Biomarkers in Irritable Bowel Syndrome: Biological Rationale and Diagnostic Value

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Abstract

Background: Patients with irritable bowel syndrome (IBS) usually suffer from nonspecific and overlapping signs that hamper the diagnostic process. In line with this, biomarkers specific for IBS could be of great benefit for diagnosing and managing patients. In IBS, the need is for apparent distinguishing features linked to the disease that improve diagnosis, differentiate from other organic diseases, and discriminate between IBS subtypes. **Summary:** Some biomarkers are associated with a possible pathophysiologic mechanism of IBS; others are used for differentiating IBS from non-IBS patients. Implementation of IBS biomarkers in everyday clinical practice is critical for early diagnosis and treatment. However, our knowledge about their efficient use is still scarce. **Key Messages:** This review discusses the biomarkers implemented for IBS diagnosis and management, such as blood (serum), fecal, immunological, related to the microbiome, microRNAs, and some promising novel biomarkers associ-

ated with imaging and psychological features of the disease. We focus on the most commonly studied and validated biomarkers and their biological rationale, diagnostic, and clinical value.

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Introduction

A wide variety of symptoms and manifestations make the definite diagnosis of irritable bowel syndrome (IBS) a continued challenge. The differential diagnosis is so extensive that it may take years to establish the correct diagnosis. Clinicians should exclude inflammatory bowel disease (IBD), celiac disease, microscopic colitis, lactose intolerance, fructose intolerance, colon cancer, gastrointestinal (GI) infections, and transthyretin amyloidosis [1] and the symptoms of hyperparathyroidism, hypothyroidism, and neuroendocrine tumors [2].

Currently, the syndrome can only be diagnosed based on accurate identification of the main clinical symptoms using modified criteria from Rome IV, 2016 (Fig. 1) [3]. According to Rome IV criteria, IBS is defined as a functional gastrointestinal disorder in which recurrent ab-

Fig. 1. Rome IV criteria for diagnosis of IBS in both adults and children (adapted from Drossman [3]). IBS, irritable bowel syndrome.

dominal pain is correlated with a change in bowel habits or defecation. Symptoms should be present during the last 3 months, and the symptom onset should have started at least 6 months before diagnosis. Abdominal bloating/distension and disordered bowel habits (i.e., constipation, diarrhea, or a mix of constipation and diarrhea) are some of the commonly observed symptoms [4]. Making an early diagnosis based on a clinical evaluation of symptoms while limiting the use of investigations is crucial for the best IBS management.

However, exhaustive investigation to exclude all organic pathology is still made in many IBS patients. Some IBS patients suffer from nonspecific and overlapping signs that cannot be associated with a specific diagnosis. In line with this, biomarkers specific for IBS could be of great benefit in the clinician's armamentarium in diagnosing IBS.

Typically, a biomarker can be simply described as a defining characteristic, measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention [5]. Biomarkers can be derived from molecular, histologic, radiographic, or physiologic characteristics of the disease. Moreover, several subtypes of biomarkers have been defined according to their putative applications.

Notably, a single biomarker may meet multiple criteria for different uses. Still, it is essential to develop evidence for each definition. In the case of IBS, while definitions for biomarkers may overlap, they also have apparent dis-

tinguishing features that specify particular uses. For example, diagnostic biomarkers, monitoring biomarkers, pharmacodynamic/response biomarkers, predictive biomarkers, prognostic biomarkers, and others can be implemented for IBS [6].

Indeed, there are several types of biomarkers for IBS aiming to improve diagnosis, to differentiate from other organic diseases and to discriminate between IBS subtypes. Some biomarkers are associated with a possible pathophysiologic mechanism of IBS, and others are used for differentiating IBS from non-IBS patients [7].

Nevertheless, an ideal biomarker should fulfill the following criteria: high sensitivity and specificity, simple to use, reproducibility, low interobserver variability, and affordable and acceptable for the patient [8]. Implementation of IBS biomarkers in everyday clinical practice is critical for early diagnosis and treatment of this functional gastrointestinal disorder. However, our knowledge about their efficient use is still scarce.

In this review, we make an overview of the biomarkers discussed for IBS diagnosis and management, such as blood (serum), fecal, immunological, microbiome, microRNAs, and some promising novel biomarkers associated with imaging and psychological features of the disease. We focus on the most common, studied, and validated biomarkers and their biological rationale, diagnostic, and clinical value (Table 1).

Blood Biomarkers for IBS Management

Serum-Based Panels of Biomarkers for IBS

Some blood-based biomarkers can provide valuable information for patients who have IBS. Although blood work can offer diagnostic clues, there are no molecules present in the blood currently recognized unique to IBS. Furthermore, although the diagnosis of IBS depends on the clinical symptoms, serological markers for diagnosis can also be utilized.

For this reason, Lembo et al. [9] conducted a study to investigate a combination of 10 blood biomarkers. These 10 biomarkers were interleukin-1 β (IL-1 β), growth-related oncogene-a, brain-derived neurotrophic factor (BDNF), anti-*Saccharomyces cerevisiae* antibody (ASCA IgA), antibody against CBir1, anti-tissue transglutaminase (tTG), tumor necrosis factor (TNF)-like weak inducer of apoptosis, anti-neutrophil cytoplasmic antibody, tissue inhibitor of metalloproteinase-1 (TIMP-1), and neutrophil gelatinase-associated lipocalin (NGAL). The authors demonstrated that this panel of biomarkers had a

Table 1. Biomarkers for IBS diagnosis and management

Type	Biomarkers	Control group	Sensitivity, %	Specificity, %	PLR	NLR	AUC
Blood biomarkers	A panel of 10 biomarkers (IL-1 β , GRO- α , BDNF, ASCA IgA, anti-CBir1, tTG, TWEAK, ANCA, TIMP-1, and NGAL) [9]	Non-IBS	50.0	88.0	4.17	0.57	0.76
	A panel of 34 serologic (including histamine, tryptase, serotonin, and substance P), gene expression markers, and psychological measurements [14]	HC	81.0	64.0	2.25	0.30	0.81
	Proteases (cathepsin L, elastase 3a, and proteasome alpha subunit-4) [21]	HC	>75.0	>85.0	5.00	0.29	0.98
	Anti-CdtB antibodies [22]	Non-IBS	43.7	91.6	5.20	0.61	0.81
	Anti-vinculin antibodies [22]	Non-IBS	32.6	83.8	2.01	0.80	0.62
Immune activation biomarkers	Inflammatory cytokines – IL-6 and its receptor, IL-8, and IL-1 β , TNF- α	n/a					
Fecal biomarkers	Calprotectin [34]	IBD	93.0	94.0	15.50	0.07	nr
	Fecal SCFA [38]	HC	92.0	72.0	3.29	0.11	0.89
	Secretogranin II [39]	HC	80.0	79.0	3.81	0.25	0.86
	A panel of 8 biomarkers (IL-1 β , IL-6, IL-12p70, TNF- α , CgA, HBD2, caproate, and calprotectin) [35]	HC	88.1	86.5	6.53	0.14	0.89
	Volatile organic metabolites [42]	HC	90.0	80.0	4.50	0.13	0.94
Microbiome	No specific microbial biomarker	n/a					
MicroRNA biomarkers	miRNA-199, miRNA-150, and miRNA-342-3p	n/a					

IBS, irritable bowel syndrome; ANCA, anti-neutrophil cytoplasmic antibody; anti-CBir1, antibody against CBir1; anti-CdtB, anti-cytolethal distending toxin B; ASCA, anti-*Saccharomyces cerevisiae* antibody; BDNF, brain-derived neurotrophic factor; CgA, chromogranin A; GRO- α , growth-related oncogene- α ; HBD2, human beta-defensin-2; IBD, inflammatory bowel disease; IL, interleukin; n/a, not applicable; NGAL, neutrophil gelatinase-associated lipocalin; NLR, negative likelihood ratio; PLR, positive likelihood ratio; SCFA, short-chain fatty acids; TIMP-1, tissue inhibitor of metalloproteinase-1; TNF- α , tumor necrosis factor alpha; tTG, anti-tissue transglutaminase; TWEAK, tumor necrosis factor-like weak inducer of apoptosis.

positive predictive value of 81%, a negative predictive value of 64%, and an overall accuracy of 70%. Moreover, the investigators concluded that the proposed diagnostic panel of 10 biomarkers could differentiate between IBS and non-IBS GI disorders.

The cytokine IL-1 β was chosen because of its central role in inflammatory diseases such as IBD. It is well known that glucocorticoids released during stress have a significant downregulatory effect on IL-1 β [10]. However, the role of IL-1 β in IBS remains uncertain, and further studies are required to investigate this potential biomarker and validate it.

Another immune factor under investigation is the growth-related oncogene. It is thought that this might be responsible for the tissue injury in IBS patients [9]. This chemokine is associated with chemotactic migration and activation of neutrophils.

BDNF is another potential biomarker under investigation; it is a member of the neurotrophin family and is thought to play a crucial role in many chronic pain conditions. It has been previously noted that patients with IBS have significant increases in total nerve fibers and damage on the mucosal nerve fibers (e.g., swollen mitochondria and nerve axons) with increased BDNF, and this correlated with the abdominal pain scores [11, 12]. This, therefore, shows potential as a biomarker for IBS patients subtype with abdominal pain.

The last biomarker in the panel was NGAL, a 25-kDa protein. NGAL participates in various roles in viscera, including molecular transportation and GI mucosal regeneration [13], with a possible role in IBS-related disruptions in the barrier function.

Another study involving 168 IBS subjects (60 IBS-C, 57 IBS-D, and 51 IBS-M) and 76 healthy volunteers aimed

to differentiate the performance of a combination of 34 serologic and gene expression markers and psychological measurements. A total of 10 serological markers were added to the original 10-biomarker panel, including histamine, trypsin, serotonin, and substance P, together with 14 gene expression markers from analysis of differentially expressed genes in IBS and healthy people, including CBFA2T2, CCDC147, and ZNF326 [14].

Results show that the panel registered a sensitivity of 81% and a specificity of 64%, together with good discrimination between IBS subtypes, the best being for the subtype IBS-C versus IBS-D. However, one of the study's limitations is that healthy volunteers were characterized as adults without any illness, active infection, or significant medical condition. Still, there was no reference to functional symptoms. Another drawback to the study is that no comparison with other organic diseases was provided. According to the investigators, their biomarker panel would best discriminate IBS from organic GI disorders [14].

Differentiating IBS from IBD

Notably, there are well-established serological markers that help differentiate IBS from IBD. These include ASCA, which facilitates the differential diagnosis of Crohn's disease (CD) and ulcerative colitis (UC), predominantly in the disease's early stages. The serum concentration of ASCA is considerably higher in patients with CD than in those with UC [15]. Thus, ASCA can be employed in differentiating organic disease from IBS. Furthermore, a study examining the levels of ASCA in CD and murine colitis concludes that the propensity to produce ASCA in a subgroup of CD patients is mostly genetically predetermined, as evidenced by their stability and lack of correlation with clinical disease activity parameters.

The other autoantibodies that can be used in distinguishing IBS from IBD are the anti-neutrophil cytoplasmic antibody. They target antigens present in neutrophils and are positive in 50–80% of the UC patients [16].

Tissue inhibitors of metalloproteinases (TIMPs), on the other hand, are endogenous protein regulators of the matrix metalloproteinase family. Altered TIMP activity can lead to disruption of the intestinal barrier and excessive immune response [17]. TIMPs have been shown to play a role in tissue degradation and remodeling. Indeed, in patients with IBD, tissue remodeling is observed due to chronic inflammation. Serum levels of TIMP-1 and TIMP-4 have been known to be related to IBD [18], but their role in patients with IBS has not yet been studied well.

A study published in 2018 followed the effects of biopsy supernatants on human and guinea pig submucous neurons with neuroimaging techniques, utilizing 7 healthy controls, 20 IBS, and 12 UC patients. The researchers differentiated by proteome analysis several expressed proteins such as proteases that were cleaving proteinase-activated receptors (PARs). PARs are proteins that are involved in protein degradation and the regulation of cell functions. The subtype PAR2 receptor is highly expressed in colonic epithelial cells and is involved in secretion, visceral sensation, inflammation, and motility [19, 20]. It is well known that the GI tract is exposed to proteases produced by the stomach, small intestine, inflammatory cells (mast cells), and bacteria.

A total of 204 proteins have been identified after a proteome analysis of the supernatants. Out of all investigated proteins, 17 proteases were differently expressed between IBS, UC, and healthy controls. Of these, significantly more abundant in IBS than healthy and UC supernatants were chymotrypsin C, an unspecified isoform of complement C3, proteasome subunit type beta-2, and proteases elastase 3a. The results indicated that the UC supernatants activated enteric neurons through protease-dependent mechanisms but without PAR1 involvement. Moreover, PAR1 antagonist SCHE79797 prevents nerve activation by IBS supernatants. The combination of cathepsin L, elastase 3a, and proteasome alpha subunit-4 reveals 98% accuracy in differentiating between IBS and healthy people. Thus, the authors conclude that proteases signaling through neuronal PAR1 may be used as IBS biomarkers [21].

In a recent study that included 2,681 subjects (2,375 IBS-D patients, 43 healthy subjects, 121 celiac patients, and 142 IBD patients), the researchers assessed the sensitivity of anti-cytolethal distending toxin B (anti-CdtB) antibodies and anti-vinculin antibodies. The results indicated that anti-CdtB demonstrated specificity and sensitivity 91.6 and 43.7% and for anti-vinculin were 83.8 and 32.6%, respectively, concerning IBS-D. These results confirm that anti-CdtB and anti-vinculin antibodies are elevated in IBS-D compared to non-IBS subjects [22].

Differentiating Celiac Disease from IBS

Anti-tTG is the gold standard in the serologic diagnosis of celiac disease [19]. However, a study published in 2019, involving 44 women and 26 men with IBS, showed that 7.1% of participants were positive for IgA and IgG anti-tTG (3 of them had diarrhea-predominant IBS [IBS-D] and 2 constipation-predominant IBS [IBS-C]). Patients diagnosed with mixed bowel habits (IBS-M) did

not give a positive result. Moreover, in patients with IBS-D, the possibility of celiac disease should be ruled out [23].

Biomarkers for IBS Related to Immune Activation

Immune activation is a known phenomenon in some patients with IBS. IBS has long been viewed as a neurological condition resulting from modifications in the brain-gut axis. However, immunological modifications are frequently reported in IBS patients, compatible with the hypothesis that there is a chronic low-grade immune activation. The mediators released by immune cells act to either depress or increase the activity of GI nerves. The release of a number of these mediators corresponds to symptoms of IBS, highlighting the significance of immune and nervous system connections [24, 25].

Critical players in gut reactions are eosinophils and mast cells. Eosinophils usually are related to allergic responses; however, no changes in numbers of eosinophils in intestinal biopsies, blood, or the in situ levels of eosinophil cationic protein are seen in IBS [26]. In line with this, several studies have linked eosinophil numbers with functional dyspepsia, a functional GI disorder associated with upper abdominal discomfort and disordered motility [27, 28]. However, little is known about the effects eosinophil-derived mediators have on GI nerves. Mast cells and their mediators from colonic mucosal biopsies may also activate sensory afferent neurons.

Several inflammatory cytokines are elevated in serum and secreted by peripheral blood mononuclear cells in IBS patients, including IL-6 and its receptor, IL-8, and IL-1 β , compared to healthy controls; however, this is not observed in all IBS patients. There are conflicting results for TNF- α , with reports of increased and decreased levels documented in IBS [29, 30].

Interestingly, there was a correlation between cytokines and symptoms; thus, lipopolysaccharide-induced TNF- α production in peripheral blood mononuclear cells was associated significantly with anxiety ($r = 0.59$, $p < 0.001$) [29]. However, a subgroup of IBS patients with elevated serum cytokine levels (IL-6, IL-8, and TNF- α) and decreased mucosal expression of IL-10 was generally associated with protection from immune activation. Those IBS patients with immune activation had a shorter transit time compared to those without immune activation. The TNF- α level was positively correlated with looser stool consistency and increased rectal sensitivity. At the same time, those with more frequent bowel movements had higher IL-6 levels [31].

At the same time, an increase in activated B lymphocytes and plasma cells and increased IgG levels in the jejunal fluid in IBS-D patients compared to healthy controls had also been shown. The immune activation is usually paralleled by mucosal mRNA upregulation of heavy immunoglobulin chains, acting as biological markers of humoral activity. The latter correlated with the consistency and number of stools per day in these patients [32].

Despite many decades of promising evidence implicating the immune system's role in IBS pathogenesis, its degree of association with clinical symptoms remains controversial. Many questions remain. Foremost is causation; what drives the immune activation – is it centrally or peripherally mediated, or a combination of both? IBS patients frequently copresent with extraintestinal and psychological comorbidities, including anxiety and depression, which correlate with altered immune function, and emotional stimuli such as chronic stress are known to alter immune function [2]. There is a need for further investigation of the immune mechanisms that might be involved in IBS development.

Fecal Biomarkers in Diagnosis and Management of IBS

Fecal biomarkers have become among the most significant biomarkers of GI diseases that allow noninvasive testing. In general, they have been developed to reflect inflammation of the intestinal mucosa. In line with this, their primary role is to identify IBD and, therefore, to exclude IBS.

Fecal Calprotectin

Fecal calprotectin (FC) is a small calcium- and zinc-binding protein that is found in abundance in neutrophilic granulocytes as well as in monocytes and macrophages [33]. FC is the most frequently studied marker for intestinal inflammation.

A study by Waugh et al. [25, 34] demonstrated that FC had a sensitivity of 93% and a specificity of 94% at a cutoff level of 50 $\mu\text{g/g}$ in differentiating IBS from IBD. In most cases, negative calprotectin rules out IBD, thereby sparing most people with IBS from having to have invasive investigations, such as colonoscopy.

A recent study with 196 IBS patients and 160 healthy controls without GI symptoms demonstrated that a panel of 8 biomarkers (IL-1 β , IL-6, IL-12p70, TNF- α , fecal chromogranin A [CgA], human beta-defensin-2, caproate, and FC) had a sensitivity of 88.1% and a specificity of 86.5% in discriminating IBS subjects from healthy people. These popu-

lations were obtained from the Maastricht IBS cohort. However, validation of this biomarker panel for the discrimination between organic GI disorders was not performed [35].

Fecal Short-Chain Fatty Acids

Fecal short-chain fatty acids (SCFAs) are derived from nondigestible carbohydrates through gut microbial fermentation [36]. They include acetate, propionate, butyrate, valerate, and caproate. Products of microbial fermentation of nondigested oligosaccharides in the colon are selected as gut intraluminal metabolic activity indicators. Since SCFAs have been associated with multiple pathological and physiological mechanisms in humans, for example, modulation of inflammation, satiety, and carcinogenesis, and are an essential energy source for colonocytes [37], they are promising biomarkers for different conditions.

To date, SCFAs have only been studied in small population (25 IBS subjects and 25 healthy controls) aimed to diagnose IBS by measuring fecal SCFA. This study noted that differences in the values of butyric and propionic acid had the best diagnostic properties, with a sensitivity of 92% and a specificity of 72% at a cutoff level >0.015 mmol/L [38].

Granins, Chromogranins, and Secretogranins

Granins are proteins distributed ubiquitously in vesicles of secretory cells of the enteric, endocrine, and immune system. It is thought that they may serve as markers for the activity of the enteric neuroendocrine system [39]. CgA was selected as a marker of intestinal neuroendocrine cell activity. This peptide is produced by enterochromaffin cells and is colocalized in storage granules with serotonin [39, 40]. Secretogranin (Sg)III, on the other hand, is found in mast cells. Öhman et al. [39] reported that CgA, SgII, and SgIII were elevated in feces samples of 82 IBS patients compared to 29 healthy volunteers. Furthermore, it was demonstrated that an SgII value of 0.16 nmol/g identified IBS with a sensitivity of 80%, a specificity of 79%, and an AUC of 0.86 on the receiver operating curve.

To date, the role of granins in the pathophysiology of IBS is not completely clear, and the reason why levels of granins are different in IBS subjects has not been understood [7, 41]. In contrast to the fecal measurements, studies of duodenal, ileal, and colonic mucosa showed reduced densities of CgA immunoreactive cells in IBS compared to healthy controls [41].

Volatile Organic Metabolites

VOCs are low-molecular-weight metabolic compounds with high vapor pressures and low boiling points, promoting evaporation at ambient temperatures. There is rising evidence that particular VOCs are unique to various disorders. In a study that explored VOM, they recruited 30 patients with IBS-D, 62 with CD, 48 with UC, and 109 healthy controls. In this study, they found 240 VOMs of interest. Univariate analysis showed that esters of SCFA, cyclohexane carboxylic acid, and its ester derivatives were associated with IBS-D, while aldehydes were significantly more abundant in IBD. With 11 key VOMs, the discriminatory model discriminated IBS-D from patients with active CD and UC with a sensitivity of 94 and 96%, respectively, and a specificity of 82 and 80%, respectively. Furthermore, IBS-D could be significantly distinguished from the healthy controls (90% sensitivity and 80% specificity) [42].

Microbiome Studies in IBS Patients

The GI tract contains a dense society of commensal bacteria. The relationship between the host and the microbe is immunologically complex, as commensals comprise a balance between beneficial and harmful strains in health. Any disruptions in this homeostasis are associated with disease states [43, 44]. The gut microbiota is most abundant in the colon, with a switch in strain predominance from Gram-positive aerobes to Gram-negative anaerobes. Therefore, the colon is the primary site of fermentation and subsequent production of organic acids and gases [43].

The microbiome has been studied in IBS with some consistent findings; among these is the demonstration of high Firmicutes and decreased *Bacteroides* in IBS patients compared to healthy volunteers [43–45]. However, there have been conflicting results with high Actinobacteria in IBS in one study and low *Bifidobacterium* (Actinobacteria) in other studies [43–45].

Such variable findings may conceivably be attributable to dietary differences in the studied patients. In a recent study, IBS patients (predominantly IBS-D and IBS-M, rather than IBS-C) had a greater *Bacteroides* enterocyte community than did healthy controls who had more *Prevotella* species, and the IBS symptom severity score (IBS-SSS) increased as the prevalence of *Prevotella* species decreased [46].

It has been proposed that these microbial alterations cause symptoms in IBS by alteration in cytokine levels.

Firmicutes and other bacteria in the microbiome produce flagellin, which is postulated to cause an inflammatory response in IBS. A significantly higher serum value of lipopolysaccharide in patients with IBS-D than controls contrasts with the higher level of antibodies to flagellin in patients with IBS (mainly driven by higher levels in IBS-D) [47].

Recently, mucosal *Brachyspira* colonization was described to be significantly more common in IBS-D than in healthy controls, which could be potentially used as a marker for differentiating IBS-D [48]. In summary, there is, at present, no specific microbial biomarker for IBS, and, therefore, there is a limited clinical utility for the use of stool microbial biomarkers for diagnosis or monitoring.

MicroRNA Biomarkers in IBS Pathogenesis

MicroRNAs (miRNAs) are involved in regulating normal biological functioning processes such as cellular development, differentiation, proliferation, apoptosis, and metabolism. Therefore, dysregulation of miRNA can result in human diseases, including GI disorders [49]. Moreover, many useful biomarkers can be established among miRNAs involved in the pathogenesis of IBS.

miRNAs are short noncoding RNA molecules that play a role in the posttranslational regulation of messenger RNAs. In addition to observations in IBS animal models, there are data on miRNAs from patients with IBS. Thus, miRNA-24 is upregulated in IBS and may aggravate IBS symptoms by inhibiting the serotonin reuptake transporter. Therefore, it may function as a clinically useful biomarker to identify a subset of IBS patients and, potentially, future treatments that can either inhibit miRNA-24 or restore normal serotonin reuptake transporter function [50].

In a study that surveyed the role of miRNAs, Zhou et al. [51] showed that miRNA-29 increases intestinal permeability by reducing claudins and nuclear factor- κ B-repressing factor levels and decreasing glutamine levels. miRNA-29 affects intestinal membrane permeability through its regulation of the glutamate-ammonia ligase gene [51, 52]. Thus, miRNA-29 could identify a subset of IBS patients with increased membrane permeability who may also benefit from glutamine treatment.

Also, there was a decreased level of colonic miRNA-199, which usually decreases visceral pain by inhibiting the signaling of the transient receptor potential vanilloid type 1. Moreover, this decrease correlated with visceral

pain in patients with IBS-D [53]. Thus, miRNA-199 may be a potential marker for visceral pain and may be a target for therapy in the future. The other 2 microRNAs, miRNA-150 and miRNA-342-3p, were found to increase in patients with IBS compared to healthy controls [54].

A recent study has reported that microRNA-related serotonin receptor gene expression control with the cisregulatory version affected this regulation and appeared to be related to the female IBS-D [55]. Serotonin receptor type 3 (5-HT3E) is an essential neurotransmitter in the gut, and abnormal 5-HT3E signaling has been implicated in several functional GI disorders, including IBS. The regulation of miRNA-510 on 5-hydroxytryptamine 3 receptor (5-HT3E) expression and the possible association between 5-HT3E single nucleotide polymorphism rs56109847 in IBS-D are exciting findings of this study. The small sample size and study population that is restricted to women are the limitations of this study. However, the serotonin receptors are an exciting potential biomarker.

Future Perspectives

The use of MRI and diffusion imaging has uncovered differences in brain structure and connectivity between IBS patients and healthy subjects. Brain connectivity differences were also found between genders among IBS patients [56, 57]. In addition to prefrontal regions, abnormalities in distinct networks in the brain were observed in patients with chronic visceral abdominal pain, including salience, emotional arousal, and sensorimotor networks. Furthermore, therapies such as educational interventions, hypnotherapy, and antidepressants can improve those functional abnormalities shown on brain imaging [58, 59]. Brain functional imaging offers powerful tools to study important pathophysiological aspects of IBS. However, it is difficult to consider such abnormalities in imaging findings as typical biomarkers. Still, they might be developed further in combination with other markers. However, their high cost and restricted availability limit their clinical and research use significantly. Thus, they are unlikely to serve as routine markers in IBS at the moment.

Psychological assessment was also used as a “marker” of illness in IBS. The addition of psychological measures, such as the Hospital Anxiety and Depression (HAD) scale, the Patient Health Questionnaire 15 (PHQ-15), and the Perceived Stress Scale, to IBS biomarker panels enhanced the ability of the panel to differentiate IBS cases from healthy volunteers, without improving the ability of

the panels to discriminate between the different subtypes of IBS [60]. Psychological markers are likely not sufficient to diagnose IBS if used alone. Still, they could improve the performance of other biomarkers. When proven in select cases, functional and structural brain dysfunction and psychological disturbances in IBS can be targeted by neuromodulators.

The overarching limitation in the development of biomarkers for IBS is that this is not 1 disease. To establish a biomarker that can identify all patients with IBS is extremely unlikely. Instead, a promising approach is recognizing IBS as multiple diseases with similar symptoms and developing biomarkers to identify the different subgroups to allow targeted therapies. Another limitation is that IBS remains a very heterogeneous group of disorders, and we are not yet great at differentiating them. There is also a vast overlap. This means that until we understand the disease better and the mechanisms that may underpin it, it may be hard to find biomarkers specific to the disease.

Conclusion

The complex and multifactorial etiology of IBS may mean that a single biomarker that can diagnose IBS with the accuracy required for a clinically useful test cannot be found. To date, symptom-based diagnostic criteria, biomarkers, and psychological markers perform modestly in predicting IBS. Moreover, IBS biomarkers are disappointing due to small study populations and the challenges of ruling out other organic diseases with modest accuracy. In terms of the positive likelihood ratio, the most effective are FC and intestinal permeability markers.

Immune biomarkers have profoundly contributed to our understanding of various aspects of IBS pathophysiology. Moreover, specific immune biomarkers correlate with disease subtypes, making it possible to distinguish the mechanisms underlying the different IBS subgroups. At present, there are several proposed biomarkers (i.e., IL-1 β , IL-6, IL-12p70, TNF- α , CgA, HBD2, calprotectin, and caproate). Still, very few of them have been validated in a diverse group of patients. Even fewer are used now in routine clinical practice. Therefore, combining symptoms with markers appears more effective and may represent the way forward in IBS diagnosis. Biomarkers need to be validated in diverse populations for them to be generalizable.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

R. Nakov had the idea to make the manuscript; R. Nakov, V. Snegarova, D. Dimitrova-Yurukova, and T. Velikova wrote the manuscript and approved the final version of the manuscript. R. Nakov and T. Velikova supervised the whole process.

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