Review Article

Serum Biomarkers in Diagnosis and Clinical Management of Inflammatory Bowel Disease: Anything New on the Horizon?

(inflammatory bowel disease / IBD / Crohn's disease / ulcerative colitis / faecal calprotectin / serum calprotectin / leucine-rich alpha-2 glycoprotein / serum biomarkers / point-of-care testing)

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Abstract. Persistent inflammation in inflammatory bowel disease (IBD) leads to progressive damage to the gastrointestinal tract, resulting in potentially severe sequelae. Diagnosis primarily relies on invasive endoscopy and monitoring of faecal calprotectin (FC), which has limitations, particularly regarding patient compliance. There is a pressing need for a new biomarker that is non-invasive, easily determinable, and possesses good diagnostic accuracy for both diagnosing and monitoring IBD. Our narrative review covers the latest developments in novel serum biomarkers, focusing on those with promising diagnostic accuracy and laboratory methods, and evaluates them in the context of established biomarkers such as FC and CRP. Serum calprotectin (SC) and leucine-rich alpha-2 glycoprotein (LRG) show the most extensive evidence and relatively good diagnostic accuracy but currently cannot replace FC due to insufficient evidence. Major limitations of the analysed studies include their monocentric nature, small sample sizes, lack of longitudinal monitoring and in some cases, missing assessments of endoscopic activity. ELISA holds a leading position among the laboratory methods; however, emerging evidence supports the potential use of point-of-care testing (POCT). Establishing these biomarkers for regular clinical application will require further validation through multicentric studies involving a larger number of patients with a longitudinal design, concurrent assessment of endoscopic activity and proactive monitoring of the biomarker. However, based on the evidence accumulated so far, SC might potentially serve as a complementary biomarker and/or in assessing the activity of extraintestinal manifestations in IBD patients, while LRG appears to be effective in evaluating endoscopic activity, especially in small bowel CD.

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Abbreviations: Anti-CBir1 – anti-CBir1 flagellin, anti-I2 – anti-*Pseudomonas fluorescens*-associated sequence I2, Anti-OmpC – anti-outer membrane porin C antibody, ASCA – anti-*Saccharomyces cerevisiae* antibodies, AUROC – area under the receiver operating characteristic curve, CD – Crohn's disease, CDAI – Crohn's disease activity index, FC – faecal calprotectin, FL – faecal lactoferrin, IBD – inflammatory bowel disease, IBS – irritable bowel syndrome, LAT – latex agglutination test, LFIA – lateral flow immunoassay, lncRNAs – long non-coding RNAs, LRG – leucine-rich alpha-2 glycoprotein, MES – Mayo endoscopic score, miRNAs – microRNAs, mSES-CD – modified simplified endoscopic score for Crohn's disease, ncRNAs – non-coding RNAs, pANCA – perinuclear anti-neutrophil cytoplasmic antibodies, PC – plasma calprotectin, RAGE – receptor for advanced glycation end products, SC – serum calprotectin, SES-CD – simplified endoscopic score for Crohn's disease, Sn – sensitivity, Sp – specificity, TLR4 – Toll-like receptor 4, UC – ulcerative colitis.

Introduction

Inflammatory bowel disease (IBD), mainly including ulcerative colitis (UC) and Crohn's disease (CD), are chronic, progressive, relapsing inflammatory conditions of the gastrointestinal tract. These are heterogeneous diseases encompassing a broad spectrum of intestinal and extraintestinal manifestations. Despite intensive re-

search, the pathogenesis of these conditions remains unclear. It is presumed that genetic predisposition, environmental factors, imbalances in the intestinal microbiota, and immune system dysregulation play roles. Over the past thirty years, the prevalence of both diseases has increased, currently affecting 0.3–0.5 % of the population in economically developed countries, with the first clinical signs increasingly appearing in younger age groups – predominantly affecting individuals aged 15–30 years. IBD significantly impacts the quality of life and the work and social activities of the affected individuals (Hlavatý et al., 2017; Ďuricová et al., 2018; Koller et al., 2018; Le Berre et al., 2023).

The diagnosis of IBD is complex, involving medical history and clinical examination, supplemented by paraclinical laboratory and imaging tests – particularly blood and stool tests, endoscopic examinations, and imaging methods such as ultrasound, MRI, or CT enterography, along with possible histopathological examination of affected sections of the gastrointestinal tract (Maaser et al., 2019). Currently, the most commonly used biomarkers of intestinal inflammation in clinical practice are FC, CRP and to a lesser extent, faecal lactoferrin (FL).

FC, elevated in IBD, is particularly useful for distinguishing IBD from IBS, with reported cut-off values < 50–150 μg/g indicating IBS. It is widely used in monitoring the activity of IBD, although a consensus on the ideal cut-off is still lacking. In controlled clinical studies, values for remission are used up to 100 μg/g, while in real-world studies, values predominantly up to 250 μg/g are common (Maaser et al., 2019; Ungaro et al., 2019; Colombel et al., 2020; D'Amico et al., 2021). The correlation coefficients of biomarkers with endoscopy ranged from 0.48–0.83 for FC and 0.19–0.87 for lactoferrin in IBD patients (Lewis, 2011; Alghoul et al., 2022). Stricter cut-off values were associated with achieving histological healing in UC and transmural healing in CD (Patel et al., 2017; Weinstein-Nakar et al., 2018; Walsh et al., 2019).

An important feature of FC is its ability to predict relapse in the patients in clinical remission. A key insight in this regard was provided by a large meta-analysis that included 672 patients (318 with UC and 354 with CD) whose data came from six prospective studies assessing FC concentrations during remission. The combined Sn and Sp for FC in predicting relapse were 78 % and 73 %, respectively. The area under the receiver operating characteristic curve (AUROC) was 0.83 and the diagnostic odds ratio was 10.31. The ability of FC to predict relapse was comparable between UC and CD. Within CD, the predictive accuracy was higher for ileocolonic and colonic localizations (Mao et al., 2012).

The currently preferred treat-to-target (T2T) strategy using FC and CRP biomarkers was evaluated in the CALM study. It was the first prospective randomized controlled study to demonstrate that the effect of tight control management on Crohn's disease via the T2T strategy using FC and CRP biomarkers (early escalation of biological therapy at ≥ 250 μg/g or CRP ≥ 5 mg/l) led to a higher rate of endoscopic remission in patients with Crohn's disease compared to a strategy based on the escalation of treatment based on clinical symptoms only. It was also shown that the combination of FC and CRP most accurately predicted the disease relapse (Colombel et al., 2017; Reinisch et al., 2020). Long-term follow-up analysis (median 3 years) further showed that patients who achieved early endoscopic remission, i.e., at the end of the first year, had significantly lower incidence of serious complications such as strictures, fistulas, or mesenteric infiltrates, and a lower need for hospitalizations and surgical treatment (Ungaro et al., 2020).

In recent studies, FC, which holds a position as an established and routinely used biomarker, has been evaluated for its utility in the remote monitoring of IBD patients through the point-of-care testing (POCT) systems. The IBD Assistant project in the Czech Republic investigated the telemedicine's potential in managing IBD care. This clinical study aimed to assess telemonitoring as an effective tool for enhancing the management and patient quality of life in IBD, with specific objectives including prediction of relapse and reducing severe complications. The study utilized a web interface combined with self-monitoring of FC, comparing home-based LFIA measurements (using the CalproSmart Diagnostic Kit® (CALPRO AS, Svar Life Science company, Lysaker, Norway) and a smartphone as a reading device with special software) to standard FIA measurements in a specialized lab. Notably, stool samples for these measurements were collected from two separate defecations within a 1–5-day interval, maintaining the same clinical status. The findings revealed a Spearman correlation coefficient of r = 0.895 (P = 0.0004) and a weighted Cohen's kappa of 0.835, indicating a high degree of consistency between the two methods. The home-based CalproSmart® test proved to be a practical tool for qualitative FC analysis in telemonitoring, though not a replacement for laboratory measurements. Patients reported a positive experience, highlighting their involvement in health monitoring, which increased their self-care and responsibility. They also valued the test's simplicity and the time and cost efficiencies it provided (Malíčková et al., 2019; Lukáš, 2021). Key aspects concerning the currently used biomarkers are listed in Table 1.

In summary, the currently preferred biomarker used to aid in diagnosis and subsequent monitoring is FC. FC can provide information about the response to treatment, detect intestinal inflammation and guide the physician in treatment with the goal of achieving better clinical and possibly endoscopic outcomes. Its importance is particularly significant in distinguishing the disease activity from functional disorders, such as irritable bowel syndrome (IBS), which may confound the diagnostic process in patients with IBD. However, its use in clinical practice has limitations: stool collection can be restrictive for patients, FC cannot reliably differentiate IBD from other intestinal inflammation processes, and there is a need for more precise correlation with the de-

Table 1. Current biomarkers used in the diagnosis and assessment of IBD activity

Biomarker	Sample	Clinical context
FC (Maaser et al., 2019; Ungaro et al., 2019; Colombel et al., 2020; D'Amico et al., 2021)	stool	• High sensitivity (Sn) for intestinal inflammation, low specificity (Sp) for distinguishing IBD from other intestinal inflammations. Differentiates between IBD and IBS, but not between UC and CD. Early detection of the onset of acute intestinal inflammation and relapses, monitoring the course (activity-remission) and response to treatment in IBD. • **Advantages:** accessible, non-invasive. • **Disadvantages:** elevated in other intestinal infections, use of certain medications (NSAIDs), colorectal cancer, diverticular disease.
FL (Guo et al., 2022; Szymanska et al., 2023)	stool	• Similar to FC, suitable for distinguishing between IBD and IBS, and monitoring disease inflammatory activity, but with lower combined Sn and Sp. • **Advantages:** rapid results, non-invasive. • **Disadvantages:** elevated in other gastrointestinal inflammations.
CRP (Maaser et al., 2019; Ungaro et al., 2019; Colombel et al., 2020)	serum	• General marker of acute inflammation, low Sn for intestinal inflammation (especially in UC), useful in CD – monitoring inflammatory activity. • **Advantages:** rapid results, non-invasive. • **Disadvantages:** low Sn.

gree of disease activity. Additionally, the methodology of the collection and processing itself remains problematic, with day-to-day variability and inconsistency in stool samples (Bernardo et al., 2019; Carlsen et al., 2019; D'Amico et al., 2021).

The goal of the treatment after inducing remission in IBD is its long-term maintenance through strict monitoring ("tight control"). The tight control is a disease management approach where treatment decisions are made based on rigorous monitoring of the disease status indicators, such as biomarkers, with the aim of achieving better treatment outcomes (Colombel et al., 2020). Particularly, endoscopic remission (also known as mucosal healing) is an important treatment goal and its timely achievement is associated with better future outcomes for the patient. This includes reductions in hospitalizations, decreased risk of disease relapse and a reduced need for surgical interventions (Colombel et al., 2020; Turner et al., 2021). Currently, IBD is monitored primarily using FC, disease activity questionnaires, but luminal assessment of inflammation in the intestines during endoscopy remains the gold standard. The need for repeated or frequent endoscopic examinations is associated with poor acceptance by patients due to the invasive nature and the need for bowel preparation, sedation and the risk of complications. Additionally, it is a costly and time-consuming examination (Bourgonje et al., 2019a; Tomaszewski et al., 2021). Clinical indices tend to reflect the symptoms reported by the patient and do not provide information on the extent or intensity of intestinal inflammation (Bourgonje et al., 2019a).

Given these facts, there is a significant need for new non-invasive biomarkers that would diagnose the disease more accurately, predict its progression and correlate more precisely with the level of endoscopic activity, thus allowing for more effective clinical management. This need has particularly been underscored in recent years based on experiences from the COVID-19 pandemic, or in connection with other possible pandemics, potentially resulting in a reduction in the number of examinations and the time a patient can spend in a healthcare facility. Over the past few years, research has therefore increasingly focused on the study of new biomarkers that could fill this gap. Current trends are characterized by intense research in the area of new faecal biomarkers (calgranulin C (S100A12), lipocalin-2 (LCN-2)), genetics, epigenetics (the importance of DNA methylation, RNA interference, miRNA) and the microbiome (Alghoul et al., 2022; Plevris and Lees, 2022).

The prevalence of patients with CD and UC is gradually increasing, with an expected rise of at least 20–30% in the next decade. This will create increased demands for patient monitoring, as well as for new ways of monitoring the treatment. In this context, telemedicine (remote monitoring using POCT and electronic health) has emerged as one of the most significant and promising solutions for patients with chronic and yet incurable diseases. This is further emphasized by the fact that a specific characteristic of IBD patients is that they are largely young people of working age, who may find the necessity of lifelong and regular visits to the doctor restrictive, but on the other hand, are interested in active participation in the care of their health and in their disease (Lukáš, 2021).

Promising strategies for the future also include the possibility of using mobile applications and wearable electronic devices (similar to glucose sensors for diabetics). Advances in technology have enabled development of wearable electronic device SWEATSENSER® (EnLiSense, Allen, Texas) for measuring CRP and interleukin 1β from the patients' sweat. This device has enabled continuous real-time measurement of these biomarkers. The Pearson correlation coefficient reached the values of $r = 0.99$ and $r = 0.95$ for IL-1β and CRP, respectively, for SWEATSENSER® compared with ELISA. Results from Bland-Altman analysis further confirmed good agreement (average bias –0.25 and –3.9 pg/ml for IL-1β and CRP, respectively) of the device with the reference

method, demonstrating its usability for real-time monitoring (Jagannath et al., 2020).

Among the other biomarkers currently under investigation are prostaglandin E-major urinary metabolite (PGE-MUM), oncostatin M, non-coding RNAs (ncRNAs), and a dominant position in the research is held by serum biomarkers of protein and peptide nature (Sakurai and Saruta, 2023). The aim of this review is to provide an overview of the current developments in this field, with a focus on the most promising serum biomarkers in terms of diagnostic accuracy and laboratory methods.

Methods

To create this narrative review, a search was conducted for published research studies indexed in scientific databases such as PubMed, Scopus, Embase and Google Scholar, using specific keywords. Studies published between 2019 and 2023 that included a minimum of 30 subjects were considered for inclusion.

Results

Serum calprotectin

Currently, significant research is focused on the study of new serum biomarkers, aiming to identify those that offer a high degree of combined Sn and Sp, are user-friendly and economically feasible for practical use. Serum calprotectin (SC) emerges as the most extensively studied serum biomarker in relation to the diagnosis of CD and UC and clinical and endoscopic activity. An important subject of research is also its correlation with other biomarkers currently used, such as FC and CRP, and possibly erythrocyte sedimentation rate, neutrophil to lymphocyte ratio, albumin and haemoglobin. Calprotectin is a heterodimeric protein belonging to the S100 protein family, consisting of S100A8 (molecular weight 10.8 kDa) and S100A9 (molecular weight 13.2 kDa) subunits, which bind calcium and zinc (Jukic et al., 2021). It is evolutionarily conserved, widely expressed in neutrophils as their major component, constituting about 45 % of their cytoplasmic proteins, and to a lesser extent in monocytes, macrophages and certain epithelial cells, and is also known for its antimycotic activity against *Candida albicans* (Andersson et al., 1988; Sohnle et al., 2000). It plays a role in several physiological processes such as cell differentiation, immunoregulation, carcinogenesis, apoptosis and inflammation (Khaki-Khatibi et al., 2020; Jukic et al., 2021). In response to inflammatory stimuli, such as bacterial antigens or cytokines such as TNF-α and IL-1β, CP expression can be induced in cells, further influencing inflammatory pathways (Kido et al., 2005; Jukic et al., 2021).

SC plays crucial roles both inside and outside cells. Intracellularly, it affects the cytoskeletal rearrangements essential for leukocyte recruitment and influences the transport of arachidonic acid to inflammation sites, thereby by modulating inflammation and tissue damage, particularly in IBD (Nielsen et al., 1987; Vogl et al., 2004). Externally, it acts through receptors such as Toll-like receptor 4 (TLR4) and the receptor for advanced glycation end products (RAGE), promoting neutrophil chemotaxis, endothelial adhesion and secretion of both pro-inflammatory and anti-inflammatory cytokines (Ma et al., 2017).

Significantly, SC impacts cell proliferation, differentiation and apoptosis and interacts with NK cells to enhance IFN-γ expression via RAGE signalling. This interaction underscores its role in linking inflammation to broader immune responses (Narumi et al., 2015). Additionally, SC's involvement in unresolved inflammation at mucosal surfaces and its influence on the tumorigenic processes indicate its critical function in both inflammation and cancer pathways. Intriguingly, despite the synergistic functions of its subunits S100A8 and S100A9, distinct biological activities can emerge depending on their configuration and interaction, particularly in inflammation and tumorigenesis (Hobbs et al., 2003; Ichikawa et al., 2011).

Through these diverse mechanisms, SC not only orchestrates an acute inflammatory environment but also plays a critical role in chronic inflammation and tissue remodelling. This highlights its potential as both a diagnostic biomarker and a therapeutic target in diseases characterized by persistent inflammation, such as IBD. The broad spectrum of activities underscores the SC's importance in both the initiation and resolution of inflammatory responses (Jukic et al., 2021).

SC is a sensitive biomarker of both acute and chronic inflammation. Recent research has confirmed that elevated levels of SC have been present in various autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, psoriasis, ankylosing spondylitis, periodontitis, as well as in malignancies such as myelodysplastic syndrome, carcinoma of the bladder, lungs, pancreas, prostate and others (Hurnakova et al., 2017; Jukic et al., 2021). SC is currently particularly useful in the diagnosis and monitoring of the disease activity in patients with familial Mediterranean fever, where it possesses relatively good Sp in detecting subclinical inflammation (Ehlers et al., 2023). SC levels were investigated among patients undergoing surgical procedures for conditions such as inguinal or umbilical hernia, cholecystolithiasis and acute issues such as appendicitis and incarcerated hernia. It was found that preoperative calprotectin levels were significantly lower in patients compared to healthy controls. However, the calprotectin levels significantly increased following surgery. In patients with acute conditions, SC levels were significantly elevated preoperatively, underscoring the calprotectin's responsiveness to surgical and acute inflammatory stresses and its potential utility in monitoring postoperative inflammation (Strohalmová et al., 2023).

Multiple pilot studies have demonstrated the significance of SC as a potentially sensitive biomarker for assessing the disease activity in IBD or for establishing a

diagnosis. One of the first significant studies on this topic, the STORI study, assessed the performance of serum calprotectin in 115 CD patients. In particular, the authors examined its correlation with clinical disease activity, endoscopic activity, CRP and FC, as well as its ability to predict CD relapse and response to treatment. Meuwis et al. (2013) found that SC had a profile very similar to CRP, with significant increases in active disease and good responsiveness to medical therapy. Findings from this study suggested that SC levels (> 5,675 ng/ml) could be complementary to CRP (> 5 mg/l) and FC (> 250 μg/g) for relapse prediction specifically after infliximab withdrawal in Crohn's disease (P = 0.0173, 0.0024, and 0.0002; HR: 3.191, 3.561, and 4.120, respectively), highlighting its potential utility in monitoring the disease activity and guiding the treatment decisions.

In recent years, numerous studies have been conducted aiming to define the potential role of serum SC in the diagnostic and treatment algorithms for IBD. Among those aiming to comprehensively assess this biomarker – not only in distinguishing IBD diagnoses from healthy individuals or evaluating clinical activity but also endoscopic activity (mucosal healing) – several are notable. Veyrard et al. (2022) evaluated 119 patients in a prospective longitudinal study by periodically monitoring SC in patients in deep remission at baseline. Patients were followed for 12 months or until relapse. SC levels were observed prior to and/or at the time of disease relapse to evaluate its diagnostic accuracy in predicting clinical relapse. The median SC levels at baseline were 3.15 μg/ml; during relapse, they increased to 4.45 μg/ml. In patients with clinical symptoms, SC demonstrated a good predictive value for relapse (AUROC 0.764), with a Sn of 72 % and a Sp of 77 % using a cut-off value of 4.45 μg/ml. SC performed better than CRP in detecting relapse but was less accurate than FC (Table 2). Overall, SC increased during clinical and endoscopic activity and enabled differentiation between active IBD and IBS in patients with clinical activity of IBD. However, SC was not elevated during the follow-up of IBD patients in clinical remission before clinical relapse.

The POCT diagnostic approach was used in the prospective monocentric study by Bernardo et al. (2019). SC was assessed as a biomarker for predicting clinical remission and mucosal healing in a cohort of 82 IBD patients. The study highlighted the SC's capacity to predict clinical remission with Sn of 65.6 % and Sp of 67.6 % at a cut-off of 5.3 mg/ml, achieving an AUC of 0.67. Notably, the SC's predictive accuracy was more favourable in UC than in CD. For mucosal healing, a threshold of 4.8 mg/ml provided a Sn of 61.9 % and a Sp of 80.9 %, with an AUC of 0.73. The SC's performance was found to be comparable to that of FC and CRP in both UC and CD.

Table 2. Diagnostic accuracy, sensitivity and specificity of biomarkers in predicting relapse (Veyrard et al., 2022)

	SC	FC	CRP
AUC	0.76	0.86	0.74
Sn	0.72	0.76	0.60
Sp	0.77	0.86	0.79

In a pilot study examining SC in adolescent IBD patients, SC demonstrated a significant positive correlation with the endoscopic score (r = 0.56, P = 0.01), symptom score (r = 0.64, P = 0.003) and CRP (r = 0.97, P < 0.0001) across 19 samples from UC patients. Additionally, a significant relationship was observed between SC and CRP in CD across 49 samples. These results underscore the SC's potential as a biomarker for monitoring the disease activity in adolescents with UC (Carlsen et al., 2019).

A prospective study by Ferrer et al. (2019) involved 53 IBD patients who underwent colonoscopy as part of routine clinical practice. The study assessed SC, FC and conventional blood test parameters. Notably, in UC patients, SC levels were significantly higher with endoscopic Mayo scores of 2/3 (median 10.39 mg/ml) compared to scores of 0/1 (median 4.07 mg/ml), underscoring the SC's effectiveness as an inflammatory biomarker. The study demonstrated the SC's high diagnostic performance in UC with an AUROC of 0.85 and Sn and Sp of 83.3 % and 81.25 %, respectively, for a cut-off of 4.4 mg/dl. However, SC did not show significant correlations with endoscopic findings in CD. Table 3 summarizes the studies of SC in the diagnosis and disease activity assessment of IBD.

Leucine-rich alpha-2 glycoprotein and other serum biomarkers

Among other serum biomarkers with more extensive evidence is serum leucine-rich alpha-2 glycoprotein (LRG), a 50 kD protein secreted by hepatocytes, neutrophils, macrophages and intestinal epithelial cells, which is released into the intestines of patients with IBD after cytokine stimulation (Alghoul et al., 2022). Its structure consists of eight domains called leucine-rich repeats and is induced by several inflammatory cytokines – TNF-α, IL-22, IL-1β and IL-6 (Sakurai and Saruta, 2023). Besides IBD, it has recently been studied as a new serum biomarker for rheumatoid arthritis (Ha et al., 2014), and its elevated levels have been observed in gastric cancer and colorectal carcinoma (Yamamoto et al., 2017; Shinozaki et al., 2018). The potential clinical use of LRG may be in two positions – monitoring activity and diagnosing mucosal healing, as evidenced by several studies, particularly by Japanese researchers.

A recent, relatively large (267 subjects) prospective study compared the diagnostic accuracy of LRG with CRP and FC in assessing clinical and endoscopic activity in IBD. LRG values significantly correlated with both clinical and endoscopic activity in CD and UC (P < 0.0001). The diagnostic accuracy of LRG in UC was significantly higher than that of CRP for assessing clini-

Table 3. Overview of clinical studies with serum calprotectin in the diagnosis and activity assessment of IBD

Study	Type	N	Laboratory methods	Results
(Veyrard et al., 2022)	OBS L	75 CD 44 UC	ELISA	SC levels were higher in active IBD and similar among groups of patients with IBS or in deep remission (3.05 µg/ml in IBS vs 2.99 µg/ml in remission vs 5.1 µg/ml in clinical relapse, P = 0.04). SC was not as effective in predicting relapse as FC.
(Carlsen et al., 2019)	2 parts: OBS CS DS and OBS L	19 UC	ELISA	In UC, a positive correlation was observed between SC and endoscopic scores, symptom scores and CRP (r = 0.56, P = 0.01; r = 0.64, P = 0.003; r = 0.97, P < 0.0001) in adolescents.
(Bernardo et al., 2019)	OBS CS DS	49 CD 33 UC	LFIA (POCT)	CR: AUROC = 0.67, cut-off of 5.3 mg/ml predicted CR with Sn of 65.6 % and Sp of 67.6 %; MH: AUROC = 0.73, cut-off of 4.8 mg/ml predicted MH with Sn of 61.9 % and Sp of 80.9 %.
(Chen et al., 2021)	OBS CS DS	64 CD 64 UC 64 HC	ELISA	For the diagnosis of CD, Sn and Sp were 87.3 % and 89.7 %, respectively. For the diagnosis of UC, Sn and Sp were 98.5 % and 75.6 %, respectively. In UC, SC levels correlated with clinical activity: 868.31 ± 375.25 ng/ml during active disease and 701.58 ± 278.88 ng/ml in remission (P < 0.05).
(Elshayeb et al., 2021)	OBS CS DS	14 CD 36 UC 30 HC	ELISA	Patients with IBD had significantly higher average SC levels compared to non-IBD subjects. The AUROC was 0.99; cut-off of 925 ng/ml predicted the disease with Sn of 98 % and Sp of 92 %.
(Ferrer et al., 2019)	OBS CS DS	26 CD 27 UC	ELISA	Patients with active UC had significantly higher average median SC levels – 10.39 mg/ml compared to patients in remission – 4.07 mg/ml (P = 0.01). The AUROC was 0.85; the cut-off of 4.4 mg/dl predicted active disease with Sn of 83.3 % and Sp of 81.25 %, no correlation in CD.
(Udegbune et al., 2022)	OBS CS DS	71 UC	ELISA	No correlation was demonstrated between SC levels and clinical activity in UC; the median was 4,534 (3,387–6,416) ng/ml for clinically active UC vs 4,031 (2,401–5,414) ng/ml for clinical remission (P = 0.1825).
(Malham et al., 2019)	OBS CS DS	84 UC	ELISA	FC and SC significantly correlated with clinical, endoscopic and histological activity in UC, while PC showed a stronger correlation – (ρ = 0.54, P = 0.002), (ρ = 0.39, P = 0.0003) and (ρ = 0.28, P = 0.01), respectively.
(Yasuda et al., 2023)	OBS CS DS	74 CD 77 UC 22 HC	ELISA	Paediatric population: average SC levels in active CD were 2,941 ng/ml, significantly higher than in remission – 962 ng/ml (P < 0.05) and healthy controls – 872 ng/ml (P < 0.05). In UC, no significant difference was observed between active UC and healthy individuals or those in remission.
(Mori et al., 2021)	OBS CS DS	105 CD 98 UC 92 HC	ELISA	Patients with UC (median 2.565 µg/ml, P < 0.001) and CD (2.565 µg/ml, P < 0.001) had higher SC levels compared to healthy individuals (1.04 µg/ml) and exhibited the highest levels during periods of high disease activity vs remission in CD, but not in UC.
(Azab et al., 2022)	OBS CS DS	20 CD 30 UC 20 HC	ELISA	SC levels correlated with the diagnosis of IBD – AUROC was 0.99, cut-off 48 ng/ml, Sn 98 % and Sp 95 %; and with clinical activity/remission in UC – AUROC was 0.88, cut-off 104 ng/ml, Sn 73 % and Sp 93 %; and in CD – AUROC was 0.97, cut-off 120 ng/ml, Sn 90 % and Sp 90 %.
(Townsend et al., 2019)	OBS DS	109 IBD	ELISA – comparison of two commercially available assays	Patients with clinically active CD had significantly higher average SC levels compared to those in remission in both assays: 5507 vs 3,830 ng/ml, P = 0.0001 (Bühlmann) and 5,131 vs. 2,994 ng/ml, P = 0.0003 (Immunodiagnostics). This was not confirmed in UC.
(Okada et al., 2019)	OBS CS DS	40 CD 61 UC 101 HC	ELISA	Patients with IBD had significantly higher average SC levels compared to non-IBD subjects – UC (1.08×10^3 µg/l) and CD (1.30×10^3 µg/l) versus healthy controls (5.28×10 µg/l). The AUROC was 0.877 with a cut-off of 5.8×10 µg/l, with Sn 79 % and Sp 82 %. In UC, SC correlated with clinical activity of the disease (r = 0.342).

Abbreviations: CR – clinical remission, Dx – diagnosis, ELISA – enzyme-linked immunosorbent assay, HC – healthy controls, LFIA – lateral flow immunoassay, MH – mucosal healing, OBS CS DS – observational cross-sectional diagnostic study with prospective parameter determination, OBS DS – observational prospective diagnostic study, OBS L – observational longitudinal prospective study, PC – plasma calprotectin, ρ – Spearman's correlation coefficient, r – Pearson's correlation coefficient.

cal activity (0.73 vs 0.63; P < 0.001) as well as endoscopic activity (P = 0.01), but significantly lower than the accuracy of FC (P = 0.009; LRG, 0.80; CRP, 0.72; FC, 0.91). In CD, there was no significant difference in accuracy among the biomarkers (clinical activity: LRG, 0.71; CRP, 0.64; FC, 0.66; endoscopic activity: LRG, 0.79; CRP, 0.78; FC, 0.81) (Shimoyama et al., 2023).

Another study evaluated a total of 286 patients and similarly focused on assessing both clinical and endoscopic activity. Median serum LRG levels were higher during active disease than during inactive disease in UC (47.95 vs. 32.27 μg/ml, P < 0.001) and in CD (89.08 vs. 47.95 μg/ml, P = 0.002). LRG levels positively correlated with the clinical activity of the disease and CRP in patients with UC and CD (r = 0.448, P < 0.001; and r = 0.392, P < 0.001) and with endoscopic activity in UC (r = 0.3, P = 0.0276) (Yoshimura et al., 2021).

Kawamoto et al. (2022) evaluated the use of LRG in diagnosing mucosal healing in the small bowel CD. Serum LRG concentrations were significantly higher in patients with mSES-CD ≥ 4 for any segment, a score indicating the presence of significant intestinal ulcers. A strong positive correlation between LRG and mSES-CD was confirmed (r = 0.745; P < 0.001), while CDAI, CRP and albumin showed only a mild correlation with mSES-CD (r = 0.568, 0.557 and –0.455, respectively; P < 0.001 for all). The diagnostic accuracy of LRG in detecting the presence of intestinal ulcers compared to CDAI and CRP was evaluated using the AUROC analysis. The AUROC for LRG was significantly higher than for CDAI (P < 0.05) or CRP (P < 0.05). Using a threshold value of 13.4 mg/ml, derived from AUROC, LRG exhibited significantly higher Sn and positive predictive value vs CRP or CDA.

The diagnostic accuracy of LRG in assessing endoscopic activity in CD, with an emphasis on remission, was also evaluated by another group of Japanese researchers. LRG showed a significant positive correlation with endoscopic activity (r = 0.619, P < 0.001), even in patients with active lesions in the small bowel (r = 0.626, P < 0.001). Multivariate logistic regression demonstrated that LRG was the only factor associated with endoscopic remission. The threshold value of LRG 8.9 μg/ml had Sn of 93.3 %; Sp of 83.3 %; positive predictive value of 96.6 %; negative predictive value of 71.4 %; accuracy of 91.7 % and AUC of 0.904 for predicting endoscopic remission (Kawamura et al., 2023).

The PLANET study aimed to evaluate the usefulness of serum LRG levels in monitoring the disease activity in IBD. Its primary endpoints were changes in adalimumab treatment-related biomarkers and the correlation between biomarker levels and endoscopic activity. LRG mean levels decreased along with improvements in clinical and endoscopic indicators after adalimumab treatment (27.4 μg/ml at week 0, 15.5 μg/ml at week 12, 15.7 μg/ml at week 24 and 14.5 μg/ml at week 52), correlating with endoscopic activity at each time point (SES-CD: r = 0.391 at week 0, r = 0.563 at week 24, r = 0.697 at week 52; MES: r = 0.534 at week 0, r = 0.429 at week 12, r = 0.335 at week 52). Endoscopic activity correlated better with LRG compared to CRP and FC in a pooled analysis at all time points (SES-CD: LRG : r = 0.636, CRP: r = 0.402, FC : r = 0.435; MES: LRG : r = 0.568, CRP: 0.389, FC: r = 0.426). The prospective PLANET study demonstrated the significance of LRG as a biomarker useful in monitoring both clinical and endoscopic activity of CD and UC in response to the treatment with adalimumab (Shinzaki et al., 2021).

Over the past few years, a relatively large number of other serum biomarkers of protein and peptide nature or compounds containing sulphhydryl groups have been studied. Their overview, including LRG, is presented in Table 4.

Serological antibodies and molecular biomarkers – from traditional to novel

Serological testing is a widely recognized diagnostic method for various immune-related diseases. In the context of IBD, its use has primarily focused on patients with an established diagnosis, with limited research exploring its potential as a primary diagnostic tool in those with suspected IBD. Among the traditional antibodies associated with IBD are anti-*Saccharomyces cerevisiae* antibodies (ASCA) and perinuclear antineutrophil cytoplasmic antibodies (pANCA), which were discovered due to the role of antibodies against gut microbiota and host antigens. These antibodies were introduced as potential diagnostic markers of IBD in the 1990s. Both ASCA and pANCA have been reported to provide clinically useful positive and negative predictive values. For example, a $pANCA^+/ASCA^-$ pattern is commonly observed in patients with UC, while a $pANCA^-/ASCA^+$ pattern is characteristic of CD (Alghoul et al., 2022). According to a meta-analysis, the sensitivity and specificity of the $ASCA^+/pANCA^-$ pattern were 0.55 and 0.93, respectively, for diagnosing CD and 0.63 and 0.93, respectively, for distinguishing IBD from non-IBD (Ruemmele et al., 1998). This pattern may thus aid in differentiating CD from UC or non-IBD conditions. However, it is important to note that the Sn of these markers is relatively low, except in paediatric CD, where the Sn and Sp of $ASCA^+/pANCA^-$ were reported as 0.70 and 0.93, respectively (Reese at al., 2006; Sakurai and Saruta, 2023). Given the fact that positive results for these antibodies are not specific to IBD and may be associated with several other gastrointestinal and inflammatory conditions, such as celiac disease, Behcet's disease, cystic fibrosis and rheumatoid arthritis (Sellin and Shah, 2012), their use is currently limited primarily to specific situations and combined serological panels. Given the above, their significance in clinical practice is gradually diminishing in favour of newer, more sensitive and specific biomarkers, among which we include antibodies to the *E. coli* outer-membrane porin C (anti-OmpC), anti-CBir1 flagellin, anti-*Pseudomonas fluorescens* CD-related protein, and mainly non-coding RNAs (such as miRNA, lncRNA).

Table 4. Overview of selected clinical studies with LRG and other new serum biomarkers in the diagnosis and activity assessment of IBD

Biomarker, study	Type	N	Laboratory methods	Results
LRG (Shimoyama et al., 2023)	OBS CS DS	64 CD 203 UC	ELISA	LRG correlated with both clinical and endoscopic activity in CD and UC, it can be an alternative to CRP for the assessment of UC.
LRG (Yoshimura et al., 2021)	OBS CS DS	96 CD 98 UC 92 HC	ELISA	LRG levels were higher during active disease than during inactive disease in CD and UC, correlated with clinical disease activity, CRP in patients with UC and CD, with endoscopic disease activity in UC.
LRG (Kawamoto et al., 2022)	OBS CS DS	108 CD	LAT (POCT)	Strong positive correlation between LRG and intestinal inflammation in small bowel CD, superior diagnostic accuracy vs CRP, CDAI.
LRG (Kawamura et al., 2023)	OBS CS DS	72 CD	LEIT	LRG showed a significant positive correlation with endoscopic activity, even in the small bowel.
LRG (Shinzaki et al., 2021)	OBS DS	34 CD 47 UC	LEIT	LRG correlated with endoscopic activity in CD and UC during the adalimumab treatment.
Serum adropin (Brnić et al., 2020)	OBS CS DS	25 CD 30 UC 50 HC	ELISA	Serum adropin levels were increased in patients with IBD and correlated with clinical activity in CD and UC.
Biomarkers of type VI collagen remodelling: C6Ma3, PRO-C65 (endotrophin) (Lindholm et al., 2021)	OBS CS DS	65 CD 107 UC 38 HC	ELISA	C6Ma3: increased in HC vs IBD, correlated with clinical and endoscopic activity in CD; PRO-C65: correlated with clinical activity in CD.
Biomarkers of elastin degradation: ELM-12, ELP-3 (Pehrsson et al., 2023)	OBS CS DS	104 CD 39 UC 29 HC	ELISA	ELP-3 levels correlated with clinical activity in CD, UC and endoscopic activity in CD. ELM-12: levels correlated with endoscopic activity in CD.
Free thiols (Bourgonje et al., 2019a)	OBS CS DS	31 CD 47 UC 50 HC	SP	Serum free thiol levels were lower in both CD and UC vs HC and correlated with endoscopic activity.
Galectins (galectin-3, galectin-9, galectin-3 binding protein – M2BP) (Cibor et al., 2019)	OBS CS DS	77 CD 48 UC 30 HC	ELISA	Galectin-3, galectin-9 levels did not differ from HC. M2BP levels correlated with clinical activity in UC.
Serum amyloid A (SAA) (Stute et al., 2023)	OBS CS DSr	166 CD 91 UC	NEPH	SAA correlated with clinical activity in CD and UC.
A combined set of four serum biomarkers: SAA, IL-6, IL-8 and eotaxin-1 (Bourgonje et al., 2019b)	OBS CS DS	64 CD 54 UC 20 HC	ECL	The combination of SAA, IL-6, IL-8 and eotaxin correlated with clinical and endoscopic activity in IBD (composite endoscopic score for CD+UC).
Homocysteine (Zhang et al., 2022)	Systematic review and SMD	1267 UC	Enzymatic tests and HPLC	Homocysteine levels were increased in patients with UC.
Serum complement C3 and α2-macroglobulin (Okada et al., 2021)	OBS CS DS	40 CD 61 UC 101 HC	HPLC, SDS-PAGE, ELISA, MS	Serum complement C3 and α2-macroglobulin levels were increased in CD and UC patients vs HC and correlated with clinical activity in UC.
Serum calgranulin C (S100A12) (El Nakeeb et al., 2023)	OBS CS DS	20 CD 50 UC 73 HC	ELISA	Serum calgranulin levels were increased in CD and UC patients vs HC and correlated with clinical activity in CD and endoscopic activity in UC.
Serum cholinesterases (Shao et al., 2020)	OBS CS DSr	60 CD 142 UC 264 HC	SP	Serum cholinesterase levels were decreased in CD and UC patients vs HC and correlated with clinical activity in both diseases.
Adipokines (leptin, ghrelin, resistin and adiponectin) (Ortega Moreno et al., 2020)	OBS CS DS	40 CD 36 HC	FCM	Resistin levels correlated with endoscopic activity in CD.
Serum nesfatin 1 (Beyaz and Akbal, 2022)	OBS CS DS	17 CD 18 UC 17 HC	ELISA	Serum nesfatin 1 levels were increased in CD and UC patients vs HC and correlated with clinical activity in both diseases.

Abbreviations: Dx – diagnosis, ECL – electrochemiluminescence, ELISA – enzyme-linked immunosorbent assay, FCM – flow cytometry, HC – healthy controls, HPLS – high-performance liquid chromatography, LAT – latex agglutination test, LEIT – latex-enhanced immunoturbidimetry, LRG – leucine-rich alpha-2 glycoprotein, MS – mass spectrometry, NEPH – nephelometry, OBS CS DS – observational cross-sectional diagnostic study with prospective parameter determination, OBS CS DSr – observational retrospective cross-sectional diagnostic study, OBS DS – observational prospective diagnostic study, SDS-PAGE – sodium dodecyl sulphate polyacrylamide gel electrophoresis, SMD – standard mean difference pooled through random effect model, SP – spectrophotometry.

Anti-outer membrane porin C antibody (Anti-OmpC) is an antibody targeting porin C, a transport protein located on the outer membrane of *E. coli*. IgA antibodies against this antigen have been predominantly detected in the sera of patients with CD, with a prevalence of 55 %. In contrast, the prevalence is significantly lower in patients with UC, non-IBD colitis and healthy controls. This indicates that anti-OmpC antibodies may serve as a useful marker to differentiate CD from these other conditions. Additionally, the presence of anti-OmpC antibodies has been linked to an increased risk of developing internally perforating CD, often necessitating surgical intervention (Chen et al., 2020; Diez-Martin et al., 2024).

Anti-*Pseudomonas fluorescens*-associated sequence I2 (anti-I2) antibodies are immune proteins that target a specific antigen, known as I2, derived from the bacterium *Pseudomonas fluorescens*. This antigen was initially identified within mononuclear cells in the intestinal mucosa of patients with CD. The presence of anti-I2 antibodies has been observed in approximately 55 % of CD patients, compared to 10 % in UC patients and 20 % in individuals with non-IBD colitis (Mitsuyama at al., 2016). Anti-I2 antibodies have been observed to be related to the stricturing behaviour, longer disease duration and early postoperative recurrence in patients with CD (Chen et al., 2020).

Anti-CBir1 flagellin (Anti-CBir1) is an antibody directed against the CBir1 flagellin, a protein forming the bacterial flagellum, which has been identified as a dominant antigen capable of inducing colitis in mice and eliciting an immune response in a subset of patients with CD. The presence of anti-CBir1 antibodies has been observed in approximately 50 % of CD patients, with their occurrence being associated with complicated forms of the disease, such as fibrostenotic and penetrating types, and early postoperative recurrence in patients with CD (Targan et al., 2005; Alexander et al., 2021).

Recent advancements in the study of non-coding RNAs (ncRNAs), particularly long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), have unveiled their significant roles in the pathogenesis, diagnosis and potential treatment of IBD. These ncRNAs are involved in regulating gene expression and immune responses, making them promising biomarkers and therapeutic targets in IBD management (Chen et al., 2020).

Emerging evidence suggests that specific lncRNAs exhibit high diagnostic accuracy in distinguishing IBD patients from healthy individuals. For instance, lncRNA H19 has shown potential as a diagnostic biomarker for both UC and CD. A recent study investigated the serum levels of lncRNA H19 and miRNA-675-5p in IBD patients, revealing significant differences compared to healthy controls, thereby highlighting their diagnostic utility (Shaker et al., 2023).

LncRNAs are implicated in various aspects of IBD pathogenesis, including regulation of the intestinal epithelial barrier, modulation of inflammatory responses and maintenance of immune homeostasis. A review by Jiang et al. (2023) consolidates existing knowledge on the mechanisms and expression patterns of lncRNAs involved in IBD initiation and progression, emphasizing their roles as immune regulators.

The regulatory effects of ncRNAs on the NLRP3 inflammasome – a critical component in the inflammatory response – suggest their potential as therapeutic targets. Research indicates that specific modulating lncRNAs can influence NLRP3 inflammasome activation, offering new avenues for IBD treatment (Abed et al., 2024).

The investigation of serum and plasma ncRNAs in IBD is a burgeoning and rapidly evolving field, offering significant promise for improving diagnostic precision and developing targeted therapies. While initial studies provide promising insights, ongoing research is essential to fully elucidate their clinical applications. Combining ncRNAs with other biomarkers could further enhance the disease evaluation and patient stratification in IBD (Chen et al., 2020).

Discussion

Our review summarizes a relatively large number of published studies focusing on the topic of potential new serum biomarkers in the clinical management of IBD, highlighting the growing importance and interest in this area. The studies vary in quality, with the majority being observational cross-sectional diagnostic studies with prospective parameter determination. The results, to varying extents within individual studies for each biomarker, confirmed their potential to distinguish IBD patients from healthy individuals and correlate with clinical and endoscopic activity, as well as other inflammation biomarkers, primarily FC and CRP. Attention is particularly focused on various serum biomarkers of protein and peptide nature or compounds containing sulphhydryl groups. In terms of both quantity and quality of data, SC and LRG appear, in our opinion, to be the most promising. However, the role of these biomarkers in the clinical management of IBD has not yet been sufficiently established.

In our review, we cover 13 studies of SC, several of which confirmed a relatively good correlation of this biomarker's levels with clinical or even endoscopic activity. However, these data also present some controversy, as several studies did not demonstrate a correlation with activity in UC (Townsend et al., 2019; Mori et al., 2021; Udegbune et al., 2022; Yasuda et al., 2023) or in CD (Ferrer et al., 2019; Chen et al., 2021). Multiple studies showed a significant correlation of SC with CRP (Carlsen et al., 2019; Malham et al., 2019; Mori et al., 2021), while other studies did not show a significant correlation between SC and FC (Meuwis et al., 2013; Fukunaga et al., 2018; Bernardo et al., 2019; Carlsen et al., 2019; Mori et al., 2021).

Considering these findings, this could suggests that SC is more of a biomarker reflecting systemic inflammation rather than intestinal inflammation, unlike FC. This assumption is further supported by the fact that SC is released from neutrophils, monocytes and macrophages as

a result of stimulation by lipopolysaccharides or various cytokines such as IL-1β and TNF-α (Mori et al., 2021).

It is known that 25–40 % of patients with IBD develop extraintestinal manifestations, including musculoskeletal, ocular, hepatopancreatobiliary, renal, dermatological complications, and others (Levine and Burakoff, 2011). Given that elevated levels of SC have also been detected in several autoimmune diseases (Jukic et al., 2021), this provides a basis for the assumption that SC could also be useful in monitoring the activity of extraintestinal manifestations in IBD.

From our perspective, SC remains one of the most promising biomarkers for potential future clinical adoption. An interesting study in terms of results and the laboratory method used is the report by Bernardo et al. (2019), who assessed SC through the currently desirable diagnostic concept – POCT. The Bühlmann Quantum Blue® (Bühlmann Laboratories AG, Schönenbuch, Switzerland) rapid test based on the LFIA principle was used, along with the 2nd generation Quantum Blue® Reader, which provided quantification of results. This prospective monocentric study demonstrated that SC is a predictive biomarker for clinical remission and mucosal healing in IBD patients. SC was not inferior in comparison to other biomarkers – CRP and FC – in terms of predicting clinical remission and mucosal healing. The study used LFIA, a method that, thanks to the use of portable reading devices and continuous improvements in the characteristics of used labels, achieves Sn comparable with ELISA (Liu et al., 2021).

In a detailed method comparison, the Bühlmann Quantum Blue® SC test, used in the study, demonstrated strong agreement with the Bühlmann SC ELISA. Analysing 29 samples within the specified measuring range of the Quantum Blue® sCAL assay, each sample was tested ten times according to the assay procedure. These results were then compared with 1–4 duplicate values obtained using the Bühlmann SC ELISA. The correlation coefficient (R^2) between the two methods was 0.94, indicating a high degree of consistency. The regression equation derived from this comparison was $y = 0.93 \times + 0.51$ µg/ml, further underscoring the strong linear relationship between the assay results obtained by the Quantum Blue® system and those derived from the ELISA method (The Bühlmann Quantum Blue® SCAL Quantitative Lateral Flow Assay 2016). Furthermore, the Bühlmann Quantum Blue® SC test method has been validated in clinical studies, demonstrating reliability and accuracy compared to the reference ELISA (Ryter et al., 2017; Park et al., 2022), while the reference method itself – Bühlmann SC ELISA – has been validated multiple times (Andrés Cerezo et al., 2011; García-Arias et al., 2013; Nilsen et al., 2015; Hurnakova et al., 2019).

On the other hand, one of the disadvantages of SC is that it has not been sufficiently effective in predicting relapse (Veyrard et al., 2022). One way to improve the diagnostic accuracy of SC is by determining it from the plasma. Malham et al. (2019) found that PC and SC significantly correlated with clinical, endoscopic and histological activity in UC, with PC showing a stronger correlation.

Another very interesting biomarker in IBD, as discussed in our review, is LRG, which is illustrated by a series of five recent studies by Japanese research teams (Shinzaki et al., 2021; Yoshimura et al., 2021; Kawamoto et al., 2022; Kawamura et al., 2023; Shimoyama et al., 2023), all of which also assessed endoscopic activity. There was a significant correlation between LRG levels and clinical and endoscopic activity in CD and UC, with the diagnostic accuracy of LRG in UC being significantly higher than CRP for assessing the endoscopic activity (Shimoyama et al., 2023). LRG also confirmed its diagnostic accuracy in detecting intestinal ulcers compared to CRP, and thus may provide better information about inflammation in UC than CRP. Other studies evaluated the performance of LRG in CD and found that LRG was quite accurately able to inform about intestinal inflammation even in the small bowel (Kawamoto et al., 2022; Kawamura et al., 2023), with diagnostic accuracy being higher compared to CRP (Kawamoto et al., 2022). The need for frequent small bowel examinations in CD can be problematic; therefore, LRG provides a perspective of a new biomarker for assessing CD activity, especially in small bowel localizations. The PLANET study highlighted LRG's effectiveness in monitoring responses to adalimumab treatment in IBD patients, with levels decreasing as patients responded to therapy, which aligns with endoscopic improvements (Shinzaki et al., 2021). This study pointed out the potential of LRG in T2T strategy similar to what was seen with FC and CRP in the CALM study (Colombel et al., 2017). The correlation coefficient with FC in the PLANET study was 0.636, yet overall, the evidence comparing LRG and FC is insufficient. Another study demonstrated that LRG can predict mucosal healing in patients with CD and UC with normal CRP levels (Yasutomi et al., 2021). The strong correlation of LRG with disease activity indicators across multiple studies suggests that it could serve as a reliable biomarker for assessing the disease activity and potentially guiding the treatment decisions.

Another possible approach in an effort to improve diagnostic accuracy is to simultaneously determine multiple biomarkers. The determination of a combination of four serum biomarkers, SAA, IL-6, IL-8 and eotaxin-1, predicted better endoscopic activity in IBD than CRP (Bourgonje et al., 2019b). The results presented from these studies are significant despite the fact that none of the biomarkers studied have yet reached the acceptance and status of FC in the diagnostic process and clinical management of IBD, which continues to be the most important biomarker, supported by recommendations (Maaser et al., 2019). The main limitations of the studies presented include their monocentric nature, a low number of patients, the absence of longitudinal monitoring and in some cases, the lack of assessment of endoscopic activity.

As for the laboratory methods used, ELISA remains predominant, but some studies with SC and LRG men-

tioned in our review were conducted using POCT (LFIA, LAT). A significant challenge associated with POCT systems in general could be to achieve analytical validation standards comparable to those in clinical laboratories. Analytical requirements should be the same as those demanded in clinical laboratories (Lukáš, 2021). ELISA methods have been widely validated, even though the protocol is quite time-consuming and the results are reported later than in POCT methods.

In POCT, while also validated, the extent and rigor can vary depending on the specific test. POCT devices are generally designed for ease of use and rapid results, often at the cost of some accuracy and Sn. Standardization can be challenging, as the simpler user interface and design can lead to greater variability between different users and environments compared to more controlled laboratory settings. POCT is optimized for speed and convenience, which can sometimes affect the limit of detection and overall precision. However, technological advancements are rapidly improving the capabilities of POCT devices, especially in Sn (Liu et al., 2021). FC, which is an established biomarker of IBD activity, already has a fairly extensive evidence record even within POCT measurements in the clinical setting (Coorevits et al., 2013; Hejl et al., 2018; Malíčková et al., 2019; Jere et al., 2021).

Recent years have been marked by intense efforts to accumulate evidence in search of the ideal biomarker for diagnosing and monitoring IBD. An ideal biomarker should be non-invasive, affordable, standardized, with rapid availability of results and high diagnostic accuracy. Current research focuses on biomarkers of various types, with serum biomarkers being a significant area of interest. Among the large number of serum biomarkers studied, LRG and SC can be considered promising for further research and potentially later implementation in practice.

Considering the evidence accumulated so far, it may seem that FC is more directly linked to intestinal inflammation, whereas SC may be more useful in assessing systemic inflammation. Its role might also be as a complementary biomarker and in assessing the activity of extraintestinal manifestations in patients with IBD. As for LRG, which has a relatively impressive evidence record and is used in clinical practice in Japan, further accumulation of evidence within the European population is particularly needed. Further research is essential for establishing regular clinical application of these mentioned biomarkers, defining standardized diagnostic thresholds and validating their use for prognosis and diagnosis, potentially replacing the widely researched FC.

Another scenario in the search for the ideal biomarker could be to demonstrate the superiority of combining multiple biomarkers. In terms of laboratory methods, there is scope for more intensive research and further validation of tests used in POCT due to their speed, economic affordability, simplicity and mobility of the reading device. The advantage of the POCT approach for patients is clear and lies in the ability to make immediate treatment decisions, thereby achieving better clinical outcomes, higher patient safety and overall satisfaction. In this regard, the continuous improvement of the tests used in POCT in terms of Sn, quantification and ability to perform multiplex detection of multiple biomarkers simultaneously within a single test is also important. A fundamental requirement for identifying the ideal biomarker and its specific application in IBD management will be to conduct further validation multicentric studies involving a higher number of patients with a longitudinal design, with assessment of endoscopic activity and proactive monitoring of the biomarkers.

Conflict of interest

The authors declare no conflicts of interest.

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