Article

Not peer-reviewed version

Inflammatory Prostatitis plus IBS-D Subtype and Correlation with the Immunomodulating Agent Imbalance in Seminal Plasma: Novel Combined Treatment

Roberto Castiglione *, Gaetano Bertino, Beatrice Ornella Vicari, Agostino Rizzotto, Giuseppe Sidoti, Placido D'Agati, Michele Salemi, Giulia Malaguarnera, Enzo Vicari

Posted Date: 9 August 2024

doi: 10.20944/preprints202408.0684.v1

Keywords: irritable bowel syndrome; inflammatory prostatitis; IBS-severity scoring system; rifaximin; probiotic VSL#3; interleukins; seminal plasma

Article

Inflammatory Prostatitis Plus IBS-D Subtype and Correlation with the Immunomodulating Agent Imbalance in Seminal Plasma: Novel Combined Treatment

Roberto Castiglione [1,*], Gaetano Bertino [1], Beatrice Ornella Vicari [2], Agostino Rizzotto [3], Giuseppe Sidoti [4], Placido D'Agati [5], Michele Salemi [6], Giulia Malaguarnera [2] and Enzo Vicari [1]

[1] Department of Clinical and Experimental Medicine, University of Catania, 95123, Catania, Italy
[2] Research Center "The Great Senescence", University of Catania, 95100 Catania, Italy
[3] Center of Rare Diseases, Policlinico Catania, University of Catania, 95100 Catania, Italy
[4] U.O.S.D. Internal Medicine Ambulatory Andrology & Endocrinology, ARNAS - Garibaldi, 95123, Catania, Italy
[5] Department "GF Ingrassia" Hygiene and Public Health, University of Catania, Catania, Italy, 95123, Catania, Italy
[6] Oasi Research Institute-IRCCS, Troina, Italy
* Correspondence: robertocastiglionen@tiscali.it - Department of Clinical and Experimental Medicine, University of Catania, 95123, Catania, Italy

Abstract: We recently demonstrated the effectiveness of long-term treatment with rifaximin and the probiotic VSL#3 at improving urogenital and gastrointestinal symptoms in patients with both chronic inflammatory prostatitis (IIIa prostatitis) and diarrhea-predominant irritable bowel syndrome (IBS-D), relative to patients with IBS-D alone. Because low-grade inflammation of the intestine and prostate may be one of the reasons for co-developing both IIIa prostatitis and IBS-D, we designed the present study to once again evaluate the efficacy of combined rifaximin and VSL#3 treatment in patients affected by IIIa prostatitis plus IBS-D, but we also measured seminal plasma pro-inflammatory (IL-6) and anti-inflammatory (IL-10) cytokines before and after treatment. We consecutively enrolled 124 patients with IIIa prostatitis and IBS-D (diagnosed using the Rome III criteria). Patients were randomized into two groups: group A (n=64) was treated with rifaximin (seven days per month for three months) followed by VSL#3, and group B (n=60) was treated with a placebo. At the beginning of the study and after three months of treatment, all patients completed NIH-CPSI and IBS-SSS questionnaires to score the severity of their urogenital and gastrointestinal symptoms, and we measured seminal plasma levels of IL-6 and IL-10 using ELISA. We defined patients who improved following the treatment as those with a ≥6-point reduction in their NIH-CPSI score or ≥50-point reduction in their IBS-SSS score. By the end of the intervention, 68.7% and 62.5% of patients from group A reported improved NIH-CPSI and IBS-SSS scores, respectively, compared to only 3.3% and 5% of the placebo group. Group A patients also had significantly lower mean seminal plasma levels of IL-6 (11.3 vs 32.4 pg/mL) and significantly higher mean levels of IL-10 (7.9 vs 4.4 pg/mL) relative to baseline, whereas levels of IL-6 and Il-10 did not change in the placebo group. The co-occurrence of two clinical phenotypes (IBS-D plus IIIa prostatitis) result in increased intestinal inflammation and altered immune activation, both of which can be balanced by treatment with rifaximin and VSL#3.

Keywords: irritable bowel syndrome; inflammatory prostatitis; IBS-severity scoring system; rifaximin; probiotic VSL#3; interleukins; seminal plasma

Introduction

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) and irritable bowel syndrome (IBS-D) are somatoform and functional disorders with a prevalence estimated at 11-16% [1,2], or

 doi:10.20944/preprints202408.0684.v1

approximately 11% of the population globally. Quantifying prevalence can be challenging because only 30% of people who experience IBS symptoms will consult a physician for their IBS symptoms [3–5]. These two syndromes, which are often associated with stress and depression [6], can coexist in approximately 30% of patients screened by andrologists and gastroenterologists [7–9]. Both syndromes are characterized by a multifactorial pathogenesis, and each condition is defined based on its clinical presentation rather than by clear diagnostic markers or findings. The diagnosis of IBS requires the administration of the Rome III questionnaire [10], while CP/CPSS is identified through the National Institute of Health's Chronic Prostatitis Symptom Index (NIH-CPSI) [11,12].

We recently demonstrated the efficacy of a long-term treatment with the probiotic VSL#3 and antibiotics (e.g., rifaximin) in patients with CP/CPPS plus IBS with diarrhea (IBS-D) compared to patients with IBS-D alone. Specifically, the probiotic/antibiotic treatment we tested resulted in greater response rate of gastrointestinal and urinary symptoms [13], as assessed with the IBS Severity Scoring System (IBS-SSS) and the NIH-CPSI. In this case, improvement was defined as a ≥50-point reduction in the IBS-SSS score [14] and a ≥6-point reduction in the total NIH-CPSI score [15]. We found that, after three months of the treatment, 77.7% of patients with NIH-IIIa prostatitis and IBS-D demonstrated a significant (≥50-point) improvement in their IBS-SSS responder rate and 71.1% of this same patient group demonstrated a ≥6-point improvement in their total NIH-CPSI score [13].

The results of our preliminary study suggested that patients with both CP/CPPS and IBS-D could have similar underlying pathophysiology, but because the diseases differed in severity, we were unable to draw any clear explanations for the treatment's mechanism of action. Although the causes of IBS-D are not fully understood, previous research has suggested that IBS-D patients have low-grade intestinal inflammation [16]. Cytokines, which are important modulators of immune responses and inflammatory reactions, can play a central role in intestinal inflammation [17]. Some studies have detected a possible imbalance of circulating cytokines in IBS patients, marked by elevated serum inflammatory IL cytokines (such as IL-6, IL-8 and TNF-α) and decreased serum anti-inflammatory cytokines (such as IL-10) relative to healthy controls [18–20]. The severity of intestinal inflammation has also been associated with the severity of IBS symptoms [21].

Thus, the primary outcome of this study was to assess the seminal plasma concentration of the pro-inflammatory cytokine interleukin 6 (IL-6) and the anti-inflammatory cytokine IL-10 before and after treatment with rifaximin and VSL#3. The secondary outcome was to compare symptom profiles between IIIa prostatitis patients with IBS-D who received the treatment and those that received a placebo. Symptom profiles were measured as the proportion of patients who demonstrated improvement as measured using validated questionnaires such as the IBS-SSS and NIH-CPSI.

Materials and Methods

We enrolled 124 male outpatients (median age: 56 years, range: 50-68 years) with a confirmed diagnosis of inflammatory chronic pelvic pain syndrome (IIIa-prostatitis) and the diarrhea-predominant IBS subtype (IBS-D) (Rome III criteria). Patients were enrolled between January 2016 and February 2019 and were consecutively recruited from the Geriatry Unit Clinic, Post-Graduate School of Geriatry, AOE Cannizzaro, Catania, and Andrology and Endocrinology Unit Clinic, Policlinic University of Catania (Catania, Italy). The diagnoses of IIIa-CPPS and IBS-D were made 24–60 months before the patients were included in this study.

Inclusion Criteria

Diagnostic Rome III Criteria for IBS

The IBS-D diagnosis was based on the presence of abdominal pain or discomfort for at least three months in the previous six months, with at least two of the following indicators: (i) pain improved after defecation; (ii) symptoms were associated with a change in the frequency of defecation; and (iii) symptoms were associated with a change in the appearance of stool. These symptoms were assessed using a simple "10-point" objective questionnaire based on the Rome III IBS module [22,23]. Patients

 doi:10.20944/preprints202408.0684.v1

were diagnosed with IBS-D if they had loose, mushy, or watery stools in the last three months, without any hard or lumpy stools (i.e., question 9 = 0 and question 10 > 0) [22,23].

Diagnostic Symptoms Suggestive of IIIa-CPPS

IIIa-CPPS symptoms were evaluated using the NIH-CPSI questionnaire. According to EAU guidelines, patients were asked if they had experienced any of the following symptoms for at least three months during the six months preceding the study [24]: pain or discomfort, unrelated to urination, in the pubic or bladder area, perineum, testis, or tip of the penis; ejaculatory pain; pain or burning during urination; incomplete emptying; and frequent urination. For a diagnosis of IIIa-CPPS, a patient's NIH-CPSI pain sub-score needed to be >8 (moderate to severe) [25]. In addition, patients were assigned to the IIIa-CPPS category if any of the following were true: the Meares-Stamey four-glass test [26] produced negative bacteriological findings, the white blood cell (WBC) count in the expressed prostate secretion (EPS) was ≥10 per high power field (HPF), or the WBC count in the VB3 was ≥5 per HPF [27].

Exclusion Criteria

Exclusion criteria were as follows: 1) history of chronic bacterial prostatitis (NIH type II) with a positive bacteriological finding on spermculture or on the Meares-Stamey four-glass test [26]; 2) evidence of IIIb-CPSS. Defined as an EPS WBC count less than 10 per HPF or a VB3 WBC count less than 5 per HPF [27]; 3) obesity (defined as a BMI ≥30 kg/m^2); 4) history of gastrointestinal bleeding or duodenal or gastric ulcers. In addition, we excluded 5) patients suffering from chronic or acute illness that could interfere with the study; 6) patients taking medications that could interfere with the study (including anti-inflammatory drugs, PPIs, antidepressants, anti-diarrhoeal, prokinetics, and antispasmodic agents); 7) patients affected by major concomitant diseases; 8) patients with known anatomical abnormalities of the urinary tract or with evidence of other urological diseases; 9) patients with residual urine volume >50 ml resulting from bladder outlet obstruction; 10) patients without evident depressive symptoms; and 11) patients who consumed antibiotics or probiotics in the four weeks prior to entering the study.

Study Design and Treatment Plan

At their first clinic visit (V0), all 124 patients were randomized into two groups using permuted-block randomization with a 1:1 allocation ratio and a block size of four. Patients were assigned random numbers in the order in which they were included and then received the prescription for their respective study group.

Group A (n=64) was prescribed the non-absorbable antibiotic rifaximin (200 mg tablets); Normix®, Alpha Wassermann, Alanno, PE, Italy) and instructed to take two tablets twice a day, seven days per month, for three months. The three-month antibiotic regiment was followed by treatment with the probiotic combination VSL#3 (VSL#3®, Ferring SpA, Milan, Italy: 450 × 10^9 CFU/day). Probiotic capsules were taken once daily.

Group B were treated with a placebo. To match the treatment provided to Group A, patients in Group B were prescribed a placebo tablet (rifaximin-placebo) to be taken seven days each month for three months. The placebo contained sodium starch glycolate (type A), glycerol distearate, colloidal anhydrous silica, talc, microcrystalline cellulose, hypromellose, titanium dioxide E171, disodium edetate, propylene glycol, and red iron oxide E172. For the second half of the intervention, when Group A patients were taking the probiotic supplement, the placebo group received a VSL#3 placebo that consisted of microcrystalline cellulose and magnesium stearate, with gelatin as the encapsulating material.

The study protocol was approved by the internal Institutional Review Board of the University of Catania (24/201517 on 17 December 2015), and informed written consent was obtained from each patient. We collected clinical histories from all patients (in both the treatment group and the placebo

 doi:10.20944/preprints202408.0684.v1

group), performed a physical examination, and administered the NIH-CPSI and Rome III questionnaires for prostatitis and IBS, respectively [30,31].

Analytical Measurements

All analytical measurements were performed using blind-coded samples (i.e., no name or personal identifiers). Measurements were taken at the time of enrollment (V0) and again after three months (V3) of the treatment or placebo. For all measurements, we obtained seminal plasma specimens from each participant after 3-5 days of the sexual intercourse. After allowing the sample to clot for at least 30 min, samples were centrifuged at 1600 g for 15 min. Serum samples were then stored at −80 °C until analysis. Seminal plasma levels of IL-6 and IL-10 were measured in duplicate using commercially available sandwich enzyme-linked immunosorbent assay kits (Human IL-6 ELISA and Human IL-10 ELISA, BD Biosciences, Milan, Italy).

Assessment of Symptoms

We assessed patient symptoms of IBS-D and NIH-IIIa prostatitis using specific validated questionnaires that patients completed regularly during the treatment period as well as through urological visits performed at the start of therapy (V0) and again three months later (V3).

IBS-D. To monitor IBS-D symptoms and changes, both groups (treatment and placebo) were asked to report their symptoms weekly using the IBS-SSS questionnaire [31]. This questionnaire includes five items on a 0-100 mm visual analogue scale: severity of abdominal pain (Question 1), frequency of abdominal pain (Question 2), severity of abdominal distension (Question 3), dissatisfaction with bowel habits (Question 4), and interference with quality of life (Question 5). The total score is used to classify subjects as having no symptoms (<75), mild IBS (75-174), moderate IBS (175-300), or severe IBS (>300), with total scores ranging from 0 to 500 mm.

Outcome measures for IBS-D. We considered an IBS-SSS score reduction of at least 50 points to be an improvement [14].

Outcome measures for NIH-IIIa prostatitis. Urogenital symptoms were assessed at V0 and V3 using the NIH-CPSI score. We defined a clinically appreciable improvement of NIH-IIIa prostatitis symptoms as a minimum six-point reduction in the total NIH-CPSI score between V0 and V3 [15]. Other endpoints were expressed as the severity of individual CP/CPPS symptoms (measured as scores on the individual NIH-CPSI subscales) as well as WBC counts on EPS after a prostate massage.

Statistical Analysis

We used SPSS 9.0 for Windows for all statistical evaluation. Quantitative data were expressed as median and range, and qualitative data were expressed as percentages. Within-group differences in NIH-CPSI or IBS-SSS questionnaire scores between V0 and V3 were analyzed using Wilcoxon's signed rank test. Mann-Whitney U-tests were used to compare the treatment and placebo groups at each time point. For all statistical tests, we considered $p<0.05$ to be significant.

Results

The two groups of IIIa prostatitis plus IBS-D patients had similar demographic characteristics and baseline clinical and laboratory results (Table 1). At the pre-treatment visit (V0), we detected elevated seminal plasma inflammatory cytokine 6 (IL-6) and decreased serum anti-inflammatory cytokine-10 (IL-10) in both groups. At V3, the mean IL-6 level was significantly lower in the treatment group relative to the placebo group (11.3 pg/mL vs. 34.8 pg/mL, respectively; $p<0.01$) (Table 2). Moreover, we found a statistically significant increase in IL-10 levels in the treatment group between enrolment (V0) and the follow-up visit (V3) (4.4 pg/mL vs. 7.9 pg/mL, respectively; $p<0.01$). In contrast, serum IL-6 and IL-10 levels were unchanged at the follow-up visit in the placebo group (Table 2).

 doi:10.20944/preprints202408.0684.v1

Table 1. Characteristics of patient groups with type IIIa chronic prostatitis plus irritable bowel syndrome (IBS-D), after randomization and before treatment.

	Type IIIa prostatitis plus IBS-D		
	Group A	**Group B**	**P values**
Patients (n)	64	60	ns
Age (years)	55 (50-68)	54 (48-68)	ns
BMI (kg/m²)	24 (21-28)	25 (21-29)	ns
Time since diagnosis (months)	37 (24-66)	34 (24-60)	ns
WBC on EPS after prostate massage	13 (10-18)	12 (10-16)	ns
NIH-CPSI total score	21.2 (15-24)	20 (13-24)	ns
IBS-SSS, mean (range)	325 (90-450)	313 (85-433)	ns
>300 (severe)	27.7 (n=18)	24.0 (n=18)	
175-300 (moderate)	56.9 (n=37)	58.7 (n=44)	
75 - 175 (mild)	15.4 (n=10)	17.3 (n=13)	
<75 (no IBS)	0 (n=0)	0 (n=0)	
Seminal plasma cytokine levels (pg/mL), mean (range)			
IL-6	30.6 (8-67)	28.7 (8-62)	ns
IL-10	4.4 (2.1-9.3)	4.7 (2.7-10.2)	ns

Irritable bowel syndrome = IBS; BMI= body mass index. Values were expressed as mean and range (in parentheses); NA= not applicable; Ns= not significant.

Table 2. Primary and secondary outcomes in patients with chronic prostatitis (Type IIIa) plus IBS-D, assessed before the treatment (V0) and 3 months afterward (V3) treatment with (R+VSL#3) (group A) or placebo (group B).

	Type IIIa prostatitis plus IBS-D			
	Group A (treatment group)		**Group B (placebo)**	
Study time-point	**V0 (n=64)**	**V3 (n=64)**	**V0 (n=60)**	**V3 (n=60)**
Primary outcome measures: seminal plasma cytokine levels (pg/mL)				
Mean IL-6 (range)	32.4 (10-74)	11.3 (5-20)#	31.8 (10.5-68)	34.8 (12.8-67)
Mean IL-10 (range)	4.4 (2.1-9.3)	7.9 (4.9-16)#	4.7 (3.3-8.8)	5.2 (3.5-10)
Secondary outcomes measures: symptom severity (responder rate)				
Outcomes measures related to III-a prostatitis				
Prostatitis symptoms (NIH-CPSI score) Total score	21.2 (15-24)	16.4*° (10-21)	20 (13-24)	19.7 (13-23)
Pain subscale	11.9 (8-15)	9.0*° (6-11)	11.7 (8-16)	10.8 (8-11)
Urinary subscale	4.5 (3-6)	3.3*° (0-3)	4.2* (3-6)	4.0 (3-5)
Quality-of-Life subscale	4.8 (3-7)	3.8*° (2-6)	4.8 (3-8)	4.2 (3-7)

 doi:10.20944/preprints202408.0684.v1

NIH-CPSI responder rate (≥6 point decline) No. / total No. (%)	NA	44/64 (68.7)	NA	2/60 (3.3)
WBC on EPS after prostate massage	13 (10-18)	7*° (5-9)	12 (10-16)	10.0 (8-12)
Outcomes measures related to IBS-D				
Mean IBS severity score	298.4 (180-410)	162.5*° (115-338)	307.5 (195-425)	272.5 (175-408)
IBS responder rate (≥50 point decline) No./total No. (%)	NA	40/64 (62.5)	NA	3/60 (5.0)

Irritable bowel syndrome = IBS. Values were expressed as mean and range (in parentheses); NA= not applicable; #p<0.01 vs. pre-treatment matched –values, and matched-values of placebo group patients; *p<0.05 vs. pre-treatment matched –values; °p<0.05 vs. matched-values of placebo group patients.

We also found that total NIH-CPSI scores decreased significantly (p<0.05) in IIIa prostatitis patients during the treatment, from a baseline mean score of 21.2 to a score 16.4 at V3. All subscales of the NIH-CPSI assessment (i.e., pain, urination, and quality-of-life) had also improved. Overall, 68.7% of the treatment patients (44 out of 64 patients in group A) demonstrated clinical improvement, defined as a ≥6-point reduction in total NIH-CPSI score. In contrast, patients from the placebo group did not show any significant improvement in their NIH-CPSI scores, either the total score or any subscales, during the treatment (Table 2). The percentage of placebo patients that demonstrated improvement (3.3%; 2 out of 60 patients in group B) was correspondingly significantly lower than in the treatment group.

With respect to gastrointestinal symptoms, symptom severity scores were similar between group A and group B at the baseline visit (Table 1). However, at V3, the IBS-SSS score had decreased significantly in the treatment group (mean 162.5, range 115-338) but remained unchanged in the placebo group (mean 272.5, range 175-408). The significant improvement from baseline IBS-SSS scores in the treatment group was associated with a response rate of 62.5% (i.e., 40 of the 64 patients demonstrated a ≥50-point decline), which was also significantly higher than the response rate in placebo group (5.0%, or 3 of the 60 patients).

Finally, we found that mean leukocyte counts on EPS after a prostate massage decreased significantly in the treatment group (from 13 to 7, p< 0.05) relative to the placebo group.

Compliance

All patients in both groups completed the treatment as planned, and no significant adverse events were reported by any patient during the intervention. However, 7.8% (five out of 64) of the subjects in the rifaximin group, and 9.4% (six out of 64) of the subjects in the matched-placebo group, reported at least one mild adverse event during the first half of the study period (i.e., antibiotic treatment). For this study, mild adverse events were defined as mild and transient symptoms of the gastrointestinal or respiratory tract that could have a possible connection with the study. During the second half of the study period (i.e., probiotic treatment), only 8 of the 64 subjects (12.5%) in the treatment group and 10 of the 64 subjects in the placebo group (15.6%) reported at least one adverse event.

Discussion

Comorbidities are common in IBS patients. These comorbidities can, like IBD, affect the gastrointestinal tract, including functional chest pain, heartburn, dyspepsia, and/or abdominal pain [35], but they can also produce extra-intestinal symptoms, in which case the clinical presentation can be classified in a phenotyping system based on which of the following six domains are affected: urinary, psychosocial, organ-specific, infection, neurologic/systemic, and tenderness (UPOINT) [36–38].

 doi:10.20944/preprints202408.0684.v1

In a previous study, we found that IBS presents with chronic prostatitis in 31.2% of patients who seek medical advice for PS or IBS in andrological or gastroenterological settings [7]. These patients also had more severe urinary and gastrointestinal symptoms than patients with either chronic prostatitis or IBS alone, which we measured as significantly higher scores in the NIH-CPSI score (total score and the pain subscale) and the Rome III questionnaire [7]. These results agree with other reports [8,9].

Despite intensive study over the past decade, clinical trials have failed to identify effective therapies for IBS patients. Some therapeutic interventions for IBS-D are designed to target presumed IBS-induced alterations in the gut microbiota; these treatments include rifaximin, medical food serum-derived bovine immunoglobulin, prebiotics, probiotics, and dietary modification [39]. Rifaximin has been shown to be an effective option for IBS-D treatment [40]: beyond its direct bactericidal effects, rifaximin dampens host pro-inflammatory responses to bacterial products and has demonstrated antibiotic efficacy against isolates derived from patients with small intestinal bacterial overgrowth [39]. The effects of another treatment option, probiotics, have been less clear; although probiotic supplementation with different (multi) species pools [41] had beneficial effects on global IBS symptoms and abdominal pain, studies have been unable to draw definitive conclusions about their efficacy [42].

On the other hand, patients with CP/CPPS traditionally receive empirical treatment. The most common CP/CPPS treatments in clinical practice include antimicrobial agents, alpha-adrenergic receptor antagonists [25,27,43], or a combined, multi-modal therapy [44–46].

The results of our controlled study reported here are consistent with the findings from our previous study using a similar treatment [13]: only the rifaximin- and probiotic-treated group had improved symptoms and reduced mean leukocyte counts on EPS after prostate massage. Thus, we demonstrated that in patients with both NIH IIIa prostatitis plus IBS-D, the administration of rifaximin followed by VSL#3 over a three-month period is effective at alleviating symptoms. For example, 68.7% of the treatment group registered a ≥ 6-point reduction in their total NIH-CPSI score compared to only 3.3% of the placebo group. Notably, the percentage of our treatment cohort that demonstrated improvement even exceeds the placebo effect of ~64% reported by long-term studies [15]. In addition, the therapeutic intervention also resulted in a significant improvement in IIIa prostatitis symptoms: 62.5% of the treatment group registered a >50-point decline in IBS-SSS scores compared to only 5% of the placebo group. Although there is no consensus on what exactly constitutes a clinically meaningful improvement as a result of a therapeutic, a 50% improvement in the primary end point and a 10–15% improvement in the global outcome measure (relative to a placebo group) has been suggested as clinically significant [41].

The relief of urinary and gastrointestinal symptoms relief, significant reduction of leukocyte counts on EPS after prostate massage, and significantly alleviated oxidative stress that we observed after treatment with rifaximin plus VSL#3 implied that an altered gut microbiome was at least partly associated with the pathogenesis of inflammatory prostatitis plus IBS-D. Our combined clinical findings therefore suggested that the therapeutic approach of rifaximin and probiotics should be incorporated into the treatment plan for patients with these coexisting conditions.

Cytokines are important modulators of immune responses and inflammatory reactions and therefore may be involved in the pathogenesis of IBS [17]. Specifically, low-grade inflammation of the intestine combined with dysbiosis in the gut microbiota may lead to the development of IBS-D and coexisting inflammatory prostatitis. Some studies have suggested that these comorbidities are marked by an imbalance of circulating cytokines, with IBS patients showing elevated serum inflammatory cytokines (such as IL-6, IL-8 and TNF-α) and decreased serum anti-inflammatory cytokines (such as IL-10) relative to healthy controls [18–20]. Our results agree with previous data documenting significantly alleviated oxidative stress after probiotic supplementation; in our study, probiotic supplementation significantly reduced serum concentrations of pro-inflammatory cytokines (such as TNF-a, IL-6, and others) and increased serum concentrations of anti-inflammatory IL-10 [47].

 doi:10.20944/preprints202408.0684.v1

In agreement with a recent systemic review and meta-analysis, documenting after probiotic supplementation a significant reduction of serum concentration of pro-inflammatory cytokines (such as TNF-a, IL-6, and others) and anti-inflammatory IL-10 [47], in our present study conducted on a group of patients who complained IIIa-prostatitis plus IBS-D subtype, we documented, in parallel to an improvement of urogenital and gastrointestinal symptoms, in their seminal plasma, after treatment with rifaximin and subsequent probiotic VSL#3 supplementation, a significant reduction of pro-inflammatory IL-6 and increased of anti-inflammatory IL-10. Therefore, our data indicate that a combined treatment with rifaximin and probiotic supplementation helps to alleviate symptoms and correct their seminal plasma cytokine imbalance.

Author Contributions: All authors contributed in the same way to research and writing of the manuscript.

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

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank dr. Emanuele Gaetano Schilirò, Department of Humanities, University of Catania, Italy, for the accurate linguistic editing.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Roberts, R.O.; Lieber, M.M.; Rhodes, T.; Girman, C.J.; Bostwick, D.J.; Jacobsen, S.J. Prevalence of a physician-assigned diagnosis of prostatitis: The Olmstead County study of urinary symptoms and health status among men. *Urol 1998;* 51: 578–584.
2. Collins, M.M.; Meigs, J.B.; Barry, M.J.; Corkery, W.E.; Giovannucci, E.; Kawachi, I. Prevalence and correlates of prostatitis in the health professionals follow-up study cohort. J Urol 2002; 167: 1363–1366.
3. El-Salhy, M. Irritable bowel syndrome: diagnosis and pathogenesis. World J Gastroenterol 2012; 18 (37): 5151–5163.
4. Canavan, C.; West, J.; Card, T. The epidemiology of irritable bowel syndrome. Clin Epidemiol. 2014 Feb 4; 6: 71-80.
5. Soares, R.L. Irritable bowel syndrome: a clinical review. World J Gastroenterol 2014; 14; 20 (34):12144-60.
6. Han L.; Zhao L.; Zhou Y.; Yang C.; Xiong T.; Lu L.; Deng Y.; Luo W.; Chen Y.; Qiu Q.; Shang X.; Huang L.; Mo Z.; Huang S.; Huang S.; Liu Z.; Yang W.; Zhai L.; Ning Z.; Lin C.; Huang T.; Cheng C.; Zhong LD L.; Li S.; Bian Z.; Fang X. Altered metabolome and microbiome features provide clues in understanding irritable bowel syndrome and depression comorbidity. ISME J 2022; 16(4): 983-996.
7. Vicari, E.; La Vignera, S.; Arcoria, D.; Condorelli, R.; Vicari, L.O.; Castiglione, R.; Mangiameli, A.; Calogero, A.E. High frequency of chronic bacterial and non-inflammatory prostatitis in infertile patients with prostatitis syndrome plus irritable bowel syndrome. PLoS One 2011; 6: e18647
8. Bullones Rodríguez, M.A., Afari, N.; Buchwald, D.S. National Institute of Diabetes and Digestive and Kidney Diseases Working Group on Urological Chronic Pelvic Pain. Evidence for overlap between urological and nonurological unexplained clinical conditions. Urol 2009; 182: 2123-2131.
9. Liao, C.H.; Lin, H.C.; Huang, C.Y. Chronic Prostatitis/Chronic Pelvic Pain Syndrome is associated with Irritable Bowel Syndrome: A Population-based Study.Sci Rep. 2016 May 26; 6: 26939.
10. Drossman, D.A.; Camilleri, M.; Mayer, E.A.; Whitehead, W.E. AGA technical review on irritable bowel syndrome. Gastroenterol 2002; 123: 2108–2131.
11. Litwin, M.S.; McNaughton-Collins, M.; Fowler, F.J. Jr,; Nickel, J.C.; Calhoun, E.A.; Pontati, M.A.; Alexander, R.B.; Farrar, J.T.; O'Leary, M.P. The NIH Chronic Prostatitis Symptom Index (NIH-CPSI): Development and validation of a new outcomes measure. J Urol. 1999; 162: 364–368.
12. Collins, M.M,; Pontari, M.A.; O'Leary, M.P.; Calhoun, E.A.; Santanna, J.; Landis, J.R.; Kusek, J.W.; Litwin, M.S. Quality of life is impaired in men with chronic prostatitis: the Chronic Prostatitis Collaborative Research Network. J Gen Intern Med 2001; 16: 656-662.
13. Vicari, E.; Salemi, M.; Sidoti, G.; Malaguarnera, M.; Castiglione, R. Symptom Severity Following Rifaximin and the Probiotic VSL#3 in Patients with Chronic Pelvic Pain Syndrome (Due to Inflammatory Prostatitis) Plus Irritable Bowel Syndrome. Nutrients. 2017;9 (11): 1208.
14. Francis, C.Y.; Morris, J.; Whorwell, P.J. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther 1997; 11: 395–402.
15. Propert, K.J.; Litwin, M.S.; Wang, Y.; Alexander, R.B.; Calhoun, E.; Nickel, J.C.; O'Leary, M.P.; Pontari, M.; McNaughton-Collins, M. Responsiveness of the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) Qual Life Res 2006; 15: 299–305.

16. Vicario, M.; González-Castro, A.M.; Martínez, C.; Lobo, B.; Pigrau, M.; Guilarte, M.; de Torres, I.; Mosquera, J.L.; Fortea, M.; Sevillano-Aguilera, C.; Salvo-Romero, E.; Alonso, C.; Rodiño-Janeiro, B.K.; Söderholm, J.D.; Azpiroz, F.; Santos, J. Increased humoral immunity in the jejunum of diarrhoea-predominant irritable bowel syndrome associated with clinical manifestations. Gut. 2014; 64: 1379–1388.
17. Choghakhori, R.; Abbasnezhad, A.; Hasanvand, A.; Amani, R. Inflammatory cytokines and oxidative stress biomarkers in irritable bowel syndrome: association with digestive symptoms and quality of life. Cytokine. 2017; 93: 34–43.
18. Chen Q, Zhang H, Sun CY, He QY, Zhang RR, Luo BF, Zhou ZH, Chen XF. Evaluation of two laboratory model methods for diarrheal irritable bowel syndrome. Mol Med. 2023; 12: 29 (1):5.
19. Bashashati, M.; Rezaei, N.; Shafieyoun, A.; McKernan D.P.; Chang, L.; Öhman, L.; Quigley, E.M.; Schmulson, M.; Sharkey, K.A.; Simrén, M. Cytokine imbalance in irritable bowel syndrome: a systematic review and meta-analysis. Neurogastroenterol Motil. 2014; 26: 1036–1048.
20. Seyedmirzaee, S.; Hayatbakhsh, M.M.; Ahmadi, B.; Baniasadi, N.; Bagheri Rafsanjani, A.M.; Nikpoor, A.R.; Mohammadi, M. Serum immune biomarkers in irritable bowel syndrome. Clin Res Hepatol Gastroenterol 2016; 40: 631-637.
21. Di Nardo, G.; Barbara, G.; Cucchiara, S.; Cremon, C.; Shulman, R.J.; Isoldi, S.; Zecchi, L.; Drago, L.; Oliva, S.; Saulle, R., Barbaro MR, Stronati L. Neuroimmune interactions at different intestinal sites are related to abdominal pain symptoms in children with IBS. Neurogastroenterol Motil. 2014; 26: 196–204.
22. Longstreth, G.F.; Thompson, W.G.; Chey, W.D.; Houghton, L.A.; Mearin, F.; Spiller, R.C. Functional bowel disorders. Gastroenterol 2006; 130:1480–91.
23. Anbardan, S.J.; Daryani, N.E; Fereshtehnejad, S.M; Taba Taba Vakili, S.; Keramati, M.R; Ajdarkosh, H Gender role in irritable bowel syndrome: a comparison of irritable bowel syndrome module (ROME III) between male and female patients. J Neurogastroenterol Motil 2012; 18: 70–7.
24. Kranz J, Bartoletti R, Bruyère F, Cai T, Geerlings S, Köves B, Schubert S, Pilatz A, Veeratterapillay R, Wagenlehner FME, Bausch K, Devlies W, Horváth J, Leitner L, Mantica G, Mezei T, Smith EJ, Bonkat G. European Association of Urology Guidelines on Urological Infections: Summary of the 2024 Guidelines. Eur Urol. 2024; 86(1):27-41.
25. Nickel, J.C.; Downey, J.; Johnston, B.; Clark. J. Canadian Prostatitis Research Group. Predictors of patient response to antibiotic therapy for the chronic prostatitis/chronic pelvic pain syndrome: a prospective multicenter clinical trial. J Urol 2001; 165: 1539 -1544.
26. Meares, E.M.; Stamey, T.A. Bacteriologic localization patterns in bacterial prostatitis and urethritis. Invest Urol 1968; 5: 492–518.
27. Schaeffer, A.J.; Knauss, J.S.; Landis, J.R.; Propert, K.J.; Alexander, R.B.; Litwin, M.S.; Nickel, J.C.; O'Leary, M.P.; Nadler, R.B.; Pontari, M.A.; Shoskes, D.A.; Zeitlin, S.I.; Fowler, J.E Jr.; Mazurick, C.A.; Kusek, J.W.; Nyberg, L.M. Leukocyte and bacterial counts do not correlate with severity of symptoms in men with chronic prostatitis: the National Institutes of Health Chronic Prostatitis Cohort Study. J Urol 2002; 168: 1048-1053.
28. Hungin, A.P.; Chang, L.; Locke, G.R.; Dennis, E.H.; Barghout, V. Irritable bowel syndrome in the United States: prevalence, symptom patterns and impact. Aliment Pharmacol Ther. 2005; 21: 1365–1375.
29. Vicari, E.; La Vignera, S.; Castiglione, R.; Condorelli, R.A.; Vicari, L.O.; Calogero, A.E. Chronic bacterial prostatitis and irritable bowel syndrome: effectiveness of treatment with rifaximin followed by the probiotic VSL#3. Asian J Androl 2014; 16(5): 735-9.
30. Krieger, J.N.; Nyberg, L. Jr; Nickel, J.C. NIH consensus definition and classification of prostatitis. JAMA 1999; 282: 236-237.
31. Drossman DA. Rome III: the new criteria. Chin J Dig Dis. 2006;7(4):181-5
32. Roalfe AK.; Roberts LM.; Wilson S. Evaluation of the Birmingham IBS symptom questionnaire. BMC Gastroenterol 2008; 23; 8: 30.
33. Frissora, C.L.; Koch, K.L. Symptom overlap and comorbidity of irritable bowel syndrome with other conditions. Curr Gastroenterol Rep 2005; 7(4): 264–271.
34. Shoskes, D.A.; Nickel, J.C.; Rackley, R.R.; Pontari, M.A. Clinical phenotyping in chronic prostatitis/chronic pelvic pain syndrome and interstitial cystitis: a management strategy for urologic chronic pelvic pain syndromes. Prostate Cancer Prostatic Dis 2009; 12: 177-183.
35. Pena N.V.; Engel N.; Gabrielson T.A.; Rabinowitz J.M.; Herati S.A. Diagnostic and Management Strategies for Patients with Chronic Prostatitis and Chronic Pelvic Pain Syndrome. Review Drugs Aging 2021; 38(10): 845-886.
36. Ichihara K, Takahashi S, Hiyama Y, Masumori N, Nagae H, Ito S, Wada K, Betsunoh H, Hamasuna R, Togo Y, Shigemura K, Takeyama K. Distribution of the positive UPOINT domain in patients with chronic prostatitis or chronic pelvic pain syndrome: A multicenter observational study. Observational Study J Infect Chemother. 2022; 28(5): 631-634.
37. Stern, E.K.; Brenner, D.M. Gut Microbiota-Based Therapies for Irritable Bowel Syndrome. Clin Transl Gastroenterol. 2018 Feb 15; 9(2): e134.

 doi:10.20944/preprints202408.0684.v1

38. Pimentel. M.; Lembo, A.; Chey, W.D.; Zakko, S.;Ringel. Y.; Yu, J.; Mareya, S.M.; Shaw, A.L.; Bortey, E.; Forbes, W.P.; TARGET Study Group. Rifaximin therapy for patients with irritable bowel syndrome without constipation. N Engl J Med 2011; 364: 22–32.
39. Lembo, A.; Pimentel, M.; Rao, S.S.; Schoenfeld, P.; Cash, B.; Weinstock, L.B.; Paterson, C.; Bortey, E.; Forbes, W.P. Repeat treatment with rifaximin is safe and effective in patients with diarrhea-predominant irritable bowel syndrome. Gastroenterology 2016; 151: 1113–1121.
40. Pimentel, M. Review article: potential mechanisms of action of rifaximin in the management of irritable bowel syndrome with diarrhoea. Aliment Pharmacol Ther 2016;43 Suppl 1: 37-49.
41. Kajander, K.; Myllyluoma, E.; Rajilić-Stojanović, M.; Kyrönpalo, S.; Rasmussen, M.; Järvenpää, S; Zoetendal, E.G.; de Vos, W.M.; Vapaatalo, H.; Korpela, R. Clinical trial: multispecies probiotic supplementation alleviates the symptoms of irritable bowel syndrome and stabilizes intestinal microbiota. Aliment Pharmacol Ther. 2008 Jan 1; 27(1): 48-57.
42. Ford, A.C.; Harris, L.A.; Lacy, B.E.; Quigley, E.M.M.; Moayyedi, P. Systematic review with meta-analysis: the efficacy of prebiotics, probiotics, synbiotics and antibiotics in irritable bowel syndrome. Aliment Pharmacol Ther. 2018 Nov;48(10): 1044-1060.
43. Bjerklund Johansen, T.E.; Gruneberg, R.N.; Guibert, J.; Hofstetter, A.; Lobel, B.; Naber, K.G.; Palou Redorta, J.; van Cangh, P.J. The role of antibiotics in the treatment of chronic prostatitis: a consensus statement. Eur Urol 1998; 34: 457–466.
44. Shoskes, D.A.; Hakim, L.; Ghoniem, G.; Jackson, C.L. Long-term results of multimodal therapy for chronic prostatitis/chronic pelvic pain syndrome. J Urol 2003; 169: 1406-1410.
45. Lackner JM, Clemens JQ, Radziwon C, Danforth TL, Ablove TS, Krasner SS, Vargovich AM, O'Leary PC, Marotto T, Naliboff BD. Cognitive Behavioral Therapy for Chronic Pelvic Pain: What Is It and Does It Work? J Urol. 2024; 211(4):539-550.
46. Youn, C.W.; Son, K.C.; Choi, H.S.; Kwon, D.D.; Park, K,; Ryu, S.B. Comparison of the efficacy of antibiotic monotherapy and antibiotic plus alpha-blocker combination therapy for patients with inflammatory chronic prostatitis/chronic pelvic pain syndrome. Korean J Urol 2008; 49: 72–76
47. Milajerdi, A.; Mousavi, S.M.; Sadeghi, A.; Salari-Moghaddam, A.; Parohan, M.; Larijani, B.; Esmaillzadeh A. The effect of probiotics on inflammatory biomarkers: a meta-analysis of randomized clinical trials. Eur J Nutr. 2020; 59(2): 633-649.