients with values under 250 mg/kg only 12% had a relapse in the same time-period (25). An Italian investigation has found similar results (10). In concordance with this, patients with low-grade infiltration of inflammatory cells in the intestinal mucosa were found to have a larger risk of relapse (26). CRP had no predictive value in these studies. In a study from Aker University Hospital we found that normalization of calprotectin levels in patients with inflammatory bowel disease was a strong indicator for healing of the mucosa (27). Colonoscopy was used to examine 45 patients with inflammatory bowel disease and normal calprotectin levels in faeces (< 50 mg/kg). Of these, 44 had normal mucosa (as assessed by endoscopy), and 38 patients had a completely normal histology. Seven patients only had a light infiltration of leucocytes, but without affection of the mucosa or crypts. Normalization of the mucosa is an important measure in treatment of inflammatory bowel disease, because it implies a lower occurrence of relapses, fewer hospital admissions and a reduced risk for complications and surgical procedures (28–30).

Practical use of calprotectin measurements

Studies vary with respect to faecal calprotectin's ability (sensitivity, specificity and predictive values [positive and negative]) to discriminate between different or-

Table 1 Calprotectin in faeces –diagnostic precision for discrimination between various clinical conditions by different cut-off values

	Patients (n)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Reference
Chronic diarrhoea with an organic cause. Cut-off 50 µg/g	120	66	84	83	68	Carroccio (31)
Chronic diarrhoea with an organic cause. Cut-off 100 µg/g	120	46	93	90	59	Carroccio (31)
Organic versus functional conditions in the gastrointestinal tract. Cut-off 50 µg/g	239	83	82	90	71	Costa (10)
Crohn's disease versus irritable intestinal syndrome. Cut-off 150 µg/g	190	100	97	89	100	Tibble (18)
Chronic diarrhoea with colorectal inflammation with. Cut-off 100 µg/g	110	83	95	63	94	Limburg (11)
Colorectal inflammation with gastrointestinal symptoms. Cut-off 50 µg/g	36	95	93	95	93	Fagerberg (32)

ganic and functional conditions in the gastrointestinal tract (tab 1) (10, 11, 18, 31, 32). This is mainly caused by differences in patient populations such as age, known diagnosis, symptomatology, disease prevalence and inflammatory activity. In one of the studies with low sensitivity, coeliac disease was the diagnosis for one third of the patients who had an organic cause for the diarrhoea and a normal value of calprotectin in faeces (31). In a meta-analysis of 13 studies (including 1 595 patients), one found that calprotectin in faeces is 89–98 % sensitive and 81–91 % specific for discrimination of inflammatory bowel disease from a functional condition (33).

Choice of cut-off value is also of importance. Tibble and collaborators have shown that the optimal cut-off value for discrimination between irritable bowel syndrome and inflammatory bowel disease is 150 mg/kg (18). If no alarming symptoms are present (such as bleeding or weight loss), a Finnish study and our own experience suggest that slightly elevated calprotectin values (50–200 mg/kg) should be confirmed with a new test before determining whether further assessment with colonoscopy is necessary (20).

As the negative predictive value (with normal values of calprotectin in faeces) in patients with abdominal pain is close to 100 % in inflammatory bowel disease, correct use of the test may result in a reduced number of colonoscopies. It is important to avoid unnecessary colonoscopies, because this is a resource-demanding and invasive investigation with potential complications. In addition, colonoscopy is unpleasant for the patient. In our opinion, patients with symptoms compatible with irritable bowel syndrome and normal values of calprotectin in faeces, also have normal colonoscopy findings. At King's College in London, where the test has been used for many years, one has estimated a saving of 600 000 euros per consultant per year in unnecessary endoscopies and imaging (Ingvar Bjarnason, personal communication).

At Oslo University Hospital, Aker, measurement of calprotectin in faeces has been used routinely for follow-up of patients with inflammatory bowel disease for many years.

Conclusion

Calprotectin in faeces is an objective criterion for inflammation and disease activity in inflammatory bowel disease, and is suitable for assessment of disease course and monitoring of treatment effect in these patients. Irritable bowel syndrome is a chronic disorder and frequent cause for consultation to both general practitioners and specialists. This patient group has normal values of calprotectin in faeces. An increasing number of people are referred for colonoscopy. With correct use of calprotectin measurements in faeces we believe that many of these colonoscopies can be avoided.

Disclosed conflicts of interest: Jørgen Jahnsen and Erling Aadland have not disclosed any conflicts of interest. Arne G. Røseth has patent rights covering commercial use of calprotectin in faeces in some countries.

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