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ORIGINAL ARTICLE

Concomitant Irritable Bowel Syndrome Does Not Influence the Response to Antimicrobial Therapy in Patients with Functional Dyspepsia

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Abstract

Background and Aims Antimicrobial therapy improves symptoms in patients with irritable bowel syndrome (IBS), but the efficacy in functional dyspepsia (FD) is largely unknown. While FD and IBS frequently overlap, it is unknown if concomitant IBS in FD alters the response to antimicrobial therapy in FD. Thus, we aimed to assess and compare the effect of antimicrobial therapy on visceral sensory function and symptom improvement in FD patients with and without IBS.

Methods Adult patients with FD with or without IBS received rifaximin 550 mg BD for 10 days, followed by a 6-week follow-up period. The total gastrointestinal symptom score as measured by the SAGIS (Structured Assessment of Gastrointestinal Symptoms) questionnaire and subscores (dyspepsia, diarrhea, and constipation), symptom response to a standardized nutrient challenge and normalization of the glucose breath tests were measured.

Results Twenty-one consecutive adult patients with FD and 14/21 with concomitant IBS were recruited. Treatment with rifaximin resulted in a significant ($p=0.017$) improvement in the total SAGIS score from 34.7 (±15.4) at baseline to 26.0 (±16.8) at 2 weeks and 25.6 (±17.8) at 6 weeks post-treatment. Similarly, compared to baseline there was a statistically significant improvement in SAGIS subscores for dyspepsia and diarrhea (all $p<0.05$) and effects persisted for 6 weeks post-treatment. Similarly, the symptom score (and subscores) following a standardized nutrient challenge improved significantly ($p<0.001$) 2 weeks post-treatment. The presence of concomitant IBS did not significantly influence the improvement of symptoms after antibiotic therapy (all $p>0.5$).

Conclusions In FD patients, the response to antimicrobial therapy with rifaximin is not influenced by concomitant IBS symptoms.

Keywords Rifaximin · Functional gastrointestinal disorders · Functional dyspepsia · Glucose breath test · Small bowel bacterial overgrowth · Irritable bowel syndrome

Introduction

Functional dyspepsia (FD) and irritable bowel syndrome (IBS), conceptualized as disorders of gut-brain interactions [1], are the most common functional gastrointestinal disorders (FGIDs) with a worldwide prevalence between 5 and 20% [2]. Although IBS and FD are both heterogeneous diseases, they appear to share similar underlying pathophysiological mechanisms including altered gastrointestinal motility or sensory function, increased intestinal permeability, low-grade mucosal inflammation, microbial dysbiosis, and dysfunction of the brain-gut axis with psychiatric comorbidities [3].

Supporting the role of gut microbial dysbiosis in the pathophysiology of FGIDs, small intestinal bacterial overgrowth (SIBO)—defined by excessive and/or abnormal types of bacteria in the small bowel—is one of the most widely recognized forms of microbial dysbiosis [4]. Our recent systematic reviews and meta-analyses have

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validated that there is an increased prevalence of SIBO in patients with IBS [5] and FD [6] compared to controls. In the routine clinical setting, SIBO is diagnosed utilizing breath tests and presents with symptoms that often overlap with FGIDs making it unclear if it is the cause, consequence, or an epiphenomenon with these conditions [7]. Treatment of SIBO with antibiotic therapy results in the improvement of symptoms and normalization of a positive breath test in a substantial proportion of patients [8] and supports the increasing interest in the role of microbiome-based therapies for the effective treatment of FGIDs.

Treatment with an oral, non-absorbable broad-spectrum antibiotic, rifaximin (as compared to placebo), has been associated with significant improvement in symptoms in patients with non-constipated IBS, but the gain over placebo is modest (~ 10%) [9]. In a recent randomized placebo-controlled study from Hong Kong, Tan *et al* [10] reported that rifaximin treatment was associated with significant improvement in global dyspeptic symptoms in FD subjects, although the mechanism of action remained uncertain, and these observations are yet to be confirmed or tested in patients from Western countries.

We have recently demonstrated that in FD patients with and without IBS, the intensity of meal-related symptoms (a surrogate marker for visceral sensitivity) and impairment of quality of life is closely related to the density of the bacterial colonization of the upper gastrointestinal tract [11, 12]. It is now well recognized that in the clinical setting FD and IBS symptom overlap is common. In a recent study, 22.5% of all FGID patients reported overlap of FD and IBS [13]; 48.9% of FD patients reported IBS, and 56.1% of IBS patients also reported FD, followed by belching disorder (12.8%) and functional heartburn (5.1%). Moreover, overlap is associated with more severe FGID manifestations [2].

Nearly all studies to date have focused on the effect of rifaximin in patients with IBS, but none have studied its effect in FGID patients with overlapping disorders or compared the effects in FD patients with or without concomitant IBS. Also, there are no clinical trials on the effect of antibiotic therapy on visceral sensory function. For these reasons, we have conducted a prospective audit in patients with FD with or without concomitant IBS to determine (a) the effects of rifaximin on gastrointestinal symptoms and visceral sensory function (assessed as symptom response to a standardized nutrient challenge) and; (b) if the presence of SIBO, diagnosed utilizing the glucose breath test (GBT) and presence of concomitant IBS predicts response to rifaximin therapy.

Methods and Materials

Subject Recruitment and Study Design

This is a single-center prospective audit conducted between September 2019 to January 2020 at a tertiary care Gastroenterology outpatient clinic (Princess Alexandra Hospital, Brisbane, Australia). After obtaining written informed consent, we recruited 22 consecutive adult patients, aged 18–80 years with chronic or relapsing gastrointestinal symptoms. All patients were referred because of failure to respond to established therapies for FGIDs including acid inhibitory drugs such as proton pump inhibitors (PPIs), low dose psychotropic agents, and prokinetics. All eligible patients had a normal endoscopy and had tested negative for *H pylori* on histopathological examination. Diagnosis and categorization of FD with or without concomitant symptoms of IBS were determined following the Rome IV criteria [1]. Briefly, FD patients were categorized as having epigastric pain syndrome (EPS) and/or postprandial distress syndrome (PDS) [14] and the IBS patients were further subtyped into constipation dominant (IBS-C), diarrhea dominant (IBD-D), and mixed (IBS-M) [15]. All demographic and clinical data were obtained from the state-wide integrated Electronic Medical Record (iEMR). All clinical data (including tests initiated by any healthcare providers) available on the system were reviewed to confirm/establish the patient's diagnosis.

Exclusion criteria included another diagnosis or organic lesions that could explain the gastrointestinal symptoms, severe psychiatric disease as the dominant clinical problem, recent (within 2 months) antibiotic or probiotic use, any gastric or intestinal surgery, being unsuitable for therapy due to any medical conditions, drug allergies, or inability to attend follow-up appointments.

Study Design

Patients meeting the selection criteria were consented and considered eligible for treatment with rifaximin if they reported at least moderate symptoms (defined as symptoms that could not be ignored) utilizing a validated questionnaire after a 2-week run-in period. Before initiation of treatment with rifaximin, all enrolled patients underwent a glucose breath test and a standardized nutrient challenge test. All patients were treated with rifaximin (Xifaxan, Norgine Pty Ltd, Australia) (550 mg 2 times daily) for 10 days. At the study center, rifaximin is not routinely available for the treatment of patients with FGIDs. Thus, all patients were naïve to treatment with rifaximin. Gastrointestinal symptoms were reassessed 2 weeks and 6 weeks after completion of rifaximin therapy (Fig. 1).

Assessment of Gastrointestinal Symptoms

The severity of gastrointestinal and extraintestinal symptoms, bowel habits, and psychiatric comorbidities was assessed utilizing SAGIS (Structured Assessment of Gastrointestinal Symptoms [16]) questionnaire. Any potential adverse events related to the study medication were also recorded.

Glucose Breath Test (GBT) for the Diagnosis of SIBO

The GBT was utilized to diagnose SIBO. GBT was performed in all patients at baseline and 2 weeks post-treatment in all those who were hydrogen and/or methane positive for SIBO at baseline according to a standardized protocol (described in supplementary materials and methods). GBT-positive status is defined as (i) an increase of > 20 ppm above the baseline in the hydrogen concentration by 90 min and/or (ii) an increase of > 10 ppm above baseline in the methane concentration. The patients were diagnosed with SIBO if they satisfied any of these criteria.

Standardized Nutrient Challenge Test

Visceral sensitivity was assessed by a standardized nutrient challenge test performed at baseline and 2 weeks post-treatment, according to our previously published protocol [17] (protocol described in the supplementary materials and methods).

Outcome Measures

The primary outcome of this study was the improvement in the total SAGIS score at 2 and 6 week after the completion of antibiotic treatment. Secondary outcomes included reduction in the SAGIS subscores, nutrient challenge symptom score and subscores, and normalization of a positive GBT.

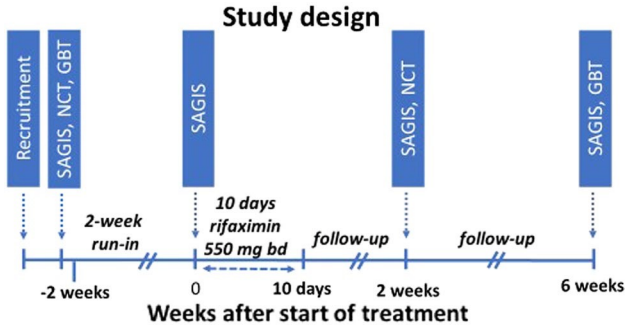

Fig. 1 Study design

Statistical Analysis

Mean values and frequencies of patient characteristics including age, gender, body mass index (BMI), underlying gastrointestinal disease and their subtypes, medical comorbidities, medications, positive GBT status including hydrogen and methane values (peak and baseline), and gastrointestinal symptoms scores are presented. Categorical data were compared between groups using the Chi-square test, while continuous data were compared using the t test or Mann–Whitney U test. Descriptive statistics for variables are reported as means ± standard deviation or proportions. Wilcoxon signed-rank test was used to compare the variable at two time points (before and after treatment). Differences or associations were considered significant if $p < 0.05$. All calculations used SPSS version 26 (IBM Inc., Armonk, NY, USA). Because of the exploratory nature of the study, no sample size calculation was conducted. The available sample size provides adequate statistical power (0.8) at the 0.05 (two-tailed) level of statistical significance for Cohen d effect sizes of 0.8 and above. An exception to this is the comparisons made among the subset of patients who underwent the glucose breath test (Tables 1, 2, 3, 4), where an effect size of $d = 1.64$ is required to provide the same statistical power.

Results

Characteristics of the Study Population

During September 2019—January 2020, 22 consecutive patients who fulfilled the eligibility criteria were enrolled and completed the study. All patients who underwent GBT, NCT, and completed the SAGIS questionnaire. The study design is outlined in Fig. 1. Of the 22 patients, 21 were included in the final analysis. One patient did not commence treatment and was not available for follow-up, and hence was excluded from the final analysis.

The demographic and clinical characteristics of the study population are summarized in Table 1. The majority of the patients in this study were females 13 (61.9%), and the mean age of these patients was 51.2 (± 18.8) years. 11/21(52.4%, 95% CI 29.8–74.3) patients were on PPI therapy. The majority 14/21(66.7%, 95% CI 43.0–85.4) of patients had an overlap of FD and IBS, and 7 (33.3%, 95% CI 14.6–57.0) had FD alone.

The GBT test for SIBO suggested 4/21 were positive based on our diagnostic criteria for hydrogen and methane (19.1%, 95% CI 5.5–41.9). There was no statistically significant difference between the baseline GBT-positive vs. -negative groups (Table 4), (all $p > 0.3$) with regard to the demographic characteristics, PPI use, total SAGIS score,

and SAGIS subscores. With regard to the NCT, the total symptom score was higher in the GBT positive- vs. -negative group but failed statistical significance ($p = 0.21$). However, the abdominal pain subscore was significantly higher in the GBT-positive subjects (128.9 ± 86.5) as compared to the GBT negative group (32.4 ± 44.3, $p = 0.004$), while the fullness subscore showed a trend for higher scores in GBT-positive subjects (170.5 ± 112.8 vs 88.1 ± 65.7, $p = 0.063$).

Outcomes

Effect of Rifaximin on Gastrointestinal Symptoms

Overall, treatment with rifaximin was associated with a significant ($p < 0.005$) improvement in total SAGIS score and the SAGIS dyspepsia (epigastric symptom, nausea/vomiting) and diarrhea subscores (Table 2, Figs. 2, and 3). Analyzing the subscores, there were significant (all $p < 0.05$) reductions in the SAGIS dyspepsia score and subscores (epigastric symptom, nausea/vomiting) at 2 and 6 weeks after the completion of rifaximin, as shown in Fig. 3. Treatment with rifaximin was associated with significant improvement in the SAGIS diarrhea subscore (5.6 ± 3.1 at baseline vs 3.9 ± 3.9 at 6 weeks post-treatment, $p = 0.039$, see Fig. 3c) but not the SAGIS constipation subscore ($p = 0.240$, see Fig. 3d). Importantly, no significant differences in the SAGIS total score and subscores at baseline, 2-, and 6-week post-rifaximin therapy were observed in FD patients with and without IBS (Table S1 &Fig. 2b) suggesting no effect from IBS on patient FD symptom response to rifaximin.

Effect of Rifaximin on Symptom Response to a Standardized NCT (Visceral Sensory Function) and GBT in SIBO Positive Subjects

As compared to the baseline total symptom score (345.4 ± 346.7), rifaximin treatment resulted in a 90% reduction in NCT total symptom score (40.8 ± 26.4, $p = 0.001$, Fig. 4a) and a > 85% reduction across all individual subscores (all $p < 0.005$) at the 2-week follow-up NCT (Table 3, Fig. 4b and C). However, the improvements in the symptom response to the nutrient challenge test after treatment with rifaximin were not different for GBT-positive versus -negative subjects.

After antibiotic treatment, both the total SAGIS score (and subscores) and the NCT score (and subscores) were not significantly different in FD patients with or without IBS, (all $p > 0.5$, Table S1, and Fig. 2b). Only 2/4 SIBO positive FD patients underwent a follow-up GBT 2 weeks after completion of rifaximin therapy, and both subjects had normalization of their GBT.

Safety

None of the patients reported any adverse events during the treatment phase (10 days) or during the follow-up period (6 weeks).

Discussion

This is the first study to compare the efficacy of rifaximin in FD patients with and without IBS. We found that treatment with rifaximin resulted in a statistically significant (> 25%) reduction in the total SAGIS score and the SAGIS dyspepsia- and the diarrhea subscores 2 weeks after completion of rifaximin treatment, and this response was maintained at

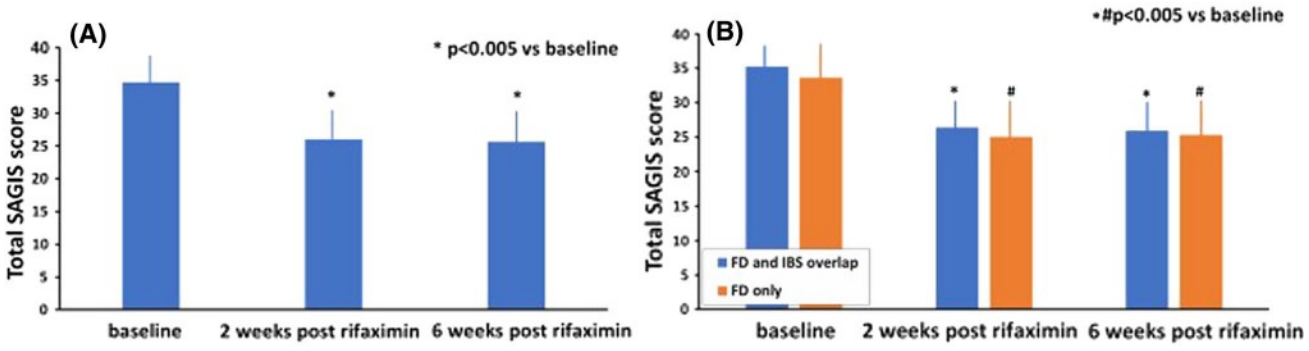

Fig. 2 **a** Total SAGIS (structured assessment of gastrointestinal symptom) score at baseline and 2 and 6 weeks post-completion of rifaximin therapy. As compared to the baseline, there is a statistically significant improvement (25.1%) in the total SAGIS score at the 2-week assessment and this improvement is maintained at the 6-week assessment. **b** SAGIS Total score, % of baseline in FD patients with and without IBS)

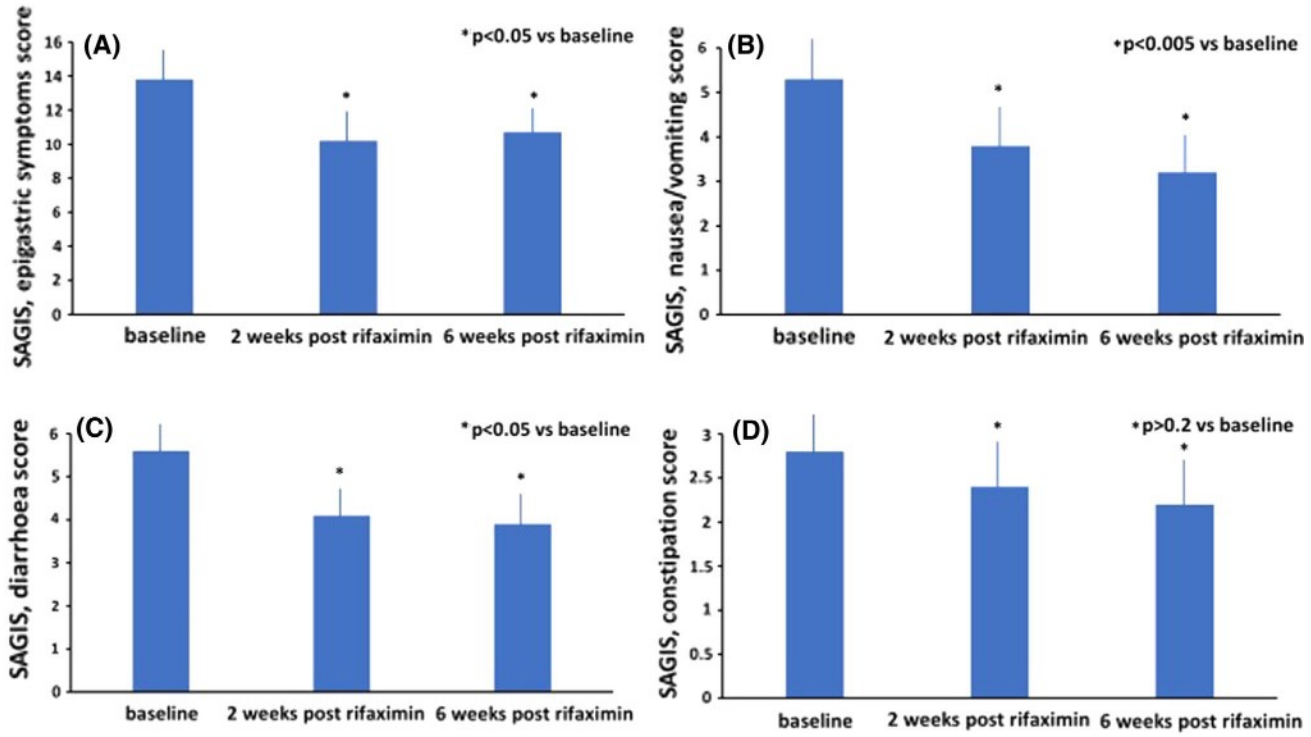

Fig. 3 **a** SAGIS, Epigastric symptom score at baseline and 2 and 6 weeks post-rifaximin therapy. As compared to the baseline, there is a statistically significant improvement (26.1%) in the epigastric pain SAGIS score at 2 weeks post-rifaximin therapy and this improvement is maintained at the 6-week assessment. **b** SAGIS, Nausea/vomiting score at baseline, and 2 and 6 weeks post-completion of rifaximin therapy. As compared to the baseline, there is a statistically significant improvement (28.3%) in the SAGIS, nausea, and vomiting score at the 2-week assessment, and this improvement is maintained at the 6-week assessment. **c** SAGIS, diarrhea score at baseline, and 2 and 6 weeks post-completion of rifaximin therapy. As compared to the baseline, there is a statistically significant improvement (26.7%) in the SAGIS, diarrhea score at the 2-week assessment, and this improvement is maintained at the 6-week assessment. **d** SAGIS, Constipation score at baseline and 2 and 6 weeks post-completion of rifaximin therapy. As compared to the baseline, there is no significant improvement (14.2%) in the SAGIS, constipation score at 2 and 6 weeks post-completion of rifaximin therapy

the 6-week assessment. Similarly, treatment with rifaximin resulted in a greater than 90% reduction in the symptom response to a standardized NCT. Subgroup analyses revealed that FD patients with a positive baseline GBT had a significantly higher symptom response to the standardized nutrient challenge for abdominal pain while the fullness subscore just failed statistical significance. The improvement of the total score and the respective subscores was not significant comparing GBT-positive and -negative subjects. All patients who underwent GBT after rifaximin treatment had normalization of their breath test. Most importantly, the presence of concomitant IBS did not affect the primary or the secondary outcome parameters. Finally, treatment with rifaximin was not associated with adverse events during the treatment phase or during the follow-up.

One of the strengths of our study is the simultaneous assessment of the improvement in gastrointestinal symptoms and changes in the visceral sensory function in response to antibiotic therapy in FD patients. It is well established that in both FD and IBS patients, symptoms are frequently related to meals and can include abdominal pain, bloating, early satiety, fullness, belching, and nausea [18]. Patients with FD show visceral hypersensitivity (i.e., increased symptom responses after standardized stimulation within the gastrointestinal tract) [19] and the symptom response to a standardized nutrient challenge is higher in FD patients as compared to healthy asymptomatic controls [20]; further, visceral hypersensitivity correlates with symptom severity [21]. In this study, the abdominal pain score during the baseline nutrient challenge was significantly higher in patients with a positive GBT, and overall, treatment with rifaximin was associated with marked improvement in the symptom response following a standardized nutrient challenge. This is indeed aligned with previous studies [11] that have demonstrated a positive link between the density of the mucosa-associated microbiome and the symptom response to a nutrient challenge.

Another important aspect of this study is the comparison of the efficacy of rifaximin in FD patients with and without IBS, as compared to other studies that have assessed the effect of antimicrobial therapy in patients with FD [10] or IBS [22] alone. FGIDs often exist with

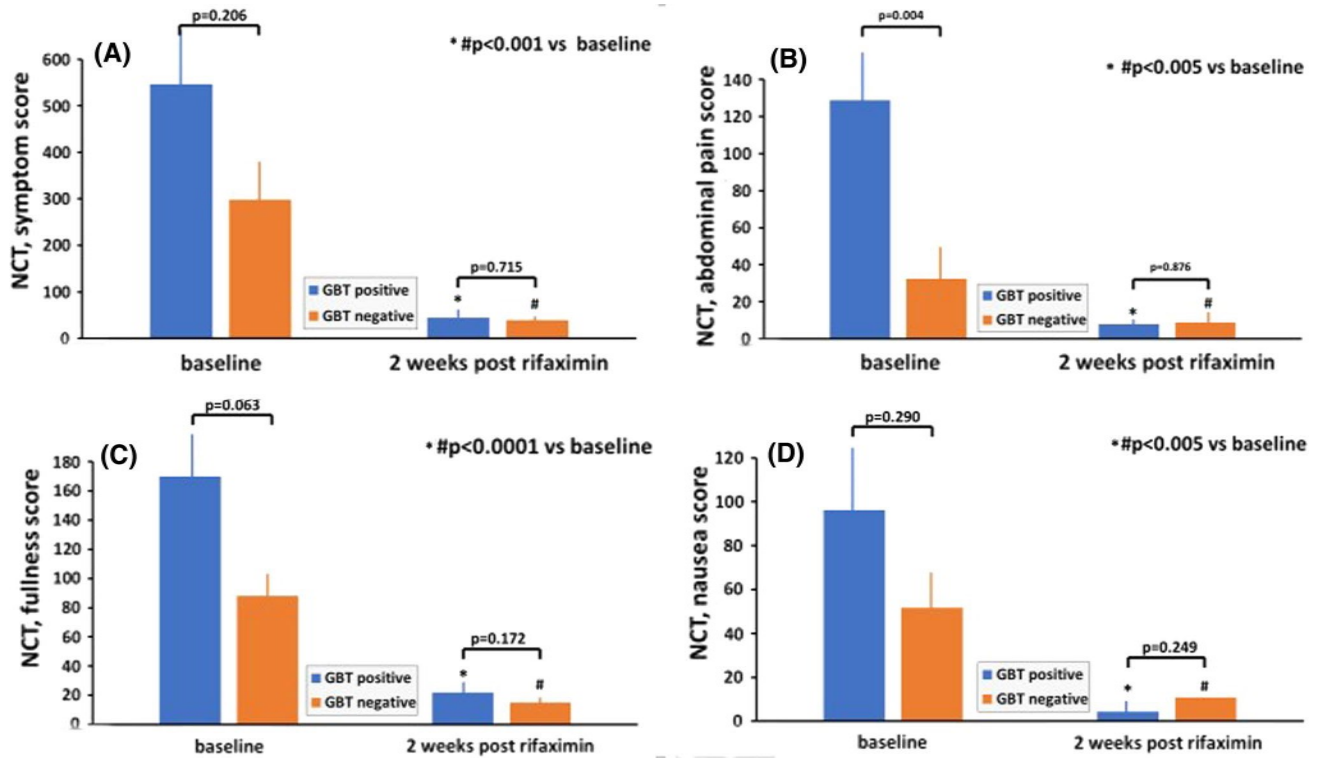

Fig. 4 **a** Symptom score following a standardized nutrient challenge test at baseline and 2 weeks post-completion of rifaximin therapy. As compared to baseline, there is a statistically significant reduction (91.7%) in symptom score 2 weeks post-completion of rifaximin therapy. SIBO-positive FGID patients have a higher symptom score at baseline as compared to FGID patients who are negative for SIBO on GBT. **b** Abdominal pain score following a standardized nutrient challenge at baseline and 2 weeks post-completion of rifaximin therapy. As compared to baseline, there is a statistically significant reduction (93.8%) in abdominal pain score at 2 weeks post-completion of rifaximin therapy. SIBO-positive FGID patients have a significantly higher abdominal pain subscore at baseline as compared to FGID patients who are negative for SIBO on GBT. **c** Fullness score following a standardized nutrient challenge at baseline and 2 weeks post-completion of rifaximin therapy. As compared to baseline, there is a statistically significant reduction (87.05%) in fullness score at 2 weeks post-completion rifaximin therapy. SIBO-positive FGID patients have a higher fullness score at baseline as compared to FGID patients who are negative for SIBO on GBT. **d** Nausea score following a standardized nutrient challenge at baseline and 2 weeks post-completion of rifaximin therapy. As compared to baseline, there is a statistically significant reduction (95.4%) in nausea score at 2 weeks post-completion of rifaximin therapy. SIBO-positive FGID patients have a significantly higher nausea score at baseline as compared to FGID patients who are negative for SIBO on GBT

a spectrum of symptoms, and that overlap of symptoms in FD and IBS could potentially be explained by intestinal dysbiosis, including changes in the density [12] and taxonomic composition of the mucosa-associated [13] and stool [23] microbiota. In this study, we found that in FD patients, the presence of concomitant IBS-type symptoms did not predict response to rifaximin with regard to gastrointestinal symptoms and visceral sensory function. This potentially suggests that small intestinal dysbiosis plays a role in the manifestation of symptoms in both FD and IBS. While antimicrobial therapy significantly improved gastrointestinal symptoms and the symptom response to a standardized nutrient challenge, the effect on extraintestinal symptoms including self-reported anxiety and depression was minimal. Thus, it is unlikely that the changes of the gastrointestinal microbiome result in alterations of the gut-brain axes. This could highlight the importance of bidirectional interaction of the gut-brain axis in FGIDs and that integrated multi-modality treatment approach—pharmacological and non-pharmacological—may be required in at least a subset of FGID patients.

This study is not without limitations. While antimicrobial therapy is established in IBS and FD, we have not included a placebo control arm since the chosen study design was thought to be appropriate to answer the study question. While this study aimed to compare the response of FD patients with and without IBS, there were no data to guide a formal power calculation for this comparison. However, the very small difference suggests that even if a difference exists, the difference is unlikely to be clinically relevant. It also needs to be noted that this study is not designed and powered to assess the precise mechanism of action of rifaximin including effects on stool, colonic, or small intestinal mucosa-associated microbiome. Consistent

Table 1 Demographic and baseline characteristics of study patients

Groups	$n=21$
Demographic characteristics	
Age (years)*	51.2 (±18.8)
Gender (female), *n* (%)	13 (61.9)
BMI (kg/m [2])*	26.6 (±5.9)
Current smokers, *n* (%)	2 (9.5)
PPI users, *n* (%)	11 (52.4)
Prokinetic users, *n* (%)	1 (4.8)
Anti-depressant, *n* (%)	7 (33.3)
Glucose breath test	
Glucose breath test positive, *n* (%)	4 (19.0)
Methane- & hydrogen-positive SIBO, *n* (%)	4 (19.0)
Methane baseline, ppm*	15.9 (±16.3)
Methane peak, ppm*	24.0 (±26.9)
Hydrogen baseline, ppm*	5.0 (±3.6)
Hydrogen peak, ppm*	11.8 (±11.0)
Clinical diagnosis	
FD Only, *n* (%)	21 (100.0)
IBS Only, *n* (%)	14 (66.7)
FD/IBS Overlap, *n* (%)	14 (66.7)
FD subtype	
EPS, *n* (%)	0
PDS, *n* (%)	4 (19)
EPS/PDS Overlap, *n* (%)	17 (81.0)
IBS subtype	
IBS-D, *n* (%)	4 (19)
IBS-C, *n* (%)	2 (9.5)
IBS-M, *n* (%)	8 (38.1)

Statistically significant *p* values ($p<0.05$) are highlighted in bold

*Indicates values expressed as mean (±standard deviation)

BMI; body mass index, PPI; proton pump inhibitor, IBS; irritable bowel syndrome, IBS-C; IBS with constipation, IBS-D; IBS with diarrhea, IBS-M; mixed IBS, FD; functional dyspepsia, EPS; epigastric pain syndrome, PDS; postprandial distress syndrome, SIBO; small intestinal bacterial overgrowth, ppm; parts per million, *n*; number

with placebo-controlled studies in IBS, in the current study, treatment with rifaximin significantly improved gastrointestinal symptoms in FD patients and the symptom response to a standardized nutrient challenge. At least in subjects with a positive GBT, rifaximin potentially corrects gut microbial dysbiosis in the proximal small intestine, which would explain its efficacy in improving dyspepsia symptoms and symptoms of bloating and belching in FD patients. A recent study by Rezaie et al., [22] also demonstrated the utility of a positive breath test as a predictor of response to rifaximin in patients with IBS-D. All these point toward the antimicrobial effect of rifaximin on the proximal small intestinal (rather than colonic) dysbiosis. This could potentially explain the effect of rifaximin in SIBO and a subgroup of patients with FD and IBS, but its exact mechanisms of action remain unknown. Moreover, in a recent study in patients with non-constipating IBS [24], treatment with rifaximin (as compared to placebo) was associated with only a modest change in the stool microbial diversity. In an elegant animal study by Xu *et al* [25], treatment with rifaximin resulted in the improvement of small intestinal (ileal) dysbiosis and subsequent prevention of mucosal inflammation, barrier impairment, and visceral hyperalgesia in response to chronic psychological stress.

The majority of the patients recruited for this study had previously or currently been on PPI therapy, which is considered as a risk factor for SIBO [26]. The overuse of PPI therapy is common in both FD and IBS. A very elegant article Chey *et al* [27] proposed the "PPI hypothesis": that PPIs can exacerbate IBS via alteration of the intestinal microbiota in a subclinical manner. As such, PPIs are potentially a confounder in the link between SIBO and FGIDs, and SIBO positivity on breath tests may reflect a PPI-induced dysbiosis in FGID subjects. Hence, the clinical benefits of antimicrobial therapy in FGIDs could, at least in part, be a result of a temporary reversal of PPI-related dysbiosis and subsequent symptoms superimposed on underlying FGIDs. Such a consideration is bolstered by our recent findings that microbial load on duodenal tissue is greatest in FD subjects, and particularly so for FD subjects that are PPI users [11]. However, the small sample size of the current study does not allow to assess the effect of rifaximin treatment in FGIDs patient with and without PPI therapy.

In this study, we used GBT to diagnose SIBO. A recent study by Rezaie *et al* [22] identified a positive baseline breath test as a predictor for improvement in gastrointestinal symptoms following treatment with rifaximin in patients with IBS. In our study, FD patients with a positive GBT at baseline had a significantly greater improvement in the symptom response to a standardized nutrient challenge. This suggests that small intestinal dysbiosis at baseline influences the response to antibiotic therapy.

Breath tests have significant methodological limitations and limited sensitivity and specificity as diagnostic tests to assesses SIBO in patients with various gastrointestinal [5, 28] and extraintestinal disorders [29]. In contrast, small bowel aspirate and culture are widely considered the gold standard for SIBO diagnosis but are invasive and the appropriate thresholds of microbial density for SIBO diagnosis are still debated [4]. In our recent study [11], utilizing a novel molecular technique we have shown that FGID patients had significantly higher small intestinal bacterial loads as compared to healthy controls. This once again highlights the lack of a true "gold standard" for diagnosis of SIBO. Thus, future clinical trials would ideally need a head-to-head comparison of the available tests to diagnose SIBO, to better characterize the small intestinal

Table 2 Gastrointestinal symptoms, assessed utilizing SAGIS (structured assessment of gastrointestinal symptom) questionnaire in patients pre- and post-completion of rifaximin therapy (after 2 weeks and 6 weeks)

Gastrointestinal symptoms (n = 21)	Pre-rifaximin treatment	Post-rifaximin treatment (2 weeks)	Post-rifaximin treatment (6 weeks)	*p* Values			
				Overall	Pre- vs post-rifaximin treatment (2 wks)	Pre- vs post-rifaximin treatment (6 wks)	Post-rifaximin treatment 2 wks vs 6 wks
Total SAGIS score	34.7 (±15.4)	26.0 (±16.8)	25.6 (±17.8)	**0.017**	**0.005**	**0.005**	0.811
SAGIS, Epigastric symptoms	13.8 (±5.3)	10.2 (±7.9)	10.7 (±8.0)	**0.042**	**0.034**	0.075	1.000
SAGIS, Constipation	2.8 (±2.4)	2.4 (±2.2)	2.2 (±2.2)	0.240	0.957	0.290	0.926
SAGIS, Diarrhea	5.6 (±3.1)	4.1 (±3.2)	3.9 (±3.9)	**0.039**	**0.036**	**0.045**	1.000
SAGIS, Nausea/vomiting	5.3 (±5.0)	3.8 (±4.4)	3.2 (±4.0)	**0.008**	0.078	**0.015**	0.071
SAGIS, acid regurgitation/gas	4.2 (±2.7)	2.8 (±2.4)	3.2 (±2.8)	0.064	0.065	0.323	0.804
SAGIS, Extraintestinal symptoms	3.1 (±1.7)	2.7 (±1.5)	2.5 (±1.6)	0.154	0.171	0.306	1.000

Statistically significant *p* values ($p < 0.05$) are highlighted in bold. All values are expressed as mean (±standard deviation)

Table 3 Gastrointestinal function test results (nutrient challenge test) in patients pre- and post-completion of rifaximin therapy

Gastrointestinal function testing, ($n = 21$)	Pre-rifaximin treatment	Post-rifaximin treatment (2 weeks)	*p* value
Nutrient challenge test, symptom score	345.4 (±346.7)	40.8 (±26.4)	**0.001**
Nutrient challenge test, abdominal pain score	50.8 (±64.8)	8.5 (±8.6)	**0.008**
Nutrient challenge test, fullness subscore	103.6 (±80.4)	16.9 (±8.2)	**0.0001**
Nutrient challenge test, nausea score	60.2 (±9.7)	9.7 (±10.0)	**0.005**

Statistically significant *p* values ($p < 0.05$) are highlighted in bold. All values are expressed as mean (±standard deviation)

dysbiosis in FGID patients and ideally predict response to antimicrobial therapy. Finally, not all patients completed the follow-up glucose breath test. However, both patients who had repeat breath test post-completion of rifaximin therapy tested negative for SIBO. In this study, FD patients (with and without IBS), treated with antimicrobial therapy, had sustained improvement in their gastrointestinal symptoms at the 6-week assessment. Beyond doubt, future trials with longer follow-up period are now required. In addition, some studies [30] suggest that a combination of rifaximin and neomycin is superior to rifaximin alone in methane-positive SIBO. Thus, antimicrobial interventions with combination antibiotics might be beneficial.

In conclusion, this is the first study that reveals that concomitant IBS symptoms appear not influence the response to antimicrobial therapy in patients with FD. Treatment with rifaximin is well tolerated and associated with sustained improvement in the gastrointestinal symptoms also evidenced by the reduced symptom response to the nutrient challenge. A positive baseline GBT is associated with an increased pain score during the nutrient challenge, and this difference disappears after the antimicrobial therapy. Rifaximin likely targets the small intestinal dysbiosis/SIBO which may play an important role in the pathophysiology in at least a subset of FGID patients.

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Author's contributions *AS and GH*were involved in study idea, concept and design, data analysis and interpretation of data, drafting of the manuscript. *RG and MM* helped in data analysis, interpretation of data, drafting of the manuscript, and review of final manuscript. *MJ* contributed to data analysis and review of final manuscript. *NT, MW, and GC* were involved in interpretation of data, drafting of the manuscript,

Table 4 Group comparisons based on glucose breath test results

Groups	Glucose breath test positive (either hydrogen (H_2) or methane (CH_4))	Glucose breath test negative (either hydrogen (H_2) or methane (CH_4))	*p* value
Demographic characteristics			
Age (years)*	58.3 (±6.5)	49.5 (±20.5)	0.418
Gender (female, $n=13$), *n* (%)	1 (25)	12 (70.6)	0.253
BMI (kg/m^2) *	29.3 (±4.5)	25.9 (±6.07)	0.308
Current smokers, ($n=2$), *n* (%)	1 (25)	1 (5.9)	0.352
PPI ($n=11$), *n* (%)	3 (75)	8 (47.1)	0.586
Glucose breath test results			
Total *n* (%)	4 (19)	17 (81)	
CH_4 baseline, ppm*	**40.0 (±20.5)**	**10.2 (±8.5)**	**0.0001**
CH_4 peak, ppm*	**65.0 (±24.7)**	**14.4 (±16.5)**	**0.0001**
H_2 baseline, ppm*	5.5 (±3.8)	4.9 (±3.6)	0.764
H_2 peak, ppm*	11.8 (±11.6)	11.8 (±11.2)	0.991
Gastrointestinal function testing pre-rifaximin therapy			
Nutrient challenge test, symptom score	546.2 (±473.7)	298.1 (±309.4)	0.206
Nutrient challenge test, abdominal pain score	**128.9 (±86.5)**	**32.4 (±44.3)**	**0.004**
Nutrient challenge test, fullness score	**170.5 (±112.8)**	**88.1 (±65.7)**	**0.063**
Nutrient challenge test, nausea score	96.1 (±131.1)	51.7 (±56.5)	0.290
Gastrointestinal function testing post-rifaximin therapy			
Nutrient challenge test, symptom score	45.3 (±22.7)	39.8 (27.7)	0.715
Nutrient challenge test, abdominal pain score	7.9 (±6.3)	8.7 (±9.2)	0.876
Nutrient challenge test, fullness score	22.0 (±9.9)	15.7 (±7.6)	0.172
Nutrient challenge test, nausea score	4.4 (±5.6)	10.9 (v10.6)	0.249
Gastrointestinal symptoms (n=21) pre-rifaximin therapy			
Total SAGIS Score	32.5 (±11.6)	35.2 (±16.5)	0.764
SAGIS, Epigastric symptoms	13.8 (±5.3)	13.5 (±6.1)	0.916
SAGIS, Constipation	3.5 (±1.9)	2.6 (±2.5)	0.509
SAGIS, Diarrhea	5.0 (±4.2)	5.7 (±3.0)	0.695
SAGIS, Nausea/vomiting	4.3 (±3.5)	5.6 (±5.3)	0.640
SAGIS, Acid regurgitation/gas	3.5 (±2.4)	4.4 (±2.8)	0.585
SAGIS, Extraintestinal symptoms	2.7 (±1.4)	3.1 (±1.8)	0.710
2 weeks post-rifaximin therapy			
Total SAGIS Score	34.5 (±22.9)	23.9 (±15.2)	0.269
SAGIS, Epigastric symptoms	15.8 (±10.0)	8.9 (±7.0)	0.122
SAGIS, Constipation	3.0 (±2.4)	2.3 (±2.2)	0.578
SAGIS, Diarrhea	5.3 (±6.7)	3.8 (±2.1)	0.422
SAGIS, Nausea/vomiting	3.0 (±3.5)	3.9 (±4.7)	0.712
SAGIS, Acid Regurgitation/gas	4.5 (±3.4)	2.4 (±2.1)	0.127
SAGIS, Extraintestinal symptoms	3.0 (±1.4)	2.6 (±1.5)	0.624
6 weeks post-rifaximin therapy			
Total SAGIS Score	34.3 (±22.6)	23.7 (±16.7)	0.295
SAGIS, Epigastric symptoms	15.8 (±9.6)	9.5 (±7.4)	0.162
SAGIS, Constipation	3.0 (±2.4)	2.0 (±2.2)	0.433
SAGIS, Diarrhea	5.8 (±7.8)	3.4 (±2.5)	0.289
SAGIS, Nausea/vomiting	3.4 (±4.3)	2.5 (±2.4)	0.692
SAGIS, Acid regurgitation/gas	4.3 (±3.6)	3.0 (±2.6)	0.412
SAGIS, Extraintestinal symptoms	3.0 (±1.4)	2.4 (±1.7)	0.530

Statistically significant *p* values ($p<0.05$) are highlighted in bold. All values are expressed as mean (±standard deviation)

BMI; body mass index, PPI; proton pump inhibitor, ppm; parts per million, SAGIS; structured assessment of gastrointestinal symptoms

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Declarations

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