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Application of Fecal Calprotectin in Inflammatory Bowel Disease

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Abstract

In the practice, a distinction between organic and functional bowel disease and assessment for endoscopic examination is required daily for gastrointestinal symptoms. In modern gastroenterology, the evaluation of disease activity, prognosis and control of therapy in patients with chronic Inflammatory Bowel Disease (IBD) are a challenge. The search for non-invasive laboratory markers for monitoring of their chronic-recurrent course and management of treatment is an important part of ensuring a good quality of life for patients. In recent years, faecal calprotectin has become a useful non-invasive biomarker. The focus of this review is on current guidelines for the use of calprotectin, with particular emphasis on faecal calprotectin, as a tool in the treatment and diagnostic management of IBD. Publications and expert clinical guidelines from the last six years on the use of calprotectin were reviewed. We have summarized the critical evaluations in the application, also the advantages and disadvantages of using faecal calprotectin (FC) in gastroenterological practice.

Conclusion: FC is a non-invasive diagnostic tool that enables physicians to more accurately, timely diagnose and predict the course and course of patients with IBD.

Keywords: Calprotectin; Faeces; Inflammatory bowel disease

Introduction

Chronic Inflammatory Bowel Disease (IBD) that includes Crohn's Disease (CD) and Ulcerative Colitis (UC) is a social and medical problem. Its frequency in industrial societies is growing, affecting mainly young people and people in their creative age. IBD has a chronic-recurrent course, which leads to impaired quality of life and high health care costs. According to statistics, in 2017, there were 6.8 million cases of IBD worldwide [1]. The prevalence of Crohn's disease is approximately 50/100,000, and for Ulcerative colitis 40 to 100/100,000 people. The highest reported values are in Europe (ulcerative colitis 505 per 100,000 in Norway; Crohn's disease 322 per 100,000 in Germany) and in North America (ulcerative colitis 286 per 100,000 in the United States; Crohn's disease 319 per 100,000 in Canada) [2]. In my country (Bulgaria), the total number of patients with IBD (UC + CD) is about 21,000 people (300/100000). According to WHO data, the cases of IBD have increased five times over the period between 1950 and 2016. Only 18-20% (1100 people with CD and 2515 with UC) of these patients receive treatment. The reason is mild forms of the disease, misdiagnosis or late diagnosis. Often the process of diagnosis is delayed up to 24 months for CD and up to 12 months for UC.

Characteristics of IBD

IBD are chronic spontaneously recurrent inflammatory disorders of the gastrointestinal tract. Etiopathogenetic CD and UC are the results of a combination of environmental, immune and bacterial factors in genetically predisposed individuals. Crohn's disease can cause inflammatory changes in all parts of the gastrointestinal tract, while the inflammation in ulcerative colitis is limited to the colon [3]. The peak of IBD usually occurs in the second to fourth decade of life. These diseases lead to many complications such as strictures, stenoses, abscesses, fistulas, malignant degeneration, perforation, massive haemorrhage or megacolon, which all require surgery. Extraintestinal manifestations such as arthritis, uveitis, oral, and skin diseases (erythema nodosum, pyoderma gangrenosum) also can occur.

Diagnosis and Monitoring of IBD

Diagnosis, prognosis, assessment of disease activity and severity and also the effect of IBD therapy are still challenges for practitioners. In gastroenterology, a combination of subjective and objective criteria, laboratory parameters, radiology and endoscopic examination with histology is most commonly used [4]. Clinical features of IBD include persistent (≥ 4 weeks) or recurrent (≥ 2 episodes in six months) abdominal pain and diarrhoea, rectal

bleeding, weight loss, or anaemia [5]. The gold standard in diagnosis is the endoscopic examination and histological evaluation. The chronic course and frequent activation in IBD patients increase the need for the introduction of less invasive, accessible and inexpensive laboratory tests. They must reliably reflect the inflammatory changes in the intestinal mucosa. Laboratory tests used in the diagnostic and treatment process are Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), leukocyte count, platelet count, coagulation status, ionogram [6]. These indicators express the inflammation and metabolic changes of the whole organism in the periods of relapse. They are less specific for the local severity of the inflammatory process in the intestine. The primary inflammatory markers used, show low sensitivity in patients with mild to moderate forms of IBD [7] and do not always correlate with existing endoscopic lesions [7,8].

The ideal biomarker should be specific for IBD and be able to distinguish IBD from non-IBD diseases. It should be able to objectively measure the activity of the disease, to predict its course (recurrence or complication) and to have prognostic value in assessing morbidity/mortality. In addition, it should be fast, non-invasive or minimally invasive, easy to perform and relatively inexpensive. Last but not least, the ideal biomarker should serve in monitoring therapy [9]. Unfortunately, there is still no such marker to cover all these requirements.

Nowadays, ESR, C-reactive protein, faecal calprotectin, faecal lactoferrin play a significant role as laboratory indicators for the assessment of inflammation, activity and monitoring of the disease [10]. C-reactive protein is a sensitive acute-phase protein, but its use alone is inappropriate as a diagnostic or monitoring biomarker in CD and UC. There is significant heterogeneity in the CRP response between UC and CD, and it is weaker in patients with UC. Approximately 30% of people do not express CRP due to genetic polymorphism and lack of an adequate response [11]. Erythrocyte Sedimentation Rate (ESR) is a non-specific inflammatory indicator. Its value depends on the erythrocyte/leukocyte ratio, erythrocyte shape and size, lean protein concentration and electrolyte imbalance. It is a sluggish indicator that increases and normalises more slowly in the course of the inflammatory reaction. ESR has low sensitivity which makes it inappropriate to monitor the clinical condition and therapeutic improvement. It also correlates better with the activity of the process in the localisation of inflammation in the colon, and much less in the localisation only in the small intestine [12].

The presence of several types of antibodies in the sera of patients with IBD determines its immune-mediated nature. The tissue damage and production of antibodies to specific microorganisms occur as a result of impaired innate immunity based on a gene mutation [13]. Antineutrophil Cytoplasmic Antibodies (pANCA) are thought to be induced by cross-reactivity with intestinal bacterial antigens. pANCA is found in 60%-70% of UC cases and about 10%-15% of CD cases. Anti-*Saccharomyces cerevisiae* Antibodies (ASCA) are antiglycan antibodies against mannan on the surface of the cell wall of the yeast *S. cerevisiae*. They have been found in patients with CD. A prospective study among unclassified cases of IBD showed that 64% of patients with UC were ASCA-/pANCA + [11]. Although two antibodies contribute to the discrimination of CD by UC, they are not associated with the pathogenesis or clinical activity/remission of the disease. Immune-mediated tissue damage is driven by a variety of cytokines associated with the inflammatory pathways. Proinflammatory cytokines play a critical role in the immune response in IBD. Research and their use to assess inflammatory activity are also limited. Often their serum levels remain within the reference range, regardless of the inflammatory activity of the intestinal disease [14].

The purpose of this manuscript is to provide an overview of the use of the inflammatory protein calprotectin. In particular, the paper looks into the faecal calprotectin, as a non-invasive marker in the diagnosis, assessment of activity/remission, prognosis and monitoring of chronic inflammatory bowel disease.

Calprotectin-An Inflammatory Protein

Calprotectin was discovered in 1980. It belongs to the family of S100 proteins. S100 proteins are small, acidic proteins located in the cytosol that bind calcium. They have a wide range of intracellular and extracellular functions. These proteins are called S100 because of their solubility in 100% saturated ammonium sulfate solution at neutral pH [15].

Calprotectin (CP) has a heterotrimeric structure - it consists of one light and two heavy polypeptide chains, with molecular mass 36.5 kDa. Each subunit binds two calcium and two zinc ions. It makes up about 60% of the total protein in the cytosol of neutrophils, in smaller amounts in monocytes and activated macrophages. CP is an acute phase reactant [16]. It is found in several biological fluids - plasma, saliva, urine, cerebrospinal fluid, and faeces. This protein is resistant to heat and proteolysis.

Main Biological Functions of Calprotectin

alprotectin is involved in the regulation of energy metaboCalprotectin is involved in the regulation of energy metabolism, calcium balance and protein phosphorylation. The zinc in its molecule is key to its bactericidal and mycostatic action. Calcium plays an important role in the binding of polyunsaturated fatty acids, such as arachidonic acid [17]. Calprotectin synthesis is induced by various lipopolysaccharide factors, IL-6, IL-1b, C5a, TNF-α, and inhibits the activity of Matrix Metalloproteinases (MMPs) [18]. CP plays an essential role in the pathophysiology of inflammation associated with tissue destruction, apoptosis and growth. It inhibits antiapoptotic genes and activates p53, caspase-3, and caspase-9 [19]. Elevated levels of calprotectin in local inflammatory sites have been shown to recover slowly and enhance necrotic effects on surrounding tissues. Calprotectin interacts with kinases and is a damage-related molecule (DAMPs) capable of initiating a non-infectious inflammatory response [20]. CP causes the breakdown of tightly bound endothelial cells, which induces the migration of neutrophils and monocytes to the site of inflammation. It plays the role of a signalling molecule, activates specific genes and pathways in tumorigenesis, stimulates tumour growth and metastasis [19,21].

Calprotectin as a Laboratory Indicator

Calprotectin is found in various biological fluids:

Diagnostic application of serum calprotectin

Neonatal sepsis is one of the causes of morbidity, mortality and prolonged hospital stay in the neonatal period. Several studies point to CP as a promising early, sensitive, and specific marker of sepsis. It reflects the protective and physiological mechanisms of the immune system by activating granulocytes and phagocytes [21,22]. Serum CP levels have been associated with several common risk factors for CVD, including highly sensitive C-Reactive Protein (hsCRP). It is a useful marker in atherosclerosis because its increase is an active secretory response of phagocytes in atheromatous plaque [23]. CP is a potential biomarker for cardiovascular disease, insulin resistance and obesity. Screening studies in healthy subjects have shown that elevated plasma concentrations of calprotectin predict the risk of Acute Coronary Syndrome [24]. Its levels have been suggested to increase earlier than other cardiac markers of necrosis (myoglobin, creatine kinase MB and troponin) [25]. Joint infiltration with inflammatory cells in rheumatoid arthritis is a source of secretion of a wide range of cytokines [26]. High concentrations of calprotectin in synovial fluid correlate positively with increased plasma levels [27]. In osteoarthritis, CP concentrations in both biological materials remain low [26]. Several studies have determined the serum calprotectin to be a new biomarker in IBD. There is no consensus on its clinical utility, and some researchers have found a significant relationship between serum calprotectin and disease severity, prognosis, and recurrence [21]. The origin of the serum CP is predominantly from circulating leukocytes and reflects more the systemic than the intestinal inflammation [21,28,29].

Calprotectin in CSF

High levels of CP in the cerebrospinal fluid are of diagnostic importance in neuroinflammation, although it cannot distinguish bacterial from viral meningitis [17]. In 64.7% of patients with Multiple Sclerosis (MS), CP was detected in CSF within two weeks of the onset of symptoms. Data on its high expression in brain tissue in Alzheimer's disease have been reported [30]. Has been suggested that FC aggregation results in a toxic form of the protein with a sticky hydrophobic surface that promotes the formation and aggregation of β-amyloid [17].

Calprotectin in urine is associated with renal impairment. Its low concentrations in the urine are characteristic of prerenal Acute Renal Failure (ARF), while the increased concentrations are characteristic of the renal genesis of ARF [31].

Our focus in this article is to examine in more detail the diagnostic, monitoring, and prognostic significance of faecal calprotectin in patients with chronic inflammatory bowel disease.

Faecal Calprotectin (FC)

Faecal calprotectin is one of the most sensitive non-invasive markers for distinguishing Inflammatory Bowel Disease (IBD) from functional disorders (IBS). In IBD patients, FC increased more than 100 times as a direct consequence of neutrophil degranulation in the damaged, inflamed mucosa. It reflects local changes, facilitates the diagnostic process, monitors the activity of inflammation, correlates with endoscopic and histopathological findings, helps monitor therapy and predicts recurrence.

Methods for Investigation of FC

- Qualitative- Rapid immunochromatographic plaque tests are used, often combined with the determination of other markers for IBD (Lactoferrin, haemoglobin, transferrin). There is good compatibility and comparability of results between tests from different manufacturers. Cut off 50 µg/g.

- Different methods can be used to quantify FC. Most are based on Enzyme-Linked Immunosorbent Assay (ELISA), but Chemiluminescent (CLIA), Fluoroenzyme Immunoassay (FEIA) and latex-enhanced turbidimetric immunoassay (PETIA) have also been introduced. The values obtained from the quantification of FC are methodologically dependent. The reason for this is that test companies use different calibrators and antibodies (monoclonal, polyclonal) for the immunochemical reaction. There is still no international standardization. As a result, the FC values of one method cannot be compared with the values of another. Therefore, it is recommended to use the same test to monitor the patient's condition.

FC Reference Values

Although there is variation depending on age, numerous studies have found that healthy adults have a mean FC level of 25 µg/g [32]. Nine studies in adult and pediatric populations to distinguish IBD from non-IBD patients showed mean sensitivity and specificity of FC of 93% and 94%, respectively, at the 50 µg/g limit [33,34]. However, the better diagnostic accuracy of the IBD test has been demonstrated at cut off 100 µg/g than at 50 µg/g [35]. As the cut-off value increases, the sensitivity becomes lower, and the specificity higher [16].

Biological Variations

Calprotectin levels are higher in early childhood for up to 5 years. The probable cause is the increased permeability of the intestinal mucosa and a difference in the intestinal flora. Children have increased trans-epithelial migration of granulocytes, as well as the inability to regulate the microbial intestinal flora associated with the immaturity of mucosal barrier function [36-38]. Higher values are also observed in adults over 60 years of age, probably due to altered microflora and reduced intestinal passage (Table 1) [33]. Not only the interday variations were observed, especially at high FC values, but also the daily faecal samples [32].

Patient age	Upper limit	Unit
2-9 years	166	µg/g
10-59 years	51	µg/g
>60 years	112	µg/g

Table 1: Age-dependent reference limits for calprotectin, according to Joshi et al. [39].

Recommendations for the Use of FC as a Laboratory Indicator

Regular use of Anti-Inflammatory Drugs (NSAIDs) can cause enteropathy in some individuals affecting FC levels. It is recommended to stop NSAIDs and test after four weeks [32]. The type of faecal sample is also important, as excessive mucus-liquid and solid samples are unacceptable for testing. Dobrzanski, et al. confirmed that variability is more pronounced in active IBD, especially in UC, where stools contain large amounts of mucus and blood [37]. For reliable results, the first sample of the day (the most concentrated) and a mixed sample collected from three consecutive days are suitable for testing. FC is stable in faeces and is not subject to proteolytic degradation. Samples can be stored for 72 hours at room temperature and up to seven days at 2-8℃. After extraction, they can be frozen [38].

Application of Fecal Calprotectin

Differentiation of IBD From IBS

Inflammatory Bowel Disease (IBD) and Irritable Bowel Syndrome (IBS) have very similar symptoms. Modern stressful lifestyle, poor diet and frequent use of drugs (Corticosteroids, NSAIDs) lead to functional changes in the intestinal tract with symptoms similar to IBD, but without the inflammatory component. While IBS does not require specific treatment, but rather symptomatic. IBD develops rapidly and requires active therapy and monitoring. Identifying low-risk patients will reduce the number of unnecessary invasive endoscopic procedures (colonoscopy). Since 2000, faecal calprotectin has been evaluated in numerous diagnostic studies in adults and children [40]. There is conclusive evidence that faecal calprotectin may play a screening role, differentiating IBD from non-IBD and reducing the number of colonoscopies by 67% in adults and 35% in children [40].

There are still unclarified problems, and there are no clear recommendations for the use of FC in clinical practice. In 2018, the Belgian IBD Research and Development Group (BIRD) conducted a critical review of the literature on the potential use of FC in different situations. The expert group of gastroenterologists and gastro-paediatricians within BIRD consensus gives the following recommendations. In case of discrimination of IBD from other forms of inflammation [16], a cut off of 50 µg/g is recommended for adults and children over four years of age. FC values <60 µg/g have a 100% negative prognostic value for IBD [41].

Von Roon, et al. determined the sensitivity and specificity of FC in a study of 1267 people with IBD vs no-IBD at the discriminant 50 µg/g and 328 with 100 µg/g (Table 2).

| Cut off	Sensitivity (%)	Specificity (%)
50 µg/g	89%	81%
100 µg/g	98%	91%

Table 2: Sensitivity and specificity of FC in discrimination of IBD and IBS [16].

In their meta-analysis Lin JF, et al. evaluate the sensitivity and specificity of frequently applied interrupt values using a random-effects model [42] (Table 3).

Cut off	Sensitivity (%)	Specificity (%)
50 µg/g	92%	60%
100 µg/g	84%	66%

Table 3: Sensitivity and specificity of frequently applied cut-off values in IBD [42].

FC can be elevated in many other gastrointestinal conditions - infections, diverticula, malignancies and the use of nonsteroidal anti-inflammatory drugs (Figure 1) (Table 4).
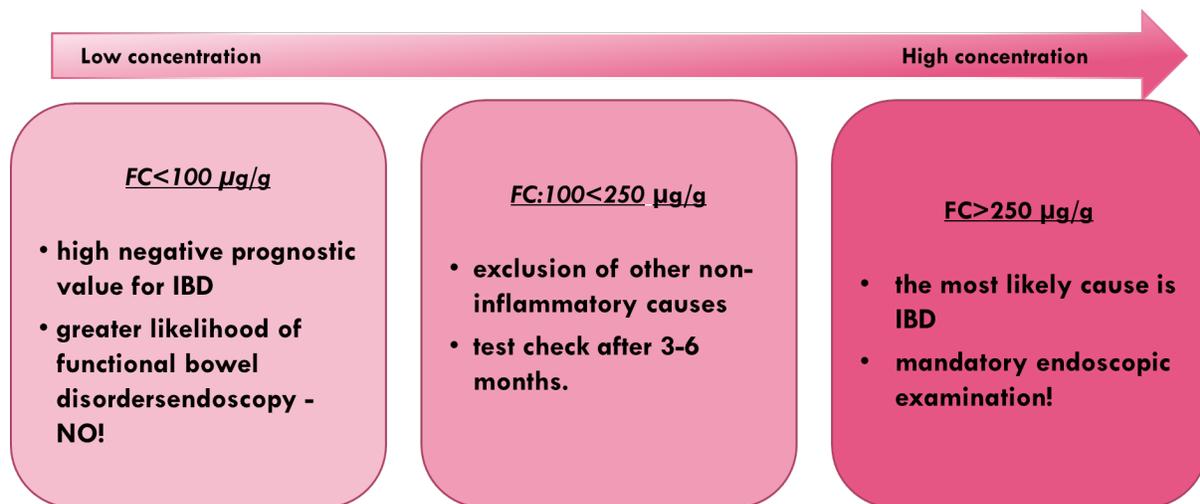
Figure 1: FC-IBD screening algorithm.

Illness	Calprotectin values
Infectious diseases of the intestine	188.2 µg/g (41.4-591.6)
Diverticula	150 µg/g
Celiac disease	57.7 µg/g
Carcinoma	159 µg/g (57-215)
IBS	50 µg/g
IBD	466 µg/g
Healthy people	12.21 µg/g

Table 4: Calprotectin levels in other diseases affecting the intestine [36].

Monitoring the Activity in Patients with Diagnosed IBD

Li F, et al. suggest a cut off of 250 µg/g of FC as an indication for IBD remission [36]. FC was found to correlate strongly with mucosal inflammatory activity, proven endoscopically (r=0.655), as in CD (sensitivity 61%; specificity 80%) and also in UC: sensitivity 71%; 100% specificity [43] (Figure 2).

Figure 2: FC-follow-up of symptomatic patients with IBD.

FC should be measured when IBD is diagnosed or before major treatment changes. Serial FC measurements every 3-6 months during treatment aim to assess response to therapy and to predict long-term remission. Normalization of FC is a marker for morphological healing of the mucosa and the onset of clinical remission. Evidence suggests that FC appears to reflect UC activity better than CD [44]. When the disease is localized in the small intestine, FC values correlate less with the inflammatory response than in the case of inflammation of the colon [45]. There is a better correlation with the histological activity (r=0.699) of the disease than with the macroscopic inflammation assessed by endoscopy [46]. Recent studies show that even in the absence of endoscopic signs of disease activity, FC levels are predictable for long-term outcomes. Consensus according to BIRD cut-offs of FC below 250 µg/g are an indication of IBD remission. In CD, FC concentration of ≥170 µg/g predicts endoscopic activity with 77.6% sensitivity, 95.5% specificity, values ≤71 µg/g predict mucosal healing with sensitivity 95.9%, specificity 52.3% [46]. Endoscopy remains the gold standard for assessing disease activity, and major therapeutic changes based on FC alone are not recommended.

Monitoring the Effectiveness of the Therapy

Mucosal healing is recommended as a therapeutic goal in IBD, which is why evaluating the response to treatment is extremely important. Symptoms do not always accurately reflect the inflammatory process, and endoscopic assessments cannot be performed frequently. Decreased FC levels at week 2 of initiation of infliximab treatment by 80% predicted endoscopic remission by week 10 (specificity 67% and sensitivity 54%) [47]. FC values <100 µg/g after induction therapy with anti-TNFα predicted prolonged clinical remission in patients with CD and UC. Insufficient reductions in FC have suspected resistance to therapy and the presence of antibodies to infliximab [48]. Serial FC measurements in the weeks after initiation of treatment are recommended to assess the therapeutic response and predict clinical remission.

Prediction of Recurrence of the Disease

Many patients with IBD in clinical remission still have subclinical mucositis. Prospective studies indicate that FC may differentiate patients at higher risk for future recurrence [49]. FC>50 µg/g predicted clinical recurrence over the next 12 months with a sensitivity of 90% and a specificity of 83%. FC>150µg/g for CD gives a double risk of recurrence, with a sensitivity of 87% but a specificity of only 47% [16]. In patients receiving maintenance therapy with Infliximab (IFX), detection of FC levels> 160µg / g has an expected probability of recurrence higher than 60% over the next eight weeks [16].

Monitoring of Postoperative Recurrence

IBD is a chronic recurrent inflammatory disease, and complications often occur that require surgical procedures. Both approximately 80% of patients with CD up to 20 years after diagnosis and 10%-30% of patients with UC up to 25 years require colectomy [50]. FC values three months after surgery can identify patients with early endoscopic recurrence. At FC values <100 µg/g, 6 to 12 months after surgery, there is a 90% negative prognostic value for endoscopic recurrence [48]. However, at FC values> 150 µg/g postoperatively within three months, endoscopic recurrence is predicted within one year, with 69% sensitivity and 70% specificity [51]. FC is a useful marker in the management of IBD, but cannot replace endoscopy six months after surgery.

Summary and Conclusion

Faecal calprotectin is a non-invasive marker that reflects local intestinal inflammation. It is a non-invasive diagnostic tool that enables physicians to more accurately and timely diagnose. This biomarker is also used to predict the course and effect of treating patients with IBD. FC has several advantages, but it also has its disadvantages. A positive test requires clarification and endoscopy. It correlates well with endoscopic and histological findings, but cannot accurately locate inflammatory changes and has relatively low specificity for distinguishing UC from CD. A negative predictive value defines IBD from IBS and excludes intestinal inflammation in both primary and secondary supervision. This helps reduce unnecessary endoscopies by 2/3 and reduces the cost of health diagnostic procedures. A disadvantage of FC is its increase in non-IBD intestinal inflammation and tumor processes. The lack of international standardization and methodologically dependent values leads to unsatisfactory comparability of values between different methods. Faecal calprotectin is the most sensitive non-invasive marker of inflammation, but cannot replace clinical, endoscopic or radiographic findings.

Conflict of Interest

I declare a lack of conflict of interest and the lack of financial commitment to the survey and its institutions.

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