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Journal ImmunoDiagnostics

Scientific news, opinions and reports

Fecal calprotectin in inflammatory bowel diseases

The level of fecal calprotectin correlates directly to the degree of inflammation in the intestines. As such, it is specifically elevated in inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis. A negative calprotectin result in a patient without alarm symptoms is reason enough to avoid endoscopy while a positive result can prioritize invasive and expensive procedures such as intestinal biopsy. Fecal calprotectin can now be measured with a fast, fully automated test, leading to improved operational efficiency and minimized costs: EliA Calprotectin.

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Change is the only constant

We all experience change in our everyday lives and our daily routines. And for the company Phadia a lot has changed since summer 2011 when it was acquired by Thermo Fisher Scientific. Phadia then became the immunodiagnostics division of Thermo Fisher Scientific's Specialty Diagnostics Group. As part of the Thermo Fisher family, exciting opportunities opened up – the company's leading positions in multiple segments of analytical technologies and laboratory products, and its global reach and depth of capabilities complemented Phadia's strengths very well. Thermo Fisher's mission is to enable its customers to make the world a healthier, cleaner and safer place. Like Phadia, Thermo Fisher's culture is driven by a strong commitment to innovation and excellent customer service. As part of Thermo Fisher Scientific, we will continue to publish a scientifically oriented journal for our customers but, to continue the theme of change, we decided to move from the autoimmunity-specialized EliA Journal to a periodical which may cover topics in both, either autoimmunity or allergy diagnostics. With the change of content also came the change of name – the EliA Journal became the ImmunoDiagnostics Journal.

This first issue of the ImmunoDiagnostics Journal from Thermo Fisher Scientific deals with calprotectin as a useful aid in the diagnosis of the inflammatory bowel diseases Crohn's disease and ulcerative colitis. A comprehensive overview of these diseases can be found on page 3. Maurice Russel et al. describes on page 6 fecal calprotectin as a potent marker for the diagnosis of inflammatory bowel diseases, which may replace the need for invasive colonoscopy or radio-labeled white cell scanning in many clinical scenarios. In January 2012, Thermo Fisher Scientific introduced the first fully automated calprotectin stool test: EliA Calprotectin. Please find a short introduction of this assay on page 11.

Hope you enjoy the journal,

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Inflammatory bowel diseases

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Introduction

Inflammatory bowel disease (IBD) is a general term for a heterogeneous group of gastrointestinal diseases, including Crohn's disease (CD) and ulcerative colitis (UC). Both disorders are life-long with periods of remission and relapse. CD is characterized by an asymmetric and segmental transmural inflammation which may affect any part of the gastrointestinal (GI) tract. In 30% of cases, the site of inflammation is the small bowel (Crohn's ileitis). Twenty percent of cases show inflammation of the colon only (Crohn's colitis). In 50% of cases, inflammation of the ileum and the colon is found (Ileocolitis). Upper GI involvement in the oesophagus, stomach, duodenum or jejunum can coincide with all three locations. The disease behaviour can be stricturing, penetrating or neither. [1]
UC, on the other hand, is characterized by a diffuse mucosal inflammation which is limited to the colon. Depending on the extension, the sub phenotypes of UC are proctitis, left-sided colitis and pancolitis, with the inflammation limited to the rectum, extending to the flexura sinistra, and involving the total colon, respectively. Many similarities exist between CD and UC, leading to the lack of a definite diagnosis in approximately 10% of patients with colon-limited IBD. These patients are (temporarily) diagnosed with colitis-type unclassified or indeterminate colitis. [2] (Table 1)

IBD is most often diagnosed in patients between 15 and 30 years, with a second incidence peak at ages above 40.
The pathogenic causes of IBD are still unknown. It is hypothesised that IBD is an immunologically mediated disorder in a genetically susceptible host. IBD is thought to result from an inappropriate and ongoing immune response and loss of tolerance to the normal luminal flora. This aberrant response leads to chronic inflammation of the gut and is most likely facilitated by defects in barrier function of the intestinal epithelium and the mucosal immune system.

IBD occurs worldwide, but a markedly higher incidence is observed in the industrialised areas of the world (Europe and the USA). The average annual incidence of CD in Europe and North America is rising and is estimated at 5-10/100,000. The annual incidence of UC is estimated at 10-20/100,000. The prevalence of CD and UC is between 200 and 500 per 100,000.

Diagnostic measurements for experts

Diagnosis of IBD is mainly based on eliminating other possible causes of the symptoms including (bloody) diarrhoea and severe abdominal pain. There is no gold standard, but the diagnosis mainly depends on a combination of endoscopic, histological, radiological and/or biochemical examinations. Initial laboratory investigations usually include markers for acute or chronic inflammation (erythrocyte sedimentation rate (ESR), C reactive protein (CRP)), anaemia (haemoglobin level, complete blood count), fluid depletion and signs of malnutrition/malabsorption (electrolyte abnormalities). Stool samples should be collected for microbiological testing. IBD-specific antibody tests include the detection of antibodies to autoantigens and microbial antigens. Perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) are antibodies directed to neutrophils that are detected in the serum of 60 to 80% of UC patients, but also in 5-25% of CD patients. Antibodies against saccharomyces cerevisiae (ASCA) are detected in 50 to 80% of CD patients, and in less than 10% of UC patients. However, at present, these autoantibodies are not routinely screened for in patients suspected of IBD because of their moderate sensitivity and specificity.

Ulcerative colitis	Crohn's disease
Rectum ± colon	Mouth to anus
Continuous	Discontinuous
Mucosal	Transmural
(fissure, abscess, fistula)	76 (39-100)
Muscular thickening	Fibrosis (stenosis)
Mucin depletion	Lymphoid ulcers, aggregates
Glandular damage	Granuloma (50-70%)
pANCA antibodies	ASCA antibodies

Table 1: Structural distinctions between ulcerative colitis and Crohn's disease

To establish the diagnosis in patients suspected of CD, ileocolonoscopy with biopsies of the ileum and colon for microscopic examination is the preferred procedure. In case of severe, active disease, flexible sigmoidoscopy is safer and better to prevent bowel perforation. A plain abdominal radiograph is valuable in the initial assessment of possible bowel dilatation, calcified calculi, sacroiliitis or the impression of mass in the right iliac fossa. Fluoroscopic examinations (small bowel follow-through, small bowel enema) are the current standard for assessing the small intestine. Barium studies can be helpful, but they are subject to several factors that can influence the quality of the result. Computed tomography (CT), mostly performed in severe cases, provides additional information on bowel thickening, changes in vascularity and mesentery. In case of obstruction or bowel narrowing, small bowel enema and double contrast enema are the procedures of choice to assess disease extent and location. For detection of extramural complications (fistula or abcess), ultrasound, CT and magnetic resonance imaging (MRI) can be performed. Histological examination of endoscopic biopsies searches for signs of patchy chronic inflammation, focal crypt irregularity and granulomas, as these are the generally accepted microscopic features of CD. In ileal samples, irregular villous architecture can be detected. [1]

To establish the diagnosis in patients suspected of UC, colonoscopy, preferably with ileoscopy and segmental biopsies, is the procedure of choice. In case of a severe attack, abdominal radiography and sigmoidoscopy are recommended. Other techniques that can be used to assess (the severity of) UC, including hydrocolonic ultrasound, Doppler ultrasound, virtual colonography, leukocyte scintigraphy etc. are of secondary value in the diagnosis of UC. Histological examination of endoscopic biopsies reveals basal plasmacytosis (presence of plasma cells around or below the crypts), an increase in heavy, diffuse transmucosal lamina propria cells and widespread distortion of the mucosa or crypt architecture. These features indicate UC. [2]

Requirements for family practitioners

IBD are chronic diseases with periods of active disease and remission. Symptoms heavily depend on disease activity (remission or active disease), but also on the subtype of IBD (UC or CD), and the severity of the disease (Table 2).
Medical history of a patient should include questioning about the onset and recurrence of symptoms, including rectal bleeding or bloody diarrhoea, abdominal pain, urgency, nocturnal diarrhoea. Furthermore, smoking habits, recent travel, food intolerance, recent medication, and family history should be explored.
Physical examination should evaluate general well-being, pulse rate, body temperature, blood pressure, body weight, abdominal examination for distension and tenderness, oral inspection and check for extraintestinal manifestations, including ocular, oral, joint, or skin lesions. However, physical evaluation may be normal in case of mild or moderate disease. Strongly suggestive symptoms include bloody diarrhoea lasting for more than 1 week, non-bloody diarrhoea lasting for more than 3 weeks, or severe abdominal pain with significant weight loss.
Initial laboratory testing should include complete blood count, electrolyte, blood urea nitrogen, creatinine, liver enzymes, iron studies, and CRP. Furthermore, examination of stool samples could eliminate the presence of infectious agents.
For definite diagnosis, medical history and physical examination should be complemented with endoscopy and/or histological findings in segmental biopsies. Rapid awareness of possible IBD and referral to a specialist for endoscopy can significantly decrease the time to diagnosis and therefore improve the prognosis of the patient [1,2].

Follow Up

Clinical observations

During treatment, symptoms gradually improve and patients reach clinical remission. Treatment is, if possible, gradually decreased to avoid dependence and/or intolerance.

Expectations

IBD patients have variable prognoses; some patients reach

	Crohn's disease	Ulcerative colitis
Persistent diarrhoea	Abdominal pain and cramping	
	Persistent diarrhoea	
	Perianal disease	Blood in the stool
	Loss of appetite	Rectal tenesmus
	Fissures*	Faecal urgency/ incontinence
Non-Intestinal Symptoms	Fever	
	Malaise	
	Anorexia*	
	Arthropathy*	
	Weight loss	Episcleritis*
	Delayed growth in children	Erythema nodosum*
	Eye irritations*	

* Symptom found in a minority of cases

Table 2: Signs and symptoms of the diseases

remission and remain in remission for several months or years, while others never reach a state of remission. If treatment fails to induce remission, surgery can be an option. Most CD patients will eventually have surgery. One in four UC patients will have surgery within ten years of diagnosis. Patients with extensive disease (pancolitis) have a higher risk for surgery. Patients with severe disease have increased risk for developing colon cancer.

Blood tests

Routine laboratory tests, including C-reactive protein determination, can be used to evaluate the response to treatment and to assess clinical improvement. Normalisation of routine laboratory test values and relief of symptoms are indicative of remission. However, complete clinical remission is defined by complete resolution of symptoms and endoscopic mucosal healing in UC patients, and as a drop in Crohn's disease activity index (CDAI) to <150 in CD patients. Complete clinical remission must be assessed by a thorough clinical exam and endoscopy.

Management

The main treatment for IBD aims at inducing and maintaining a state of remission. For each patient, the most effective treatment is determined by considering the disease activity, site of inflammation, disease behaviour, response to previous medications and the preferences of the patient. IBD is mostly treated with aminosalicylates (mesalazine, sulfasalazine), corticosteroids, immunomodulators (thiopurines (azathioprine, mercaptopurine), methotrexate, cyclosporine, tacrolimus) and/or biological therapies (anti-TNF antibodies (Infliximab, Adalimumab)).

Budesonide, a corticosteroid, is the preferred treatment for mildly to moderately active CD. Severe disease should be treated with systemic corticosteroids, possibly complemented with azathioprine/mercaptopurine in case of a relapse, or methotrexate in case of azathioprine/mercaptopurine intolerance. In case of dependence or intolerance to corticosteroids and/or immunomodulators, Infliximab or Adalimumab can be added, but surgery can also be an option. [3]

In mild to moderate UC, mesalazine is the preferred initial treatment, topical and/or oral. Severe UC should be treated in the hospital with intravenous corticosteroids. Immunomodulators should be started in steroid-dependent or steroid-refractory patients. Patients dependent or intolerant to corticosteroids and/or immunomodulators could be treated with biological therapies. If the disease persists, surgery is an option. [4]

The treatment options described here are considered the standard treatment. However, treatment has to be evaluated for each patient.

Diagnostic tests

The presence of pANCA antibodies in the serum of patients is evaluated by means of indirect immunofluorescence with neutrophils as a substrate. Three distinct staining patterns can be detected; a cytoplasmic staining pattern, a perinuclear staining and an atypical perinuclear staining, characterized by a broad inhomogeneous labelling of the nuclear periphery along with multiple intra-nuclear fluorescent foci. The atypical perinuclear staining pattern (atypical pANCA) is found in 60-80% of UC patients and in 5-25% of CD patients.

The presence of ASCA antibodies in the serum of patients is evaluated by means of enzyme-linked immunosorbent assay (ELISA). These antibodies are detected in 50-80% of CD patients, compared to less than 10% of UC patients and less than 5% of the controls.

Other antibodies described in IBD are antibodies to pancreas, anti-OmpC (E. coli) antibodies, anti-I2 (pseudomonas fluorescens) antibodies, anti-CBirl (Clostridium) antibodies and several anti-glycan antibodies (ACCA, ALCA, AMCA). These antibodies still need confirmation and are currently only used in experimental settings. [5]

Testing methods

Several limitations are associated with pANCA/ASCA testing for IBD. Both antibodies have relatively low sensitivities and specificities, which make them less accurate in the diagnosis of IBD. Furthermore, pANCA is detected with indirect immunofluorescence, which is associated with high interassay and interobserver variability. Therefore, pANCA and ASCA are not routinely tested in every patient suspected of IBD. [5]

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Noninvasive diagnostic tools for diagnosing and monitoring inflammatory bowel disease patients

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Introduction

Inflammatory bowel disease (IBD) is a group of chronic inflammatory diseases of the gastrointestinal tract that includes both ulcerative colitis (UC) and Crohn's disease (CD). Both diseases are characterized by periods of active inflammation, spaced by asymptomatic periods which can last from weeks to many months, or even years. Relapses, causing an increase in symptoms, are usually due to increased bowel inflammation. Fatigue, abdominal pain and depression are however frequently ascribed to IBD itself in spite of the absence of disease activity. Fibrotic stenoses without inflammation, regularly occurring in CD, may furthermore mimic active bowel inflammation.

Since medical therapeutic interventions are aimed at diminishing inflammation or prolonging disease remission, it is important to be able to distinguish between those conditions. The golden standard in clinical practice is traditionally gastrointestinal endoscopy. More simple and cheap methods such as laboratory tests can however also help distinguish IBD from other conditions, such as Irritable Bowel Syndrome (IBS), and monitoring the effects of medical therapies in IBD. During recent years fecal biomarkers have proven to be a new valuable tool for evaluating people with symptoms of IBD and managing those diagnosed with IBD.

Diagnosing IBD

Clinical suspicion is raised in patients with persistent abdominal pain and diarrhea. Rectal bleeding, weight loss, or anemia additionally increase the probability of this inflammatory process. Endoscopy with histopathological sampling are generally considered necessary in the investigation of patients with suspected IBD. In active UC the most typical appearance is a diffusely erythematous, friable, and granular mucosa, with loss of the normal vascular pattern. The lesions begin at the anorectal junction and spread in a homogeneous fashion aborally. [1] The mucosal lesions in CD are patchy, asymmetrical, and heterogeneous. Ulcers might be aphtoid, superficial or deep and are frequently surrounded by normal mucosa.

	Crohn's disease		Ulcerative colitis	
	Inactive N = 56	Active N = 37	Inactive N = 52	Active N = 8
Calprotectin (mg/l)*	96.5 (6 – 1703)	145.5 (6 – 7115)	58 (6 – 6905)	275 (6 – 3103)
CRP (mg/l)	11.8 (6 – 50)	13.4 (10 – 62)	10.52 (10 – 23)	10.25 (10 – 12)
EQ-5D score	0.86 (0.36 – 1)	0.72 (0.09 – 1)	0.87 (0.41 – 1)	0.83 (0.69 – 1)
VAS score	76 (49 – 100)	66 (30 – 94)	76 (39 – 100)	63 (40 – 90)
IBDQ score	186 (118 – 220)	156 (76 – 218)	190 (132 – 220)	159 (121 – 199)
Bowel symptoms	58 (39 – 70)	50 (25 – 70)	60 (41 – 70)	49 (29 – 57)
Systemic symptoms	26 (12 – 34)	21 (9 – 33)	27 (9 – 34)	23 (13 – 32)
Social function	32 (14 – 35)	27 (8 – 35)	32 (19 – 35)	27 (16 – 34)
Emotional function	70 (70 – 35 – 83)	58 (33 – 80)	71 (49 – 84)	60 (42 – 76)

* Calprotectin values are based on the geometric mean and range.

Table 1: Laboratory and quality of life parameters at time of inclusion in the active and inactive groups by disease type. The data are presented as mean and range. Active disease is a HBI score (CD) > 5 or a SCCAI score (UC) > 4.

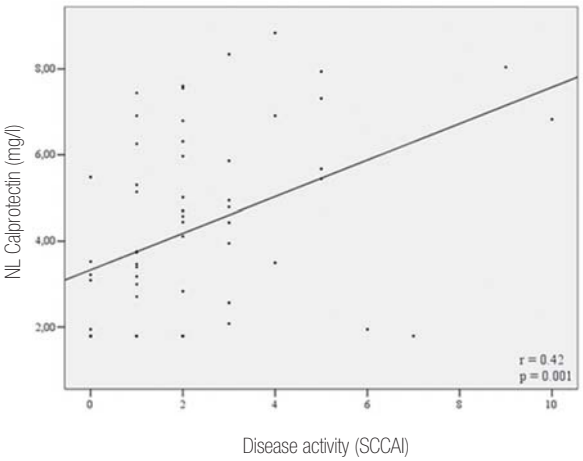

Figure 1: Correlation between fecal calprotectin concentration and disease activity in subjects with ulcerative colitis.

Inflammatory lesions in CD may be found throughout the whole gastrointestinal tract, however, in most cases are restricted to the terminal ileum and colon. Histological investigation is often non-specific but supportive to the diagnosis of IBD and can differentiate between UC and CD. Approximately one-third of patients who present with bloody diarrhea and suspected IBD actually have an infectious etiology and stool cultures and histological examination can be helpful to distinguish IBD from infectious causes. [2] Diagnosis of CD of the upper gastrointestinal tract might sometimes be a challenge. During recent years video capsule and enteroscopy have been introduced for investigating the small bowel in detail. Diagnostic imaging modalities such as MRI and CT are capable of establishing the diagnosis of IBD.

In a relatively large proportion of people with suspected IBD the results of endoscopy will be negative, most of these patients will be diagnosed with IBS. Identification of low risk patients would reduce the number of unnecessary invasive endoscopic procedures. Use of a simple, non-invasive, and cheap screening test to make a presumptive diagnosis of IBD would help to reach these goals. Determination of fecal markers in stool could be a good screening method. A number of neutrophil derived protein markers have been studied, including lactoferrin, lysozyme, elastase, myeloperoxidase, S100A12 and calprotectin.

Calprotectin is the most commonly utilized fecal marker and may be used for reliable assessment of bowel inflammation and disease activity. Calprotectin belongs to a group of calcium-binding proteins of the S100 family. [3] The protein is stable in stool samples for up to seven days at room temperature and one sample of less than 5 g is sufficient for reliable measurement. These qualities allow for stool sample collection at home and potential delays in transport to the laboratory. It is found in abundance in neutrophil granulocytes, in which it accounts for 5% of total protein and 60% of the protein in the cytosolic fraction. The presence of calprotectin in stool can be seen as a direct representation of neutrophil migration to the gastrointestinal tract. Lower concentrations of calprotectin are found in monocytes and reactive macrophages. [4] Calprotectin was initially called leukocyte L1 protein, after being discovered by Fagerhol et al. in the search for a plasma marker of increased granulocyte turnover. [5] In view of the biological activity of L1, the name calprotectin was introduced to describe this antimicrobial protein with calcium-binding properties. [6]

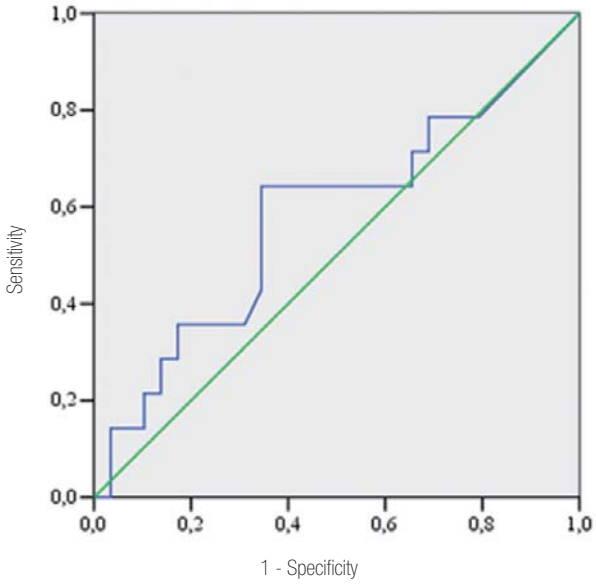

Figure 2: Receiver operator characteristics (ROC) curve for fecal calprotectin in predicting the relapse rate in Crohn's disease. The area under the curve was 0.58 (95% confidence interval (CI) 0.39 – 0.77). p = 0.4

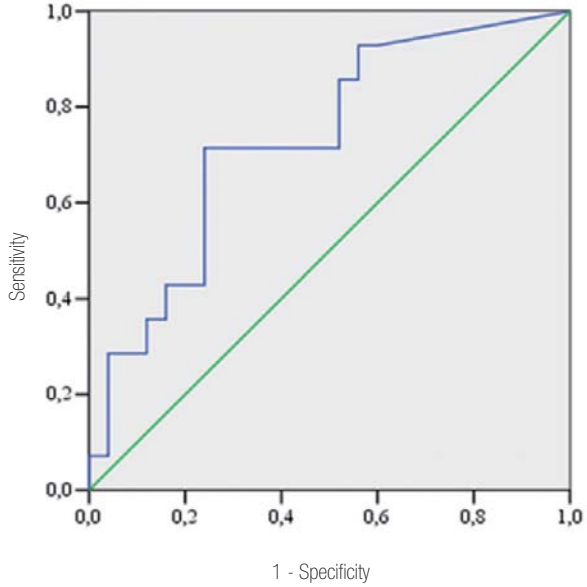

Figure 3: ROC curve for fecal calprotectin in predicting the relapse rate in ulcerative colitis. The area under the curve was 0.73 (95% CI 0.57 – 0.9). p = 0.018

Roseth et al. developed a method for extraction of calprotectin in feces and quantification by an enzyme linked immunoassay. [7] Direct assessment of intestinal inflammation using fecal calprotectin has shown to correlate with disease activity (as assessed by endoscopy, histology, Tc-scan and fecal excretion of 111-indium leucocytes) in patients with IBD. [8,9] Although fecal calprotectin measurement is a sensitive test of intestinal inflammation, it is not specific, because any cause of increased intestinal neutrophils will result in increased fecal calprotectin. Elevated levels of calprotectin have been described with sepsis, colorectal cancer, bacterial gastrointestinal infections and in non-steroidal anti-inflammatory drug induced enteropathy. [10,11,12]

During the last decade numerous studies have evaluated fecal calprotectin as a diagnostic tool in patients with suspected IBD. A meta-analysis was carried out by van Rheenen et al. to evaluate whether adding fecal calprotectin testing to the investigation of patients with suspected IBD reduced the number of unnecessary endoscopies. [13] Thirteen studies were included: six in adults (n=670), seven in children and teenagers (n=371). IBD was confirmed by endoscopy in 32% (n=215) of the adults and 61% (n=226) of the children and teenagers. In the studies of adults, the pooled sensitivity and pooled specificity of calprotectin was 0.93 (95% confidence interval 0.85 to 0.97) and 0.96 (0.79 to 0.99) and in the studies of children and teenagers it was 0.92 (0.84 to 0.96) and 0.76 (0.62 to 0.86). The lower specificity in the studies of children and teenagers was significantly different from that in the studies of adults (P=0.048). Screening by fecal calprotectin levels would result in a 67% reduction in the number of adults requiring endoscopy. Three of 33 adults who undergo endoscopy will not have IBD but may have a different condition for which endoscopy is inevitable. The downside of this screening strategy is delayed diagnosis in 6% of adults because of a false negative test result. In the population of children and teenagers, 65 instead of 100 would undergo endoscopy. Nine of them will not have IBD, and diagnosis will be delayed in 8% of the affected children. The authors conclude that testing for fecal calprotectin is a useful screening tool to identify patients who are most likely to need endoscopy for suspected inflammatory bowel disease.

Disease activity

Assessing disease activity is important when making decisions regarding disease management in IBD. Currently, colonoscopic examination with histology is still regarded as the most accurate objective measure of colonic inflammation. This is, however, an invasive and expensive procedure, and not suitable for regular practice. Patient symptoms can be an important indicator of inflammation and disease activity, but are subjective and may be influenced by noninflammatory factors, such as strictures and social factors. Clinical disease activity indices, such as the Ulcerative Colitis Disease Activity Index (UCDAI), Simple Clinical Colitis Disease Activity Index (SCCAI), Crohn's Disease Activity Index (CDAI) and the Harvey-Bradshaw Index (HBI) are based on clinical variables, physical examination and laboratory markers. [14-17] Although they have been thoroughly validated, these indices have a significant interobserver variability and the correlation with invasive endoscopic measurements is poor. [18,10]

Several standard laboratory markers are used to aid in the diagnosis and monitoring of inflammatory bowel disease. These include erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), platelets, and other acute-phase proteins. [18,19] In comparison to subjective symptoms, they are potentially an

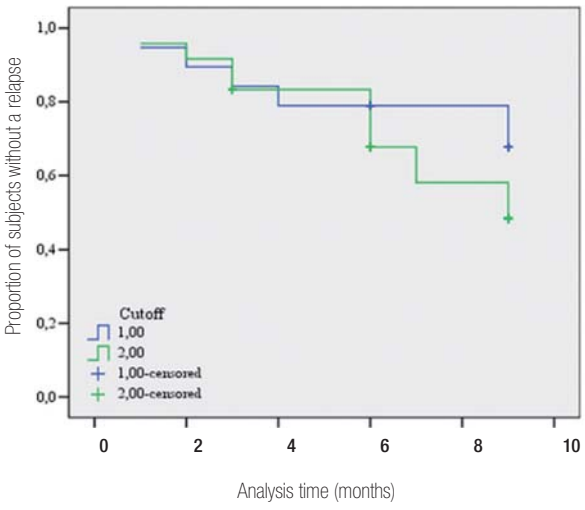

Figure 4: Kaplan-Meier survival curve for subjects with Crohn's disease, with values for calprotectin above and below 85 mg/l. Log rank for equality functions, p value = 0.38

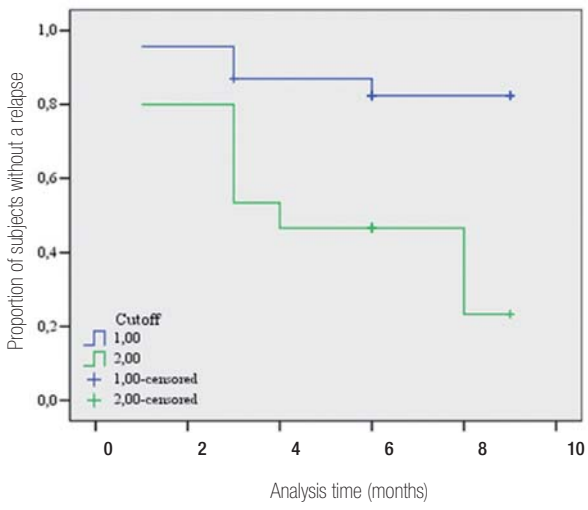

Figure 5: Kaplan-Meier survival curve for subjects with ulcerative colitis, with values for calprotectin above and below 85 mg/l. Log rank for equality functions, p value = 0.006

objective method of bowel inflammation assessment. CRP is increased in most patients with active CD and approximately half of patients with active UC. It has a sensitivity of 48-68% and a specificity of 58-91% when used to identify endoscopically active inflammation. [20] Compared with CRP, ESR will peak less rapidly and may take several days to decrease, even if the clinical condition of the patient or the inflammation has improved. Platelet count will also increase as part of the acute phase response and is therefore an indication of inflammation, without being a specific marker. [21]

Fecal biomarkers like lactoferrin and calprotectin tend to perform better when assessing bowel inflammation than CRP, being more specific and sensitive. [22] Calprotectin has a sensitivity of 80-100% and a specificity of 44-100% and lactoferrin of 66-80% and 67-100%, respectively, when used to identify active disease in IBD. [20] Sensitivity and specificity values depend on the cut off values used. Both appear to be more specific and sensitive in colonic disease compared to small bowel inflammation, but the extent of colonic inflammation does not seem to be important. [23]

We investigated at our institution (data presented at the Dutch Society of Gastroenterology, October 2006) the relationship between fecal calprotectin concentration and disease activity in subjects with known inflammatory bowel disease. Disease activity was primarily assessed using clinical activity indices, and also by generic and disease-specific health-related quality of life instruments, and a CRP measurement. The calprotectin concentration correlated significantly with disease activity (SCCAI) in UC ($r=0.42$, $p=0.001$) (Figure 1). There was no correlation between calprotectin concentration and disease activity (HBI) in subjects with CD ($r=-0.005$, $p=0.96$). In both subjects with CD and UC there was no correlation between CRP concentration in blood and fecal calprotectin (CD: $r=0.24$, $p=0.27$; UC: $r=0.72$, $p=0.587$). The reason for not finding a significant correlation between disease activity and calprotectin in CD in this study is probably related to the subjective properties of the activity indices poorly correlating with the inflammatory activity measured by 111In-labeled neutrophils and endoscopic indices, both objective markers of disease activity.

One should realize that fecal biomarkers are not specific to inflammation due to IBD, but also react to other bowel disorders amongst these colonic cancer and mucosal damage due to non-steroidal anti-inflammatory drugs. [10,11,12] The major disadvantages of fecal biomarkers are the higher cost compared to blood derived markers and the need to collect a fecal sample by patients.

Mucosal healing

An increasingly important treatment goal in CD and to a lesser extent in UC is mucosal healing, being associated with a better long-term disease outcome. Roseth et al. demonstrated that IBD patients who had remission following medical therapy had large reductions in levels of fecal calprotectin. [24] In a study by Sipponen et al. both calprotectin and lactoferrin normalized in 4 of 5 patients with mucosal healing whereas calprotectin levels remained unchanged in 8 of 9 and lactoferrin in 6 of 9 patients without mucosal response. [25] Development of new biomarkers as well as better understanding of those already at our disposal will probably decrease the need for endoscopic evaluation to identify mucosal healing in the future.

Predict disease course and relapse

Symptoms of IBD often appear to be the direct consequence of the inflammatory process itself. Most patients with inflammatory bowel disease have a low-grade inflammation and it is possible that symptomatic relapse only occurs when the inflammatory process reaches a critical intensity. [26] Direct assessment of the level of inflammatory activity may provide a pre-symptomatic measure of an imminent disease relapse. A raised fecal calprotectin has consistently been shown to be associated with an increased risk of relapse in UC, [20] whereas CRP and ESR have not. In CD some, but not all, studies report a positive correlation between increased levels of CRP and ESR and disease relapse. [27] Data regarding fecal biomarkers in CD are somewhat conflicting in this respect, however, the majority identify relapse rates of 80% or more within a year of a raised calprotectin measurement. [20]

At our institution an observational prospective cohort study was carried out examining the role of intestinal inflammation in relapsing IBD and if fecal calprotectin could be used as a predictor in CD and UC (data presented at the Dutch Society of Gastroenterology, October 2006). Subjects were followed for a period of 9 months, in which regular three-monthly clinical evaluations were performed. Approximately half of all the subjects (82 of the 148 subjects) met the in- and exclusion criteria for the follow up period. Fourteen of the forty-three (33%) subjects with CD had a relapse within the nine month period, with a mean relapse time of five months. In the UC group, fourteen of thirty-nine (36%) subjects had a relapse, with a mean relapse time of three months. Table 1 shows laboratory and quality of life instrument parameters in the relapse and nonrelapse groups. When assessing the various laboratory, quality of life, clinical and demographic parameters, only fecal calprotectin gave significant risk of relapse. Mean calprotectin concentration in the CD relapse group (132.8 mg/l, SD 7.4) was non-significantly higher ($P=0.37$) compared to the nonrelapse group (76.5 mg/l, SD 6.2).

In UC the mean calprotectin concentration was significantly higher (P=0.014) in the relapse group (153.9 mg/l, SD 7.6) compared to the non-relapse group (30 mg/l, SD 6.1).

Receiver operator curves for fecal calprotectin as a predictor of relapse in IBD are shown in figure 2 and 3. In the subjects with UC analysis showed that for a cut-off level of 85 mg/l the sensitivity of calprotectin predicting relapses was 71% with a specificity of 76%. A fecal calprotectin concentration of 85 mg/l gave a sensitivity of 64% with a specificity of 52% in predicting relapse in subjects with CD. Using the Cox proportional hazard model, fecal calprotectin levels above 85 mg/l gave a Hazard ratio of relapse of 1.6 (95% confidence interval (CI) 0.5 – 4.8, p=0.395) in subjects with CD and 4.2 (95% CI 1.3 – 13.8, p=0.017) in subjects with UC. The plots of time-to-relapse are shown in figure 4 and 5. Multivariate analysis using the Cox model selected only fecal calprotectin concentration above 85 mg/l as a predictor of disease relapse in subjects with UC.

Conclusions

Diagnosis of IBD is sometimes rather difficult and endoscopic examination of the gastrointestinal tract and histopathological examination are generally considered necessary. Studies have shown that new fecal biomarkers potentially reduce the need of these expensive procedure and the for patients cumbersome preparation in differentiating IBD from IBS and other non-inflammatory conditions. Furthermore, once a diagnosis of IBD has been established, fecal biomarkers like calprotectin and lactoferrin, can serve as markers of disease activity and as predictors of relapse of inflammation reducing the need for endoscopic assessment. Further advances, such as the recent validation of a home testing calprotectin kit, may allow an expansion of the use of biomarkers, for example as a tool in self-management programmes. [28]

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EliA Calprotectin brings efficiency to stool testing

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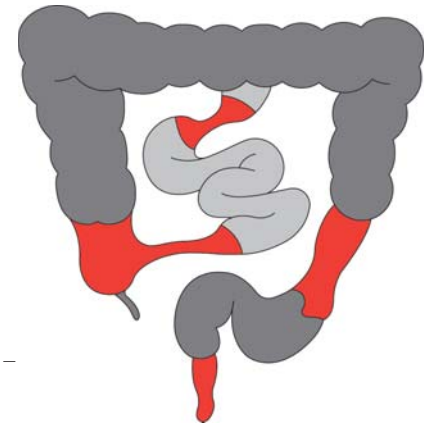
The high clinical usefulness of fecal calprotectin causes a constant increase in the requests for this test. This is a challenge for many laboratories, as not only stool extraction is laborious and time consuming, but also running a calprotectin ELISA requires manual handling. In addition, due to the need of a full standard curve per run, calprotectin ELISA tests can only be performed cost-efficiently if there are enough samples to test. This can now be overcome by EliA Calprotectin, the first fecal calprotectin test which brings automation and efficiency to fecal calprotectin testing. After routine stool extraction the test can be run fully automated on four Phadia® Laboratory Systems (Phadia 100, Phadia 250, Phadia 2500 and Phadia 5000), which are designed to meet the need of laboratories of all sizes. Short hands-on-time, a true walk away process and random access sample loading on the three high capacity Phadia Laboratory Systems reduce the workload for the lab personnel, minimize operational costs and optimize the workflow. The stored calibration curve, which is valid for one month, makes quick testing of single samples cost-efficient and improves the service of the laboratory for the test requester.

On top of this, EliA Calprotectin shows an excellent clinical performance, which was assessed in an internal study, using stool samples from 132 patients with inflammatory bowel diseases (IBD) and 59 patients with irritable bowel syndrome and other functional bowel disorders, which served as controls. Because of the test's high sensitivity, a negative result can be used to rule out IBD, while the test's high specificity assures a clear identification of IBD patients without generating many misleading false positive results (Table 1).

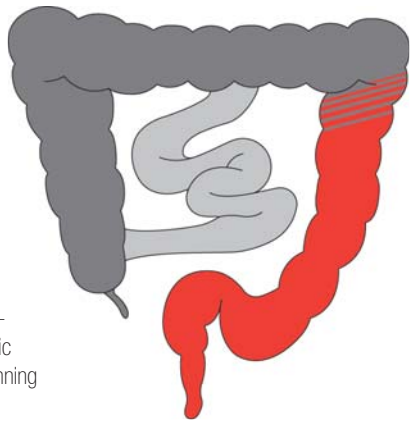
The clinical usefulness of EliA Calprotectin is underlined by the excellent likelihood ratios (LR), expressing how reliable the test result is for either identifying (LR+) or ruling out IBD (LR-). A positive likelihood ratio (LR+) of 10 and greater, and a negative likelihood ratio (LR-) of 0.1 and lower, indicate a high diagnostic accuracy. EliA Calprotectin shows a LR+ very close to ten, while the LR- is far below 0.1, highlighting the capability of the test to provide early diagnostic guidance in the diagnostic process.

EliA Calprotectin thus combines high clinical usefulness with the requirements of a modern clinical laboratory.

EliA Calprotectin	
Sensitivity	97.7 %
Specificity	89.8 %
Positive predictive value (PPV)	0.96
Negative predictive value (NPV)	0.95
Positive likelihood ratio (LR+)	9.58
Negative likelihood ratio (LR-)	0.03



Crohn's disease – Skip lesions



Ulcerative colitis – Continuous colonic involvement beginning in rectum

Crohn's disease can affect any part of the gastrointestinal tract, from mouth to anus (so-called skip lesions). The majority of cases starts in the terminal ileum. In contrast, ulcerative colitis is restricted to the colon and the rectum.

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- Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, are chronic gastrointestinal diseases with periods of active disease and remission.
- In a relatively large proportion of people with suspected IBD the results of endoscopy will be negative, most of these patients will be diagnosed with irritable bowel syndrome. Identification of low risk patients would reduce the number of unnecessary invasive endoscopic procedures.
- Calprotectin is the most commonly utilized fecal marker and may be used for reliable assessment of bowel inflammation and disease activity.
- The high clinical usefulness of fecal calprotectin causes a constant increase in the requests for this test.
- EliA Calprotectin is a fast, fully automated test and combines high clinical usefulness with the requirements of a modern clinical laboratory.

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