Review

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Fecal calprotectin in inflammatory bowel diseases: update and perspectives

Abstract: Inflammatory bowel diseases (IBDs) are chronic diseases that result from the inflammation of the intestinal wall, suspected in any patient presenting with intestinal symptoms. Until recently, the diagnosis was mainly based on both clinical and endoscopic arguments. The use of an easy, fast, reliable, non-invasive, and inexpensive biological assay is mandatory not only in diagnosis but also in evolutionary and therapeutic monitoring. To date, the fecal calprotectin is the most documented in this perspective. This marker allows the discrimination between functional and organic bowel processes with good performance. The determination of the fecal calprotectin level contributes to the evaluation of the degree of disease activity and to monitoring of therapeutic response.

Keywords: calprotectin; Crohn's disease; inflammatory bowel diseases; irritable bowel syndrome; stools.

Introduction

Functional bowel disorders are one of the most common causes of chronic diarrhea. Irritable bowel syndrome (IBS), which is a subgroup of the functional bowel disorders, has no identifiable cause. Treatment is symptomatic. In clinical practice, distinguishing between IBS and organic gastrointestinal diseases, notably chronic inflammatory bowel disease (IBD), is challenging. Sensitive, accurate and noninvasive markers that can help in the diagnosis of IBD are thus mandatory. Furthermore, monitoring IBD

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patients to adapt treatment in case of relapse or aggravation is also of crucial importance.

This review focuses on fecal calprotectin as a primary tool for differential diagnosis of IBS and IBD and follow-up of IBD patients.

What is IBS?

IBS affects 10%–15% of the adult population in Europe and North America, mostly individuals younger than 50 years and with a marked predilection for females [1–3]. The classical symptoms of IBS are colicky abdominal pain, bloating, and bowel dysfunction, including diarrhea, constipation, or alternating subtypes. The heterogeneity of the disorder together with a symptomatic overlap with more serious organic diseases complicates the diagnosis.

What are the IBD?

IBD includes Crohn's disease (CD), ulcerative colitis (UC) and inflammatory bowel disease unclassified (IBDU). Chronic inflammation of the wall of the digestive tract is common to all these diseases [4]. IBD evolves through inflammatory attacks, alternating with remissions.

In CD, the inflammatory disease can affect the whole digestive tract, from the mouth to the anus. Lesions occur in patches and leave intervals of healthy mucosa between inflammatory areas [5]. Conversely, in UC, lesions are continuous from the colon to the rectum [6]. IBD's etiology is only partially understood, although its pathogenesis seems to involve genetic predisposition, dysregulation of the immune system, and environmental factors (e.g. bacterial antigens).

IBD is most often diagnosed in young people aged 20–30 years [7], and 15% of patients are children [8].

The incidence of IBD is increasing over time around the world [9]; 2.5 million Europeans and about 1 million persons in the USA have these diseases [10, 11].

During inflammatory episodes, IBD is characterized by abdominal pain, sometimes bloody diarrhea, or anal fissure or abscess accompanied by asthenia, anorexia, and fever, or even extra-intestinal manifestations [7].

Abscess or fistulas need surgery in more than 50% of CD patients and 20% of UC patients.

Finally, IBD is associated with an increased risk of colorectal cancer. The risk of colon cancer is 2- to 2.5-fold after 10 years of evolution [12].

Current IBD treatments allow the prolonged disease's control and improve the quality of life. Intestinal mucosal healing appears desirable to avoid long-term complications [13].

Both long-term treatment and treatment of acute attacks are used.

Long-term treatment with immunosuppressive drugs, (azathioprine, mercaptopurine, or methotrexate) may be used [14]. Attacks may be treated by amino-salicylates (5ASAs), corticosteroids, and biological therapy [14]. Tumor necrosis factor α (TNF) plays a central role in the pathogenesis of IBD; its concentration increases in the stools, peripheral blood, and lamina propria of patients. Anti-TNF drugs, used in severe active forms, bind to soluble and membrane forms of TNF, which induce cell apoptosis [15].

Finally, surgical treatment is reserved for patients resistant to observed therapy or following the appearance of complications [16].

Diagnostics of IBD

The current diagnosis of IBD is multidisciplinary, and based on clinical and biological criteria. First, physical examination includes oral and perianal inspections and anthropometric measurements. Non-specific clinical biology investigations include the measurement of acute and/or chronic inflammation markers of erythrocyte sedimentation rate (ERS) in the first hour and C-reactive protein (CRP) and platelet count. These non-specific biological parameters of inflammation increased in active IBD [17]. Biological diagnosis of anemia, which is due to iron deficiency, inflammation, and malnutrition, is frequently performed [18].

Endoscopy is usually prescribed following an initial assessment of a diagnosis to confirm the diagnosis, differentiate between UC and CD, and evaluate the disease extent. This examination allows the detection of lesions and the realization of histologic samples for surveillance of dysplasia or neoplasia. The use of endoscopic video vignettes or small bowel capsules may also participate in the diagnosis. Double-contrast barium enema and small bowel magnetic resonance imaging may help estimate the extent of lesions.

Endoscopic scoring systems have been developed such as the Crohn's Disease Endoscopic Index of Severity (CDEIS), the Ulcerative Colitis Endoscopic Index of Severity (UCEIS), or the Simple Endoscopic Score for Crohn's Disease (SES-CD) [17–22].

The Crohn's Disease Activity Index (CDAI) is the most widely used [23]. A CDAI index below 150 is observed in inactive disease, 150–450 in active CD, and upper 450 in severe CD [24]. The Mayo Score is the best-characterized disease activity index for UC [25].

Biological markers in IBD

Fecal leukocyte degranulation markers (lactoferrin, polymorphonuclear elastase and myeloperoxidase) have been proposed as diagnostic biomarkers for IBD but offer modest sensitivity for detecting recurrent disease [26].

Calgranulin C (S100A12 or EN-RAGE) is a Toll-like receptor 4 ligand, amplifying monocyte activation and thus contributing to organ-specific as well as systemic inflammation. A prospective study of Wright et al. has evidenced fecal calgranulin as a sensitive marker of CD with low specificity and as a negative predicting value in CD after intestinal resection [27].

Intestinal inflammation has also been associated with a significant decrease in fecal α1-antitrypsin, a serine protease inhibitor of pro-inflammatory mediators. Select studies considered α1-antitrypsin as a marker of CD and UC disease activity, as its levels positively correlate with disease severity [28, 29]. Recently, elevated serum levels of granulocyte macrophage colony-stimulating factor (GM-CSF) autoantibodies were also associated with disease activity and relapses in CD [26].

The combination of anti-*Saccharomyces cerevisiae* antibodies (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA) may discriminate between CD and UC, but the sensitivity of both biomarkers is poor [30].

Fecal calprotectin

General information

A loss of homeostasis of the immune system, initiating inflammation in the intestinal mucosa, is observed in IBD.

Calprotectin is a calcium- and zinc-binding heterodimer of 36.5 kDa that belongs to the S100 family. The protein complex is composed of two hydrophobic regions S100A8/A9 non-covalently linked.

Calprotectin is primarily found in granules of polynuclear neutrophils (PN) where it represents 5% of total proteins and 60% of cytosolic proteins in monocytes and macrophages and is expressed at a lower concentration in epithelial cells [31].

Its antimicrobial activity involves a Zn^{2+} sequestration resulting in impairment of zinc-dependent enzymes, with consequent inhibition of bacterial growth. Calprotectin has been shown to exert antimicrobial activity against *Escherichia coli*, *Klebsiella* sp.*, Staphylococcus aureus*, as well as fungistatic activity. Similarly, cells that express calprotectin can resist invasion by *Listeria monocytogenes* and *Salmonella enterica*.

Calprotectin is present on the surface of monocytes and macrophages, facilitating their recruitment to the site of inflammation. The synthesis of calprotectin is increased during the inflammatory process. It also provides an immunoregulatory role by interaction with the zinc-dependent metalloproteinases responsible for the activation of proinflammatory cytokines such as TNF. Its fecal excretion is highly correlated to that of 111-indium-radiolabeled leukocytes scanning, the gold standard for measurement of severity of intestinal inflammation [32].

Fecal calprotectin increased in patients with IBD [33]. Its concentration in stools correlated with the infiltration of the intestinal mucosa by PN. In IBD, calprotectin is highly correlated with clinical and histopathological activity [33, 34]. Calprotectin has the advantage of being useful in the diagnosis, relapse prediction, and therapeutic monitoring.

Technical principle and analytical performances

Pre-analytics

Fecal calprotectin is resistant to intestinal proteolysis, which makes it stable for several days at room temperature [35]. An assay requires about 100 mg of stools collected in clean containers without additive, preferably obtained at the same time of the day in case of iterative samples. The patient must avoid any accidental dilution of stools with urine or water. The collection of the stool samples could be done at home and then sent to the laboratory. However, one study has shown that the concentration of fecal calprotectin decreases from the third day after collection at room temperature [36]. Before assay, samples could be stored at 2–8 °C for 6 days. The sample may be stored at –20 °C for up to 4 months [37].

Extraction

The first step of the analytical process is to extract proteins from stools. Stools samples have to be homogenized; long fibers may be removed to avoid bias in the extraction process.

The reference technique is to weight stools. However, the use of a standardized volume of buffer added with calibrated amount of stools is quite common. The extraction step is carried out using an extraction device, which varies depending on the supplier. The stool extract is diluted and homogenized by prolonged use of a vortex. Depending on stool texture (normal, hard or liquid), the protocols may vary. Particularly for liquid stools, a standardized volume of liquid could be diluted into the extraction buffer. An additional centrifugation may be mandatory to sediment the larger particles in the suspension.

Methods for measurement of fecal calprotectin

Table 1 lists the different methods and commercial kits. Briefly, all these techniques are enzyme immunoassays, based on the use of monoclonal or polyclonal antibodies targeted to the dimeric calprotectin molecule. Chemiluminescent, fluorescent, or immuno-turbidimetric methods allow detection.

Quantitative enzyme-linked immunosorbent assay (ELISA) is the most frequently used method.

Those techniques are time-consuming and mostly suited for analyzing samples in batch rather than as it comes some automated methods have been developed (Liaison and Liaison XL, Diasorin; Elia Calprotectin, Thermoscientific).

Some point-of-care and rapid tests are available. The rapid tests are based on lateral flow immunochromatography, resulting in a line on the test strip if the calprotectin is present in the sample. It can produce quantitative results, as the density of the test line may be evaluated by an automated reader or a semi-quantitative test, with a visual interpretation.

Recently, point-of-care tests (IB*Doc*, Bühlmann; QuantOn Cal, Preventis) that can be used for calprotectin home testing have been proposed to patients. Smartphone application allows scanning the test cassette and calculates calprotectin concentration. The physician immediately gets the result.

Only a few methods correlated between each other, and substantial quantitative differences between the

Table 1: To supress assays for fecal calprotectin.

Assay	Manufacturer	Method	Antibodies	Measuring range[a], µg/g
Quantitative				
fCAL Elisa	Bühlmann	ELISA	Monoclonal	10–600
				30–1800
fCAL Turbo	Bühlmann	Turbidimetric immunoassay	Monoclonal	2–2000
Calprolab and PhiCal	Calpro	ELISA	Polyclonal	25–2500
			Monoclonal	
Liaison and liaison XL calprotectin	Diasorin	Chemiluminescent immunoassay	Monoclonal	5–8000
Calprest	Eurospital	ELISA	Polyclonal	0–3000
			Monoclonal	
IDK calprotectin	Immundiagnostik	ELISA	Monoclonal	3–2100
Orgentec calprotectin	Orgentec	ELISA	Polyclonal	0–1000
Ridascreen calprotectin	r-Biopharm	ELISA	Monoclonal	20–800
Calprotectin EliA	Thermo fisher scientific	Fluorescence enzyme immunoassay	Monoclonal	15–5000
Semi-quantitative				
CerTest	Biotec	Immunochromatography	Monoclonal	50
				200
Quantum blue fCAL	Bühlmann	Immunochromatography	Monoclonal	30–300
				30–1000
				100–1800
Calfast	Eurospital	Immunochromatography	Polyclonal	50–300
			Monoclonal	
CalDetect	Preventis	Immunochromatography	Monoclonal	50
				200
Qualitative				
CalScreen	Preventis	Immunochromatography	Monoclonal	>50

[a]According to the manufacturer.

assays have been observed [38]. Due to this variation, it is impossible to use those methods interchangeably to follow-up IBD.

Values in healthy individuals

Calprotectin reflects gastrointestinal inflammation and is not affected by lifestyle changes in adults, with the exception of non-steroidal anti-inflammatory drugs treatment, which should be stopped 2 weeks before sampling.

Using both ELISA and rapid tests, common values are assumed to be below 50 µg/g for adults and children older than 4 years. In younger children, the concentration of calprotectin is physiologically higher [39]. In a Danish cohort, 75 stools samples of healthy infants from 1 month to 4 years old were collected. Three cut-off levels were established based on the 97.5% percentiles of calprotectin in different age groups: 538 µg/g (1–6 months), 214 µg/g (6 months to 3 years), and 75 µg/g (3–4 years) [40].

In a Chinese cohort of very young infants from 1 to 18 months, using Bühlmann ELISA, the authors

found median values of 375 µg/g (1–3 months), 218 µg/g (3–6 months), and 100 µg/g (6–18 months) [41]. Among children <1 year old, this study evidenced a higher fecal calprotectin level in breastfed infants.

In the elderly, few data are currently available. Mindemark et al. have evidenced that patients older than 65 years frequently had calprotectin values higher than 100 µg/g [42]. In a study including patients between 50 and 70 years old, Poullis et al. evidenced that near 25% had calprotectin values higher than 65 µg/g, suggesting that in those patients, a reference value could be at 100 µg/g or even higher [43].

Table 2 summarized those results.

Diagnostic value of fecal calprotectin in IBD

The primary interest of calprotectin is the differential diagnosis of IBD and IBS without endoscopy investigation [44], avoiding useless invasive procedures.

Table 2: Reference values of fecal calprotectin according to the age in European populations [40, 43].

Age	Normal values, µg/g
1–6 months	<538
7 months to 3 years	<214
3–4 years	<75
4–49 years	<50
50–70 years	24.7% To supress – higher than 65
Above 70 years	No data; probably higher than 100

In a systematic review of Waugh et al. including seven different studies, sensitivity was always high (usually 100% at levels of <50 µg/g, ranging from 83% to 100% at a cut-off of 50 µg/g) and specificity ranged from 51% to 100% [44].

Several recent meta-analyses confirmed that in adults and children, specificity is sometimes lower [45–47]. High levels of fecal calprotectin were found in some other gastrointestinal tract disorders and patients treated with non-steroidal anti-inflammatory drugs or proton pump inhibitors (Table 3) [44, 45, 48].

The negative predictive value (NPV) ranges from 0.5 to 0.91 according to the literature [49]. Values higher than 50 µg/g and below 200–250 µg/g ("borderline area" or "gray zone") are associated with gastrointestinal inflammation of small intensity of various etiologies (e.g. infectious, neoplastic, inflammatory). In this case, repeating the measurement and additional tests are recommended. Waugh et al. suggest a simple biological monitoring for values between 50 and 150 µg/g, as these values relate exceptionally to serious illness and can be found in patients with IBS [44]. Values above 250 µg/g are usually associated with an active organic type of sickness [33].

Table 3: False-positive results reported for fecal calprotectin assays [44, 45, 48].

False-positive results of fecal calprotectin
Gastric cancer
Colo-rectal carcinoma
Celiac disease (untreated)
Infectious gastroenteritis
Diverticular disease
Microscopic colitis
Cystic fibrosis
Autoimmune enteropathy
Nutritional allergy (untreated)
Liver cirrhosis
Aspirin or non steroidal anti-inflammatory drugs
Proton pumps inhibitors

Disease location should also be taken into account when interpreting fecal calprotectin levels. Indeed, patients with ileal CD may have large or vast ulcers even in the absence of markedly elevated fecal calprotectin levels. Consequently, cut-off values for ileal CD may differ from those with ileocolic disease [50].

Correlation between fecal calprotectin and other biological diagnostic and monitoring tools of IBD

The interest of fecal calprotectin in the diagnosis is greater than other biological parameters such as the CRP elevation, platelets, hemoglobin, and blood leukocytes values [51]. Correlation of fecal calprotectin with endoscopy and histology is superior to its correlation with clinical disease activity indices that are mainly based on subjective data. Therefore, calprotectin can be used to detect subclinical inflammatory activity in asymptomatic disease [21].

Raised calprotectin in stools correlated with all the major endoscopic evaluation scores – SES-CD [23], UCEIS [52], and Mayo Endoscopic Subscore (MES) [53].

Identification of IBD relapse

In retrospective studies, it has been shown that high level of fecal calprotectin allows prediction of relapse of UC and CD [54], Table 4 presented some studies investigating fecal calprotectin predicting relapse in IBD. All the cohorts studied adult IBD patients, and ELISA served to detect fecal calprotectin.

Several studies have shown that patients with elevated calprotectin values are at risk of relapse within 3 to 6 months following the calprotectin increase [58, 59].

Few studies illustrate the longitudinal course of fecal calprotectin in the evolution of the disease, and predictive or decision cut-off level differ according to the authors [55, 60]. Tibble et al. reported that elevated calprotectin level (cut-off level 50 mg/L) showed good predictive value for clinical relapse during the following 12-month period [55]. A meta-analysis of 672 patients from six prospective studies shown that fecal calprotectin predicts relapse of IBD, with a sensitivity of 78% and a specificity of 73%. Ability to predict relapse was comparable between CD and UC [46].

Table 4: Selected studies concerning fecal calprotectin measurements in predicting clinical relapse of IBD.

Study	IBD type	Test used	Subjects, n	Relapsed subjects, n	Calprotectin mean level - relapsed subjects, μg/g (range or ±SD in brackets)	Proposed Cut-off level, μg/g or [a]mg/L
Tibble et al. [55]	CD	Elisa	43	25	122 (98–229)	50[a]
	UC	test	37	19	123 (79–353)	50[a]
Costa et al. [34]	CD	Calprest	38	15	220 (22–348)	150
	UC		41	19	207 (86–355)	150
D'Inca et al. [56]	CD	Calprest	65	20	207 (14–1846)	130
	UC		97	37	190 (16–2104)	130
Gisbert et al. [57]	CD	PhiCal	89	13	266 (±158)	169
	UC		74	13	213 (±142)	164
Garcia Sanchez et al. [58]	CD	Calprest	66	18	524 (101–883)	200
	UC		69	21	298 (34–883)	120

SD, standard deviation.

In patients with quiescent CD, fecal calprotectin had a sensitivity of 80% and specificity 91% for predicting clinical relapse, with a threshold of 150 μg/g [61]. In a prospective study, Naismith et al. ascertained using Bülhmann ELISA assay that a cut-off value of 240 μg/g had a significant predictive value of a high risk of clinical relapse within 12 months with an NPV of 96.8% [62].

Fecal calprotectin appears to be less useful in predicting relapse in patients with ileal CD compared with UC or colonic/ileocolic CD [56, 58].

In a recent review, Musci et al. provided an extensive discussion of the utility of fecal calprotectin and others biomarkers for the prediction of relapse in IBD according to the available literature. In summary, they evidenced that the predictive values in predicting relapse are lower than that usually observed for IBD diagnosis. However, larger studies are now needed, since most of the studies cited by Musci et al. only included a small number of patients. Finally, UC and CD patients have to be examined separately to increase the performance [26].

Therapy monitoring

Fecal calprotectin is also used in the follow-up of therapeutic response in IBD. A decrease of calprotectin parallels mucosal healing, evidencing a good response to treatment that can lead to complete remission of the disease. Conversely, maintained high levels suggest resistance to the treatment. The use of this biomarker in therapeutic follow-up should naturally take into account the pharmacokinetic properties of the treatment and the average time required for a response: usually faster for biotherapy than for immunosuppressants (some days to weeks and weeks to months, respectively).

The study of Molander et al. evidenced that calprotectin values below 250 μg/g were observed in clinical remission in 50 patients treated for IBD by anti-TNF antibodies [63]. This threshold is, however, not consensual and may fluctuate depending on the technique used.

The rapid decrease in fecal calprotectin in patients receiving anti-TNF antibodies was significantly correlated with the intestinal endoscopic remission [64–66]. Few studies have evaluated the interest of determination of fecal calprotectin after bowel resection: Lamb et al. published that the value of fecal calprotectin normalizes after 2 months of uncomplicated intestinal resection [16]. Fecal calprotectin can be conveniently measured 3 months after surgery [67]. Calprotectin levels remain high among patients with recurrence observed at endoscopy [68]. In a recent study, a decision algorithm of follow-up of patients, especially after surgery, defined phenotypic stratification the respective place of fecal calprotectin assays and endoscopy [69]. Larger cohorts are needed to confirm those results.

One or several thresholds: that is the question

One current point of interest is the threshold to recommend in clinical practice.

Almost all manufacturers recommend a threshold of 50 μg/g of the stools for IBD diagnosis in adults.

However, several difficulties arose from this choice. First, the different methods currently used in diagnosis do not give the same quantitative result, which is not rare in clinical practice. Some efforts have now to be made to ensure commutability of results.

Second, according to the clinical context, thresholds seem to vary, since the differential diagnosis of IBD and IBS and the prediction of a relapse in UC or CD are not associated to the same fecal calprotectin values.

Moreover, two recent studies have evidenced a high intra-individual variability, up to 40%, suggesting that a single determination of calprotectin may be insufficient to monitor relapse risk or treatment [36, 70]. This high variability may be partly due to the variability of the extraction process since the relative quantity of water in stools may vary across the day. As a consequence, iterative determinations of fecal calprotectin could be useful in the setting of follow-up, but also in patients presenting values comprised in the gray zone.

In a large study including 900 patients, Kennedy et al. evidenced a sensitivity of 0.97, a specificity of 0.74, an NPV of 0.99, using a threshold of ≥50 µg/g for IBD vs. functional disease [60].

In another large study in primary care settings, Pavlidis et al. showed that an increase of the threshold from 50 to 150 µg/g using an ELISA method (Bülhmann) led to a 1% decrease of the NPV, increasing the positive predicting value from 28% to 71% [71]. As a consequence, it should be recommended to establish a cut-off value for each kit and clinical utilization to optimize the performance of this marker. Results will have to take into consideration the assay variability together with the intra-individual variability.

Medico-economic aspects

The use of fecal calprotectin assay allowed a reduction in the cost of care for patients with suspected IBD [44]. Following intestinal resection, the use of fecal calprotectin to select patients for endoscopy could reduce the cost of around 600 euros per year [27]. This biomarker changes the patient's care and improves the quality of life by limiting invasive procedures. The benefit/risk imbalance and cost/efficacy ratio have been considered: the test is already refunded in some European countries. Therefore, fecal calprotectin is a non-invasive marker that can objectively assess mucosal healing, and its use is now systematically recommended not only for IBD diagnosis [72] but also in protocols evaluating new treatments [31, 73].

Conclusions

As summarized in Figure 1, in current practice, the dosage of the fecal calprotectin has two primary interests:
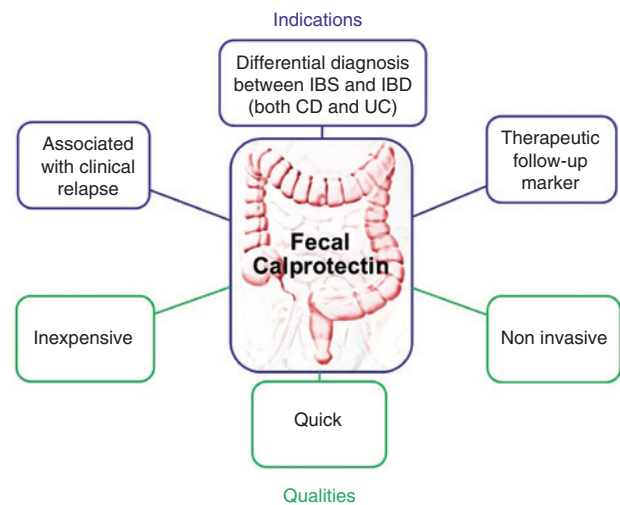

Figure 1: Schematic representation of calprotectin indications in clinical practices and main advantages.

-   To differentiate organic from functional intestinal diseases, both in adult and children;
-   To monitor IBD therapy and predict relapses [74, 75].

Calprotectin is a non-invasive, inexpensive, and efficient parameter for the diagnosis of IBDs (CD and UC), with a high NPV. Measurement of its concentration in stools is used to distinguish inflammatory diseases and tumors. Patient with increased fecal calprotectin can be referred early to an endoscopic examination. Calprotectin is also a useful marker for therapeutic monitoring and early detection of disease relapse of these types of inflammation. Further harmonization studies are currently needed to permit the comparison of different commercially available methods and to clarify the source of intra-individual variations, apart from active IBD and treatment effect. Some efforts must be followed to establish thresholds in all possible clinical settings.

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