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**Title:**
**Small intestine bacterial overgrowth (SIBO) breath test does not predict symptom severity in gut-brain interaction disorders: role of anxiety, depression, and inflammatory biomarkers**

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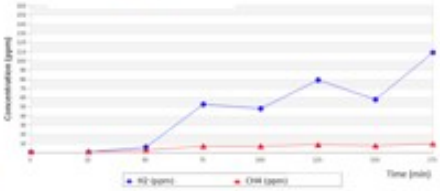
**70 pacientes del servicio de Aparato Digestivo a los que se les solicitó un test de SIBO**

**1-Cuestionarios**
**2-Biomarcadores en sangre**
**3-Test de SIBO**

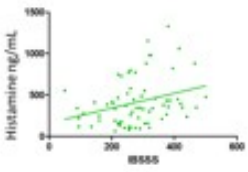
**IBSSS, dolor abdominal y distension se asociaron a...**

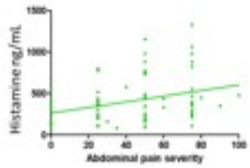
Roma IV
IBSSS
HAD-A
HAD-D

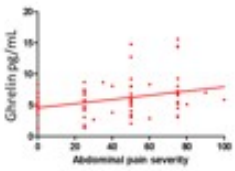
Histamina
Citrulina
Grelina
I-FABP
TRPV-1

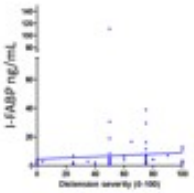


- Pacientes más jóvenes
- Ansiedad y depresión autopercibidas
- Algunos biomarcadores en sangre...




... pero no se asociaron al resultado del test de SIBO.

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**TITLE**:

SIBO BREATH TEST DOES NOT PREDICT SYMPTOM SEVERITY IN GUT-BRAIN INTERACTION DISORDERS: ROLE OF ANXIETY, DEPRESSION, AND INFLAMMATORY BIOMARKERS.

**RUNNING TITLE:**

SIBO TEST AND GASTROINTESTINAL SYMPTOMS

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**Conflict of interest:** The authors declare no conflicts of interest.

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**Key Words:** Disorder of the gut-brain interaction, SIBO, histamine, ghrelin, I-FABP

**List of abbreviations:**

DGBI: Disorders of the gut-brain interaction

SIBO: Small intestine bacterial overgrowth

IBS: Irritable Bowel Syndrome

ASENEM: Asociación Española de Neurogastroenterología y Motilidad

SEPD: Sociedad Española de Patología Digestiva

I-FABP: Intestinal-Fatty Acid Binding Protein

TRPV-1: Transient Receptor Potential Cation Channel Subfamily V, Member 1

IBD: Inflammatory bowel disease

CD: Celiac disease

IBSSS: Irritable Bowel Syndrome Severity Score

HAD-A and HAD-D: Hospital Anxiety and Depression subscales

PCR: C reactive protein

FC: fecal calprotectin

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**Abstract:**

Background: The high prevalence of disorders of the gut-brain interaction (DGBI), the availability of breath tests for the diagnosis of small intestine bacterial overgrowth (SIBO) together with some confusion about the concept of SIBO are leading to an increase in the number of SIBO diagnoses.

Objective: We aimed to analyze the factors associated with the severity of gastrointestinal symptoms in patients undergoing a SIBO breath test.

Methods: A cross-sectional observational study including 70 patients who underwent a SIBO test with lactitol and completed questionnaires including the ROME IV criteria for Irritable Bowel Syndrome (IBS), the Irritable Bowel Syndrome Severity Score (IBSSS), and the HAD anxiety and depression scales. Additionally, blood levels of histamine, citrulline, ghrelin, Intestinal-Fatty Acid Binding Protein (I-FABP) and Transient Receptor Potential Cation Channel Subfamily V, Member 1 (TRPV-1) were measured.

Results: The mean age was 45±16 years and 70% were women. Abdominal pain and/or abdominal distension were present in 85% of patients. 44% met IBS Rome IV criteria. IBSSS total score had a correlation with age (-0.354, $p<0.001$), HAD-A (0.391, $p<0.001$) and HAD-D (0.409, $p<0.001$) scores, and histamine levels (0.279, $p=0.019$). Abdominal pain correlated with levels of histamine (0.320, $p<0.05$; 0.282, $p<0.05$) and ghrelin (0.252, $p<0.05$, 0.347, $p<0.05$), while abdominal distension correlated with I-FABP levels (0.314, $p<0.05$). The SIBO test was positive in 75% but did not correlate with symptom severity.

Conclusion: We hereby unveiled some factors associated with the severity of abdominal pain and distension such as age, auto-perceived anxiety and depression and some biomarkers but not SIBO test result.

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**Lay summary:**

The small intestinal bacterial overgrowth breath test is proposed for the management of some gut-brain interaction disorders such as irritable bowel syndrome and symptoms like flatulence. The wide availability and overdemand of small intestinal bacterial overgrowth breath tests may lead to an increase in the number of diagnoses and antibiotic prescriptions.

We aimed to analyze the factors that are associated with the severity of gastrointestinal symptoms in patients who, by clinical practice, a small intestinal bacterial overgrowth breath test is performed.

We included 70 patients in the study, who underwent a small intestinal bacterial overgrowth test, as well as questionnaires on gastrointestinal symptoms related to their irritable bowel syndrome and scales assessing anxiety and depression. Additionally, blood levels of various molecules, such as citrulline, ghrelin, histamine, Intestinal-Fatty Acid Binding Protein (I-FABP) and Transient Receptor Potential Cation Channel Subfamily V, Member 1 (TRPV-1), considered markers of inflammation, were measured.

In patients who, a SIBO breath test is requested due to clinical practice, the intensity of symptoms is not associated with the results of the test but with age and the patient's autoperception of anxiety and depression. Molecules such as histamine, ghrelin and I-FABP correlate with the intensity of some gastrointestinal symptoms in this group of patients.

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**Key summary:**

Established knowledge on this subject:

The small intestinal bacterial overgrowth (SIBO) breath test is proposed for the management of some gut-brain interaction disorders (GBID) such as irritable bowel syndrome (IBS) and symptoms like flatulence.

The wide availability and overdemand of SIBO breath tests may lead to an increase in the number of diagnoses and antibiotic prescriptions.

New findings of this study

In patients who, a SIBO breath test is requested due to clinical practice, the intensity of symptoms is not associated with the results of the test but with age and the patient's autoperception of anxiety and depression.

Molecules such as histamine, ghrelin and I-FABP correlate with the intensity of some gastrointestinal symptoms in this group of patients.

**Ethic statements**

Funding: Not applied.

Data availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest: The authors declare no conflicts of interest.

Ethics approval: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Hospital Clínico Universitario from Valladolid (Spain) with code: PI 22-2877

Patient consent: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: Not applied.

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**INTRODUCTION:**

The high prevalence of disorders of the gut-brain interaction (DGBI) [1], the availability and simplicity of breath tests for the diagnosis of SIBO, together with consensus documents or reviews that assume that a positive SIBO test could explain symptoms, such as abdominal distension, or irritable bowel syndrome (IBS) [2-5], are leading to an increased number of SIBO diagnosis and some confusion among patients and doctors. In Spain, ASENEM (Asociación Española de Neurogastroenterología y Motilidad, Neurogastroenterology and Motility Spanish Association) and SEPD (Sociedad Española de Patología Digestiva- Digestive Pathology Spanish Society) have published a position document defining the circumstances when SIBO must be considered [6]. Other authors have recently published an interesting critical review about SIBO, breath tests and DGBIs [7].

Patients suffering from DGBI may have intense symptoms that limit their quality of life. These disorders are associated with high auto-perceived levels of anxiety and depression [8, 9]. So, when faced with a patient suffering from DGBI, the first approaches should be explaining to him/her his condition, proposing changes in habits and diet in addition to making a psychological assessment of the patient [10].

On the other hand, despite the low-grade inflammation described in the gastrointestinal mucosa of patients with IBS and other DGBIs, there are still no biomarkers that facilitate the diagnosis. Molecules such as citrulline [11], ghrelin [12], histamine [13, 14], intestinal-fatty acid binding protein (I-FABP) [15-17], and Transient Receptor Potential. Cation Channel Subfamily V, Member 1 (TRPV-1) [18], have been analyzed as biomarkers of enteropathies of varying severity.

We think that to facilitate the diagnosis, management and monitoring of DGBI patients it is necessary to delve deeper into the pathogenesis of these disorders in order to identify related factors and biomarkers which could be useful in monitoring patients and searching for new therapies.

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**AIMS:**

We aim to identify factors and novel biomarkers that may aid in managing patients with nonespecific gastrointestinal symptoms in which, by clinical practice, a breath test is requested for the diagnosis of SIBO. Some molecules, not usually measured or used in clinical practice: citrulline, ghrelin, histamine, I-FABP and TRPV1; anxiety and depression levels; and result of the SIBO test will be analyzed.

**MATERIAL AND METHODS:**

**Patients demographics**

The observational cross-sectional study included 70 adult patients prospectively recruited in the *Hospital Clínico Universitario* of Valladolid Gastroenterology outpatient clinics. They all underwent a breath test with lactitol for the diagnosis of SIBO due to abdominal distension or bloating, abdominal pain, diarrhea or constipation from September 2022 to September 2023. Patients with a new diagnosis of inflammatory bowel disease (IBD), celiac disease (CD), colon cancer or other organic diseases were excluded.

All patients had received prior written instructions to perform the test correctly [2, 3]. On the day of the test and before ingesting 75 g of lactitol, a blood sample was obtained.

The ethics committee of the Valladolid Este Health Area authorized this study with code: PI 22-2877.

**Clinical variables**

Patients completed a structured questionnaire that included the ROME IV criteria for IBS diagnosis [19], the Irritable Bowel Syndrome Severity Score (IBSSS) [20] and the HAD, Hospital Anxiety and Depression subscales (HAD-A and HAD-D) [21]. The IBSSS includes different items such as the severity and frequency of abdominal pain, the severity of abdominal distension (the IBSSS Spanish translation details: “swollen, inflated or tense belly”), the degree of dissatisfaction, and the influence of symptoms on quality of life. Based on the total IBSSS, we established: IBSSS<75 as in remission;

from 75 to 150 as mild intensity symptoms; 150 to 300 as moderate intensity symptoms; and >300 as severe symptoms [20]. Auto-perceived anxiety and depression scores were also classified: HAD 0-7, normal cases; 7-11, borderline; >11, significant anxiety and depression [22].

History referring to previous intestinal surgery, diseases causing intestinal motility disorders such as connective tissue diseases, diabetes, neurological diseases or small intestine diverticulosis was obtained from the electronic medical record (JIMENA IV).

The diagnosis of SIBO was established with spectrometry (Isomed Diagnostics) if hydrogen in exhaled air was greater than or equal to 20 ppm over the baseline value before 90 minutes, or methane determination was above 10 ppm over the basal value at any point of the curve, based on previously published reference values [2].

**Laboratory determinations**

Basic laboratory values (hemoglobin (g/dL), platelet (*$10^3$/uL), ferritin (ng/mL), folates (ng/mL), vitamin $B_{12}$ (pg/mL), c reactive protein (CRP) (mg/dL), fecal calprotectin (FC) (ug/g) in the last 3 months were also obtained in the context of the normal clinical practice.

Serum soluble levels of citrulline, ghrelin, histamine, I-FABP and TRPV1 were determined with enzyme immunoassay (ELISA) techniques following manufacturer´s instructions (Quimigen SL, Invitrogen for I-FABP kit).

**Statistical analyses**

Continuous variables with parametric distribution are shown as mean and standard deviation while those with non-parametric distribution or scales are shown with medians and ranges. Discontinuous variables are shown in absolute and relative frequencies. Continuous variables were compared with Student's t test (parametric) and with the Mann Whitman and Kruskal Wallis U tests (non-parametric). Cualitative variables were compared with the Chi square test with Fisher's correction. For correlation studies, the Spearman test was used. The p-value <0.05 has been considered significant. SPSS and GraphPad programs have been used.

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**RESULTS:**

**Patient demographics**

Clinical parameters from the 70 prospectively recruited patients are summarized in Table 1. Briefly they were 45.7 (16.2) years old and 70% were women. Only ten of them (14.3%) suffered from previously diagnosed scleroderma (3 patients), IBD (2 patients), CD (one patient), or gastrointestinal surgery (2 patients) that could predispose them to a SIBO. Sixty (85.7%) patients acknowledged having abdominal pain and/or abdominal distension or bloating. Only 44.3% of them met ROME IV criteria for the diagnosis of IBS [19, 23]. Pain occurred on 6 (0-10) days out of 10. The bloating severity score was 50 (0-100), the degree of dissatisfaction was 65 (0-100), and the interference of symptoms on quality of life was 70 (20-100). More than half of the patients had a borderline or significant HAD-A score and more than a quarter of patients had a borderline or significant HAD-D score. SIBO test was positive in 75% of patients, 28% based on hydrogen production and 72% considering methane production (Table 1).

**IBSSS total score**

The IBSSS total score was inversely correlated with the age of the patients (-0.354, $p<0.001$) and positively with HAD-A (0.391, $p<0.001$) and HAD-D (0.409, $p<0.001$) scores (figure 1). The IBSSS did not correlate with routine clinical laboratory values nor did it show a difference in relation to the SIBO test result (Table 2).

Histamine levels were positively correlated with the IBSSS total score (0.279, $p=0.019$) (figure 2), with ascending histamine levels in patients with mild, moderate and high IBSSS, 232.6 (294.2), 334.7 (733.6) and 415.4 (2095.6) ng/mL respectively ($p=0.027$) (Table 2).

**IBSSS items scores**

The severity of abdominal pain was negatively correlated with age (-0.442, $p>0.001$), and positively with the HAD-A (0.405, $p<0.001$) and HAD-D (0.444, $p<0.001$) scores, and with ghrelin (0.252, $p=0.035$) and histamine (0.320, $p=0.007$) values (figure 2).

The frequency of abdominal pain was correlated with the HAD-A (0.359, p=0.002) and HAD-D (0.424, p<0.001) scores as well as with ghrelin (0.347, p=0.003) and histamine (0.282, p=0.018) levels.

The severity of abdominal distension or bloating was negatively correlated with age (-0.364, p=0.002), and positively with HAD-A (0.296, p=0.013) and HAD-D (0.239, p=0.046) scores, and with I-FABP levels (0.314, p=0.008) (figure 2).

Life disruption by gastrointestinal symptoms score had a positive correlation with the HAD-A (0.414, p<0.001) and HAD-D (0.516, p<0.001) scores. No item showed differences in relation to the result of the lactitol SIBO test nor when the positivity was due to hydrogen or methane production (table 3).

**Biomarkers, HAD and SIBO test**

No correlation has been found between the levels of citrulline, ghrelin, histamine, I-FABP and TRPV-1 and HAD-A and HAD-D subscales scores (mat supl.table 5). I-FABP levels showed a positive correlation with the platelet count (0.283, p=0.019) and a negative correlation with ferritinemia (-0.354, p=0.004). TRPV-1 levels correlated positively with ferritinemia (0.379, p=0.004) (mat. Suppl table 6).

In the correlation study among the 5 biomarkers, histamine had a negative correlation with citrulline (-0.334, p=0.004) and a positive correlation with ghrelin (0.241, p=0.045). Ghrelin was negatively correlated with TRPV-1 (-0.287, p=0.016) (mat. Suppl. table 7).

The result of the SIBO test was not associated with differences with the values of the 5 biomarkers, nor when the positivity was due to hydrogen or methane production (mat. Suppl. table 4).

**DISCUSSION:**

DGBIs can affect 30% of the general population [1, 24] and it is the most frequent reason for consultation in our gastroenterology unit. The availability and simplicity of SIBO breath test, as well as the influence of social networks and economic interests are, in our opinion, creating false expectations for patients and causing confusion in the medical community and in society, explaining in a simplified way the symptoms of

these disorders [7, 25].

Some publications have emerged reviewing aspects such as the concept of SIBO, the limitations of breath tests in SIBO diagnosis, the relationship of the supposed SIBO with DGBIs, the dubious benefits of a SIBO diagnosis and antibiotic treatment in patients with gastrointestinal symptoms such as abdominal distension [6, 7]. Some medical societies do not recommend SIBO test for patients with IBS or non-specific symptoms [10, 26-28] but others do with low level of evidence and recognizing limitations [2-5].

Seventy five percent of the 70 patients who were requested a SIBO test, mainly because of abdominal pain, and distension or bloating, had a positive result. It was mostly because methane production. Methanogens are usually found in the healthy colon and a fast orocecal transit with the arrival of lactitol to the colon may explain partially such a high positive rate. Besides, a higher rate in IBS patients has been reported before [29].

Our results show that a positive SIBO test result did not associate with more severe gastrointestinal symptoms (nor when the positivity was due to hydrogen or methane production). However other factors such as age, mood, and some mediators did.

We found a negative correlation between the severity of symptoms and age. In our country, DGBIs are very prevalent at any age [1], but in our experience they are, among young patients, the most frequent reason for consultation in a gastroenterology unit.

The association of some DGBIs and auto-perceived anxiety and depression levels has already been demonstrated with different studies [8, 9]. We found a positive and statistically significant correlation of auto-perceived anxiety and depression with total IBSSS and with some IBSSS items such as the severity and duration of abdominal pain, the severity of abdominal distention or bloating, and the disruption of the symptoms on daily life. This is why we think that the initial DGBI treatment must include explaining the patient, in an understandable way, the relationship between the nervous system and the digestive tract. A psychological approach is often necessary in these patients, and neuromodulators, and even behavioral treatment and hypnosis, may be part of the recommended treatment of more resistant forms of IBS [10, 28].

The necessity to identify pathogenic mediators and biomarkers of these disorders has been highlighted by different authors [15, 18, 30, 31]. We are not looking for diagnostic tools but biomarkers which may explain different DGBI symptoms, and help us in treating and monitoring patients. The correlations found in our work are weak but statistically significant. Our results, showing a correlation between histamine and IBSS total score, pain severity and pain frequency, are in line with studies that confirm the effectiveness of a mast cell stabilizer such as ketotifen [18] and histamine receptor blockers such as ebastine in reducing the symptoms of abdominal pain of IBS [14].

Ghrelin, a hormone of mainly gastric origin, but also synthesized in the duodenum and pancreas, has been proposed as a marker of IBD inflammatory activity [12]. We have observed higher levels of ghrelin with more severe and persistent abdominal pain and a positive correlation with histamine has also been found (0.241, p=0.045).

Our results confirm also a weak but statistically significant correlation between I-FABP levels and abdominal distension or bloating that we previously reported [32]. I-FABP is a protein released into the blood circulation from the cytosol of mature enterocytes in case of epithelial damage. Its levels are elevated in CD and Crohn's disease [17]. Elevated levels have been demonstrated in the case of IBS with increased intestinal permeability, but without reaching the levels of patients with Crohn's disease [15]. The low-FODMAP diet in patients with IBS reduces I-FABP levels, in addition to leading to symptomatic and psychological improvement [16].

Citrulline is a non-proteinogenic amino acid rarely present in the diet, which blood levels are reduced when intestinal function is compromised [11]. It has been proposed as a biomarker of CD, with lower levels associated with villous atrophy [33]. We have not found any relationship between citrulline levels and patients' symptoms, nor with anxiety or depression levels, but they have been negatively correlated with histamine levels (-0.344, p=0.004).

We have not found a correlation between TRPV-1 and the IBSSS score and subscores, nor with anxiety or depression, but inversely with ghrelin levels (-0.287, p=0.016). TRPV-1, present in the rectal submucosal plexus, is activated in the presence of histamine or by its metabolite inidazolacetaldehyde, and mediates pain sensitivity in some patients with IBS.

We think SIBO breath test is not recommended in the management of patients with nonspecific gastrointestinal symptoms or DGBIs. As Kashyap et al, we think SIBO is a confusing concept, and there is not strong enough evidence relating SIBO, SIBO breath test, DGBIs and gastrointestinal symptoms [7]. Management of DGBI patients must focus on explanation of symptoms, recommendations in habits and diet, a psychological approach and symptomatic treatment. In the case of a positive SIBO breath test, the clinical benefits obtained in patients with IBS treated with antibiotics are not superior to those achieved with other approaches [7].

We are aware of some of the limitations of the work. 1-We meassured intensity of symptoms but patients were not classified in base of different DGBI criteria but IBS. 2- We used lactitol for breath test, being glucosa the recommended sugar by some experts avoiding the oro-cecal transit interference [3, 6, 34]. 3- We did not measure abdominal perimeter of the patients. Instead we have correlated auto-perceived, often co-existing, abdominal distension and bloating [20, 35]. 4- We did not analyze the response to treatment. Patients were recruited prospectively but it is a cross-sectional study. Very different approaches were proposed in base to symptoms, SIBO test result, and patients and doctors preferences and experience.

In conclusion, SIBO test result is not associated to a higher severity of gastrointestinal symptoms and we consider it is not a useful test when managing patients with nonspecific symptoms or DGBI patients. The auto-perceived anxiety or depression scales and some biomarkers, mainly histamine, are associated and correlate with more severe symptoms so we think therapeutic and research efforts should focus on mood and biomarkers.

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Table 1. Characteristics of the patients.

| | |
|---|---|
| Age (years) | 45.7 (16.2) |
| Sex : Female/ male | 49 (70%)/ 21 (30%) |
| Background predisposing SIBO | 10 (14.3%) |
| IBS Roma IV criteria | 31 (44.3%) |
| Total IBSSS | 275 (450) |
| -IBSSS healthy <75 | 1 (1.4%) |
| -IBSSS mild disease 75-150 | 8 (11.4%) |
| -IBSSS moderate disease 150-300 | 33 (47.1%) |
| -IBSSS severe disease >300 | 28 (40%) |

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|---|---|
| HAD-A | 8.0 (19) |
| -HAD-A: <7; 7-11;>11 | 33 (47.1%); 22 (31.4%); 15 (21.4) |
| HAD-D | 4.0 (17) |
| -HAD-D:<7; 7-11; >11 | 53 (75.7%); 12 (17.1%); 5 (7.1%) |
| Hemoglobin (g/dL) (n=69) | 14 (7) |
| Platelets (*$10^3$/uL) (n=69) | 254 (300) |
| Ferritin (ng/mL) (n=57) | 84 (623) |
| Folic (ng/mL) (n=53) | 6.0 (18) |
| Vitamine $B_{12}$ (pg/mL) (n=56) | 475 (668) |
| RCP (mg/dL) (n=31) | 1 (39) |
| FC (ug/g) (n=28) | 29.5 (1548) |
| Positive SIBO test (n=68) | 51 (75%) |
| -Hidrogen production | 19 (27.9%) |
| -Methane production | 49 (72.1%) |
| Citruline (pg/mL) | 266.6 (3329.6) |
| Ghrelin (pg/mL) | 5.5 (14.2) |
| Histamine (ng/mL) | 347.3 (2095.6) |
| I-FABP (ng/mL) | 2.9 (111.0) |
| TRPV-1 (ng/mL) | 38.3 (40.5) |

FC, fecal calprotectin; HAD, Hospital Anxiety and Depression scale; IBS, Irritable bowel Syndrome; IBSSS, Irritable Bowel Syndrome Severity Scale; I-FABP, Intestinal-Fatty Acid Binding Protein; RCP, Reactive C Protein; TRPV-1, Transient Receptor Potential Cation Channel Subfamily V, Member 1.

Table 2. Variables associated to total IBSSS.

| | Total IBSSS | P | Mild IBSSS (75-150) | Moderate IBSSS (150-300) | Severe IBSSS (>300) | P |
|---|---|---|---|---|---|---|
| Age | -0.354 | <0.001 | 64.0 (35) | 44.0 (49) | 39.5 (47) | 0.002 |
| Female/ male | 275.0 (420)/ 290 (405) | 0.863 | 3/5 | 26/7 | 19/9 | 0.126 |
| Sibo predisposing background yes/ no | 277.5 (420)/ 265.0 (355) | 0.669 | 2/6 | 4/29 | 4/24 | 0.791 |
| HAD-A | 0.391 | <0.001 | 3.5 (7.0) | 7.0 (15) | 10.0 (16) | <0.00 |

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|---|---|---|---|---|---|---|
| | | | | | | <0.001 |
| HAD-D | 0.409 | <0.001 | 1.5 (8) | 4.0 (14) | 6.5 (17) | 0.009 |
| Hemoglobin (g/dL) | 0.015 | 0.904 | 14.7 (3) | 13.6 (5) | 14.2 (6) | 0.105 |
| Platelets (*$10^3$/uL) | 0.116 | 0.343 | 189 (221) | 254 (297) | 268 (269) | 0.223 |
| Ferritin (ng/mL) | 0.138 | 0.307 | 84.0 (197) | 57.5 (266) | 108.0 (618) | 0.211 |
| Folate (ng/mL) | -0.182 | 0.191 | 6.2 (6) | 6.8 (18) | 5.1 (9) | 0.072 |
| Vit. $B_{12}$ (pg/mL) | 0.029 | 0.830 | 532.0 (599) | 471.0(661) | 442.5 (525) | 0.701 |
| PCR (mg/dL) | 0.139 | 0.398 | 1.0 (5) | 1.0 (29) | 2.0 (8) | 0.633 |
| FC (ug/g) | -0.128 | 0.418 | 105.0 (1494) | 48.0 (1548) | 20.0 (313) | 0.298 |
| SIBO test +/- | 275.0 (380)/ 300.0 (405) | 0.994 | 6/2 | 25/7 | 51/17 | 0.836 |
| Hidrogen +/- | 270.0 (295)/ 290.0 (410) | 0.854 | 1/7 | 11/21 | 7 /21 | 0.422 |
| Methane +/- | 275.0 (380) / 300.0 (405) | 0.929 | 6/2 | 24/8 | 19/9 | 0.812 |
| Citruline (pg/mL) | 0.027 | 0.826 | 288.6 (699.2) | 265.4 (427.4) | 255.4(3326.6) | 0.891 |
| Ghrelin (pg/mL) | 0.224 | 0.063 | 5.3 (5.7) | 5.5 (13.4) | 5.8 (12.9) | 0.452 |
| Histamine (ng/mL) | 0.279 | 0.019 | 232.6 (294.2) | 334.7 (733.6) | 415.4 (2095.6) | 0.027 |
| I-FABP (ng/mL) | 0.066 | 0.589 | 2.7 (5.5) | 2.9 (110.1) | 2.9 (38.8) | 0.664 |
| TRPV-1 (ng/mL) | 0.019 | 0.878 | 38.8 (3.5) | 37.8 (16.4) | 38.6 (29.7) | 0.550 |

FC, fecal calprotectin; HAD, Hospital Anxiety and Depression scale; IBS, Irritable bowel Syndrome; IBSSS, Irritable Bowel Syndrome Severity Scale; I-FABP, Intestinal-Fatty Acid Binding Protein; RCP, Reactive C Protein; TRPV-1, Transient Receptor Potential Cation Channel Subfamily V, Member 1. Spearman test. U Mann Whitney test. Kruskall Wallis test.

Table 3. Variables associated to IBSSS items.

| | Abd. pain severity | Abd. pain duration | Distension severity | Insatisfaction | Life disruption |
|---|---|---|---|---|---|
| Age | -0.442** | -0.235 | -0.364* | 0.036 | -0.127 |
| HAD-A | 0.405** | 0.359* | 0.296* | -0.044 | 0.414** |
| HAD-D | 0.444** | 0.424** | 0.239* | -0.037 | 0.516** |
| Hemoglobin (g/dL) | 0.048 | -0.902 | -0.021 | 0.121 | 0.061 |
| Platelets (*$10^3$/uL) | 0.115 | 0.067 | 0.305* | -0.040 | 0.042 |

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|---|---|---|---|---|---|
| Ferritin (ng/mL) | 0.100 | 0.055 | -0.085 | 0.231 | 0.130 |
| Folate (ng/mL) | -0.246 | -0.119 | -0.100 | 0.040 | -0.183 |
| Vit $B_{12}$ (pg/mL) | -0.102 | -0.031 | 0.065 | -0.044 | -0.062 |
| PCR (mg/dL) | 0.060 | -0.010 | 0.015 | 0.328* | 0.083 |
| CF (ug/g) | -0.243 | -0.151 | -0.166 | 0.303 | -0.174 |
| SIBO test +/ - | 50.0 (90)/ 50 (100) | 6.0 (10)/ 6.0 (10) | 50 (100)/ 75 (100) | 65.0 (100)/ 65.0 (75) | 70.0 (80)/ 70.0 (70) |
| Hidrogen +/ - | 50.0 (80)/ 50.0 (100) | 5.0 (10)/ 6.0 (10) | 60 (96)/ 50 (100) | 50.0 (80)/ 65.0 (100) | 70.0 (75)/ 70.0 (80) |
| Methane +/ - | 50 (90)/ 50 (100) | 6.0 (10)/ 6.0 (10) | 50 (100)/ 75 (100) | 70.0 (100)/ 50.0 (80) | 70.0 (80)/ 70.0 (70) |
| Citruline (pg/mL) | 0.041 | 0.041 | 0.135 | 0.010 | -0.153 |
| Ghrelin (pg/mL) | 0.252* | 0.347* | 0.157 | -0.024 | 0.029 |
| Histamine (ng/mL) | 0.320* | 0.282* | 0.153 | 0.037 | 0.155 |
| I-FABP (ng/mL) | 0.132 | 0.033 | 0.314* | -0.199 | -0.018 |
| TRPV-1 (ng/mL) | 0.078 | -0.020 | 0.014 | -0.114 | -0.055 |

FC, fecal calprotectin; HAD, Hospital Anxiety and Depression scale; IBS, Irritable bowel Syndrome; IBSSS, Irritable Bowel Syndrome Severity Scale; I-FABP, Intestinal-Fatty Acid Binding Protein; RCP, Reactive C Protein; TRPV-1, Transient Receptor Potential Cation Channel Subfamily V, Member 1. Spearman test. U Mann Whitney test. *<0,05; **<0,001.

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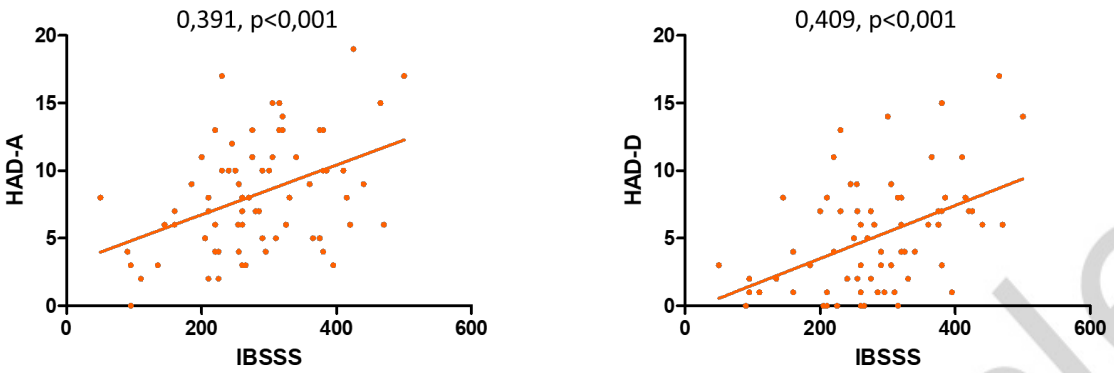


Figure 1. Gastrointestinal symptoms severity correlates with HAD-A and HAD-D. HAD, Hospital Anxiety and Depression scale; I-FABP, Intestinal Fatty Acid Binding Protein; IBSSS, Irritable Bowel Syndrome Severity Scale. Spearman test.

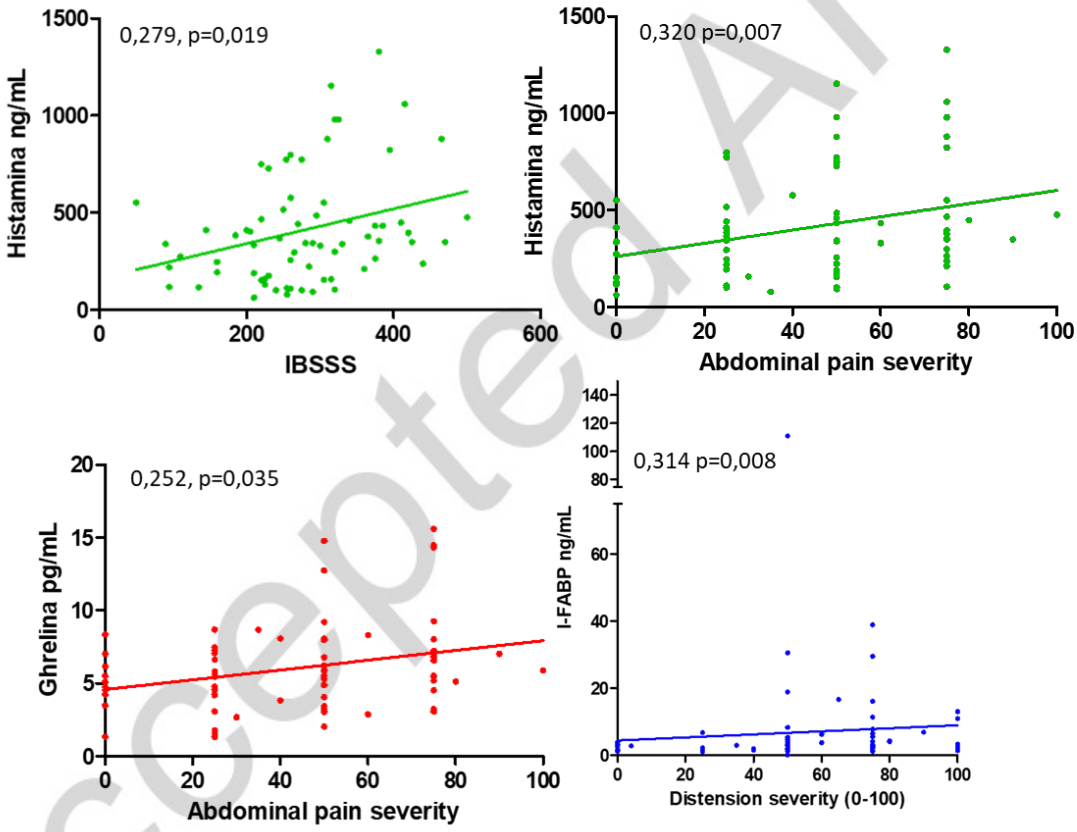


Figure 2. Gastrointestinal symptoms severity correlates with histamine, ghrelin and I-FABP levels. IBSSS, irritable bowel symptoms severity scale; I-FABP, Intestinal Fatty Acid Binding Protein. Spearman test.