Biomarkers in management of inflammatory bowel disease

Andrzej Moniuszko[1], Anna Wiśniewska[1], Grażyna Rydzewska[1,2]

[1]Department of Internal Medicine and Gastroenterology, Central Clinical Hospital of Ministry of Home Affairs, Warsaw, Poland
[2]Faculty of Health Studies, Jan Kochanowski University of Humanities and Sciences, Kielce, Poland

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Address for correspondence: Prof. Grażyna Rydzewska MD, PhD, Department of Internal Medicine and Gastroenterology, Central Clinical Hospital of Ministry of Home Affairs, 137 Woloska St, 02-507 Warsaw, Poland, e-mail: grazyna.rydzewska@cskmswia.pl

Abstract

In recent years the use of faecal and serologic biomarkers has been evaluated in the diagnosis and management of inflammatory bowel disease (IBD). Faecal calprotectin (FC) has been proposed as a surrogate marker for intestinal inflammation; elevated concentrations in IBD patients have been confirmed in numerous studies. Already available rapid calprotectin tests help to differentiate between IBD and irritable bowel syndrome. Faecal calprotectin greatly correlates with endoscopic activity scales and reflects the mucosal healing; thus in patients in clinical remission high levels of it correlate with increased risk of disease relapse in the following 12 months. Adapting the calprotectin assay as a screening test before colonoscopy enables a significant reduction in endoscopic procedures. ANCA/ASCA antibodies have been used in IBD diagnosis and to distinguish CD from ulcerative colitis (UC). Lactoferrin and S100A12 protein were also used to assess the disease activity. This review aims to present the actual potential of biomarker assays for faster diagnosis of IBD and their ability to monitor the disease course, predict exacerbations and improve the way IBD is managed.

Introduction

Inflammatory bowel disease (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC), is a heterogenic group of recurrent and relapsing disorders that can be characterised by chronic inflammation in the gastrointestinal tract. The immunological dysregulation of the digestive system provokes many unspecific symptoms and exerts a serious impact on the patient's health [1–3]; therefore proper diagnosis and precise monitoring play a crucial role in treating IBD patients. Traditionally used laboratory parameters indicating systemic inflammation such as C-reactive protein or white blood cell count do not have enough sensitivity and specificity when it comes to diagnosing IBD or taking therapeutic decisions, as they poorly correlate with clinical indices and endoscopic activity of the disease. That is why to confirm IBD, its remission or exacerbation, performing endoscopy and histological sampling or biopsy remain a gold standard. However, these procedures are invasive, expensive and carry the risk of complications, associated morbidity and mortality.

In the last decade thorough investigations have been conducted in search for biomarkers that would reflect the actual advancement of the disease and thus would play an important role in IBD management. The ideal one should be non-invasive, easily accessible and repetitive, economical, highly specific and sensitive.

In this paper the most important biomarkers have been described, with a special focus on calprotectin, the enzyme with the broadest clinical application.

Calprotectin

Faecal calprotectin is a cytosolic protein in neutrophils, which is released from damaged or activated/stressed cells in the inflammatory state. It is found in cerebrospinal fluid, colonic biopsies, saliva, plasma, synovial fluids, urine and faeces [4]. However, only faecal calprotectin seems to be a suitable marker for intestinal inflammation, as it is not influenced by inflammation occurring outside the gut. Enzyme elevation during non-steroidal anti-inflammatory drug (NSAID) [5, 6] and proton pump inhibitor (PPI) treatment has been established [7]. Furthermore, elevated levels of faecal

calprotectin (FCP) occur not only in IBD, but also in infection, colorectal cancer, untreated coeliac disease, microscopic colitis and diverticulitis [8–10], as these states are also connected with the release of neutrophils. The influence of age on enzyme concentration is presented in Figure 1.

Calprotectin is stable in faeces for up to 7 days at room temperature and the test can be performed on 1–2 g of stool (because of its homogeneous distribution in faeces [11]), making sample collection possible at home and potentially also its delivery to the laboratory by post [12, 13]. Consequently, it means that the IBD patient coming to the control outpatient visit may bring the sample and thanks to the quick tests available on the market the calprotectin level may be measured (see 'Rapid calprotectin tests' section).

Diagnosing of inflammatory bowel disease

As the inflammatory bowel diseases are a heterogenic group of illnesses, and the symptoms that patients present are highly unspecific, the initial diagnosis of IBD takes months or even years. Traditional laboratory findings such as C-reactive protein level, leukocyte count, erythrocyte sedimentation rate (ESR) etc. are highly unspecific and do not correlate with inflammatory lesions in the gut. Vavricka *et al.* [14] assessed the diagnostic delay (time from the appearance of the first symptoms to diagnosis) of IBD. Seventy-five percent of CD patients were diagnosed within 24 months compared to 12 months for UC and 6 months for IC patients.

Delayed diagnosis implies not only the subsequent development of the inflammatory state in the bowel, but also the elevated risk of complications such as strictures, perforations or fistulas. Especially in children a quick diagnosis is even more important, because of IBD's negative effect on growth and maturation [15, 16].

The quantitative meta-analysis of von Roon *et al.* [17] performed on prospective studies, comparing FC levels against the histological diagnosis, showed that FCP levels in patients with IBD were higher by 219.2 µg/g compared with normal patients ($p < 0.001$). Patients with colorectal neoplasia had non-significantly higher FC levels by 132.2 µg/g compared with non-cancer controls ($p = 0.18$). It appeared that calprotectin level higher than 100 µg/g provided 98% sensitivity and 91% specificity for distinguishing between IBD and non-IBD cases.

According to the differentiation between CD and ulcerative colitis, a difference was also found in FCP levels between patients with CD and UC (FCP was 55.79 µg/g higher than in those with UC); however, this is of limited clinical use since the range of values in both groups is large, making the test not useful for differentiating between the two conditions [17]. Quail *et al.* [18] looked at faecal calprotectin concentrations in Scottish children with a diagnosis of IBD; there was no statistical difference in calprotectin concentrations between CD and non-Crohn's patients (UC or IBD type unspecified).

Irritable bowel syndrome (IBS) may present similarly as IBD with symptoms such as abdominal pain and diarrhoea. Most recent studies [19] have used 50 µg/g as the cut-off to define a positive test result that excluded IBS. Patients with elevated calprotectin and abdominal discomfort had endoscopy performed in order to rule out IBD or other organic pathologies. Langhorst *et al.* confirmed that FCP is a valuable tool in differentiating between IBD and IBS patients (Figure 2) and between active and inactive IBD [20]. Calprotectin was able to identify active IBD more accurately than CRP and activity indices and better reflected the endoscopic inflammation.

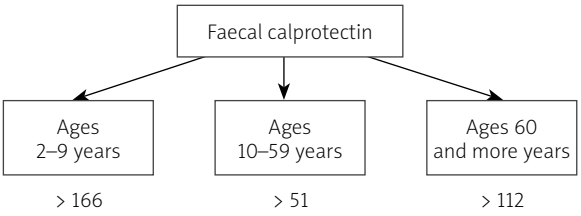

Figure 1. Faecal calprotectin (adapted: NCGC Crohn's disease. Management in adult, children and young people. Clinical Guideline 152. Methods, evidences and recommendations. 10 October 2012)

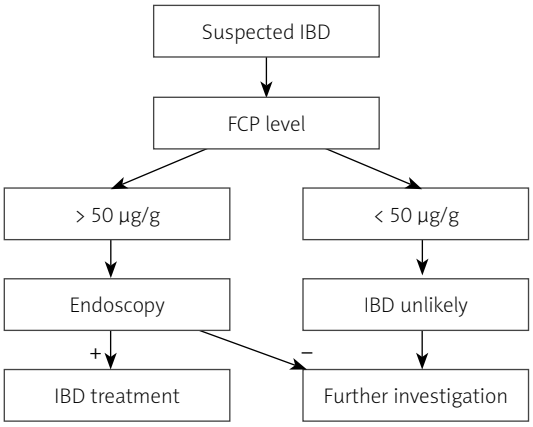

Figure 2. A diagnostic algorithm for the evaluation of patients with suspected inflammatory bowel disease that includes faecal calprotectin measurement before endoscopy (adapted: Burri E, Beglinger C. Faecal calprotectin – a useful tool in the management of inflammatory bowel disease. Swiss Med Wkly 2012; 142: w13557)

Faecal calprotectin correlates highly with the severity of the bowel inflammation; an elevated level of calprotectin helps to rule out irritable bowel syndrome and to distinguish between IBD and other abdominal pathologies.

Calprotectin and endoscopy

Calprotectin has been widely described as a biomarker that greatly correlates with the endoscopic results (Tables I, II). From the clinical practice, the disease activity indices such as CDAI or Harvey-Bradshaw index do not perfectly reflect the actual state of the patient, and are rarely used in everyday life. Possibly this may explain why the correlation of faecal calprotectin tends to be stronger with endoscopic activity than with aforementioned clinical indices [21–23].

In a recent study conducted by Ricane*k et al.*, faecal calprotectin levels correlated very well with endoscopic activity scores in patients with suspected IBD, but were not consistent with the CDAI score in CD patients [24]. Schoepfer *et al.* reported similar results in patients with UC [25]. Some studies even demonstrated no relationship between calprotectin level and the clinical indices [22, 26].

Localisation of the disease

As CD is characterized by non-linear distribution in the whole digestive tract, and highly specialist diagnostic procedures assessing the exact localization of the lesions are not readily available, researchers posed the following question: Does the different localization affect the results of the faecal calprotectin assay?

Table I. Application for selected biomarkers (adapted: Lewis JD. The utility in the diagnosis and therapy of inflammatory bowel disease. Gastroenterology 2011; 140: 1817-26)

	FCP	Lactoferrin	S100A12	CRP	Serologies
IBD vs. other disease	+	+	+	+	+
UC vs. CD					+
Risk of complications					+
Active disease vs. remission	+	+	+	+	
Assess mucosal healing	+	+	+	+	
Predict relapse	+	+	+	+	
Predict response to therapy	+	+	+	+	+

Note: The table includes both confirmed and theoretical roles for these biomarkers

Table II. Correlation of biomarkers with disease activity, determined by endoscopy (adapted: Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. Gastroenterology 2011; 140: 1817-26)

Patient population	Assessment of endoscopic disease activity	Calprotectin (correlation coefficient)	Lactofferin (correlation coefficient)	CRP (correlation coefficient)	References
IBD	Saverymuttu *et al.* [27]	0.52			Fagerberg *et al.* [28] 2007
CD	SES-CD	0.72	0.76	0.46	Jones *et al.* [29] 1997
	SES-CD	0.48	0.19		D'inca *et al.* [30] 2007
	CDEIS	0.73	0.77	0.55	Sipponen *et al.* [31] 2008
	SES-CD	0.64	0.63	0.52	Siponnen *et al.* [32] 2008
	CDEIS	0.83	0.87	0.61	Siponnen *et al.* [33] 2008
	CDEIS	0.75		0.53	Schoepfer *et al.* [34] 2010
UC	Mayo score	0.57			Roseth *et al.* [35] 1997
	Matt's index	0.81			Hanai *et al.* [36] 2004
	Mayo score	0.51	0.35		D'Inca *et al.* [30] 2007
	Rachmilewitz index	0.83		0.50	Schoepfer *et al.* [37] 2009

CDEIS – Crohn's Disease Endoscopic Index of Severity, SES-CD – Simple Endoscopic Score for Crohn's Disease

In fact, several studies proved that the level of faecal calprotectin correlates better with colonic CD rather than the phenotype limited to the ileum [22, 23, 38–40]. Moreover, it better reflected the inflammatory rather than the stricturing and/or penetrating phenotype [23, 38], which may be explained by the fact that the enzyme is secreted in the actively inflamed tissue. Sipponen *et al.* [21] assessed this relationship in active CD (endoscopic scale CDEIS ≥ 3); faecal calprotectin concentrations were significantly higher in colonic compared to ileal CD. Furthermore, in CD limited to the ileum, faecal calprotectin failed to correlate with endoscopic activity. In a study by the same author [41], it was found that calprotectin had a low utility for predicting CD localized in the small bowel on wireless capsule endoscopy – the sensitivity was low (only 59%) with a specificity of 71% using a cut-off of 50 µg/g. On the other hand, in the Scandinavian paper of Jensen *et al.* [42] the results were different, despite similar methods used (capsule endoscopy). Forty patients with CD underwent numerous diagnostic procedures including ileocolonoscopy, capsule endoscopy, magnetic resonance imaging (MRI) or computed tomography (CT) enterography. Faecal calprotectin turned out to be equally sensitive in CD, affecting the small bowel and colon at the cut-off set at 50 µg/g.

What is the relationship in colitis ulcerosa patients then?

Ricanek *et al.* [43] showed that the median faecal calprotectin concentration was significantly lower in patients with proctitis compared to extensive and left sided disease distribution (86 µg/g and 740 µg/g, 2106 µg/g, respectively). No statistically significant difference in calprotectin concentration was found between extensive and left sided distribution of CU.

Calprotectin reflecting mucosal healing

Controlling IBD activity is one of the biggest challenges in the management of patients suffering from CD and UC. Currently the most commonly used tools are clinical indices such as CDAI; however these scales usually reflect the patients' subjective well-being rather than the degree of mucosal inflammation [22, 26]. During the early relapse stage, when the inflammation process starts to develop, the clinical symptoms are usually not present. It has been widely assessed that complete disease control can only be achieved by (complete) mucosal healing (MH) both in CD and UC, and there is a growing consensus that the ultimate goal of IBD therapy is to stop disease progression by obtaining MH.

In most IBD patients in clinical remission it seems that residual mucosal inflammation exists and it appears that measuring faecal calprotectin can detect subclinical inflammation and thus identify patients who might suffer a relapse of the disease.

In a large population-based, cohort study with 495 patients (141 CD, 354 UC) who had endoscopy after 1 and 5 years, mucosal healing was associated with a 60% reduction in surgery among CD patients, and correlated well with a lower need of colectomy in UC patients (2% vs. 7%) [44]. Similarly, Baert *et al.* showed that after 2 years of treatment endoscopic activity of mucosal inflammation in CD could predict the clinical course for the next 2 years [45], and in the group with MH the risk of relapse was lower than in patients with residual inflammation (32% vs. 65%). Røseth *et al.* [46] demonstrated that normalisation of faecal calprotectin concentration corresponds to endoscopic mucosal healing. Forty-four out of 45 patients who had clinical remission with faecal calprotectin < 50 mg/l who underwent colonoscopy with histological assessment of the biopsies had inactive mucosal disease.

Mucosal healing seems to reflect IBD activity better than the clinical scales. It correlates well with sustained remission and predicts the clinical outcome. Data on faecal calprotectin as a surrogate marker of MH are emerging, but are still not conclusive.

Monitoring the course of the disease/ predicting the risk of relapse

One of the major goals of treatment for IBD is to prevent complications and keep the disease relatively quiescent. The clinical course of CD and UC tends to be chronic, relapsing and somewhat surprising with the development of new lesions in the GI tract. The goal is to specifically identify the patients before the complications occur and to individually tailor the therapies that can effectively prevent a relapse.

As calprotectin has been proved to reliably predict relapse in IBD, this biomarker may help clinicians to focus the resources effectively by avoiding aggressive treatment in those less likely to relapse and by intensifying the treatment in patients at highest risk of recurrence, earlier than the symptoms would present. Mao *et al.* [47] recently performed a meta-analysis of the predictive capacity of faecal calprotectin in IBD relapse. The authors after having analysed 6 studies (a total of 672 IBD patients – 318 UC and 354 CD) found a pooled sensitivity of 78% and specificity of 73% in predicting relapse. The results were comparable between CD and UC patients. Unfortunately, because of the insufficient number of patients the predictive value of faecal calprotectin in the ileal localization of CD was not as-

sessed. As a conclusion the authors showed that the measurement of faecal calprotectin seemed to be more accurate in ileocolonic and only colonic CD.

What is interestingly emerging from the clinical trials is the fact that faecal calprotectin was found to be less useful for predicting the disease recurrence in patients with CD limited to the ileum compared with patients with ulcerative colitis or colonic/ileocolonic CD [39, 40].

Differentiating quiescent from active disease

In patients suffering from CU and UC faecal calprotectin has been proved to differentiate quiescent from active disease [21, 26, 48, 49]. In a study conducted by Shoepfer *et al.* [38] faecal calprotectin correlated closely with SES-CD and was the only biomarker that could reliably discriminate inactive from mild, moderate, and highly active disease, which proves the utility of calprotectin in monitoring the disease activity.

Predicting colectomy

Faecal calprotectin turned out to be able to predict colectomy in patients suffering from acute severe UC. Ho *et al.* [50] showed that in patients with acute severe UC that required hospitalization and *i.v.* administration of steroids faecal calprotectin was significantly higher in patients who did not respond to this therapy and underwent colectomy than those who did not. At a cut-off of 1922.5 µg/g the maximum likelihood ratio for colectomy was 9.23 with specificity of 97.4%. Overall in the study faecal calprotectin concentrations were high with 86% of patients having levels of > 500 µg/g (median 1020 µg/g).

Pouchitis

Pouchitis is inflammation of the pouch and may affect up to 45% of patients after restorative proctocolectomy. Faecal calprotectin has been shown to reliably differentiate between inflamed and non-inflamed pouches. A correlation between severity of pouchitis and FC levels has been described [51–53]. As a result it may reduce the need for endoscopic procedures in these patients.

In patients in clinical remission high faecal calprotectin levels correlate with increased risk of disease relapse in the following 12 months. Thus it may be useful in long-term management of the disease. Moreover, faecal calprotectin can predict the need for colectomy in UC patients and it correlates well with the severity of the pouchitis.

Response to therapy

As previously mentioned, FCP's ability to monitor the course of the disease may help the clinician to assess the choice of the treatment, whether it is adequate for the particular individual. Every time the practitioner considers the change in the IBD treatment, it should be assessed that undertreatment of patients from the high-risk group may lead to clinical relapse, but also that overtreatment of low-risk patients may provoke side-effects and it generates unnecessary costs.

Wagner *et al.* [54] assessed the value of FCP in 38 patients with active IBD (11 CD and 27 UC) treated with various forms of therapy, from 5-aminosalicylic acid (5-ASA) to different combinations of 5-ASA, prednisone, and azathioprine. None received anti-tumour necrosis factor α (TNF-α) agents. After 8 weeks of therapy, 82% of patients had normal endoscopy and the levels of calprotectin were normalized, being 100% predictable for complete response to treatment. The study conducted Kohle *et al.* [55] analysed the ability of calprotectin to reflect the response to glucocorticoid therapy in a group of 15 children suffering from active IBD, who were followed up endoscopically. The calprotectin was measured at 0, 2 and 4 weeks and at 4-week intervals until one month after discontinuation of the therapy. The results showed FCP decreasing in line with the clinical improvement, but without the normalization of its value. Shortly after discontinuation of therapy there was a rapid increase in the level of FCP, suggesting a flare-up of the intestinal inflammation. Louis *et al.* [19] investigated in the STORI prospective study 115 patients with CD who were in steroid-free remission for at least half a year. Patients were treated with infliximab and an antimetabolite for at least 1 year. After the infliximab was discontinued, patients were on combined maintenance therapy with antimetabolites. An elevated calprotectin level above 300 µg/g correlated with the risk of relapse of the disease.

In turn, opposite results were published by Ho *et al.*, who studied the role of FCP in predicting which from among 90 patients with acute severe CU would require colectomy or would not respond to corticosteroid or infliximab treatment [56]. The results showed that calprotectin levels were higher only in the group requiring colectomy ($p = 0.04$), but not in corticosteroid ($p = 0.08$) and infliximab non-responder groups ($p = 0.06$). The results of two studies [57, 58] conducted on children showed that the Pediatric UC Activity Index (PUCAI) more accurately predicts treatment response and long-term outcome than faecal calprotectin levels.

Economy – reduction in colonoscopies

Calprotectin may be used as a sort of screening method in the management of patients presenting with

symptoms suggestive of IBD. This would result in a limited number of colonoscopies being performed, and thus would prevent complications and save costs. Being economical is an important aspect of calprotectin, a useful discriminator between patients who require a colonoscopy, and those who do not.

In 2010 Rheenen *et al.* [10] published a meta-analysis, summarising data of 1041 patients (670 adults, 371 children), in which they compared the diagnostic accuracy of FCP in the evaluation of patients with suspected IBD. Pooled sensitivity was 93% and specificity was 96%, while for children and teenagers the specificity was significantly lower (76%). The authors proved that measuring faecal calprotectin reduced the number of unnecessary endoscopies that would be performed in patients with symptoms suggestive of IBD. The number of adults requiring endoscopy would decrease by 67% due to the use of FCP for screening. However, it was concluded that 6% of patients received a false negative result that led to a delayed diagnosis. Swedish investigators [59] were also interested in the problem of unnecessary endoscopies. After having analysed almost 3,500 cases of patients, they proved that the use of a 50 µg/g calprotectin cut-off resulted in a 49.7% reduction in unnecessary colonoscopies, while the use of a 100 µg/g cut-off resulted in a reduction of almost 66.9%, which would lead to savings of around 1.5 and 2 million euro, respectively.

Rapid calprotectin tests

Rapid calprotectin tests that allow the repetitive measurement of FCP in order to monitor activity of the disease are already available on the Polish market. One of them is Quantum Blue provided by Buhring. It is a point-of-care, quick test, designed for the conditions of primary care. It is especially useful in situations when the result should be obtained in a short period of time. In comparison with the typical ELISA test the correlation between calprotectin concentration results is strong (87–90%) [60–63].

Currently on the market a rapid test for qualitative determination of calprotectin in faeces is also available. It is designed for screening for IBD, especially in the differentiation with irritable bowel syndrome. Due to the ease of performance and relatively low cost it can be used in outpatient clinics. The cut-off point is set at 50 µg/g.

Because of the very good negative predictive value, rapid calprotectin tests can be used in primary care by general practitioners to rule out IBD. It may result then in reduction of referrals to a specialist and reduction in unnecessary colonoscopies.

ANCA/ASCA antibodies

Perinuclear anti-neutrophil cytoplasmic antibodies (P-ANCA) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) are the most widely investigated serological markers for IBD [64, 65]. An increased level of P-ANCA is associated with UC or those with CD who had UC-like pancolitis, while p-ASCA are more common among patients with CD [66].

The role of these serological markers in establishing IBD diagnosis and their predictive value was assessed by Solberg *et al.* [67], based on the results of the population-based Norwegian IBSEN cohort. A total of 526 patients were followed up 10 years after the initial diagnosis (UC, n = 357 and CD, n = 169). ASCA in the CD group had 27% sensitivity, 95% specificity and 73% positive predictive value (PPV). P-ANCA in patients with UC had sensitivity, specificity and positive predictive value (PPV) of 31%, 86%, and 82% respectively.

The combination of ANCA and ASCA was used to differentiate between CD and CD, resulting in 84% of PPV. A meta-analysis of 60 studies showed low sensitivity (40–50%) and high specificity (90%) of the combination of p-ANCA and ASCA tests for distinguishing patients with CD from those with UC. However, the ASCA test had lower sensitivity for CD and differentiated worse between CD and UC when the analysis was limited to the population of patients with colonic manifestation of the disease [68]. From the practical point of view, for this particular group of patients an effective discriminating marker would be the most relevant.

Lactoferrin

Lactoferrin, an iron-binding molecule, is also a protein secreted by mucosal membranes, being found in neutrophil granules and serum. Its resistance to proteolysis and degradation makes it a promising marker reflecting the intensity of the inflammatory process in the intestines. Several studies but not all [69, 70] have proved that faecal lactoferrin's sensitivity and specificity range to detect an active mucosal disease is similar to FCP. Kane *et al.* [71] investigated the use of lactoferrin in identifying intestinal inflammation in 104 CD patients, 80 UC patients, 31 IBS patients, and 56 healthy controls. The results showed that lactoferrin's specificity in identifying inflammation in patients with active IBD is 90% and in ruling out IBS 100%. Gisbert *et al.* collected and analysed data from multiple studies and 1001 patients, showing that the faecal lactoferrin test identified patients with IBD with relatively high mean sensitivity of 80% and specificity of 82% [72].

Other biomarkers

100A12 protein has also been a subject of research in recent years. 100A12 is a S100 protein similar to calprotectin that can be measured in faecal samples and serum. According to the latest research faecal levels of S100A12 have high sensitivity and specificity to distinguish between IBS and IBD in both children and adults [73, 74]. It has also been proved that serum S100A12 does not have as high a level of sensitivity and specificity as the faecal assay [75]. However, application of these rare biomarkers to everyday practice is yet to be assessed.

Conclusions

There is a growing body of evidence that calprotectin and other presented biomarkers satisfactorily correlate with the disease activity and reflect the intestinal inflammation. Recently the ability of calprotectin to detect clinical relapse, especially after anti-TNF treatment, has been investigated. Therefore we look forward to further research in this field. Currently the rapid calprotectin tests, quantitative as well as qualitative, are becoming increasingly popular and may play an important role in early diagnosis of IBD and monitoring the disease course, especially in the field of primary care if adopted by GPs and outpatient specialist clinics.

Although to date no algorithm concerning the dynamics of biomarkers in IBD treatment has been established, possibly calprotectin or another surrogate marker for gut inflammation would change the way we treat CD and UC patients in the near future.

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