Review Article

Faecal Calprotectin

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Abstract

Calprotectin is a calcium- and zinc-binding protein of the S-100 protein family which is mainly found within neutrophils and throughout the human body. The presence of calprotectin in faeces is a consequence of neutrophil migration into the gastrointestinal tissue due to an inflammatory process. Faecal calprotectin concentrations demonstrate good correlation with intestinal inflammation and faecal calprotectin is used as a biomarker in gastrointestinal disorders.

Faecal calprotectin is a very sensitive marker for inflammation in the gastrointestinal tract, and useful for the differentiation of inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS). Faecal calprotectin is used for the diagnosis, monitoring disease activity, treatment guidance and prediction of disease relapse and post-operative recurrence in IBD. There may also potentially be a role for faecal calprotectin in the management of infectious gastroenteritis, acute appendicitis, peptic ulcer disease, cystic fibrosis, coeliac disease, transplant rejection and graft versus host disease. Further studies are needed to confirm its utility in these conditions.

Analysis of faecal calprotectin consists of an extraction step followed by quantification by immunoassay. Over the past few decades, several assays and extraction devices including point-of-care methods have been introduced by manufacturers. The manufacturer-quoted cut-off values for different faecal calprotectin assays are generally similar. However, the sensitivities and specificities at a given cut-off, and therefore the optimum cut-off values, are different between assays. A reference standard for calprotectin is lacking. Therefore, assay standardisation is required for more accurate and traceable test results for faecal calprotectin.

Introduction

Calprotectin, also known as MRP8/14 and S100A8/A9, is a calcium- and zinc-binding protein of the S-100 protein family first isolated from blood leucocytes.[1] It is an oligomer of two light (11 kDa) and one heavy (13 kDa) subunits with a total molecular mass of approximately 36.5 kDa.[2-4] The genes for the calprotectin subunits are known as *S100A8* and *S100A9* and are located in chromosome 1q21.[5] MRP8 is considered as the active subunit while MRP14 prevents early degradation of MRP8.[6]

Calprotectin accounts for 60% of the cytosolic protein in neutrophils, and, to a lesser extent, in monocytes and macrophages which can be found throughout the human body mainly in plasma, urine, cerebrospinal fluid, faeces, saliva or synovial fluid.[7] Calprotectin is involved in many physiological functions including cell differentiation, immune regulation, tumourigenesis, apoptosis and inflammation.[8-10] Calprotectin plays a major role during inflammation and is considered to be a positive acute phase protein.[11] It induces cells receptor expression involved in migration, adhesion and phagocytosis of neutrophils (e.g. CD35, CD66b, CD18, CD11b), promotes chemotaxis and is implicated in the innate immune response as a damage-associated molecular pattern protein.[12-14]

Several pathological conditions can cause infection or inflammation of the gut mucosa which leads to an increase in permeability of the mucosa. This leads to enhanced migration of granulocytes and monocytes towards chemotactic substances in the bowel.[15] In addition, bacterial components derived from the intestinal lumen act as stimuli for the release of mediators such as calprotectin from granulocytes and monocytes, and

therefore the amount of calprotectin increases in the faeces.[16] Thus, the presence of calprotectin in faeces is a consequence of migration of neutrophils into the gastrointestinal tissue due to an infection or an inflammatory process.

Faecal calprotectin is extremely resistant to degradation by intestinal pancreatic secretions and intestinal proteases as well as bacterial degradation both *in vitro* and *in vivo*.[17,18] Its homogenous distribution in stools and stability in stool for up to one week at room temperature contributes to its suitability as a faecal biomarker, allowing stool samples to be transported to the laboratory for analysis.[19-23] The fact that faeces is in direct contact with the mucosa is conducive to the detection of intestinal inflammatory conditions by measurement of faecal markers far more precisely than by biomarkers measured in serum.[24] Therefore, due to its specificity for gastrointestinal tract inflammation, faecal calprotectin is superior to serum calprotectin.

Calprotectin extracted from stool can be detected easily using standard enzyme linked immunosorbent assays (ELISA). Numerous studies have shown that faecal calprotectin concentrations demonstrate a good correlation with intestinal inflammation.[25-28] This simple, non-invasive and less expensive quantitative faecal calprotectin test is the most widely used surrogate marker for monitoring intestinal inflammatory activity.[29]

Faecal calprotectin is useful for determining the cause of gastrointestinal symptoms, when it is difficult to differentiate between organic and functional causes by symptoms or clinical examination.[30] It is used in practice to differentiate inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS) where the signs and symptoms are very similar but the pathology is different. IBD is an organic disease due to the inflammation of the intestinal wall whereas IBS has a functional pathology due to gut motility disorders. An additional utility of faecal calprotectin is that changes in its levels are a good indicator of mucosal healing or recurrence of inflammation.[11,31-36] Therefore, faecal calprotectin can be used for monitoring of patients with IBD and to identify the patients at risk of relapse.

Although faecal calprotectin is a very sensitive marker for inflammation in the gastrointestinal tract, it is not a specific marker for IBD. Increased levels are also seen in gastrointestinal malignancies, infections, polyps and with the use of nonsteroidal anti-inflammatory drugs. However faecal calprotectin has many clinical advantages over other inflammatory markers such as plasma C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR).

Clinical Utility

Faecal Calprotectin in IBD

IBD are chronic diseases that result from the inflammation of the intestinal wall resulting in diarrhoea, abdominal pain, fatigue and weight loss. Crohn's disease and ulcerative colitis are two major forms of IBD. The disease course is unpredictable, punctuated with remissions and complicated by relapses and needing long term medication and, at times, surgery. The incidence of IBD is increasing in both adults and children.[37,38]

Identification of IBD

According to the World Gastroenterology Organization, the diagnosis of IBD relies on a combination of patient's history, physical examination as well as a number of diagnostic tests including laboratory analyses, stool examination, endoscopy, biopsy and imaging studies.[39] Endoscopy is the gold standard for the diagnosis of IBD.[40,41] Endoscopic procedures are unpleasant and sometimes painful, time-consuming and expensive. In addition, bowel cleansing procedures are necessary in order to ensure optimal visualisation, which may require hospitalisation of the patient. Due to these disadvantages of endoscopic examinations, there was a need for the availability of sensitive, rapid, reliable, cost effective and non-invasive biomarkers to reflect mucosal inflammation. Among biomarkers that have been proposed over the past few years, faecal calprotectin has gained an important role, with current clinical guidelines recommending faecal calprotectin determination as a part of diagnostic work-up for Crohn's disease and ulcerative colitis.[42-47]

Several studies have investigated the value of faecal calprotectin in distinguishing organic from non-organic intestinal disease in symptomatic patients, suggesting its potential role in the diagnosis of IBD.[42,43,48] Faecal calprotectin could also be used in decisions to select IBD patients for invasive endoscopic procedures and may help avoid unnecessary interventions in some patients.

Faecal calprotectin has high sensitivity and modest specificity for the diagnosis of suspected paediatric IBD and levels correlate highly with endoscopic scores of inflammatory disease activity.[49,50] From a practical point of view, faecal calprotectin measurement is cheaper, faster, and more patient-friendly than the standard endoscopic procedures.[49] In a study by Pous-Serrano *et al*., of patients who underwent small bowel resection for Crohn's disease, preoperative faecal calprotectin values were significantly associated with the degree of histologic inflammation (Chiorean's score) in lesions with IBD in the surgical specimen.[51] Therefore, it is valuable as a preoperative marker of transmural inflammatory damage in patients with IBD. Even though faecal calprotectin

is effective in differentiating between IBD and IBS, it does not help differentiate between Crohn's disease and ulcerative colitis, and does not help locate the lesion in the intestine.[52] In addition, faecal calprotectin is not totally specific to IBD and might be increased in several other organic gastrointestinal disorders, such as colorectal carcinoma and diverticulitis.

Faecal calprotectin levels may vary with age.[24] Children aged from 1 to 4 years old have lower faecal calprotectin concentrations compared with healthy infants (<1 year), and higher faecal calprotectin concentrations when compared with children older than 4 years and adults.[53] Faecal calprotectin may also have considerable day-to-day variation.[24]

Monitoring Disease Activity

Symptoms of colonic inflammation are often non-specific and thereby complicate the evaluation of the disease activity of IBD. Mucosal healing has become an accepted goal of treatment for IBD, and faecal calprotectin has been shown to correlate well with intestinal microscopic and macroscopic inflammation.[54-57] Faecal calprotectin levels increase rapidly even in the presence of the mildest signs of inflammation both in ulcerative colitis and Crohn's disease. Moreover, this test may be considered an alternative to colonoscopy in this setting because of its high correlation with endoscopic activity scores.[49] Therefore, faecal calprotectin is a sensitive marker for monitoring disease activity of IBD.

Management of small-bowel Crohn's disease is challenging because of the lack of accurate and reliable non-invasive modalities to assess the ongoing mucosal inflammation. Ileocolonoscopy is limited to visualisation of the terminal ileum. Capsule endoscopy is the most sensitive test to diagnose small bowel Crohn's disease, but may be considered to be invasive. A study done by Aggarwal *et al.* concluded that faecal calprotectin correlated well with findings of capsule endoscopy and therefore faecal calprotectin is a useful independent marker of mucosal inflammation for monitoring small bowel Crohn's disease.[58]

Studies have shown that the physiological changes that occur during pregnancy do not affect faecal calprotectin, in contrast to CRP, and indices of clinical disease activity (Harvey–Bradshaw Index and Simple Clinical Colitis Activity Index).[59] Therefore faecal calprotectin can be used to assess disease activity during pregnancy.

Response to Therapy (Monitoring of Treatment)

Medications used for IBD include aminosalicylates, corticosteroids, antibiotics, immune modifying agents and biologic agents like anti-tumour necrosis factor. Patients with IBD are often prescribed long-term 5-aminosalicylate therapy, which has been shown to reduce the frequency and severity of clinical relapse.[54-56] Traditionally, the assessment of the effect of medical treatment of IBD disease activity has been based on symptoms, clinical scores and serum markers of inflammation. However, studies have shown that faecal calprotectin consistently performed better than clinical indices and serum markers in assessing mucosal healing.[24] Faecal calprotectin levels significantly correlate with endoscopic assessment of disease extent, mucosal healing and histological activity, and reflect microscopic disease activity even in the phase of macroscopic healing.[60] Therefore faecal calprotectin can be used as a biomarker to assess the response to therapy in IBD. Many patients, especially those with ulcerative colitis, take a fixed daily dose of 5-aminosalicylate for maintenance therapy. Faecal calprotectin may identify patients, with normal calprotectin levels, who may not require maintenance treatment.[23] Patients with high faecal calprotectin levels on the other hand may benefit from an increase in therapeutic dose of 5-aminosalicylate.[23]

Studies have shown that 5-aminosalicylates use was associated with a reduced risk of colorectal carcinoma in patients with ulcerative colitis, especially in the cases with a higher average daily dose of 5-aminosalicylates use, but the chemo-preventive benefit of 5-aminosalicylates use in patients with extensive ulcerative colitis was limited.[61,62]

Faecal calprotectin is also used for biological therapy monitoring in IBD. It has been shown that an increase in faecal calprotectin predicts short-term relapse after discontinuation of antitumor necrosis factor-α therapy in patients with IBD in deep remission.[49] Measuring faecal calprotectin levels during treatment of acute relapse of the disease can confirm that the treatment is effective and the apparent improvement is not due to masking of the symptoms.[23]

According to a study by Xie *et al*., baseline faecal calprotectin levels correlated significantly with ulcerative colitis endoscopic index of severity (UCEIS) in acute severe colitis, and both faecal calprotectin and UCEIS were useful in predicting short-term outcome of corticosteroid treatment.[28] Therefore, baseline faecal calprotectin levels could be used as an alternative to UCEIS to guide the decision for early salvage therapy or colectomy and therefore reduce the adverse effects of long-term corticosteroid usage.

Prediction of Disease Relapse

Faecal calprotectin has been widely proposed as a surrogate marker to predict clinical course in patients with IBD. A patient with asymptomatic IBD with a high faecal calprotectin level has an 80% chance of a clinical relapse in the next 6 months.[23] Some authors suggest that rather than the baseline

levels, faecal calprotectin values measured during treatment are more useful to predict recurrence of active disease.[24]

Heida *et al*. concluded that two consecutively elevated faecal calprotectin values are highly associated with disease relapse, indicating a consideration to proactively optimise IBD therapy plans.[63] They were not able to identify the best faecal calprotectin cut-off for monitoring purposes. As ulcerative colitis and Crohn's disease are diseases with different inflammatory patterns, the value of faecal calprotectin in predicting relapse may be different for these two diseases.[64] Faecal calprotectin exhibited a better correlation with the relapse of ulcerative colitis compared with relapse of Crohn's disease.[65]

Post-Operative IBD Recurrence

Surgical treatment may not cure Crohn's disease. Postoperative recurrence is a feature of the clinical course of the disease. After resection of the terminal ileum in Crohn's disease, aphthous lesions at the anastomosis can be demonstrated in most patients within weeks, and up to 80% have endoscopic evidence of recurrence within 12 months.[66] Clinical relapse will occur in a third of patients within 3–5 years. Current recommendations include colonoscopy 6–12 months after surgery; but colonoscopy is invasive, costly and often not appreciated by the patient.[47] Lamb *et al*. showed that calprotectin values decreased significantly after surgery and normalised in patients with an uncomplicated course.[67] They found that faecal calprotectin elevation predicts early postoperative recurrence in patients with Crohn's disease.

A longitudinal study done by Lopes *et al*. evaluated the accuracy of faecal calprotectin and faecal lactoferrin to predict disease recurrence in asymptomatic postoperative Crohn's disease patients with an anastomotic stricture.[68] Faecal calprotectin and faecal lactoferrin levels accurately predicted endoscopic recurrence in the presence of anastomotic stricture and thus may guide the need for endoscopic balloon dilation in this context.

Faecal Calprotectin in Colorectal Carcinoma

The expression of calprotectin in colorectal carcinoma tissue, tumour-adjacent mucosa and adenomatous polyps in colonic biopsies has been investigated by Luley *et al*.[69] Tissue levels of calprotectin were higher in colorectal carcinoma and adenomatous polyps compared with those of mucosa from healthy controls and tumour-adjacent mucosa. Additionally, there was an excellent correlation between calprotectin staining and neutrophil infiltration in colonic tissue. Therefore, calprotectin expression seems to be an early step in the neoplastic transformation during colorectal carcinogenesis.[24]

A meta-analysis of early studies with faecal calprotectin found a pooled sensitivity and specificity for colorectal carcinoma of 87% and 76%, respectively.[48] More recently, a study by Damms and Bischoff confirmed that faecal calprotectin assays accurately identify active IBD and colorectal carcinoma (both had 100% sensitivity and 79% specificity at 50 µg/g cut-off in ELISA assay).[70] Faecal calprotectin is a poor marker for differentiating colorectal carcinoma from adenoma as well as the adenoma from control group. Therefore, faecal calprotectin is not recommended as a screening marker for colorectal carcinoma in asymptomatic patients.

Faecal Calprotectin in Other Intestinal Disorders

Infectious Gastroenteritis

In a large study of patients with acute diarrhoea Shastri *et al*. found that faecal calprotectin identified bacterial infection with a sensitivity and specificity of 83% and 87%, respectively at the cut-off of 15 mg/L and showed better diagnostic accuracy than faecal lactoferrin and occult blood testing.[71] Faecal calprotectin values were higher in acute bacterial diarrhoea compared with viral diarrhoea both in adults and children and increased with severity of diarrhoea.[72,73] They concluded that faecal calprotectin may be included in management algorithms for patients with suspected infective diarrhoea since a raised faecal calprotectin would support a presumptive diagnosis of bacterial diarrhoea and decision to culture stool samples for confirmation.

Acute Appendicitis

Acute appendicitis is an acute suppurative infection which can cause systemic inflammatory symptoms when left untreated. Unnecessary appendectomies pose surgical risks to patients and waste resources. Identification of non-invasive, inexpensive, easily available and accurate diagnostic markers of acute appendicitis would be clinically important in this context.[26]

A study done by Sarsu *et al*. concluded that faecal calprotectin may be a useful marker for differentiating acute uncomplicated appendicitis from other causes of abdominal pain and from complicated appendicitis.[26] Moreover, their levels may be helpful for the clinicians to judge the severity of the condition. However faecal calprotectin analysis is rarely available in the time-frame needed to make diagnostic decisions in this setting.

NSAID-Induced Small Bowel Enteropathy

Gastrointestinal toxicity affects up to 70% of patients with long-term nonsteroidal anti-inflammatory drug (NSAID) use but might be underestimated in clinical practice, as the majority of such patients remain asymptomatic.[74] Additionally, small bowel damage is more difficult to diagnose than damage to

the stomach and proximal duodenum.[24] A study by Maiden *et al*. in 40 healthy subjects showed 75% of subjects had elevated faecal calprotectin levels after two week's treatment with diclofenac.[75] However, its role in the management of NSAID-induced small bowel enteropathy is unclear.

Acid-Related Diseases / Peptic Ulcer Disease

Elevated faecal calprotectin values have been reported in peptic ulcer disease.[16] In a study by Manz *et al*., faecal calprotectin with 50 μg/g as cut-off predicted endoscopic findings in the upper gastrointestinal tract with 59% sensitivity and 82% specificity.[76] Faecal calprotectin values also increased with the disease severity.[24] Manz *et al*. concluded faecal calprotectin added value to clinical decision making in patients presenting with abdominal discomfort, to decide on endoscopy, as it predicted the presence of oesophageal and gastric mucosal lesions. However, its ability to predict positive endoscopic findings in the upper intestinal tract was not as good compared to findings in the colon (sensitivity of 84% and specificity of 92% for the latter). Faecal calprotectin is not useful as a screening test in asymptomatic patients.

Cystic Fibrosis

Faecal calprotectin levels differ in infants with cystic fibrosis compared with those of healthy infants, indicating that an altered intestinal environment is present from early life.[77] Faecal calprotectin is known to be elevated in patients with cystic fibrosis, particularly in patients with pancreatic insufficiency.[78,79] Furthermore, faecal calprotectin levels correlated with quality of life questionnaire scores in cystic fibrosis.[80] A study by Garg *et al*. concluded that faecal calprotectin in children with cystic fibrosis has distinct age-related variations compared to healthy subjects and may be reliably used as a marker of gastrointestinal inflammation in children older than four years of age.[77]

Coeliac Disease

The use of faecal calprotectin in coeliac disease has been investigated both in adult and paediatric patients.[81-83] Montalto *et al*. compared faecal calprotectin values of 28 consecutive adult patients with results from 23 healthy controls and found them to be identical.[81] However, in children, mean faecal calprotectin values in coeliac disease were higher compared with healthy controls and levels were related to the severity of histopathologic findings and response to gluten-free diet.[82] Therefore, faecal calprotectin may be used as a marker for diet adherence and improvement in gastrointestinal inflammation in children with coeliac disease.[83] The mechanisms leading to increased faecal calprotectin in coeliac disease remain unclear.

Transplant Rejection and Graft Versus Host Disease

Intestinal transplantation has become standard therapy for patients who suffer life-threatening complications from intestinal failure. Diarrhoea is one of the most common clinical presentations after intestinal transplantation and it may be due to rejection of the allograft. Sudan *et al*. concluded that low faecal calprotectin correlates well with a low risk for intestinal allograft rejection.[84]

Studies have investigated faecal calprotectin as a non-invasive test to replace endoscopic histology sampling in gastrointestinal graft versus host disease (GvHD) following allogeneic stem cell transplantation.[85,86]

Studies of faecal calprotectin have examined its sensitivity for detecting gastrointestinal involvement in GvHD, and to its ability to predict response to steroid therapy. O'Meara *et al*. have reported that faecal calprotectin was significantly higher in patients with gut GvHD than in patients with other organ involvement and correlated with histological findings, helping to discriminate between gut GvHD and other causes of diarrhoea in such patients.[87] Moreover, they found that high levels of faecal calprotectin at the onset of gut GvHD showed a correlation with higher GvHD grade and correlated with steroid refractory gut GvHD.

Analysis of Faecal Calprotectin

Over the past few decades, several assays for the detection and measurement of faecal calprotectin including point-of-care methods have been introduced by various manufacturers.[88,95] They all involve a first stage extraction. Methods for extraction and quantification of faecal calprotectin have evolved significantly since Røseth and colleagues developed the first method by enzyme linked immunosorbent assay (ELISA) in 1992.[19] A new simplified faeces sample preparation method was developed by Tøn and colleagues in 2000.[20] The Tøn extraction system uses high urea concentration plus vortexing which enhance the release of calprotectin from faeces to yield a high percentage of calprotectin extraction. More automated assays which allow high throughput such as enzyme-linked immunoassays (ELISA), chemiluminescence immunoassays (CLIA), fluoro enzyme immunoassays (FEIA) and particle enhanced turbidimetric immunoassays (PETIA) are now being used for the measurement of faecal calprotectin (**Table 1**).[47,96-98] The most popular method currently in use to measure faecal calprotectin is based on the ELISA principle.

ELISA kits are available with monoclonal as well as polyclonal antibodies. The Calpro ELISA (CALPRO AS, Lysaker, Norway) test uses polyclonal antibodies and the Calprotectin fCAL® ELISA (Bühlmann Laboratories AG, Schönenbuch, Switzerland) uses monoclonal antibodies.[21] The EliA™

Table 1. Currently available commercial assays and platforms for faecal calprotectin.

Platform	Faecal Calprotectin Assay
ELISA (polyclonal antibodies)	CALPRO AS, Lysaker, Norway
ELISA(monoclonal antibodies)	Calprotectin fCAL ELISA, BÜHLMANN LABORATORIES AG, Switzerland
CLIA (monoclonal antibodies)	DiaSorin Liaison®, Italy
PETIA (polyclonal antibodies)	fCAL™ turbo assay, BÜHLMANN LABORATORIES AG , Switzerland

Calprotectin immunoassay, run on the ImmunoCAP platform (Thermo Fisher Scientific, Uppsala, Sweden) was possibly the first commercialised automated faecal calprotectin assay. The DiaSorin Liaison® Calprotectin assay is a CLIA using a monoclonal antibody developed for the Liaison® and Liaison® XL platforms. The fCAL™ Turbo assay is a PETIA using a polyclonal antibody reagent and it is commercialised as an open channel assay suitable for general clinical chemistry analysers.[99]

A study of between-assay variability of faecal calprotectin ELISA kits was done by Whitehead *et al*.[22] Imprecision, linearity, recovery, drift and limit of quantitation of three different faecal calprotectin assays (Bühlmann, Immunodiagnostik and Eurospital) were evaluated and between-assay variability was assessed. The three faecal calprotectin ELISA assays showed acceptable intra- and inter-batch imprecision, good calprotectin recovery and exhibited dilution linearity across the range of concentrations tested. The evaluation data were in agreement with specifications by the respective manufacturers and, therefore, all methods were deemed suitable for routine laboratory measurement of faecal calprotectin. The diagnostic accuracy for IBD and between-assay variability were assessed by Whitehead *et al*. using four different faecal calprotectin assays (Bühlmann, Eurospital, Immundiagnostik and Thermo Fisher Scientific EliA).[100] This study concluded that diagnostic sensitivities of the faecal calprotectin assays were similar despite inter-kit variability in absolute values and highlighted the need for assay standardisation.

Oyaert *et al*. performed a study on analytical performance and diagnostic accuracy of six different faecal calprotectin assays in IBD.[101] In this study, currently available faecal calprotectin assays (EliA Calprotectin, Diasorin Calprotectin, Inova QUANTA FlashR, Bühlmann fCAL Turbo, Orgentec Calprotectin and Euroimmun Calprotectin) were evaluated and investigated to examine whether quantitative results of different assays were comparable. Generally, all evaluated faecal calprotectin assays demonstrated good analytical performance. Within-run and total imprecision were found to be higher for the ELISA/FEIA techniques (Euroimmun, Orgentec and Phadia) compared to the CLIA/PETIA assays (Diasorin, Inova and Bühlmann fCAL Turbo). Correlations between the methods from the different manufacturers were found to be good, but quantitative agreement was poor, which means that the result of one method cannot be replaced by the result of another method. The lack of interchangeability of results from different assays may be due to several possible reasons: the antibodies used in the different assays are directed against different complexes of the faecal calprotectin protein; the methods use different antibodies (monoclonal vs. polyclonal) of different origins (recombinant vs. native) and different immunoassay techniques (ELISA vs. PETIA vs. CLIA vs. FEIA); finally, the assays use different calibrators. So far there is no reference extraction procedure, reference preparation or reference measurement procedure for faecal calprotectin and this contributes to the lack of agreement between assays. With the introduction of new faecal calprotectin assays, steps need to be taken towards standardisation or harmonisation in order to improve agreement between assays.

Few method comparison studies have included the ELISA from CalproAS, yet this assay was used in many of the earlier clinical studies. Further comparison studies including this kit may be warranted to enable direct comparison with the early studies.

Point-of-Care Tests

As an ELISA test has many disadvantages – such as its time-consuming nature, a long turnaround time and a need for scientific expertise – point-of-care (POC) tests for faecal calprotectin have become popular in clinical settings although they still require the stool sample to be extracted, which reduces the utility of this approach. The method frequently used in POC testing is quantitative immunochromatography.[21] Quantum Blue-Bühlmann-Alere® is a POC test which is based on a quantitative sandwich lateral flow immunochromatography method.[98] IBDoc (Bühlmann Laboratories AG, Schönenbuch, Switzerland) POC test comes with a software application for the measurement of faecal calprotectin based on lateral flow chromatography that turns a smartphone camera into a results reader.[102] This rapid test allows patients to measure their own stool calprotectin values at home.

Over the past few years several studies have been done to compare POC faecal calprotectin assays with established immunoassay methods. Coorevits *et al*. carried out a comparative study of the Quantum Blue rapid test and an established ELISA method.[21] They concluded that the POC test can serve as a reliable alternative to the time-consuming ELISA in the differential diagnosis between functional and organic bowel disease. Furthermore, it also seems to be reliable in the follow-up of IBD patients.

An evaluation of the Quantum Blue rapid test for faecal calprotectin by Wassell *et al.* showed the Quantum Blue method was a suitable screening test for excluding IBD.[91] A similar study was done by Damms and Bischoff who compared the assay characteristics of a new faecal calprotectin rapid test (Prevista GmbH & Co KG, Munich, Germany) with an ELISA (MRP8/14, ELISA kit, Bühlmann Laboratories AG, Schönenbuch, Switzerland).[70] They showed that both faecal calprotectin rapid test and ELISA test were effective in identifying active IBD and colorectal carcinoma.

Sample Collection and Extraction Methods

Faecal calprotectin measurements require a small sample of faeces which can be obtained relatively easily using universal containers with a spoon attached to the interior of the screw cap or other commercially available devices.[103] Morning sample has been suggested for standardisation purposes.

Over the past few decades various types of extraction devices were introduced by manufacturers in an effort to reduce the time and effort required in sampling the faecal specimen. According to the literature there is limited agreement between different extraction methods, and laboratories should be aware of the lack of standardisation of the extraction procedure. Oyaert *et al*. compared two devices for extraction of faecal calprotectin (Thermo Fisher extraction device and the Smart Prep extraction device from Roche Diagnostics).[88] This study showed that Thermo Fisher extraction devices gave lower values of faecal calprotectin concentrations when compared to the Roche extraction device.

The two extraction methods on the Liaison® analyser (weighing protocol and extraction device protocol) were compared by Delefortrie *et al*., and calprotectin measured based on the extraction device protocol was found to be higher.[98] Whitehead *et al*. evaluated and compared the performance of the Roche, Immunodiagnostik and ScheBo Biotech commercial faecal extraction devices. In contrast to Delefortrie *et al*., they found that the commercial extraction devices led to a 7.8–28.1% under-recovery of faecal calprotectin in comparison to the manual weighing method.[22] This was particularly noticeable with liquid stool samples, for which most sampling device manufacturers still recommend weighing out.

Intra-Individual Variability and Standardisation of the Sampling Procedure

Significant day-to-day variability of faecal calprotectin in Crohn's disease patients with mild to moderate clinical disease activity has been described by Moum *et al*.[104] Kristensen *et al*. found high intra-individual faecal calprotectin variability in IBD patients, demonstrated by a high CV (mean 39.4%) from three samples obtained from bowel movements during morning – evening – morning.[105] Lasson *et al*. studied faecal calprotectin diurnal and day-to-day variability in 18 ulcerative colitis patients with active disease verified by endoscopy.[106] They found large intra-individual variability both during the day (median CV 52%) and from one day to the next (median CV 40.8%). Since faecal calprotectin increased with increasing interval between the bowel movements, the authors found it more appropriate to analyse samples from the first bowel movement of the day.

Cut-off Values

The manufacturer-quoted cut-off values for most faecal calprotectin assays are similar. Most laboratories have taken 50 μg/g faeces as the recommended cut-off. But studies have shown that the sensitivity and specificity vary with the set cut-off value in different faecal calprotectin assays. An early meta-analysis, carried out when only two faecal calprotectin assays were available, found that whereas many studies had used a cut-point of 50 μg/g, 100 μg/g may have greater diagnostic efficiency.[48] Nevertheless, 50 μg/g is usually taken to be the upper limit of normal.

A study by Oyaert *et al*. showed that IBD was ruled out in 106 (80.3%) and 99 (75.0%) of 132 non-IBD patients by the EliA assay (<37 μg/g) and Bühlmann POC test (<54 μg/g), respectively.[88] On the other hand, the diagnosis was confirmed in 30 (58.8%) and 38 (74.5%) of 51 IBD patients by the EliA (>257 μg/g) and POC assay (>242 μg/g), respectively.

A study by Coorevits *et al*. concluded that the Quantum Blue POC test could serve as a reliable alternative to ELISA (Bühlmann Laboratories AG), using a cut-off value of 30 μg/g and a grey zone of 30–110 μg/g in between functional and organic bowel disease.[21]

The results of a study carried out by De Sloovere *et al*. to assess analytical and diagnostic performance of automated faecal calprotectin immunoassays for detection of IBD in patients with a clinical suspicion are shown in **Table 2**.[99] Analysis of likelihood ratios (LR) for different faecal calprotectin result intervals demonstrated that faecal calprotectin concentrations

Table 2. Diagnostic performance of different faecal calprotectin assays in 136 patients with ileocolonoscopy and histopathology proven diagnoses at manufacturer's and optimum cut-offs .The optimum cut-off values were based on highest sum of sensitivity and specificity. [99]

	Diasorin Liaison®	BÜHLMANN fCAL™ turbo	BÜHLMANN ELISA
Manufacturer's Cut-off (µg/g)	50	50	50
Sensitivity at Manufacturer's Cut-off (%)	95.0	100.0	100.0
Specificity at Manufacturer's Cut-off (%)	65.6	53.1	43.8
Optimum Cut-off (µg/g)	78	243	311
Sensitivity at Optimum Cut-off (%)	90.0	87.5	90.0
Specificity at Optimum Cut-off (%)	80.2	80.2	79.2

Table 3. Sensitivities and specificities of different faecal calprotectin assays at the optimum cut-off, the manufacturer's cut-off and cut-off with fixed specificity of 75% and 90%.[101]

	EliA Calprotectin 2	Inova QUANTA Flash®	Diasorin Calprotectin	BÜHLMANN fCAL turbo	Euroimmun Calprotectin	Orgentec Calprotectin
Manufacturer's Cut-off (µg/g)	50	50	50	50	50	50
Sensitivity at Manufacturer's Cut-off (%)	100	100	100	100	100	100
Specificity at Manufacturer's Cut-off (%)	66.2	72.9	78.5	66.2	58.4	58.6
Optimum Cut-off (µg/g)	376	115	111	285	371	477
Sensitivity at Optimum Cut-off (%)	85.7	100	100	95.2	95.2	100
Specificity at Optimum Cut-off (%)	100	86.4	96.9	95.4	96.9	98.5
Cut-off (µg/g) at Fixed Specificity of 75%	102.0	61.6	41.3	71.9	125.0	142.9
Cut-off (µg/g) at Fixed Specificity of 90%	250.0	208.7	110.0	284.5	345.4	411.9

between 34 and 211 μg/g (LR = 0.80), between 83 and 385 μg/g (LR = 0.88) and between 164 and 919 μg/g (LR = 1.25) for the Liaison® Calprotectin assay, fCAL™ turbo assay and the Bühlmann Calprotectin ELISA, respectively were of limited clinical usefulness and cannot be used to rule out or suggest a diagnosis of IBD. Results outside these ranges were likely to be useful.[99]

In a similar study, Oyaert *et al.* compared analytical and diagnostic properties of six different faecal calprotectin assays in patients being evaluated for possible IBD, characterised on clinical grounds including endoscopy and histology as IBD or non-IBD.[101] The test results are summarised in **Table 3**. Cut-off values calculated from ROC analyses for detection of IBD were considerably higher for the Orgentec assay (477 μg/g) than for the other assays, compared to the cut-offs recommended by manufacturers (all 50 μg/g faeces). The sensitivities and specificities of all assays were comparable when using the optimal cut-offs. The study found that all assays had a sensitivity of 100% at the manufacturer's cut-off (i.e. 50 μg/g) but specificity at this cut-off value differed between the assays (ranging from 58.4% to 78.5%). The optimal cut-offs in this study are broadly in line with those of the same assays in the study of De Sloovere *et al*.[99]

A drawback of the use of a single cut-off value is that this diagnostic information is lost when results are interpreted as positive/negative only. For example, a calprotectin result higher than 477 μg/g (Orgentec) does not mean the patient has IBD; neither does a result lower than 477 μg/g rule out the diagnosis of IBD, but the probability is different. This interpretative ambiguity, together with the fact that there is not yet a standardised method for faecal calprotectin measurement, indicates that recommended cut-off values would have to be determined depending on the clinical setting in which they are used.

In a different approach Vasquez-Moron *et al*. examined faecal calprotectin cut-offs for predicting endoscopic activity scores and mucosal healing in patients with known Crohn's disease using an ELISA assay (Calprest® Eurospital, Trieste, Italy).[107] A faecal calprotectin cut-off of ≥170 μg/g (sensitivity 77.6%, specificity 95.5% and LR +17.06) predicted a high probability of increased endoscopic activity score, and a cut-off of ≤71 μg/g (sensitivity 95.9%, specificity 52.3% and LR -0.08) predicted a high probability of mucosal healing. Three clinical groups identified according to the data obtained are endoscopic activity (calprotectin ≥170 μg/g), mucosal healing (calprotectin ≤71 μg/g) and uncertainty (71 μg/g > calprotectin < 170 μg/g).[104] As different calprotectin assays tend to give different results for the same sample, these cut-offs will not be directly transferable to other assays.

A comparison study of the Liaison® Calprotectin kit with a well-established POC test (Quantum Blue – Bühlmann-Alere®) in terms of analytical performances and ability to detect relapses amongst a Crohn's disease population in follow-up was carried out by Delefortrie *et al*.[98] The optimal cut-offs and associated negative predictive values (NPV) for detecting relapses were:

- Quantum Blue® 183.5 μg/g and NPV of 100%
- Extraction device protocol + Liaison® analyser 124.5 μg/g and NPV of 93.5%
- Weighing protocol + Liaison® analyser 106.5 μg/g and NPV of 95%.

This confirms that different cut-points are needed for different assays.

A study of validation and clinical significance of a new calprotectin rapid test (Prevista GmbH & Co KG, Munich, Germany) for the diagnosis of gastrointestinal diseases was performed by Damms and Bischoff.[70] This study included 140 patients with lower gastrointestinal symptoms. Faecal calprotectin was analysed by two methods, ELISA kit (MRP8/14, Bühlmann Laboratories) and calprotectin rapid test (Prevista GmbH & Co KG, Munich, Germany). The

Table 4. Assay characteristics of faecal calprotectin determined by two methods. Calprotectin ELISA test cut-off 50 μg/g.[70]

	Active IBD	**Colorectal Carcinoma**	**Adenoma**
ELISA			
% Sensitivity	100	100	55
% Specificity	79	79	79
AUC	0.995	0.992	0.686
Rapid test			
% Sensitivity	89	100	52
% Specificity	80	80	80
AUC	0.896	0.948	0.666

cut-off was taken as 50 µg/g for both methods. The results of the study are shown in **Table 4**.

While other cut-points were not formally tested, the authors concluded that for screening for IBD or colorectal carcinoma, the cut-point of 50 µg/g was optimal for the ELISA and that both faecal calprotectin assays accurately identified active IBD and colorectal carcinoma.

It is difficult to directly compare the results of these studies because of small differences in the way the data are presented. However it is clear that different assays give different optimal cut-points in ROC analysis, which, in some recent studies, are 2–8 times higher than the traditionally used 50 µg/g. When the authors have generated cut-points associated with high specificity these vary between assays.

Conclusion

Faecal calprotectin is a biomarker for the diagnosis and management of inflammatory bowel disease. Immunoassays are widely used by the laboratory for the measurement of faecal calprotectin. POC testing for faecal calprotectin are also becoming popular in clinical settings. There is a major lack of agreement between the results produced by different faecal calprotectin assays even though the manufacturer cut-off values for most faecal calprotectin assays are similar. Therefore, sensitivities and specificities at manufacturer cut-off values vary between assays; the optimum cut-off values also differ between assays. At present there is no reference preparation or reference method for faecal calprotectin measurement. Assay standardisation for faecal calprotectin is needed for traceable test results and uniform cut-off values.

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