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Biomarkers for visceral hypersensitivity in patients with irritable bowel syndrome

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Abstract

Background: Increased visceral sensitivity is observed in up to 60% of patients with Irritable Bowel Syndrome (IBS). Mucosal inflammation, altered neuroendocrine activity and intraluminal metabolic processes may contribute to the development of visceral hypersensitivity. Previously, we demonstrated that biomarkers, indicative for these biological processes, were altered in IBS patients compared to healthy controls. However, how these processes relate to visceral hypersensitivity is unknown.

Aim: The aim of this study was to provide insight in biological processes associated with visceral hypersensitivity. Fecal and plasma biomarkers were measured in normosensitive and hypersensitive IBS patients.

Methods: A total of 167 IBS patients underwent a rectal barostat procedure to assess visceral sensitivity to pain. Based on the outcome, patients were classified into a normosensitive or hypersensitive group. Calprotectin, human β -defensin 2 (HBD2), chromogranin A (CgA), and short chain fatty acids (SCFAs) were measured in feces, citrulline in plasma, and serotonin and its main metabolite 5-hydroxyindoleacetic acid (5-HIAA) in platelet-poor plasma.

Key Results: Fecal markers and plasma citrulline were measured in 83 hypersensitive and 84 normosensitive patients, while platelet-poor plasma for the assessment of serotonin and 5-HIAA was available for a subgroup, i.e. 53 hypersensitive and 42 normosensitive patients. No statistically significant differences were found in concentrations of biomarkers between groups. Adjustment of the analyses for potential confounders, such as medication use, did not alter this conclusion.

Conclusions & Inferences: Our findings do not support a role for the biological processes as ascertained by biomarkers in visceral hypersensitivity in IBS patients. This study is registered in the US National Library of Medicine (clinicaltrials.gov, NCT00775060).

KEYWORDS

biomarkers, irritable bowel syndrome, visceral hypersensitivity

1 | INTRODUCTION

Visceral hypersensitivity, which can be measured by rectal balloon distention procedures,¹ is a hallmark of irritable bowel syndrome (IBS).² The prevalence of visceral hypersensitivity among IBS patients ranges between 30% and 60%.²⁻⁷ In a previous study, we found that hypersensitive IBS patients were younger, more often female, used selective serotonin reuptake inhibitors (SSRI's) more frequently and reported higher scores for gastrointestinal (GI) symptoms, when compared to IBS patients without visceral hypersensitivity.⁴ The latter may imply a role for visceral hypersensitivity in symptom generation in this subgroup of patients. However, these findings did not elucidate why some subjects with IBS are hypersensitive to artificially induced mechanical stimuli, while other IBS patients are not.

Several pathophysiological factors associated with IBS may affect nociception and thereby contribute to visceral hypersensitivity.⁸ Increased intestinal pain perception may result from low grade mucosal inflammation, immune activation, or more specifically mast cell activation,^{9,10} or may be caused by alterations in intestinal neuroendocrine activity^{10,11} and signaling to the brain.¹² Furthermore, intraluminal factors such as gut microbiota, their metabolic products or dietary compounds,¹³⁻¹⁵ and altered gut barrier function,¹⁶ may play an important role. The link between these intestinal biological processes and the development of visceral hypersensitivity has mostly been investigated using animal models.^{12-14,16-21} However, data regarding the role of these biological factors that explain the difference between IBS patients *with* and those *without* visceral hypersensitivity are still lacking.

Previously, we found that fecal calprotectin, a marker of intestinal inflammation,^{22,23} and fecal chromogranin A (CgA), a neuroendocrine secretory protein of the enterochromaffin cells,^{24,25} were increased in IBS patients compared to healthy controls.²⁶ In addition, some fecal short chain fatty acids (SCFAs) and fecal human β -defensin 2 (HBD2), as indicators of microbial activity and host-microbiome interaction,²⁷⁻³⁰ were altered in IBS compared to healthy subjects.²⁶ Furthermore, a decrease was found in IBS patients vs healthy subjects for plasma 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin (5-hydroxytryptamine, 5-HT), and the ratio of 5-HIAA and 5-HT.³¹ However, concentrations of 5-HT were not altered in IBS vs controls.³¹ Neither were differences observed between these groups in plasma citrulline concentrations,²⁶ which is a marker of effective enterocyte mass^{32,33} and may be indicative for small bowel barrier function.^{34,35} Taken together, our previous findings point to alterations in biological processes, such as mucosal immune activation and neuroendocrine and microbial activity in IBS patients vs healthy controls. However, it is not known whether these markers also differ between normosensitive and hypersensitive IBS patients.

The aim of this study was to provide more insight in biological processes associated with the presence of visceral hypersensitivity in IBS by comparing concentrations of individual biomarkers between IBS patients with and without visceral hypersensitivity.

Key Points

- Altered visceral perception is considered a characteristic of IBS. However, it is unknown why visceral hypersensitivity is found in only a subgroup of IBS patients and how these subjects differ with regard to various biological processes compared to normosensitive IBS patients.
- Calprotectin, human β -defensin 2, chromogranin A, and short chain fatty acids measured in feces, and citrulline, serotonin and its' main metabolite 5-hydroxyindoleacetic acid (5-HIAA) measured in plasma, as biomarkers indicative for mucosal inflammation, neuroendocrine and microbial activity, did not differ between hypersensitive and normosensitive IBS patients.
- Our findings do not support a role for certain biological processes as assessed by biomarkers in visceral hypersensitivity in IBS patients.

We hypothesized that markers related to inflammation and serotonin activity may be increased in hypersensitive IBS patients, while biomarkers related to mucosal integrity and gut microbiota homeostasis may be decreased, compared to normosensitive IBS patients.

2 | MATERIALS AND METHODS

This study is part of a larger cohort study on the phenotypic and genotypic characterization of patients with IBS (Maastricht IBS cohort). The study protocol has been approved by the Maastricht University Medical Centre+ (MUMC+) Ethics Committee, was executed in compliance with the revised Declaration of Helsinki (59th general assembly of the WMA, Seoul, South Korea, Oct. 2008), and was registered in the US National Library of Medicine (<http://www.clinicaltrials.gov>, NCT00775060).

2.1 | Study participants

All study participants gave written informed consent prior to participation. Adult subjects, aged 18-75 years, diagnosed with IBS by a gastroenterologist based on the Rome III criteria³⁶ were recruited via the Gastroenterology-Hepatology outpatient department of the MUMC+, a secondary and tertiary referral center. When considered indicated by the physician, GI endoscopy with biopsies, abdominal imaging by ultrasonography or CT scan, and/or blood, breath and fecal analyses were performed to exclude organic disease. History of abdominal surgery was considered an exclusion criterion, with the exception of appendectomy, laparoscopic cholecystectomy or hysterectomy. Rectal sensitivity was assessed in all IBS patients in this study using an electronic rectal barostat (Distender II; G&J Electronics, Toronto, ON, Canada, part: C7-CB-R) and a balloon of non-compliant material (Mui Scientific, Mississauga, ON, Canada, part: C7-2CB-R). The procedure

was executed according to a standardized protocol, and a VAS-score of ≥ 20 mm at pressure step 26 mmHg was used as cut-off to define the presence of visceral hypersensitivity to pain.^{2,4} All subjects completed several questionnaires as described in detail previously⁴: GI symptoms, assessed by the Gastrointestinal Symptom Rating Scale (GSRS)^{37,38} and a 14-day GI symptom diary,⁴ scores of anxiety and depression, assessed by the Hospital Anxiety and Depression Scale (HADS)³⁹ and the State and Trait Anxiety Inventory (STAI),⁴⁰ quality of life scores assessed by the 36-item Short Form Health Survey (SF-36),⁴¹ and the use of medication.⁴

2.2 | Analysis of biosamples

Fecal samples were collected of all subjects, aliquoted and stored at -80°C within 24 hours after defecation, until further analysis. Of all participants, fasting blood samples were collected from the antecubital vein using two precooled K2EDTA tubes. To avoid oxidative breakdown of serotonin, 0.1 mL 1.4% ascorbic acid (Sigma Aldrich, St. Louis, MO, USA) was added to one of the tubes, to obtain platelet poor plasma (PPP) samples after a centrifuge step for serotonin analysis. PPP samples were available for only a subgroup of study participants. K2EDTA tubes were centrifuged at 2300 g at 4°C for 10 minutes. Supernatants (i.e. plasma and/or PPP) were aliquoted and frozen at -80°C until further analysis.

Fecal and plasma biomarkers were selected based on literature to be measured as indicators of pathophysiological mechanisms in IBS. The markers were measured simultaneously to avoid additional thawing cycles, and this was carried out in blinded conditions by Medische Laboratoria Dr. Stein & Colleagues, The Netherlands. Calprotectin and human β -defensin 2 (HBD2) concentrations were determined using commercial enzyme-linked immunosorbent assay (ELISA, Büllmann, Switzerland,²⁵ and Immunodiagnostik AG, Germany,²⁹ respectively), and chromogranin A using commercial radioimmunoassay (RIA, Euro Diagnostica, Sweden).²⁵ All test kits were used according to manufacturers' instructions, the procedures were described in detail previously.^{25,29} Short chain fatty acids (SCFAs), i.e. acetate, propionate, butyrate, valerate, and caproate, were determined by gas chromatography-mass spectrometry (GCMS) according to the method described by García-Villalba et al.⁴² Citrulline was measured in plasma samples by high pressure liquid chromatography (HPLC) fluorescence detection.³³ Concentration of 5-HT and 5-HIAA were measured in PPP samples by high-performance liquid chromatography (HPLC), as described previously.⁴³

2.3 | Data and statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 22.0 (IBM Statistics for Windows, Armonk, NY, USA). Data were checked for normality using a normal probability plot. Baseline characteristics are presented as mean (\pm SD) or percentages when appropriate, for both IBS groups. Dichotomous data were analyzed using the Chi² test and continuous (parametric) data using the independent *t* test. To investigate differences in biomarker levels between

the normosensitive and hypersensitive IBS patients, Mann-Whitney *U* test was used (due to a left skewed distribution of the biomarker data). A post hoc analysis was conducted (linear regression analysis with natural log transformed biomarker data), to adjust the results for possible confounding effects of age, gender, IBS subtypes, symptoms of anxiety and depression, and medication use. Drugs that were included in the analysis were proton pump inhibitors (PPI), SSRIs, spasmolytics, prokinetics, anti-diarrheal drugs, laxatives, and non-steroid anti-inflammatory drugs (NSAIDs).

3 | RESULTS

In total, 167 IBS patients were included in the study. Based on the outcome of the rectal barostat procedure 83 (49.7%) patients were classified as visceral hypersensitive and 84 (50.3%) as normosensitive to pain. The majority (i.e. 155 subjects, 93%) of the patients included in this study, also participated in a previous study by our group,⁴ and therefore baseline characteristics (Table 1) are comparable to our previously published data.⁴

As shown in Table 1, in line with previous findings,⁴ hypersensitive IBS patients were significantly younger, more often female and had a lower mean BMI compared to the normosensitive group ($P < .05$). No differences were observed for IBS duration or IBS subtype distribution between the groups. GI symptom scores are presented in Table 2, scores for anxiety, depression and quality of life and medication use are presented Table 3.

3.1 | Biomarker levels

Fecal markers and plasma citrulline were measured in 83 hypersensitive and 84 normosensitive IBS patients, whereas 5-HT and 5-HIAA levels in PPP were available for only a subgroup of study participants, i.e., 53 hypersensitive and 42 normosensitive IBS patients (Table 4). All marker concentrations are presented as median with interquartiles,

TABLE 1 Patient characteristics, for the hypersensitive (IBS_{HYP}) compared to normosensitive IBS group (IBS_{NORM}). Differences tested with Chi-square and independent *t*-test for categorical and continuous data, respectively

Patient characteristics	IBS _{HYP} (n=83)	IBS _{NORM} (n=84)
Age (years; mean \pm SD)	37.7 \pm 15.8 ^c	46.7 \pm 16.3
Female sex (%)	81.9 ^a	69.0
BMI (mean kg/m ² \pm SD)	23.5 \pm 4.5 ^b	25.7 \pm 4.3
IBS subtype: D/C/M/U (%)	26.5/24.1/42.2/7.2	35.7/14.3/40.5/9.5
Duration of IBS symptoms (years; mean \pm SD)	13.2 \pm 12.2	11.8 \pm 14.8

^a $P < .05$, ^b $P < .01$, ^c $P < .001$ for IBS_{HYP} vs IBS_{NORM}.

TABLE 2 Gastrointestinal (GI) symptoms assessed by a 14-day GI symptom diary (presented as mean of 14 days \pm SD), based on a 1-to-5 point scale, and the Gastrointestinal Symptom Rating Scale (GSRS), based on a 1-to-7 point scale (presented for 5 subscales/syndromes)

Parameters (mean \pm SD)	IBS _{HYP} (n=83)	IBS _{NORM} (n=84)
14-day End-of-Day GI symptom diary (1-to-5 point scale)		
Abdominal pain	2.7 \pm 0.8 ^c	2.1 \pm 0.8
Abdominal discomfort	2.8 \pm 0.7 ^c	2.3 \pm 0.8
Bloating	2.5 \pm 0.9 ^b	2.0 \pm 1.0
Belching	1.8 \pm 0.8	1.6 \pm 0.7
Nausea	1.8 \pm 0.9	1.6 \pm 0.8
Flatulence	2.4 \pm 0.9	2.3 \pm 0.9
Constipation	1.8 \pm 0.9 ^c	1.3 \pm 0.4
Diarrhea	2.8 \pm 0.7	2.3 \pm 0.8
Gastrointestinal symptom rating scale syndromes (1-to-7 point scale)		
Abdominal pain	3.5 \pm 1.2	3.2 \pm 1.2
Reflux	2.2 \pm 1.2 ^a	1.8 \pm 1.2
Indigestion	4.1 \pm 1.3 ^a	3.7 \pm 1.2
Constipation	3.7 \pm 1.6 ^c	3.0 \pm 1.2
Diarrhea	3.5 \pm 1.5	3.5 \pm 1.5

^a $P < .05$, ^b $P < .01$, ^c $P < .001$ for IBS_{HYP} vs IBS_{NORM}.

due to the nonparametric distribution. There were no statistically significant differences in biomarker levels between the IBS groups.

External factors, such as the use of specific drugs, could influence the concentrations of the markers measured in this study and these could potentially mask differences between the two IBS groups. A post hoc analysis was conducted to adjust for possible confounding effects of age, gender, BMI, IBS-subtype distribution, symptoms of anxiety and depression, and medication use. However, adjustment for these parameters (Table S1), did not reveal significant differences in levels of markers between groups.

4 | DISCUSSION

In this study, we investigated biomarkers in IBS patients with and without visceral hypersensitivity. Several fecal and plasma biomarkers, which are indicative for pathophysiological processes that may be associated with increased visceral sensitivity, were measured. In contrast to our hypothesis, the concentrations of the investigated markers were not significantly altered in the hypersensitive compared to the normosensitive IBS group.

Abdominal pain is a highly prevalent symptom in IBS. Pain is the major characteristic of the Rome diagnostic criteria for IBS.³⁶ In this study, a subgroup of IBS patients (49.7%) was found to have increased visceral sensitivity to an artificial pain stimulus induced by rectal balloon distention, which is in line with previous findings.²⁻⁷ We hypothesized that factors that potentially affect visceral pain perception, such as mucosal inflammation or dysregulation of the neuroendocrine or microbiota activity, are altered in IBS patients with visceral

TABLE 3 Symptoms of anxiety and depression were assessed by the Hospital Anxiety and Depression Scale (HADS). The percentage of subjects with a score ≥ 8 on either the anxiety or depression scale were presented, which is indicative for the presence of anxiety or depressive symptoms, respectively. Trait anxiety score as assessed by the State and Trait Anxiety Inventory (STAI) presented as mean \pm SD (range 0-100). Composite scores for mental and physical quality of life of the SF-36 questionnaire presented as mean \pm SD (range 0-100). Use of medication, at least once in the 14 days prior to inclusion (self-reported and cross-checked in medical files) presented as % of users per group. The variable 'use of any medication' includes the listed drugs, but also any other medication (such as statins, oral anti-conceptive drugs, etc.)

Parameters	IBS _{HYP} (n=83)	IBS _{NORM} (n=84)
Psychological symptoms (mean \pm SD)		
HADS depressive symptoms (%)	28.7	17.6
HADS anxiety symptoms (%)	39.1	34.5
STAI trait anxiety scale	41.0 \pm 12.3	40.3 \pm 10.9
SF-36 QoL composite scores (mean \pm SD)		
SF-36 mental QoL score	45.9 \pm 10.7	47.2 \pm 10.9
SF-36 physical QoL score	38.5 \pm 10.0	41.7 \pm 10.3
Use of medication in 14 days prior to inclusion (%)		
Proton Pump Inhibitors (PPI)	25.3	30.1
Selective Serotonin Reuptake Inhibitors (SSRI)	21.5 ^a	12.3
Laxatives	20.3	20.5
Spasmolytic drugs	6.3	12.3
Prokinetic drugs	3.8	2.7
Anti-diarrheal drugs	3.8	2.7
Nonsteroid Anti-inflammatory Drugs (NSAID)	12.7	13.7
Use of any medication	77.6	81.9

^a $P < .05$, ^b $P < .01$, ^c $P < .001$ for IBS_{HYP} vs IBS_{NORM}.

hypersensitivity when compared to normosensitive patients, and can be measured by biomarkers.

Low grade immune activation has previously been associated with IBS, and may influence the process of nociception.⁸ In previous studies, fecal calprotectin has been found to be slightly though significantly increased in IBS patients compared to healthy controls.^{26,44} In this study, we investigated calprotectin, as a non-invasive marker of intestinal inflammation, in relation to visceral pain perception within the IBS population. However, fecal calprotectin concentrations could not provide leads for an explanation for the difference in visceroperception between IBS patients. This does not exclude a role for intestinal inflammation as factor contributing to hypersensitivity in IBS. Other inflammatory and immunomodulatory factors, not measured in this study, may contribute to altered pain perception; for example cytokines, chemokines, prostanoids, proteases, bradykinins, biogenic amines (eg, histamine), neuro-peptides and neuro-trophic factors

TABLE 4 Levels of fecal (CgA, calprotectin, HBD2, SCFAs) and plasma markers (citrulline, 5-HT, 5-HIAA) for subjects IBS_{HYP} or IBS_{NORM}. No statistically significant differences between groups, as tested by the Mann-Whitney *U* test

Markers (median [Q1,Q3])	IBS _{HYP} (n = 83)	IBS _{NORM} (n = 84)
Chromogranin A (CgA) (nmol g ⁻¹)	14.5 (7.4, 43.0)	16.8 (8.4, 47.9)
Calprotectin (μg g ⁻¹)	35.6 (15.0, 72.0)	33.5 (16.7, 72.4)
Human β-defensin 2 (HBD2) (ng g ⁻¹)	30.2 (14.6, 51.7)	25.9 (15.8, 45.7)
SCFA (C2): Acetate (μmol g ⁻¹)	30.6 (19.7, 45.5)	30.6 (20.6, 45.4)
SCFA (C3): Propionate (μmol g ⁻¹)	8.8 (5.1, 13.8)	9.2 [5.7, 13.4]
SCFA (C4): Butyrate (μmol g ⁻¹)	7.3 (4.1, 12.7)	7.9 [4.6, 12.2]
SCFA (C5): Valerate (μmol g ⁻¹)	1.2 (0.8, 1.6)	1.3 (0.8, 1.9)
SCFA (C6): Caproate (μmol g ⁻¹)	0.09 (0.02, 0.63)	0.05 (0.02, 0.45)
Plasma citrulline (μmol L ⁻¹)	39.8 (34.4, 49.3)	41.9 (33.5, 50.2)
Markers (median [Q1,Q3])	IBS _{HYP} (n=53)	IBS _{NORM} (n=42)
5-HT (μg L ⁻¹)	3.6 [1.7, 9.2]	4.6 (1.7, 8.4)
5-HIAA (μg L ⁻¹)	17.8 (12.3, 28.0)	22.1 (14.9, 35.4)
5-HIAA/5-HT ratio	5.0 (1.8, 10.5)	6.2 (2.2, 11.1)

(e.g., substance P, somatostatin, brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF)), reduced pH and/or increased ATP.^{5,6,8,9,19,20,45,46}

Serotonin (5-HT) is known to modulate several intestinal processes including motility, secretion, barrier function, and perception.^{10,11,47} Most 5-HT is produced by enterochromaffin cells and it can activate intrinsic or extrinsic primary afferent nerves in the intestinal mucosa.^{48,49} Previous studies have suggested that elevated serotonin activity may act as an excitatory mediator in visceral sensory pathways, in particular via increased activation of 5-HT₃ receptor.⁵⁰ Moreover, treatment with a 5-HT₃ receptor antagonist has resulted in higher perception thresholds to colonic distension in IBS patients.⁵⁰ In this study, we measured levels of 5-HT and its principal metabolite 5-HIAA in platelet-poor plasma, which was available for a subgroup of subjects. In a previous pilot intervention study of 7 hyper- vs 8 normosensitive IBS patients, 5-HIAA levels were significantly higher in the hyper- compared to the normosensitive IBS group following intake of placebo.⁶ However, in this study, the 5-HIAA levels did not differ between normo- and hypersensitive patients. This was also true for the plasma 5-HT concentrations. We can only speculate about the explanation for the differences between these study results. Firstly, it could have been a matter of chance due to the small numbers of study participants in the previous study.⁶ On the other hand, the effect of placebo and anticipation of an intervention may have affected neurotransmission processes in these subjects, which could have resulted in altered serotonin metabolism,⁵¹ compared to our baseline

measurement without an intervention. Furthermore, it should be acknowledged that differences between hyper- and normosensitive IBS patients in mucosal serotonin activity, which were not assessed in this study, cannot be excluded.

Chromogranin A, as serotonin, is produced by enterochromaffin cells, and is co-localized in storage granules with serotonin.^{24,25,52,53} Although the relation between IBS and CgA has not yet been fully elucidated, several studies point to a role of this protein in IBS and even in gut health in general. Previous reports showed that the density of CgA producing cells both in the duodenum and colon was reduced in IBS patients compared to healthy controls,⁵² while we²⁶ and others²⁵ have demonstrated that the level of fecal CgA was increased in IBS patients compared to controls. Furthermore, in a large population-based metagenomics analysis, low CgA concentrations were associated with a more diverse fecal microbiome, which is considered beneficial to gut health.⁵⁴ However, up to now, the association between CgA and visceral sensitivity in IBS patients has not been studied. Our results do not show significant differences in fecal CgA concentrations between visceral hypersensitive and normosensitive IBS patients.

Previously, we have shown that small intestinal permeability was increased in IBS-D patients, indicating small bowel barrier dysfunction in these subjects.⁵⁵ This finding was in line with several other reports.^{56,57} Furthermore, associations between impaired gut barrier function and altered visceral sensitivity have been reported.⁵⁸ We did not observe a relation between visceral sensitivity and plasma citrulline, which is a marker of effective enterocyte mass^{32,33} and may be indicative for small bowel barrier function.^{34,35}

Crouzet et al. have demonstrated that hypersensitivity to colonic distension in IBS patients can be transferred to rats via fecal transplants,¹³ indicating that luminal factors, such as microbiota, its toxins or metabolites, or dietary products, may alter visceral sensitivity of these rodents. Furthermore, Rousseaux et al. found that specific *Lactobacilli* species (i.e., *L. acidophilus* NCFM) were able to affect μ-opioid and cannabinoid receptors in intestinal epithelial cells of rats, suggesting that the intestinal microbiota may influence visceral perception.²¹ We measured fecal concentrations of HBD2, a human antimicrobial peptide produced by intestinal epithelial cells in response to bacteria.³⁰ Alterations in microbiota composition may result in changes in the intestinal mucosa response toward bacteria, possibly via HBD2. In previous studies, both increased and decreased fecal HBD2 has been observed in IBS patients compared to healthy controls.^{26,29} In this study, we did not observe significant differences in HBD2 concentrations between the hyper- and normosensitive IBS patients. We also measured several SCFAs in fecal samples. These SCFAs are products of microbial fermentation of non-digested oligosaccharides in the colon and are generally regarded as beneficial to gut health.²⁶⁻²⁸ The intraluminal SCFA concentration is affected by microbiota composition as well as dietary substrates. Previous attempts to affect visceral sensitivity using SCFA-enemas in animal models have shown varying results.^{59,60} In this study, no significant differences in fecal SCFA concentrations between the IBS groups were observed. The intestinal microbiota composition itself was not investigated in this study and is a

potential target for future research focusing on differences between hyper- and normosensitive IBS patients.⁶¹

We previously reported that several markers (i.e., calprotectin, CgA, HBD2, the SCFAs valerate and caproate, 5-HIAA and 5-HIAA/5-HT ratio) were significantly altered in IBS patients compared to healthy subjects.^{26,31} In this study, no differences were observed between the hyper- and normosensitive IBS patients with regard to these markers. Although these biomarkers were associated with the presence of IBS, they may be less important in the development or maintenance of visceral hypersensitivity in IBS patients. The heterogeneity of the IBS patient population with and without visceral hypersensitivity may have led to the overall lack of positive findings with regard to tested biomarkers in this study. It should also be taken into account that the panel of tested markers is indicative for only some of the key biological processes, which does not exclude that other intestinal factors or other markers not investigated here contribute to the presence of visceral hypersensitivity in IBS. These factors may include mucosal mast cell activation or mucosal cytokines release,^{9,17,62} alterations in the function of nociceptive transducer molecules (e.g., transient reporter potential channels (TRP) and acid-sensing ion channels (ASIC)),^{5,9,63} dysfunctional intestinal barrier function,^{55,58} alterations in microbial composition and activity^{14,61} and/or alterations in visceral nervous system signaling.^{19,20,45} Furthermore, we have measured markers in blood and fecal samples, but mechanisms on mucosal level which could initiate hypersensitivity have not been assessed. There is growing evidence for the involvement of mucosal mast cells in visceral sensitivity in IBS patients. In colonic biopsies of IBS patients, mast cell proximity to nerve cell terminals have been found to correlate to abdominal pain scores.⁶⁴ More recently, a study by Wouters et al. showed that histamine receptor H1 (HRH1) mediated sensitization of TRPV1 plays a role in visceral nociception in IBS patients. The study demonstrated that ebastine, an antagonist of HRH1, reduced visceral hypersensitivity and abdominal pain in patients with IBS, when compared to placebo.⁹ The sample size of this trial was too small for subgroup analysis for hyper- vs normosensitive IBS patients. Furthermore, mast cells are the main source of nerve growth factor (NGF) in the intestines, and a negative correlation was found between NGF gene expression in rectosigmoid biopsies of diarrhea predominant IBS patients and visceral sensitivity thresholds.⁴⁶ These studies point to mucosal mast cell function as a relevant focus for further research to investigate the differences in visceral sensitivity between IBS patients. We postulate that in line with IBS, visceral hypersensitivity in IBS is also based on a multifactorial etiology. Dysregulation of processes on intestinal level in combination with altered central pain modulation and/or mental health factors, such as stress, anxiety, depression, or somatization,^{7,51,65,66} may lead to altered pain perception and to hypersensitivity in a subgroup of the IBS population. Further research is needed on mucosal and systemic level, which may be based on a more holistic approach, using omics techniques, in combination with neurological and psychological parameters to elucidate the complex interplay between intestinal and central factors which lead to the development of visceral hypersensitivity in IBS patients.

5 | CONCLUSION

This study did not reveal significant differences between hypersensitive and normosensitive IBS patients with regard to the biomarkers: fecal calprotectin, chromogranin A, human β -defensin 2, short chain fatty acids, and plasma citrulline, serotonin and 5-HIAA. These biomarkers represent specific pathophysiological mechanisms in IBS, such as immune activation and neuroendocrine and microbial activity.

COMPETING INTERESTS

None.

AUTHOR CONTRIBUTION

ZM: data collection, analysis, interpretation and manuscript writing; DJ: study concept, data interpretation; SL: data collection and interpretation; DK: data interpretation; MH, RK, JA, ZW, CL, JK: data collection and/or processing; FS: biosample analyses, data interpretation; AM: project leader, study concept, data interpretation. All authors participated to constructive review of the manuscript and approved the final version.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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