

DOUTORAMENTO

MEDICINA

**Síndrome Doloroso Vesical/Cistite Intersticial:
Novas modalidades de tratamento e de fenotipagem**

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D

2025



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PROGRAMA DOUTORAL EM MEDICINA

Pedro Abreu Mendes

Síndrome Doloroso Vesical/Cistite Intersticial: Novas
modalidades de tratamento e de fenotipagem

Bladder Pain Syndrome/Interstitial Cystitis: Innovative
treatment options and new forms of phenotyping patients

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Tese de Doutoramento em Medicina apresentada à Faculdade de Medicina da
Universidade do Porto

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Ao Tomás

Aos meus Pais

À minha Família e Amigos

Aos meus Mestres

"I guess I've been working so hard, I forgot what it's like to be hardly working."

Michael Scott

Em obediência ao disposto no Decreto-Lei no388/70, Artigo 8º, parágrafo 2, esclareço que efetuei o planeamento e execução das experiências, observação do material e análise dos resultados e redigi as primeiras versões das seguintes publicações, que fazem parte integrante desta dissertação:

1. Abreu-Mendes, P.; Araújo-Silva, B.; Charrua, A.; Cruz, F.; Pinto, R.
Silodosin Improves Pain and Urinary Frequency in Bladder Pain Syndrome/Interstitial Cystitis Patients. J. Clin. Med. 2022, 11, 5659.
(DOI:10.3390/jcm11195659)

2. Abreu-Mendes P., Dias N., Dinis P., Cruz F., Pinto R.
Daily low-dose of Tadalafil improves pain and frequency in Bladder pain.
Turk J Urol. 2022 Jan;48(1):82-87. (DOI:10.5152/tud.2022.21292)

3. Abreu-Mendes P.; Ferrão-Mendes A.; Botelho F.; Cruz F.; Pinto R.
Effect of Intratriganal Botulinum Toxin in Patients with Bladder Pain Syndrome/Interstitial Cystitis: A Long-Term, Single-Center Study in Real-Life Conditions. Toxins MDPI 2022, 14, 775. (DOI: 10.3390/toxins14110775)

4. Lydia C.; Charrua A.; Abreu-Mendes P.; Cruz F *et al.*
Comprehensive quantitative sensory testing shows altered sensory function in women with chronic pelvic pain: results from the Translational Research in Pelvic Pain (TRiPP) Study. PAIN 164 (2023) 2528–2539
(DOI:10.1097/j.pain.0000000000002955)

5. Abreu-Mendes, P.; Costa, A.; Charrua, A.; Pinto, R.A.; Cruz, F.
The Role of Urinary VEGF in Observational Studies of BPS/IC Patients: A Systematic Review. Diagnostics 2022, 12, 1037.
(DOI:10.3390/diagnostics12051037)

6. Abreu-Mendes, P.; Pinto, R.A.; Dias, D.; Cruz F.; Charrua A.

Soluble Vascular Endothelial Growth Factor Receptor 1 (VEGFR1): A

Possible Biomarker in BPS/IC? Contenance 2025

(DOI:10.1016/j.cont.2025.101898.)

A reprodução destas publicações foi autorizada pelas respectivas editoras.

Abstract

Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC) is a unique urological entity. The etiology is yet to be known, and treatment options are lacking. Clinical and biomarker phenotyping has not evolved in recent years despite several attempts.

The main objectives of our research were to identify potential new treatment options, namely silodosin and tadalafil; to evaluate the long-term results of intratrigonal OnaBotulinum toxin A (OnaBotA), a recently introduced invasive therapy for refractory patients; assess the use of quantitative sensory testing (QST) to phenotype patients on clinical clusters; and explore the use of the Vascular Endothelial Growth Factor (VEGF) pathway as a biomarker.

A pilot study was conducted in female patients refractory to conventional treatments to evaluate the clinical effect and safety of silodosin following the evidence that alpha 1-A antagonists had a marked effect in pain and urinary frequency in BPS/IC animal models based on chronic stress and adrenergic hyperactivity. Bladder pain was the primary endpoint. Urinary frequency, maximum voided volume, the O'Leary–Sant Index (OSS), and two questions accessing stress coping were also evaluated periodically at baseline, the 4th and 12th week. Relevant clinical improvement was found in two-thirds of the patients.

Another pilot study evaluated the effect of tadalafil on safety in BPS/IC refractory patients. Patients were treated with 5 mg daily for 3 months. Evaluations were made at baseline and the 4th and 12th weeks. The primary outcomes were pain score and urinary frequency, with a concomitant evaluation

of the OSS for symptoms and problems, and life quality. A significant decrease in pain score, the primary endpoint, was observed at the end of treatment.

A retrospective cohort study was conducted to evaluate the long-term effect and safety of OnaBotA in BPS/IC female patients in real-life conditions. Data from a cohort of 47 patients treated between 2009 and 2022 was used. All patients received 100 Units (U) of OnaBotA in ten injections limited to the bladder trigone. The medical records retrieved and analyzed include bladder pain, the number of treatments, the time between injections, and the age at the first injection. Twenty-five patients, more than half of the cohort, were long-term responders. However, no predictor was identified. The time between injections was stable throughout the treatments, and the safety profile was confirmed.

A part of the European Translational Research in Pelvic Pain (TRiPP) project evaluated the somatosensory system in patients with chronic pelvic pain. Eighty-five women with and without chronic pelvic pain were separated into 5 diagnostic subgroups (healthy, "endometriosis-associated pain", "BPS/IC", "endometriosis with bladder pain", "pelvic pain without BPS/IC or endometriosis") and submitted to the QST. All BPS/IC patients were from our department, and our group performed the QST. About 95% of patients presented QST abnormalities. However, BPS/IC patients presented a distinct type of QST profile characterized by somatosensory changes compatible with "gain of function", such as changes in pressure pain threshold and mechanical pain threshold, among other changes. However, some patients presented other profiles, indicating the presence of different clusters in BPS/IC patients. The findings indicate profound modifications in the somatosensitive system of BPS/IC patients.

An observational cross-sectional study was conducted with 18 female BPS/IC patients and 19 age-related healthy volunteers to compare urinary levels of VEGF, soluble VEGF receptor 1 (VEGFR1) between groups. Furthermore, the correlation between soluble VEGFR1 and pain, OSS, and life quality parameters was explored. The VEGF levels were different between groups but without clinical relevance. The levels of VEGFR1, on the other hand, were significantly lower in the patient's urine. However, VEGFR1 levels did not correlate with the severity of the symptoms or clinical parameters.

Based on our findings, we concluded the following:

- Silodosin is an effective and safe treatment for refractory BPS/IC patients, improving pain and urinary symptoms. Particularly in patients with a personality characterized by a limited ability to cope with stress.
- Tadalafil presented a safe profile and was effective in refractory female patients.
- Repeated intratrigonal injection of 100 U of OnaBotA shows a persistent effect through multiple injections with a duration of effect longer than one year. No relevant adverse events were detected.
- We believe that QST analysis of the somatosensorial system in BPS/IC patients can be a promising non-invasive phenotyping tool.
- The VEGF pathway does not seem useful as a biomarker, but soluble VEGFR1 may be a biomarker candidate.

Resumo

A Síndrome de Dor Vesical/Cistite Intersticial (SDV/CI) é uma entidade urológica única, cuja etiologia permanece desconhecida e com opções terapêuticas limitadas. Nos últimos anos, a fenotipagem clínica é baseada em biomarcadores não apresentou avanços significativos, apesar de vários esforços.

Os principais objetivos desta investigação foram identificar potenciais novas opções de tratamento como a silodosina e o tadalafil, avaliar os resultados a longo prazo da injeção intratrigonal de toxina botulínica do tipo A (OnaBotA), analisar a utilização do *quantitative sensory testing* (QST) para fenotipar doentes em diferentes *clusters* clínicos, bem como explorar o papel da via *Endothelial Growth Factor* (VEGF) como biomarcador.

Foi conduzido um estudo piloto em mulheres com SDV/CI refratária a tratamentos convencionais para avaliar o efeito clínico e a segurança da silodosina. Este estudo baseou-se em evidências de que antagonistas alfa-1A demonstraram efeitos significativos na dor e frequência urinária em modelos animais de SDV/CI associados a stress crónico e hiperatividade adrenérgica. A dor vesical foi o *endpoint* primário. Outros parâmetros avaliados incluíram a frequência urinária, o volume máximo urinado, o índice *O'Leary–Sant Index* (OSS) e questões relacionadas com mecanismos de lidar com o stress. As avaliações clínicas ocorrem antes do início da terapêutica, na 4^a e 12^a semanas. Observou-se uma melhoria clínica relevante em dois terços das participantes.

Outro estudo piloto avaliou o efeito do tadalafil em doentes com SDV/CI refratária. As participantes foram tratadas com 5 mg diários durante 3 meses,

com avaliações no início, na 4^a e 12^a semanas. Os *endpoints* primários foram a redução da dor e da frequência urinária, enquanto o OSS e a qualidade de vida foram analisados como *endpoints* secundários. Uma redução significativa na pontuação da dor, o desfecho primário, foi observada ao final do tratamento.

Foi realizado um estudo de coorte retrospectivo para avaliar os efeitos a longo prazo e a segurança do OnaBotA em 47 mulheres com SDV/CI tratadas entre 2009 e 2022. Todas receberam 100 U de OnaBotA em dez injeções no trígono vesical. A análise incluiu dor vesical, número de tratamentos, intervalo entre injeções e idade na primeira administração. Mais de metade das doentes (25) foram consideradas respondedoras a longo prazo. Não foi identificado qualquer preditor de resposta, mas o intervalo entre injeções manteve-se estável, sem efeitos adversos relevantes.

No âmbito do projeto europeu de investigação *European Translational Research in Pelvic Pain (TRiPP)*, avaliou-se o sistema somatossensorial de 85 mulheres, com ou sem dor pélvica crónica, distribuídas em 5 subgrupos diagnósticos. Todos os doentes com SDV/CI eram seguidas no nosso serviço de urologia, e o QST foi realizado pela nossa equipa. Cerca de 95% apresentaram anomalias no QST, sendo que as pacientes com SDV/CI revelaram um perfil distinto compatível com “*gain of function*”. No entanto, alguns apresentaram outros padrões, sugerindo a existência de diferentes clusters dentro deste grupo. Estes achados indicam modificações relevantes no sistema somatossensitivo de pacientes com SDV/CI.

Um estudo de caso-controlo analisou os níveis urinários de VEGF e do recetor solúvel VEGF 1 (VEGFR1) em 18 doentes com SDV/CI e 19 voluntárias saudáveis. Embora os níveis de VEGF apresentassem diferenças entre os

grupos, estas não foram clinicamente relevantes. Contudo, os níveis urinários de VEGFR1 estavam significativamente mais baixos nas doentes. Apesar disso, não foi encontrada correlação entre os níveis de VEGFR1 e a gravidade dos sintomas.

Conclusões

- A silodosina é uma opção terapêutica eficaz e segura para doentes refratárias, especialmente aquelas com dificuldade em lidar com o stress.
- O tadalafil demonstrou um perfil de segurança favorável e eficácia em doentes refratárias.
- As injeções intratrigonais repetidas de OnaBotA (100 U) apresentam efeito persistente por mais de um ano, sem eventos adversos relevantes.
- A análise QST pode ser uma ferramenta promissora para fenotipagem não invasiva em SDV/CI.
- A via do VEGF não parece ser útil como biomarcador, mas o VEGFR1 solúvel poderá ser útil nesse papel.

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List of abbreviations

APF – Anti Proliferative Factor	MID - Minimal Important Difference
ATP - Adenosine Triphosphate	MRI - Magnetic Resonance Imaging
BPS - Bladder Pain Syndrome	NE - Noradrenaline
BDNF - Brain-Derived Neurotrophic Factor	NGF - Nerve Growth Factor
BKPyV - BK polyomavirus	NIDDK - National Institute of Diabetes and Digestive and Kidney Diseases
BPE - Benign Prostate Enlargement	NO - Nitric Oxide
BPH - Benign Prostate Hyperplasia	OAB - Overactive Bladder
CCL – Chemokine (C-C motif) ligand	OnaBotA - OnaBotulinum Toxin A
cGMP – Cyclic Guanosine Monophosphate	PDE5i - Phosphodiesterase 5 inhibitors
CGRP - Calcitonin-Genes Related Peptide	PKG - Protein Kinase G
CNS - Central Nervous System	PPS - Pentosan polysulfate sodium
DMA - Dynamic Mechanical Allodynia	PPT - Pressure Pain Threshold
e.g. - for example	PUF - Pelvic Pain, Urgency, Frequency
EAU - European Association of Urology	QST - Quantitative Sensory Testing
EGF - Epidermal Growth Factor	RCT - Randomized Control Trial
ESSIC - International Society for the Study of BPS	RNA - Ribonucleic Acid
Etio-S - Etiocholan-3 α -Ol-17-One Sulfate	RTX - Resiniferatoxin
FDA - Food and Drug Administration	OSS - O'Leary–Sant Symptom Index
GAG - Glycosaminoglycans	VEGF - Vascular Endothelial Growth Factor
GUPI - Genitourinary Pain Index	VEGFR - VEGF receptor
IASP - Association for the Study of Pain	TNF - Tumor Necrosis Factor
IC - Interstitial Cystitis	TRiPP - European Translational Research in Pelvic Pain
IGF - Insulin-Like Growth Factor	TrkA - Tropomyosin Receptor Kinase A
IL - Interleukins	TRPV1 - Transient Receptor Potential Channels Of The Vanilloid Subtype
MAPP - Multidisciplinary Approach to the Study of Chronic Pelvic Pain	VAS - Visual Analog Scale
	U - Unit

Introduction

Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC) is a unique urological entity. It has been investigated over the last century, but despite an initial successful improvement in the description and phenotyping of the disease, the etiology, pathophysiology, and treatment are still far from completely known(1).

It was first described in 1887 by Hunner as an "intense inflammatory disease affecting the bladder", updated in 1915 with the description of bladder ulcerations. The disease was initially understood as a bladder problem, which led to a bladder-centric vision: inflammation with urothelium denudation. The definition was, however, not applicable to every patient, and more specific diagnostic criteria were needed. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) was the first association to define symptom-based criteria: pain, possibly associated with frequency and urge to void, for the last 9 months (2). When present, Hunner's lesions (HL) were pathognomonic, while the occurrence of bladder glomerulations during cystoscopy and a low bladder capacity only favored the diagnosis. Later, the International Continence Society (ICS) redefined the diagnostic criteria associating the suprapubic pain related to bladder filling with the presence of lower urinary tract symptoms in the absence of other pathology (3). In 2008, the International Society for the Study of BPS (ESSIC) advanced with the first phenotyping criteria of the disease: IC should be used when HL were present, while BPS would be reserved for patients with the same symptoms but without bladder lesions (4). This division was further supported by bladder histology. In interstitial cystitis, bladder inflammation was

evident, while in BPS, bladder histology was uneventful. This separation into two different entities, the former being an inflammatory disease of the bladder associated with a painful perception and the latter a chronic pain syndrome, has since then prevailed (5,6).

The European Association of Urology (EAU) guidelines on Chronic Pelvic Pain follow the International Association for the Study of Pain (IASP) concept (7). They added the possibility of pain being referred to other regions, such as the groin, lower back, abdomen, or the perineum. It is now accepted that pain may be perceived in one single organ, the bladder, but also in other pelvic organs and outside the pelvic region (8). Furthermore, systemic symptoms or diseases may concurrently exist, such as chronic fatigue, fibromyalgia, or Sjogren syndrome (9,10). In these cases, clinicians may consider pelvic pain as part of a systemic disturbance more than a local problem.

The presence of symptoms outside the bladder, manifested through pain or dysfunctions, aligns with the rationale that BPS/IC is not a bladder-centered disease but rather a systemic disease involving the peripheral and central nervous system (CNS) (8). The condition can evolve with systemic symptoms, with both urological and non-urological symptoms, and with or without pathological changes in the bladder wall.

BPS/IC Epidemiology

Epidemiological surveys of the condition are complex, given the lack of a universal diagnostic definition. Pelvic pain may affect up to 10% of the population, but this number may probably result from including confoundable diseases (11). Cultural impact on pain perception may explain the variable prevalence worldwide (12). Epidemiology highly depends on the instrument and definition used to diagnose and identify patients (13). Recent studies suggest it affects approximately 3.3% to 7.9% of women and 1.6% to 4.2% of men in the general population (14). The condition is most commonly diagnosed in individuals between 40 and 60 years old, although it can occur at any age.

Geographical variations have been observed, with higher rates reported in Western countries compared to Asian populations. These differences may be attributed to variations in healthcare access, diagnostic practices, and cultural perceptions of chronic pain. The prevalence varies from 1.2 per 100,000 population in Japan (15) to 16,000 per 100,000 in South Korea (16). Two studies using different epidemiological tools in the United States reported distinct prevalences. One reported a prevalence of 20,000 women per 100,000 (17), while the other reported 2,700 per 100,000 (18). In Spain, the incidence of BPS/IC in women using health units was 5,400 per 100,000 (19). Based on pathological findings in the Netherlands, Bade *et al.* concluded that the prevalence was only 16 per 100,000 female patients (20). A recent survey conducted in France, Germany, Italy, Spain, and the United Kingdom evaluated the presence of pelvic pain among women during the last 12 months (21). The prevalence of bladder-related pain was 1220 per 100,000, but only 440 per 100,000 had the medical

diagnosis of BPS/IC. A balanced prevalence between 300 and 400 per 100,000 subjects seems reasonable.

BPS/IC Etiology and Pathophysiology

The etiology and pathophysiology of BPS/IC remain uncertain. Multiple hypotheses have been postulated and studied: neuroinflammation, defect of glycosaminoglycans (GAG), urothelium damage with increased permeability, infections, autonomic dysfunction, neuroendocrine dysfunction, and other hypotheses involving neuronal malfunctioning (22,23), including a central sensitization theory, which could explain the BPS/IC behavior as a systemic and auto-perpetuating condition (24,25).

The hypothesis of a urothelial defect, the first to be explored intensively, remains attractive. The rationale is that an increased permeability of the urothelium allows urinary ions, such as potassium and hydrogen, to infiltrate the bladder wall and, consequently, activate bladder nociceptors and initiate a self-perpetuating neurogenic inflammation (26,27). This infiltration would activate inflammatory cells, like mast cells, by releasing inflammatory mediators locally, such as histamine, leukotrienes, serotonin, nitric oxide (NO), and cytokines, with sensitization of bladder nociceptors (28). This inflammation can also contribute to bladder angiogenesis (29). The role of cytotoxic urinary agents causing aggression to urothelium is also postulated. Cationic metabolites harmful to human urothelial cells are significantly higher in the urine of BPS/IC patients than in controls (30).

At the bladder level, the network of suburothelial bladder nociceptors seems denser in BPS/IC patients, which might also contribute to enhanced pain intensity (31). In addition, the urothelium of IC patients releases more adenosine triphosphate (ATP) upon distention, which is responsible for

exciting suburothelial sensory nerves expressing P3X3 receptors (32–34). Interestingly, our group showed that the density of nociceptors in the human bladder is much higher in trigone than in the lateral bladder walls, and the bladder dome presents scarce fibers (35,36). Moreover, the bladder trigone is a fixed part of the bladder and does not contract during voiding, which leads to a maintained bladder emptying efficacy even when the trigone nerve fibers are pharmacologically knocked out (37). In ulcerative cases, an intense infiltration of lymphocytes T and B was identified in the bladder wall, as in Sjogren syndrome (38). These findings are in favor of an auto-immune hypothesis for the disease. Association in the prevalence of BPS/IC patients with auto-immune diseases has been widely identified, especially Hashimoto's thyroiditis, ankylosing spondylitis, rheumatoid arthritis, and Sjogren's syndrome (39,40).

Variations in the microbiota of BPS/IC patients were observed when compared to normal women. A smaller urinary microbiota diversity was identified in BPS/IC women compared to healthy females, with the *Lactobacillus* representing more than 90% of the genus analyzed in the urine of patients (41). An imbalance was observed during flare periods, with a higher prevalence of fungi (42). The BK polyomavirus (BKPyV), a ubiquitous virus that remains latent in the bladder surface, was identified in the urine of 11 out of 15 patients in one study, with all the patients positive for BKPyV presenting glomerulations or lesions in the bladder (43). This result raises the hypothesis that the bladder ulcers in the IC phenotype may be caused by a BK polyomavirus infection known to cause hemorrhagic cystitis in renal transplanted patients (43,44). Infection would lead to abnormal Tamm-Horsfall's glycosylation and reduced capacity to neutralize the toxic cations (45).

Neuronal afferents from the pelvic organs ascend similarly to the CNS in shared sensory pathways (46). This neuroanatomic phenomenon explains functional changes in an organ due to the transmission of a noxious stimulus from an adjacent organ. It may also explain the coincidence of urinary and colonic symptomatology in many BPS/IC patients (46).

The central sensitization theory originated on the idea that an initial, prolonged insult without a prompt and correct resolution may lead, as a consequence of continuous afferent input signaling, to changes in the peripheral and central nervous system that may perpetuate an abnormal pain sensation in response to innocuous stimulation (25). It was initially evidenced in other chronic pain conditions such as fibromyalgia (47). Ultimately, the decrease in the pain threshold leads to normal afferent input perception changes, transforming any stimulus into a noxious input. This theory correlates to BPS/IC if the condition is a consequence of an initial unresolved bladder insult. Given this, the normal bladder feeling is perceived as a noxious input, which is part of the definition of the condition (4). Anatomical changes in the CNS are believed to co-occur with functional changes, and these have been seen in functional brain Magnetic resonance imaging (MRI) (48,49). These central changes could explain the bladder-centric symptoms when the bladder seems normal in every diagnostic tool, except for its function (49,50).

Dysfunction of the autonomic sympathetic nervous system has been implied in this condition, with clinical studies showing patients with increased resting heart rates, high levels of urinary catecholamines, and an increased number of sympathetic fibers coursing the bladder - overall, an excess of sympathetic activity (51–53). A previous investigation from our group used the

Tilt test in BPS/IC patients and observed an increased sympathetic tonus and higher noradrenaline (NE) levels in the serum and 24-hour urine samples, supporting excessive sympathetic activity independent of patient phenotype (53). Furthermore, urinary noradrenaline levels decreased as bladder pain symptoms improved with botulin injection (54). Following these observations, our group carried out pre-clinical studies with animal stress models in rodents. These pre-clinical models induce a BPS/IC-like behavior later in life despite the animals being subjected to stressful situations early in life. In our studies, we have observed a relation between high urinary levels of NE and the development of pain behavior and increased voiding frequency, consolidating the role of the sympathetic nervous system and the development of pain behavior (53,55,56).

On the other side of the same coin, stress is an old suspect of being involved in chronic pain conditions, and it is directly related to a dysfunction of the sympathetic system (57). Increasing evidence suggests that stress is positively related to BPS/IC, with increased symptoms during stressful periods, but the idea that stress could be the origin of BPS/IC has gained some support (58–60). A large European multicentric study named IMI-Pain Care, in which our group was actively involved, evaluated the prevalence of previous traumatic events. It has shown that patients with pelvic pain had a much higher prevalence of childhood and adulthood traumatic experiences, including severe illnesses and sexual abuse. Interestingly, a history of child abuse had been associated with a specific symptomatic BPS/IC phenotype, with worse pain and more intense urinary symptoms (59).

BPS/IC Biomarkers

The lack of pathognomonic symptoms and signs for a straightforward diagnosis of BPS/IC, besides Hunner's ulcers, has pushed forward the investigation of a BPS/IC biomarker. An ideal biomarker should establish the diagnosis, orient the management and the response, predict remissions and relapses, and define the prognosis. Until today, no biomarker for BPS/IC has been found that could be used at least in some of the above-described functions (61,62). While building up the number of etiological theories, more and more biomarker molecules have been investigated as possible biomarkers (41). Some of these molecules might be therapeutic targets if involved in the etiological process or underlying symptoms. However, many molecules potentially identifiable in the urine may result from the activation of non-specific metabolic pathways.

Several molecules have been identified in abnormal levels in the urine of the patients: the epidermal growth factor (EGF), Insulin-like growth factor (IGF) - binding protein-3, interleukin (IL) 6, heparin-binding EGF-like growth factor, and cyclic guanosine monophosphate (cGMP) (63). Other markers were not significantly different in the urine of IC and control groups, including total glycosaminoglycans, epitectin, hyaluronic acid, IL-8, IL-1, and nitrates (63). Nerve growth factor (NGF) is an appealing biomarker for BPS/IC as it sensitizes nociceptors and decreases mechanical pain threshold (64). This neurotrophin released from the urothelium is responsible for peripheral afferent sensitization at both somatic and visceral levels (65). The receptor TrkA, with high affinity to NGF, has been identified at the bladder afferents. These findings can explain why pre-clinical administration of NGF causes bladder behavior and increased voiding

frequency through the Transient receptor potential channels of the vanilloid subtype (TRPV1) pathway (65). The urinary levels of NGF correlated with pain intensity, and NGF serum levels increased in ulcerative and non-ulcerative phenotypes, which may explain the systemic pain referred by patients (66,67). The potential use of specific anti-NGF antibodies was investigated as a treatment possibility in BPS/IC patients, but there have been no positive results until now (unpublished results reviewed by F. Cruz).

Emotional and psychological stress has always been postulated to be involved in the etiology of the condition, as previously mentioned, with increased urinary excretion of NE in a small cohort of BPS/IC patients (53). A similar increase was confirmed in our animal stress models, leading to the premise that urinary NE levels could be used to phenotype patients (69).

Cytokines and IL are other molecules detected in the urine of these patients at abnormal levels (68,70). In a cohort of BPS, IC, and overactive bladder (OAB) patients, 40 inflammatory urine biomarkers were evaluated (70). IL-1 α , IL-6, and chemokines, including chemokine (C-C motif) ligand (CCL) 2, CCL5, CXCL1, CXCL8, and CXCL10, were significantly increased in both BPS and IC phenotypes, compared with OAB patients. Despite none being used to diagnose BPS/IC or exclude other urinary conditions, a combination of biomarkers could be used as a diagnostic tool or to guide patient management.

The low usefulness of the biomarkers discussed above has led investigators to explore other molecules. The vascular endothelial growth factor (VEGF) molecule and its receptors exemplify such additional effort. The association is probable since the actions of VEGF are related to vessel permeability, angiogenesis, and neuronal modulation, all of which are observed

in BPS/IC: the glomerulations, the waterfall bleeding seen after bladder distention during cystoscopy, and pain. There are three known receptors. However, their role is still under study, mainly since their location in the membrane or as soluble receptors seems crucial for their function (71,72). The type 1 receptor, VEGFR1, presents a high affinity to the VEGF molecule and a little kinase effect related to the pro-nociception (73). However, there is also evidence that VEGFR1 can enhance the activity of VEGFR2 (74). The soluble isoform of VEGFR1 has the same high affinity to VEGF and no kinase effect, seeming to have the unique function of acting as a decoy, entrapping VEGF, and reducing free VEGF available to bind to other receptors in transmembrane position (71,75). The VEGFR2 is the best-characterized receptor and the one with the highest tyrosine kinase activity, having functions in angiogenesis, vascular permeability, and endothelial cell proliferation but also at the neuronal level is responsible for stimulating the sprouting of neural dendrites (71,76,77). Type 3 receptor has less tyrosine kinase activity than R2 and is mainly responsible for vascular lymphangiogenesis (77). The role of each receptor is not fully understood, and it is reasonable to say that their effect, especially R1 and R2, seems to be context-dependent.

A systematic review was necessary to add more information about the VEGF pathway in BPS/IC. Our group performed it, and it is part of this thesis. The results of this systematic review suggested that an exploratory study was warranted, evaluating the urinary levels of VEGF and VEGF receptor 1 in a cohort of BPS/IC patients and in healthy volunteers.

The NO molecule has also been implied in BPS/IC for a long time through the L-arginine-NO-cGMP pathway and its regulation (78,79). By activating the

NO-protein kinase G (PKG) pathway, NO inhibits pain signaling at the peripheral and central levels (80). The PKG molecule stimulates the opening of ATP-sensitive potassium channels and the consequent increase in the potassium current, restoring the nociceptor threshold to normal levels. (81). Regarding possible local effects, a study has observed that all the IC patients, but not BPS, presented elevated levels of intraluminal bladder NO despite not being correlated with symptom severity (82). Through an immunohistochemistry evaluation, the authors observed that the origin of NO was mainly from urothelial production. Macrophages are in higher numbers than normal cases but contribute less when compared to urothelium (83). On the contrary, the possible effect of NO-induced muscular relaxation was also postulated to have beneficial therapeutical effects in cases where bladder pain originates in pathological detrusor contractions caused by elevated levels of urinary potassium or adrenergic activity (84).

Urine metabolite profiling in women with IC/BPS revealed a steroid metabolite that can differentiate between IC/BPS patients and control subjects. A sulfoconjugated 5- β reduced isomer of testosterone, etiocholan-3 α -ol-17-one sulfate (Etio-S), was able to distinguish female BPS/IC patients from controls with 90% sensitivity and specificity in pre-selected diagnosed patients (85).

As stated, microbiota diversity is narrower compared to normal women, with *Lactobacillus* corresponding to approximately 90%, and a significant prevalence of fungi (*Candida* and *Saccharomyces*) was detected during flares (41,42). Also, BK viruria was postulated to be associated with vesical ulceration (42). Besides the findings of polyomavirus BKPyV shedding in the urine of IC patients, treating these patients with the anti-DNA cloropactin showed efficacy (43,44).

Despite all these biomarkers that have been suggested and analyzed in small studies, the only biomarker able to phenotype BPS/IC patients and broadly used these days is the presence of bladder lesions, according to the pathological classification of ESSIC, based on the cystoscopy and biopsy findings (86). However, it requires an invasive procedure that can aggravate pain, and lesions might be challenging to identify if the exam is not done under anesthesia or if performed by less experienced clinicians.

Diagnosis and Clinical Evaluation of BPS/IC

Different entities can cause the pelvic pain perceived by the bladder. The lack of specific symptoms, pathological features, or biomarkers makes excluding confoundable diseases the fundamental first step in the diagnostic pathway. Pain perceived in the bladder is the main symptom, but pain outside the pelvic area occurs in up to 75% of the patients, requiring a careful mapping of painful regions (87,88). In some cohorts, Hunner's lesions do not enhance pain intensity (89–91). Furthermore, chronic pelvic pain, a related condition, affects an estimated 14.7-24% of women of childbearing age (92). The coexistence of various comorbidities, such as endometriosis, adhesions, infection, bladder tumors, urinary stones, irritable bowel syndrome, and musculoskeletal and neuropathic conditions, including nerve entrapment, underscores the complexity of diagnosis requiring, in most cases, a multidisciplinary approach (93,94).

During diagnosis, the urge to pass urine due to the fear of pain should be distinguished from the urgency to pass urine to prevent incontinence, avoiding the misdiagnosis of OAB (95). Symptoms severity of BPS/IC fluctuates with time, presenting periods of relapse and periods of symptomatic exacerbation (96). The occurrence of flare events was studied by both the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) research group and the IMI-Pain Care consortium, and flare episodes were highly prevalent in both studies (97,98). The MAPP study group evaluated a BPS/IC cohort for twelve months. Seventy-five percent reported having at least 1 flare per year, and most of these patients had more than two per year. An average increase of 2.6 points in the visual analog scale score was observed during the flare. Stabilized BPS/IC symptoms were

associated with less probability of a flare episode with worsening urological symptoms (97). The MAPP identified the presence of *Candida* and *Saccharomyces sp.*, both fungi, in a greater prevalence in flare patients (16%) when compared to non-flare patients (4%) (42). The IMI/TRIPP consortium evaluated patients with pelvic pain from different causes, and BPS/IC patients were the group with more heterogeneity of flare characteristics (98). Flares could vary in duration from a few minutes to a few days. Stress, sexual activity, fatigue, prolonged standing position, and urinary tract infections were the main precipitating events of pain flares, which are frequently accompanied by a worsening of the urinary symptoms, frequency, and nocturia.

The classification according to the ESSIC criteria, the most used phenotype BPS/IC, based on the bladder biopsies taken during cystoscopy under anesthesia, is unspecific (4). Invasive diagnostic tools can be used more prematurely in cases of severe disease and type of complaints (6). Despite having the same symptoms, it has been postulated that IC would be a bladder-centered disease with fewer systemic symptoms and BPS, contrary to more systemic disease (99–101). More recently, another clinical phenotyping was introduced, the UPOINT and INPUT classifications of symptoms (acronyms for the distinct symptoms evaluated). This systematic and holistic patient evaluation helps streamline personalized multimodal therapy (102–105).

The severity of the condition should guide management. Validated questionnaires can be used, such as the O'Leary–Sant index (OSS), and the Pelvic Pain, Urgency, Frequency (PUF) questionnaire, which captures a wide variety of symptoms, and the Genitourinary Pain Index (GUPI), based on a chronic prostatitis index (106).

The central sensitization theory was primarily shown in other chronic pain conditions, such as fibromyalgia. After suspecting the involvement of the CNS in some patients who maintain pain after bladder anesthesia with lidocaine (107), the investigation to correlate anatomical and functional changes in the brain with pain behavior through brain MRI began (47,108). It was observed that functional brain areas (grey matter) presented changes in their relative size and activation patterns and that the tissue responsible for connection and interaction of different areas (white matter) was altered (47). The brain suffers a process of remodeling in chronic pain conditions. Following this line, the BPS/IC patients' brain function was also evaluated with functional brain MRI (49,50,109,110). Changes in BPS/IC studies were observed, as they were in other chronic pain conditions. The sensorimotor network related to the pelvis and the supplementary motor area, involved in the micturition cycle and pelvic nociception, is altered in BPS/IC patients (111). Our research group demonstrated that the default mode network represents the brain's basal activity during the resting state and presents a modified pattern of activation, which seems to be a consequence of a continuous painful state or painful input (49). Moreover, the cross-network that specifically intertwines the areas responsible for sensory input, the nociception, and the resting state presented functional aberrations that seem to be related to symptom severity (50). It has been shown in other chronic pain entities, namely knee and hip arthritis, that when central sensitization is present, patients tend to respond poorly to local treatments, which can be used to tailor therapeutic options (48). Despite the growing evidence in functional brain image studies, protocols for this evaluation are scarce and lack reproducibility validation, but brain MRI can be

used in the future for assessing CNS involvement and potentially phenotyping patients.

Chronic pelvic pain can be attributed to and occur with the central sensitization phenomenon, but it can also be attributed to the peripheral pelvic nervous system, or both systems can be involved (112). Changes in both systems can translate into abnormal sensory functions, as has been demonstrated in other chronic pain conditions (113,114). Evaluating these abnormalities in the somatosensory nervous system can be used as a proxy to evaluate different neuroanatomical pathways with specific nerve fibers (115). For this purpose, a set of standardized cutaneous tests called quantitative sensory testing (QST) is used to evaluate the detection and the pain thresholds to different stimuli, such as pressure and temperature. Patient thresholds are then classified as a sensory gain or loss of function. The combination of all the different threshold results is used to profile the patient into three possible clusters: sensory loss (mechanical and thermal sensory loss), thermal hyperalgesia (preserved sensory function with heat or cold hyperalgesia), and mechanical hyperalgesia (a loss of thermal sensation with mechanical hyperalgesia or allodynia).

In the particular case of BPS/IC patients, the QST test was never used systematically or compared with other pelvic pain conditions of different origins, which could help both in the initial diagnosis and phenotyping. Therefore, as part of the IMI-Pain Care consortium, we designed a multicenter study to compare the QST of BP/IC patients with patients with other forms of pelvic pain. The results of this collaborative study are included in this thesis since all the BPS/IC patients were from our cohort.

Imaging the bladder is indicated to be mainly used to exclude causes such as cancer or bladder stones. In recent years, with the advance of MRI technology and different chemical contrasts used, as seen at the brain level, pelvic MRI has also been investigated for BPS/IC. Changes in the bladder wall are the phenotype criteria used until today, involving the need for a surgical procedure for bladder wall biopsies, the previously stated ESSIC criteria. Recent investigations have shown that bladder wall characteristics such as bladder permeability, fibrosis with collagen changes, inflammation, and bladder relaxation patterns could be assessed using simultaneous recent chemical contrasts mixtures, given simultaneously by intravenous and intravesical routes, with different absorption timings and reactions, allowing for a more precise visualization of bladder wall layers and properties (116). Using this dynamic use of contrasts, changes in bladder wall signaling have been seen in patients phenotyped with IC but not in BPS patients. Moreover, it seems usable with low volumes of intravesical contrast instillations, which are particularly important for patients with low bladder capacity. A better characterization of bladder wall layers and urethral permeability could be significant in phenotyping patients. Using this type of bladder MRI to characterize patients before advancing with intravesical instillation therapies directed to replenish the GAG layer can be of some value. Ultimately, it can be important during the diagnosis since it can identify bladder changes, as stated, but it can also raise the hypothesis of a myofascial pelvic syndrome since muscle hypertonicity usually corresponds to muscle shortening and displacement in MRI (117). It can also confirm other non-urolological causes of pain, like pelvic masses or prolapses.

Management is complex and time-consuming. The absence of pathognomonic findings and the fact that it is a diagnosis of exclusion delay the diagnosis. As seen, several strategies for BPS/IC patients' phenotyping have been studied, but there is no clear evidence of the benefits. This problem can also be responsible for the significant number of negative clinical trials regarding treatment, as documented below.

Treatment of Bladder Pain Syndrome/Interstitial Cystitis

When treating BPS/IC patients, it is crucial to address every complaint. Considering that the main driving symptoms are bladder pain and lower urinary symptoms. However, pain might be reduced by an increase in urinary frequency, leading patients to concentrate their complaints on lower urinary tract symptoms rather than bladder pain. Other important considerations are that no treatment fits all, there is no cure, and, to make it worse, most patients experience treatment failures. Also, as previously referred, clinicians should use clinical phenotyping to decide which symptoms should be tackled in the first instance, remembering that the existence of bladder lesions does not change the treatment approach, except that the fulguration of the lesions may be considered. A step-by-step approach is recommended, beginning with lifestyle habits changes, followed by non-invasive therapy and, lastly, surgical treatment (6,118).

For the first line of treatment, conservative measures such as stress reduction, exercise, and a healthy lifestyle, including dietary changes, can improve life quality. Strategies to train the patients to accommodate higher urine volumes, such as timed voiding, controlled fluid intake, pelvic floor muscle training, and bladder training, are also used with inconstant success (119,120). Pelvic floor muscle reeducation, despite the lack of high-level evidence, is used in patients with myofascial components present (5,121). Individualized diet restriction can be tried since solid evidence that it may work in most patients is lacking (122).

Pharmacotherapy can be considered as the second line, being used if conservative measures fail. Pain treatment is the cornerstone in BPS/IC patients,

with many oral therapies that are part of the analgesic ladder, but treatments directed to the condition physiopathology are also approved (123). Pharmacotherapy can be delivered systemically, orally or intravenously, or through intravesical instillations (124). Until nowadays, despite the type of delivery, all treatment options usually have limited efficacy, are based on low-level evidence, or have significant side effects. The multiple existing options today are insufficient, as we present next.

Pentosane polysulfate sodium (PPS) is the only therapy *per os* approved by the Food and Drug Administration (FDA) to target GAG-layer replenishing, but the reported evidence shows both positive and negative trials (125–130). Recently described cases of pigmentary maculopathy are a new drawback, especially if high doses are used for long periods (131).

Immunosuppressive drugs, such as cyclosporine, have shown a symptomatic improvement in a small cohort and have also been recommended for refractory patients (132–134). However, in a small cohort, twenty percent of patients discontinued treatment due to cardiovascular and renal dysfunction (135). New immunosuppressive agents were tested, such as methotrexate, azathioprine, and montelukast, without evidence (136)

Antagonists of the histamine receptor types H1 and H2, hydroxyzine and cimetidine, are also used, and hydroxyzine has had a 55% success in patients with allergies (137). However, efficacy was not shown multiple in placebo-controlled trials (129,138,139).

Non-opioid analgesics and nonsteroidal anti-inflammatory drugs are usually the first approach (96). When the latter fails, guidelines suggest the use

of chronic neuropathic pain drugs as adjuvants, such as gabapentin and pregabalin. However, trials with gabapentin alone were negative (140,141).

Amitriptyline is a tricyclic antidepressant that presents both anticholinergic and sedative properties. Clinical guidelines almost unanimously recommend it after a study that showed the superiority of amitriptyline over placebo (118,142). A subanalysis of the initial trial showed, nevertheless, that patients receiving high doses of amitriptyline had a positive effect, although at the cost of more adverse events (143).

Given the fact that the last few presented therapies are the approved drugs and have such low efficacy, several drugs have been investigated. Several other drugs have been tried, all of them with negative results or important adverse events targeting different pathways such as the C-kit inhibitor, the anti-IL10, the tumor necrosis factor (TNF) alpha inhibitors, quercetin, anti-NGF, micro-ribonucleic acid (RNA) manipulation, selective cannabinoid type-1 receptor agonists, anti-proliferative factor (APF) antagonist or supatast (T-cell helper inhibitors) (144).

Phosphodiesterase 5 inhibitors (PDE5i) have also been tested in a randomized placebo-controlled clinical setting, enrolling 55 female patients treated with daily sildenafil (84). Patients were assessed for four months. The treatment group demonstrated statistically significant improvements in pain, urinary frequency, and bothersome symptoms during the initial two months. At the end of the trial, sildenafil patients maintained positive results in all parameters despite the improvement in pain not being statistically significant. One could speculate that sildenafil may not be the best PDE5i drug to treat lower urinary tract symptoms, given its reduced half-life. However, another PDE5i, tadalafil,

presents a better pharmacokinetic profile, with a half-life of approximately 30 hours (145), and could be a potentially better drug to use in this condition; however, data is missing. Our group conducted an open trial to investigate the efficacy and safety of tadalafil in BPS/IC female patients, which is included in this thesis. To our knowledge, it is the only trial published at the time.

The direct use of drugs in the bladder is also an approved treatment option, but the evidence is, once again, of low level. Dimethylsulfoxide (DMSO) is the only intravesical therapy approved by the FDA, although its recommendation was withdrawn in the last version of EAU Guidelines (118). It is often combined in intravesical cocktail solutions (lidocaine, heparin, and/or sodium bicarbonate). The use of alkalinized lidocaine can reduce pain for a week, but the exact phenotype that benefits is unknown (146,147). Chondroitin sulfate, hyaluronic acid, and heparin were also evaluated, but controlled clinical trials were negative or showed a high recurrence rate in 1 year, except for high molecular weight hyaluronic acid (148,149). Intravesical resiniferatoxin (RTX) was also tested to desensitize the TRPV1 receptor and deactivate C-fibers, but the only placebo-controlled trial was negative (150).

The last line of treatment is the surgical treatment. Bladder hydrodistension under anesthesia is a diagnostic procedure, and although transient improvements have been reported afterward, its therapeutic use is far from being consensual, and long-term relief of symptoms is rare. It was recently withdrawn from EAU Guidelines recommendations (118,151). The action of hydrodistention helps to distinguish between BPS and IC. When bladder lesions are seen, transurethral resection or fulguration can be used, and both techniques show a similar effect for up to 12 months (152–156). The use of electrical

neuromodulation, either through the sacral or the tibial nerve, has been investigated in the last decade (157). Overall, the studies presented mixed results, and their quality was generally poor. Furthermore, the exact technique to use is the sacral approach, unilateral or bilateral, is discussed (158). The explantation rate due to local complications is higher than in other conditions and can be as high as 50% (159).

Intratrigonal injection of OnaBotulinum toxin type A (OnaBotA) is an approved surgical third-line treatment for BPS/IC, recommended in guidelines worldwide, following the consistently positive results from placebo trials published in the last decades (160). This toxin was already recommended for use in other conditions long before being used for BPS/IC (161). Its use in BPS/IC was approved following its efficacy in reducing pain and improving urinary symptoms at short-term, 1, and 3 months (160,162) in both phenotypes (91). In placebo-controlled trials, the trigone-restricted injections of OnaBotA 100U reduced relative pain by more than 50% in more than half of the patients, contrary to saline solution, and no cases of urinary retention occurred (163). The most common adverse effects were voiding difficulty and lower urinary tract infections, but no cases of urinary retention occurred (164). Metanalyses concluded that among the different treatment options for BPS/IC, OnaBotA bladder injections have the highest probability of decreasing bladder pain (165). Later, a randomized control trial (RCT) designed and published by Pinto R. *et al.* was essential for OnaBotA to demonstrate the efficacy of repeated injections (162). In this study, patients were re-treated with 100 OnaBotA units injected in the trigone every time symptoms returned for up to three years. The duration of improvement for each treatment was approximately 9 to 10 months after each treatment. A real-life

study of the BPS/IC patients treated with intratrigonal injections of OnaBotA 100U was carried out and is part of this thesis.

Major surgical procedures involving reconstructive surgery are the last resource in treating BPS/IC patients. Its use has been progressively declining, with evidence regarding cystectomy and/or urinary diversion showing mixed or negative results (166–170).

Objectives

The main objective is to identify potential new treatment options, evaluate the long-term results of intratrighonal OnaBotA, assess the use of QST to phenotype patients on clinical clusters, and explore the use of the VEGF pathway as a biomarker.

- Evaluate silodosin's safety and efficacy in treating refractory BPS/IC patients and its relationship to a phenotype of higher stress levels.
- Evaluate the efficacy and safety of daily dose tadalafil in refractory patients.
- Evaluate the safety and efficacy of the long-term use of intratrighonal OnaBotA in BPS/IC patients and possibly predict responders.
- Clinical phenotyping of BPS/IC patients based on the respective QST findings.
- Review the literature to evaluate how the VEGF and its receptors can be involved in the BPS/IC disease. Investigate how VEGF and VEGFR1 work as a phenotyping method in our cohort.

Publications

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Publication 1

Silodosin Improves Pain and Urinary Frequency in Bladder Pain Syndrome/Interstitial Cystitis Patients

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J. Clin. Med. 2022, 11, 5659. (DOI:10.3390/jcm11195659)



Article

Silodosin Improves Pain and Urinary Frequency in Bladder Pain Syndrome/Interstitial Cystitis Patients

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Abstract: Purpose: Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC) is a bladder-related chronic inflammatory disease. Data indicate that stress enhances the excitability of bladder nociceptors through the stimulation of alpha1A-adrenoceptors. Stress is known to play a crucial role in BPS/IC patients. We aimed to assess the efficacy and safety of daily silodosin in refractory BPS/IC female patients and its correlation with stress coping. Materials and Methods: An open-label trial was conducted with 20 refractory BPS/IC patients. Evaluations occurred at baseline and the 8th and 12th weeks. Primary endpoint was bladder pain evaluated by visual analogue scale (VAS). Secondary endpoints included daily frequency, nocturia and maximum voided volume obtained from a 3-day bladder diary, the O'Leary–Sant Symptom Score, and two questions accessing stress coping. Patients initiated daily doses of 8 mg silodosin, which could be titrated to 16 mg. Median values with percentiles 25 and 75 (25; 75) were used. Wilcoxon signed-rank test was used for comparisons. A minimally important difference of 3 points for pain was established to define clinically relevant improvement. Results: Median age was 56 years. Median pain score decreased from 8.00 (6.00; 8.00) at baseline to 4.00 (2.00; 5.50) ($p < 0.001$), meaning that the primary endpoint was reached. Total urinary frequency decreased from 14.00 (13.00; 21.00) to 9.00 (7.50; 11.00) ($p < 0.05$), and all the other secondary endpoints also showed a statistically significant improvement. Eleven patients improved by ≥ 3 pain points in VAS, meaning that 65% of patients that ended the study protocol achieved clinical significant improvement or, in the full analysis set, that 55% of the 20 initial patients improved significantly. Fourteen (82%) decreased by ≥ 2 micturitions/day. Overall, the cohort's stress coping was low. Conclusions: Silodosin can be an effective and well-tolerated treatment for refractory BPS/IC female patients.

Keywords: bladder pain syndrome; interstitial cystitis; stress; alpha-blocker; silodosin



Citation: Abreu-Mendes, P.; Araújo-Silva, B.; Charrua, A.; Cruz, F.; Pinto, R. Silodosin Improves Pain and Urinary Frequency in Bladder Pain Syndrome/Interstitial Cystitis Patients. *J. Clin. Med.* **2022**, *11*, 5659. <https://doi.org/10.3390/jcm11195659>

Academic Editors: Hann-Chorng Kuo, Yao-Chi Chuang and Chun-Hou Liao

Received: 22 August 2022

Accepted: 23 September 2022

Published: 26 September 2022

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1. Introduction

Primary Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC) is a chronic disease. Suprapubic pain associated with bladder filling is the cornerstone symptom and is associated with urinary symptoms such as frequency, nocturia and persistent urge to prevent/relieve pain [1]. Effective curative treatment for BPS/IC is yet unknown. This unfortunate situation is due to the poor understanding of BPS/IC etiology. Chronic bacterial infections, the defective glycosaminoglycan layer of the bladder urothelium, the inappropriate activation of mast cells, autoimmune-mediated mechanisms, and autonomic nervous system dysfunction have been suggested [2]. As the modulation of the activity of bladder nociceptors involves complex mechanisms, different partners may participate in their activation [3]. Furthermore, systemic pain syndromes and somatic disorders such as irritable bowel syndrome, chronic fatigue syndrome, and fibromyalgia frequently coexist

with BPS/IC [4,5]. This observation has led, in recent years, to another pathophysiological paradigm in which BPS/IC is seen as a local manifestation of a systemic pain condition [2].

Increasing evidence suggests that stress plays a crucial role in BPS/IC [5,6]. Symptoms are aggravated by stressful periods [7]. Patients who have suffered early-in-life traumatic events show a distinctive symptomatic phenotype, with intense pain and a high urinary frequency [8,9]. Pain triggered or exacerbated by stress is thought to reflect an increase in NE release, particularly in BPS/IC patients [10]. The excessive stimulation of $\alpha 1_A$ -ARs enhances the responses of nociceptors, such as transient receptor potential cation channel subfamily V member 1 (TRPV1) [9], resulting in visceral hyperalgesia [10–12]. Interestingly, early-in-life insult causes erratic responses to stress later in life, with an increased release of corticotrophin-releasing factor, adrenocorticotrophic hormone, and norepinephrine (NE) [13,14]. Moreover, an excess of NE may leave an imprint on pain pathways during development, with the dysregulation of the hypothalamic–pituitary–adrenal axis [13].

In a recent pre-clinical experiment, adult female mice developed an equivalent of a BPS/IC phenotype after being subjected to an early-life water avoidance stress test (WAS). In this model, the lower abdominal mechanical pain threshold was decreased and the frequency of bladder voiding contractions during filling cystometries was increased, while urinary NE levels were multiplied by a factor of 20. Interestingly, the blockade of $\alpha 1_A$ -ARs by silodosin, although not altering NE levels, normalized the abdominal pain threshold and bladder function of rats subjected to WAS [11].

Silodosin has a safe profile, conferred by its high selectivity for $\alpha 1_A$ -ARs, limiting cardiovascular events [12]. With this in mind, we performed this single-arm study to evaluate the efficacy and tolerability of a daily dose of silodosin in 20 women with BPS/IC refractory to conventional treatments. Simultaneously, we assessed the capacity of stress coping through a brief personality questionnaire.

2. Material and Methods

2.1. Patients

This study was approved by the local ethics committee (Comissão de Ética do Centro Hospitalar e Universitário de São João) under protocol number 338-21: “Efeito da Silodosina nos doentes com Síndrome Doloroso vesical aos 3 meses”. The trial occurred in a tertiary university hospital with expertise in BPS/IC. Oral and written consent was obtained from patients after explaining the off-label drug use. Patients were informed that silodosin is licensed for the treatment of male LUTS. The rationale for this use was understood. We also stated that the side effects of this drug were extensively known.

Previously, a small cohort of patients outside the study, refractory to every other previous therapy (oral, systemic and intravesical), showed a good response to silodosin.

Twenty women with BPS/IC refractory to previous therapies were enrolled between 2019 and 2020. As a pilot study, without any available data to predict the potential efficacy, power calculations were not carried out.

During the previous follow-up, patients had been advised to adjust their diet, increase physical activity, avoid stressful events, and stop smoking. Patients under any oral treatment in the last two months, other than simple oral analgesia (such as acetaminophen or ibuprofen), were excluded. Patients who received intravesical treatments in the previous six months were also excluded.

2.2. Study Design

We designed a 12-week single-arm exploratory study to evaluate the efficacy of silodosin against BPS/IC symptoms.

Patients were evaluated at baseline, at week 8 (8w), and at week 12. The primary outcome was the pain intensity at 12w, assessed by a 0–10 VAS score (in which 10 was the worst pain ever). The secondary outcomes included the daily urinary frequency, the nocturia, and the MVV recorded in a 3-day bladder diary; the OSS problems and symptoms index (0 to 34, being 34 the worst); and the QoL evaluated with the question used in the

IPSS questionnaire (from 0 to 6, with six being the worst quality of life). For the definition of a relevant clinical improvement, we considered a decrease in 3 points in the VAS score as the minimally important difference (MID). Decreases of 2 micturitions in total daily urinary frequency and 1 micturition in nocturia were defined as the MID to consider a relevant improvement in these parameters.

Two questions were addressed to the patients at the beginning of the study in order to assess their capacity to cope with stress: the first question was answered yes/no, “Does stress aggravates or initiate BPS/IC symptoms?”, and a second question, “I have a relaxed personality, coping well with stress”, could be answered on a scale from 1 to 5, with 1 being “I totally disagree with it” and 5 being “I totally agree with it”. After consenting to enter the study, patients began with an 8 mg daily oral dose of silodosin. This dose could be titrated to 16 mg at 8w. Adverse events were assessed and recorded.

2.3. Statistical Analysis

Data analysis was performed with the statistical package for social sciences (SPSS version 28.0). Data are presented as medians, with the 25 and 75 percentiles (25; 75). Differences assessed by a non-parametric Wilcoxon signed-rank test ($p < 0.05$) were considered statistically significant. The analysis was carried out *per-protocol*, comparing the data from the baseline with that from 12w in patients completing all 12 weeks. The primary end-point was also evaluated, taking into account the dropouts. Patients who left the study before the 12w were excluded from the final analysis but were included for safety exploration.

3. Results

3.1. Demographics

Patients had a median age of 56 (46; 66) years old. Three patients presented Hunner lesions at cystoscopy. Fifteen of them had previously received at least one treatment of intra-trigonal injection of onabotulinum toxin A six or more months before. Dose escalation to silodosin 16 mg was requested by four patients at 8w.

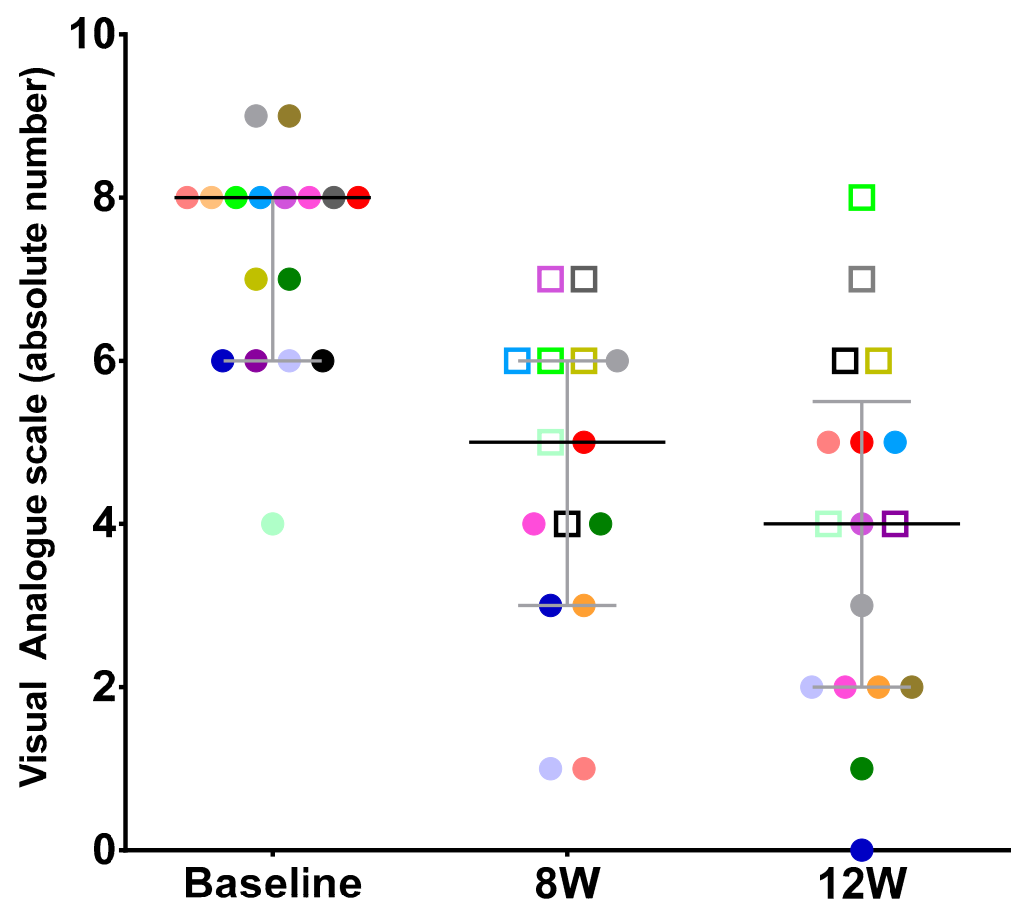
A total of three patients abandoned the study. Two patients abandoned therapy at week 8 due to lack of effect, without trying dose escalation despite the clinician’s effort to try medication until the 12th week. The third patient was excluded due to the diagnosis of a UTI during the study period, which caused a symptomatic flare up. Two patients failed to attend the 8w visit due to the COVID-19 pandemic but were observed at 12w.

3.2. Outcomes

The characteristics of the population at baseline are shown in Table 1. The median value of pain intensity ascertained by VAS was 8.0 (6.0; 8.0); total urinary frequency and nocturia were 14.0 (13.0; 21.0) and 4.0 (2.0; 4.8), respectively. The MVV was 175 mL (150; 250); OSS was 26.0 (24.0; 28.0); and QoL was 5.0 (4.3; 6.0). Concerning the primary endpoint, pain score at 12w, the median VAS score from the 17 patients available was 4.0 (2.0; 5.5), which indicates a significant reduction when compared to the baseline value of 8.0 (6.0; 8.0) (non-parametric Wilcoxon signed-rank test $p = 0.001$). A total of 11 out of 17 patients (65%) had an improvement of 3 or more points from baseline to 12w, as seen in Figure 1. This reflects an MID improvement in 11 out of 17 patients (65%) at 12w, in the *per-protocol* analysis. When evaluated taking into account the full analysis set, the percentage of clinically significant improvement was 55% (11/20).

Table 1. Outcome comparison between baseline and 12w.

	Baseline Median (P25, P75)	W12 Median (P25, P75)	<i>p</i> -Value
Pain VAS	8.00 (6.00; 8.00)	4.00 (2.00; 5.50)	0.001
Total Voiding Frequency	14.00 (13.00; 21.00)	9.00 (7.50; 11.00)	0.001
Nocturia	4.00 (2.00; 5.50)	2.00 (1.00; 3.00)	0.001
MVV	175.00 (150.00; 250.00)	250.00 (200.00; 350.00)	0.03
OSS	26.00 (24.00; 28.00)	19.00 (14.00; 22.50)	0.001
QoL	5.00 (4.25; 6.00)	3.00 (2.00; 4.00)	0.001

**Figure 1.** Median and (p25–p75) intervals and individual pain score on the VAS at baseline and weeks 8 and 12. Each patient is identified by a specific color and squares mean that MID was not reached.

At 12w, the total urinary frequency decreased to a median of 9.0 (7.5; 11.0), which was significantly lower than the baseline median of 14.0 (13.0; 21.0) micturition (non-parametric Wilcoxon signed-rank test $p = 0.01$). A total of 14 patients out of the 17 (82%) achieved the MID of a decrease of at least two micturition from baseline to 12w. The total urinary frequency variation per patient result is shown in Figure 2.

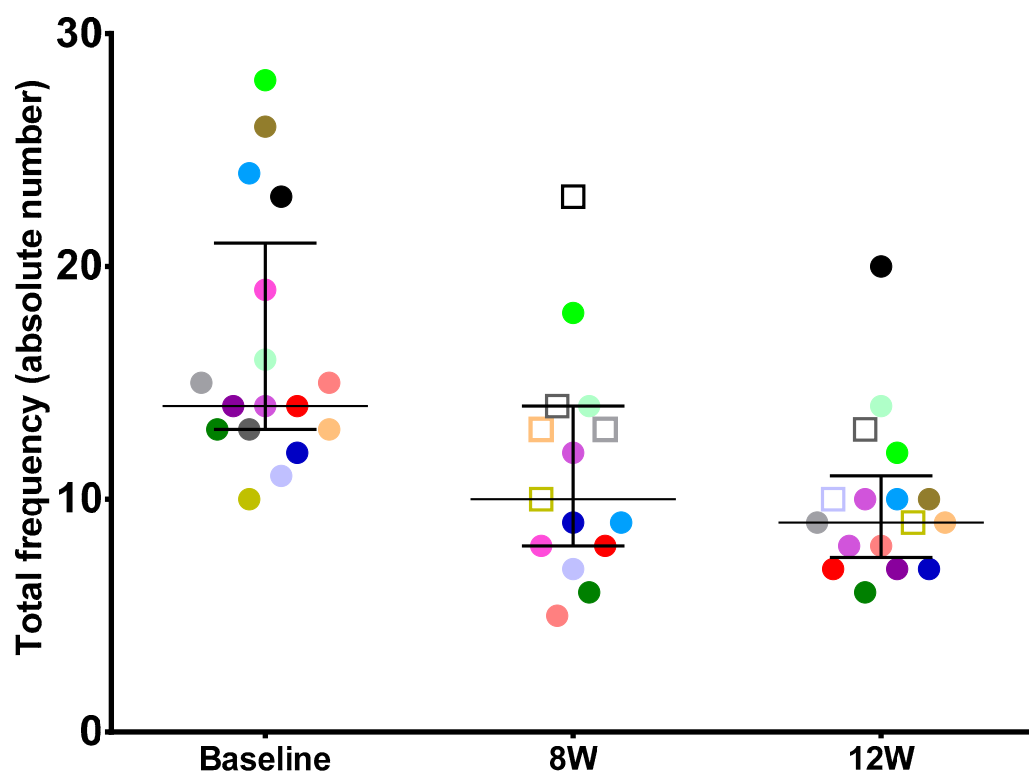


Figure 2. Median and (p25–p75) intervals and individual total daily frequency at baseline and weeks 8 and 12. Each patient is identified by a specific color, and, when represented as a square at 8w or 12w, this means that MID was not reached.

Nocturia decreased to 2.0 (1.0; 3.0) at 12w, with the baseline being 4.0 (2.0; 5.5) micturition per night, and this is a statistically significant difference (non-parametric Wilcoxon signed-rank test $p = 0.01$). A total of 13 (76%) patients achieved an MID of one episode per night between baseline and 12w.

Assuming that greater satisfaction relies upon an important improvement in both pain and urinary frequency, we analyzed the simultaneous decrease in pain and total frequency according to the defined MID. A total of 10 out of 17 patients (59%) presented this simultaneous improvement, as shown in Figure 3. Five patients achieved an MID improvement in only one of the parameters and only 2 patients did not reach an MID improvement in any of the two outcomes.

When it comes to the other secondary endpoints, at 12w the median MVV increased to 250 mL (200; 350) mL; OSS total score decreased to 19.00 (14.00; 22.50) with a and QoL decreased to 3.00 (2.00; 4.0). All these data were statistically significant when compared to baseline values through the non-parametric Wilcoxon signed-rank test (MVV $p = 0.03$; OSS $p = 0.01$ and QoL $p = 0.04$) shown in Table 1.

At 8w, some improvement could already be detected. The median VAS pain score was 5.0 (3.0; 6.0), indicating a significantly early pain reduction (non-parametric Wilcoxon signed-rank test $p = 0.001$). Moreover, at this earlier time point, the total frequency had already significantly decreased to 10.0 (8.0; 14.0) micturition when compared to baseline (non-parametric Wilcoxon signed-rank test $p = 0.03$) (Figures 1 and 2).

In an exploratory attempt to find possible predictors of a positive response to silodosin, we compared the demographics between patients who presented an improvement of more than 3 points in the VAS pain intensity and the patients who did not reach this improvement. The compared demographics were: age, pain intensity, urinary frequency, nocturia, MVV, OSS, and QoL. The results and the comparison between groups are presented in Table 2. None of the above characteristics show a statistically significant difference.

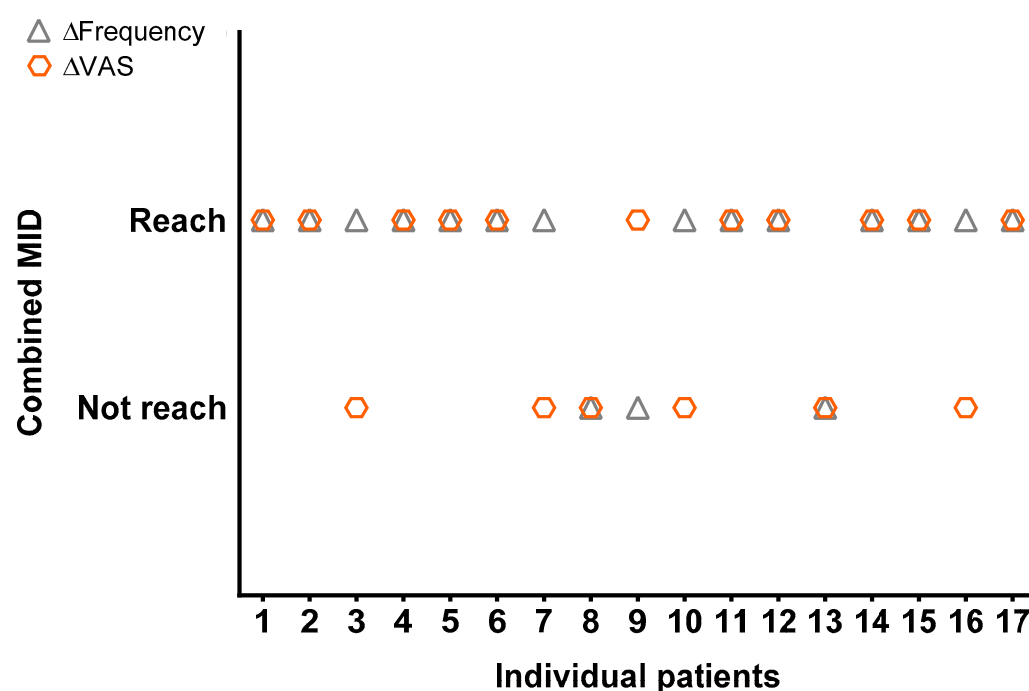


Figure 3. Individual combined evaluation of MID in VAS score and total urinary frequency at week 12.

Table 2. Comparison of demographic characteristics between patients that reached the primary endpoint (responders) and those who did not (non-responders).

Demographics	Responders	Non-Responders	<i>p</i> -Value
Age	49.0 (35.0; 67.0)	58.8 (49.0; 64.0)	0.52
Baseline VAS	8.00 (7.00; 8.00)	6.50 (5.50; 8.00)	0.09
Total Urinary Frequency	14.00 (13.00; 19.00)	15.00 (12.50; 24.50)	0.81
Nocturia	4.00 (3.00; 5.00)	3.00 (1.75; 8.00)	0.81
MVV	150.00 (150.00; 250.00)	200.00 (150.00; 265.00)	0.78
OSS	24.00 (27.00; 29.00)	25.00 (23.50; 27.50)	0.24
QoL	5.00 (4.00; 6.00)	4.50 (6.00; 3.80)	0.40

Of the 17 patients, 13 answered to the question “Does stress aggravate or initiate BPS/IC symptoms?” with yes, as opposed to 4 patients who denied an association between stress and symptoms worsening. The median answer to the second question “I have a relaxed personality, coping well with stress” was 2.0 (1.0; 2.75) from 0 to 5, which means that patients tend to have a low capacity for coping with stress.

No adverse events were registered, not even in the dropouts.

4. Discussion

The most important finding of the present pilot study was the clinical confirmation that the blockade of α_{1A} -Ars with silodosin, at a dose of 8 to 16 mg daily, induced a substantial improvement in pain, the primary endpoint. Although the endpoint was defined at 12 weeks, relevant improvement in pain intensity was also observed at week 8. In addition, secondary outcomes such as daily voiding frequency and bladder functional capacity were also substantially improved after 12 weeks of therapy. All these findings certainly contributed to the marked improvement in QoL observed. The effect of silodosin is also in agreement with a previous finding of this group, which consisted of the demonstration of an exaggerated sympathetic activity and high levels of urinary NE excretion [11].

We statistically evaluated the cohort with medians comparisons and with individual treatment responses given the low number of enrolled patients. This way, we weakened the bias that great responses could have in lower responders and at the same time evaluated responses through MID, assuming that a complete resolution of complaints would be unrealistic; rather, a satisfying level of improvement was sought.

All the parameters evaluated improved significantly. This could indicate that silodosin could have a place in the oral pharmacology treatment for BPS/IC patients. These improvements were obtained both in objective and subjective parameters.

In total, 11 out of 17 patients achieved an MID of 3 points of pain in the VAS score, which is a significant result since the main complaint of this condition is bladder pain. These results could be biased by the single-time evaluation of pain, a parameter that could fluctuate over time. When comparing the demographic parameters between responders and non-responders, none of these parameters were found to be helpful in identifying a possible characteristic or predictor of positive results, as seen in Table 2. The urinary frequency, both daytime and night-time, also significantly improved. Although the urge to pass urine in BPC/IC patients is usually caused by pain or the fear of bladder pain, in some patients the high urinary frequency persists despite pain improvement [13], and for this reason the improvement of this parameter could be of equal importance when compared to pain. A total of 14 in 17 patients had an improvement of at least 2 micturitions in total urinary frequency, and 8 of the 17 patients had an MID improvement in nocturia.

The analysis of treatment response *per patient*, using the MID, evaluated the most bothersome features for these patients: pain and urinary frequency. Fifty-nine per cent (10 out of 17) of the patients significantly improved simultaneous in terms of pain and urinary frequency, and this study could strongly support a future randomized clinical controlled trial (RCT), which we are planning to perform. According to the calculations, a total of 70 patients (35 per group) should be randomized to placebo or silodosin in a ratio of 1:1.

The present clinical data support previous experimental observations. Matos et al. showed in rodents that bladder pain, voiding bladder contractions, and histologic bladder features similar to those seen in BPS/IC patients, could be triggered by the systemic administration of an alpha-adrenergic agonist, and that such effect could be prevented by α_{1A} -ARs [14,15]. Moreover, it has also been shown that the activation of peripheral α_{1A} -ARs could enhance bladder pain through the pain receptor TRPV1 by increasing the amount of ATP released to bladder distension, suggesting the existence of crosstalk between the sympathetic nervous system and nociceptive pathways.

In addition, in the WAS model of stress, Dias et al. showed increased levels of noradrenaline in the urine of rodents [16]. The WAS model in rodents seems to replicate the signs of nociception and bladder changes seen in BPS/IC patients [11]. Rothrock as well as Lutgendorf have shown that higher levels of stress are related to greater pain and urgency in patients with IC but not in controls [17,18]. Stress threshold differs inter- and intra-individually, as it can be dependent on the circumstances in which the questionnaire is answered. In our study, the answers to both questions seem to reflect a group of patients with a usually low capacity to cope with stress, recognizing symptom aggravation with stressful events. These observations should be considered when selecting BPS/IC patients for alpha-1A blocker treatment and in patient inclusion in RCT.

Silodosin is a very safe drug. The absent effect on α_{1B} -AR lowers the risk of dizziness or orthostatic hypotension. In women, we confirmed the safety of silodosin use, even when escalated to 16 mg. Two patients abandoned the study before week 4 at a very early phase of the treatment, eventually indicating that the effect of silodosin on pain is not immediate.

This single-arm study has obvious limitations: the lack of a control group and not being blinded. The number of enrolled patients was small, with three of them dropping out. Intrinsic to this condition, pain is a subjective symptom which depends on different variables, and its intensity can fluctuate over short periods. This last one is specifically true in chronic pain conditions, which can present unprovoked flares. Another limitation was

the assessment of self-perception of stress coping, which was based on two questions, and the answer was always modulated by the feeling at that moment.

5. Conclusions

In conclusion, daily silodosin could be an effective and well-tolerated treatment for refractory BPS/IC females. The treatment effect was sustained for 12 weeks. Personality type does not seem to be an independent factor for treatment response. More robust studies should be conducted to assess the role of silodosin in BPS/IC patients.

Author Contributions: Conceptualization, R.P.; Data curation, B.A.-S.; Formal analysis, B.A.-S., A.C. and R.P.; Funding acquisition, B.A.-S. and A.C.; Investigation, F.C.; Methodology, F.C.; Project administration, F.C.; Software, R.P.; Supervision, P.A.-M., F.C. and R.P.; Validation, P.A.-M. and R.P.; Writing—original draft, B.A.-S.; Writing—review & editing, P.A.-M., A.C. and F.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Centro Hospital e Universitário de São João (protocol code 338-4, 25 February 2022).

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

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy issues.

Acknowledgments: This abstract was previously presented at the Portuguese Urological Association Annual Congress and the EAU Annual Congress 2022.

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

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Publication 2

**Daily low-dose of Tadalafil improves pain and frequency in
Bladder Pain Syndrome/Interstitial Cystitis patients.**

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Turk J Urol. 2022 Jan;48(1):82-87 11, 5659. (DOI:10.5152/tud.2022.21292)

Daily low dose of tadalafil improves pain and frequency in bladder pain syndrome/interstitial cystitis patients

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Cite this article as: Abreu-Mendes P, Dias N, Simões J, Dinis P, Cruz F, Pinto R. Daily low dose of tadalafil improves pain and frequency in bladder pain syndrome/interstitial cystitis patients. *Turk J Urol.* 2022; 48(1): 82-87

Abstract

Objective: Bladder pain syndrome/interstitial cystitis (BPS/IC) is a chronic disease, with consequent high morbidity. Increasing evidence suggests that bladder afferent hyperexcitability, through neurogenic bladder inflammation and urothelial dysfunction, plays a key role in the pathophysiology of BPS/IC. The rationale of using phosphodiesterase type 5 inhibitors (PDE5i) would be to decrease bladder afferent hyperactivity. Detrusor relaxation, improvement of microcirculation, and a decrease in adrenergic nociceptive overactivity would be other effects in bladder tissue. We aimed to evaluate the efficacy, tolerability, and safety of a daily low dose of 5 mg tadalafil in refractory BPS/IC patients.

Material and methods: A total of 14 refractory BPS/IC female patients, previously evaluated with a physical examination, bladder diary, bladder-pain related visual analogue score, O'Leary-Sant Scores (OSS) for symptoms and problems, and quality of life (QoL) question from International Prostate Symptom Score, were treated with 5 mg of tadalafil, for 3 months. Re-evaluations occurred at 4 and 12 weeks. Adverse events were assessed and recorded.

Results: Urinary frequency, OSS, and QoL were significantly improved at 1-month follow-up (10 ± 2.5 , 21.9 ± 4.1 , and 4 ± 1.5 , respectively, $P < .05$). Pain intensity and volume voided were significantly improved at a 3-month follow-up (3.5 ± 2 and 266.7 ± 60.5 , $P < .05$). Patients referred to urinary frequency as the most important parameter improved at 4 weeks, and pain at 3 months. No differences between ulcerated and nonulcerated patients were observed. Two patients dropped out due to unsatisfactory results and two due to persistent headache and/or tachycardia, but both events were resolved after discontinuing the drug.

Conclusion: Daily low-dose tadalafil is an easy, well-tolerated, and effective treatment for refractory BPS/IC in women.

Keywords: Cystitis; drug therapy [E02.319]; interstitial [C12.777.829.495.500]; phosphodiesterase 5 inhibitors [D27.505.519.389.735.500]; tadalafil [D03.633.100.473.155.500].

Introduction

Bladder pain syndrome (BPS) was defined in 2008's consensus from International Continence Society (ICS), as a chronic (>6 months) pelvic pain, pressure, or discomfort perceived to be related to the bladder, accompanied by at least one other urinary symptom such as a persistent urge to void or frequency.¹ The term interstitial cystitis (IC) should be reserved for a specific phenotype in cystoscopy and histologic features: the presence of

Hunner's lesions (HLs).^{2,3} During a transition period, International Society for the Study of Bladder Pain Syndrome (ESSIC) has agreed to include IC in the overall term (BPS/IC) to facilitate the transition of terminology.¹ BPS/IC has an overall prevalence of 10.6/100,000, affecting more women (6:1) than men.^{4,5}

According to the American Urologic Association (AUA) guidelines, the impact of BPS/IC on psychosocial health and quality of life (QoL) is so severe that it could ultimately ruin work-

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Submitted:
06.10.2021

Accepted:
01.12.2021

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life, psychological well-being, personal relationships, and general health.⁶ All the guidelines agree that the initial step should be the assessment and management of pain.^{6–9} The following steps are still controversial. According to ESSIC, the second step should be bladder biopsies with hydrodistention, while the AUA suggests undergoing this invasive procedure only in more complex or refractory cases.^{1,6} The findings in the urothelium during bladder hydrodistention (normal vs glomerulations vs the presence of HL) and the findings from the biopsied urothelium are needed to phenotype according to the ESSIC criteria.¹⁰ The two phenotypes are with HL (used for IC, or ESSIC classification 3X) and the non-HL or BPS.

When referring to the physiopathology of these conditions, little is known but one of the main actors in nociception seems to be the L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway.^{11,12} This pathway is responsible for signal transduction both in the central and peripheral nervous systems being especially responsible, through the activation of protein kinase G (PKG), for the inhibition of the nervous pain signaling.¹³ The mechanism of action seems to be directly related to the effect of PKG in stimulating the opening of ATP-sensitive K⁺ channels, and the consequent restoring of the nociceptor threshold.¹⁴ cGMP, the final product of the L-arginine-NO pathway, seems to be utterly important for the regulation of the nociceptor fibers threshold. The enzymes phosphodiesterase (PDE) 5, 6, and 9 inhibits the GMP degradation to 5-GMP, potentially favoring their antinociceptive effect, being PDE 5 the most relevant enzyme.¹⁵

According to the AUA guidelines, most treatments are targeted at symptom control and no treatment works overtime for the majority of patients.⁶ The treatment approach should be therefore patient-tailored to optimize life quality.^{7,8}

A previous multicentric, double-blinded, randomized controlled trial evaluated the efficacy and safety of low-dose silde-

nafil, improved in terms of urinary frequency complaints, both daytime and nocturn, although the results in terms of pain were not significant.¹⁶ Following a previous study, we aimed to assess the efficacy, tolerability, and safety of an oral daily low dose of tadalafil (5 mg) in refractory BPS/IC patients, once tadalafil presents a better pharmacokinetic profile, with and half-life of approximately 24–36 hours.¹⁷

Material and Methods

Patients

After obtaining clearance from the local ethics committee (Protocol number 40-20: Papel do tadalafil na Cistite Intersticial), 14 females, previously diagnosed with BPS/IC, according to ESSIC criteria, and refractory to previous medical and intravesical therapy, entered this single-arm trial, during 2019 Centro Hospitalar e Universitário de São João, Porto, Portugal. Patients were asked if they accepted to take tadalafil as an off-label treatment for this kind of Lower Urinary Tract Symptoms (LUTS) and if they consent to have their data analyzed and shared at the end of the 12 weeks of treatment.

Patients were aware of the type of drug and the possible adverse events (AEs). The subjects were subdivided, during the analyses of the results, according to the presence or absence of the HL. Patients have been previously advised to change their negative lifestyle choices, such as excessive drinking and quitting smoking before starting this trial.

Study Design

This was a single-arm exploratory trial. Patients who accepted to participate received an oral daily low dose of tadalafil (5 mg) for 12 weeks. There was no dose titration option. The evaluation of symptoms and complaints was performed at baseline, in the 4th week, and the 12th week.

Evaluation

The evaluation compromised a 3-day bladder diary to evaluate functional bladder capacity (CC) the week previous to the appointment; and during the appointment, the investigators evaluated: bladder-pain related visual analogue score (VAS) from 0 to 10 points representing the perception of mean pain during the period since last evaluation, O'Leary-Sant Scores (OSS) for symptoms and problems from 0 to 43 (with 43 representing being the worst symptoms possible), and QoL was evaluated through the last question from International Prostate Symptom Score (from 0 to 6 points, being 6 the worst QoL imaginable). Urinary tract infections and other AE were also screened.

Statistical Analysis

Data analysis was performed with a Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM SPSS Corp.;

Main Points

- BPS/IC patients usually underwent several lines of therapy before reaching the best therapeutic approach (mono or multimodality).
- Tadalafil, an inhibitor of the phosphodiesterase 5, seems to act in the pain pathway.
- In patients' refractory to previous modalities of treatment, tadalafil 5 mg seems to have positive results in the pain and urinary frequency symptoms.
- The already known adverse events of this drug can be limiting and should be carefully explained to these patients.

Table 1. Patient's Demographics

	n/HL	HL
Age	39 ± 9	48 ± 16
N	7	7
Previous local therapies	2	6
Compatible histology (ESSIC class C)	2	6
>6 months disease time	7	7
N/HL, non-Hunner's lesion; HL, Hunner's lesion.		

Armonk, NY, USA). Data evaluation at two-time points was deltas against the baseline and presented as mean and standard deviation. Differences were assessed by appropriate exact parametric testing: T-test when normality was observed and non-parametric Mann-Whitney U test ($P < .05$ was considered statistically significant). The relative reduction was calculated by a simple comparison between the two evaluations, in percentage.

Results

Demographics

The trial included 14 female patients, with a mean age of 46 ± 15 years. Seven patients presented HLs, being six of them classified as 3C according to ESSIC classification and one patient had an inconclusive biopsy and was classified as 3B. The remaining patients were classified as 2C. Regarding previous treatments, all were refractory to systemic and/or intravesical therapies. Eight patients who had received an intratrigonal injection of 100 units of onabotulinum toxin A and decided to participate in the trial had the injection at least 12 months apart from the beginning of the trial. Patients' demographics are presented in Table 1.

Outcomes (VAS/Frequency/OSS/CC/QoL)

The improvements in all measured outcomes are represented in Table 2. At baseline, pain intensity in VAS was 6.7 ± 1.4 , OSS was 25.7 ± 4.3 , and day and night frequency were 12.6 ± 5.2 and 4.4 ± 2.7 , respectively. Functional bladder capacity was 185.7 ± 56.9 mL, and QoL score (0-6) was 5.0 ± 1 .

Pain intensity was majorly improved at the 12th week, with a reduction of 6.7 ± 1.4 to 3.5 ± 1.9 (relative reduction of 48%) and was statistically significant at that time point ($P < .01$). At 3 months, eight of the 10 patients had a reduction in VAS of more than two points, the other two patients maintained the same score.

OSS decreased through the 3 months' study, from 25.7 ± 4.3 to 17.8 ± 5.9 (reduction of 31%), and the difference was statistically significant in the transition between baseline and week 4 with a relative reduction of 15% ($P = .01$), and at week 12 ($P < .01$), with a relative reduction from baseline of 31%.

Compared with baseline, the urinary frequency was significantly improved at week 4 with a reduction of 16.9 ± 7 to 9.9 ± 2.6 (relative reduction of 42%). At week 12, the mean urinary frequency was 8.3 ± 2.6 , with a relative risk reduction of 51% from baseline. This trend toward reduction was maintained at the 12th week. Differences were statistically significant between baseline and week 4 and week 12 ($P < .01$, in both). At the 3rd month, all 10 patients reduced the number of micturitions. The minimum reduction was from 13 to 8, and the maximum difference was from 36 to 13 micturitions per day.

QoL scale presented a gradual improvement, from 5.1 ± 0.9 to 4.1 ± 1.5 at week 4 (relative reduction of 20%), with a final score of 3 ± 1.3 (relative reduction of 42%) at 12 weeks, and differences were statistically significant along with the trial ($P = .01$ and $P < .01$, respectively).

Table 2. Study Results

	Baseline	W4	RR (Base-W4)	P value (Bas-W4)	W12	RR (Base-W12)	P value (Bas-W12)
VAS	6.7 ± 1.4	5.3 ± 1.9	21%	.056	3.5 ± 1.9	48%	.001
Freq	16.9 ± 7.2	9.9 ± 2.5	42%	.01	8.3 ± 2.6	51%	.001
CC	185.7 ± 56.9	238.9 ± 48.6	28%	.07	266.7 ± 60.5	43%	.01
QoL	5.1 ± 0.9	4.1 ± 1.5	20%	.01	3 ± 1.3	42%	.001
OSS	25.7 ± 4.3	21.9 ± 4.1	15%	.01	17.8 ± 5.9	31%	.001

W4, week 4; W12, week 12; RR, relative reduction; base, baseline; VAS, visual analogue scale; Freq, frequency; CC, functional bladder capacity; QoL, quality of life; OSS, O'Leary-Sant.

CC, evaluated through micturition in the 3-day bladder diary, also experienced a major improvement, with an increasing volume of voided urine per micturition (185.7 ± 56.9 to 266.7 ± 60.5). This difference was only statistically significant at week 12 ($P = .01$), with a relative increase in 43%.

When comparing patients with and without HL at baseline, no statistical differences between the groups were noted except the QoL score, significantly worse in the HL group.

A more pronounced improvement in nonulcer patients, when compared with HL patients, is seen, but this difference is not statistically significant in all variables measured ($P > .05$ when compared to the different values). Frequency improvement was always statistically significant in non-HL patients ($P < .05$), while it was only statistically significant at 3 months of follow-up on ulcer patients ($P = .04$). VAS and OSS improvements were statistically significant only at 3 months of follow-up, in both phenotypes. CC improved with a statistical significance in non-HL, while in ulcerated patients, this improvement was not significant—expected given the fibrotic course of this specific phenotype. At 3 months, none of the particular phenotype seems to benefit more than the other.

According to patients, while the urinary frequency was the most important reported parameter improved at 1-month follow-up, the pain improvement was the most important at 3 months.

Adverse Effects

After 1 month in therapy, four patients (30% of the participants) dropped out of the study: two (15%) due to the lack of symptom improvement—treatment failure, and two (15%) had a persistent headache and/or tachycardia. Both of the patients presented minor AEs, not life-threatening, and were resolved after tadalafil discontinuation without the need of consulting a doctor for this purpose.

Discussion

The absence of unanimous guidelines in terms of which therapeutic escalation should be used, allied to a large number of patients who do not respond to the usual first lines (analgesic, amitriptyline, and pentosane sulfate), explains the reason why pain control is usually achieved after experimenting several drugs, usually with a massive burden of pain-killers. This motivates us to search for off-label treatments. 5PDE-i(s) were initially used for pulmonary hypertension and, after observing their AEs, began to be used for erectile dysfunction given their vasodilator effect. Later, it was observed that this class of

drugs could improve LUTS, and tadalafil was the drug with a more favorable (longer) half-lifetime. Nowadays, its use in voiding and storage LUTS are official as European Association Urology Guidelines demonstrate.¹⁸

The first and only placebo-controlled trial evaluating tadalafil in BPS/IC patients was published in 2014 by Chen et al¹⁶ The group evaluated the efficacy of daily sildenafil (25 mg) for 3 months. The authors concluded that sildenafil improved the OSS and the urodynamic index (first desire to void, strong desire to void, and functional bladder capacity). No significant change in the VAS score was observed between the two groups, except at week-12 in the treatment group.

Although pain is the hallmark symptom of BPS/IC and it can be located anywhere in the pelvis or external genitalia, most patients experience urgency and/or urinary frequency.¹⁹

The primary outcomes in our trial were pain and urinary frequency. Our patients reported urinary frequency as the most important reported parameter improved at 1-month follow-up, and pain the most important at 12-week follow-up. No significant differences were registered in terms of improvement between ulcerated and nonulcerated patients in these parameters.

Jain et al¹⁵ conducted a preclinical trial exploring the role of the NO-cGMP pathway in nociceptive conditions. Peripheral nociception was assessed chemically while central nociception was assessed by tail-flick and hot-plate methods. In this trial, the use of sildenafil, a member of the PDE5i drug class like tadalafil, induced a dose-dependent antinociception. This analgesia was blocked by methylene blue, a guanylate cyclase inhibitor (inhibits the formation of cGMP) as tadalafil.

Minagawa et al²⁰ showed that tadalafil dose-dependently decreased single afferent activities in both A δ and C-fibers during intravesical saline instillation. Tadalafil inhibited the acrolein-induced hyperactivity of both fibers, showing that systemic administration of tadalafil reduces mechanosensitive afferent activities. A long way must be walked to understand the real function of PDE5i in bladder nociception. Nevertheless, our hypothesis relies on PDE5i as a new possible drug to control pain in BPS/IC patients' refractory to other therapies. Tadalafil is commercially available in low-dose, 5-mg, with an optimal half-life of approximately 17 hours (the reason for being the only PDE5i licensed for male LUTS) and is the ideal drug to start the experimentation in these patients.¹⁸

BPS/IC patients also experience urinary frequency and urgency. In our study, the urinary frequency was the parameter with a higher improvement in the 4th-week evaluation.

This symptom can be caused by increased sensitivity of detrusor muscle due to elevated urinary potassium or adrenergic nociceptive overactivity.^{21,22} These are probably mediated by an increased RhoA/Rho-kinase (ROCK) signaling, and it is known that this pathway, in the vascular soft muscle, is disabled by the GMPc-protein kinase G. Fibbi et al²³ investigated the PDE5 tissue distribution and activity in human lower urinary tract tissues (urethra, prostate, and bladder), concluding that the highest expression and biological activity of PDE5 were found in the bladder. Morelli et al²⁴ demonstrated that the increase in GMPc in the bladder, by the use of a PDE5i, can block ROCK signaling. Bittencourt et al²⁵ showed sildenafil-induced smooth muscle relaxation, demonstrating that there is a dependence of the NO and GMPc formation pathways for bladder relaxation.

Vasquez et al²⁶ showed that bladder overcontraction caused by deficient bladder vascularity in the setting of endothelium dysfunction could be reverted with the use of 5PEDi. It was also shown that sildenafil can modulate the number of endothelium progenitor cells, which can explain the effects of this drug on endothelium reparation and normal function.

From all these preclinical and clinical studies, it seems that tadalafil mechanisms of action can be related to BPS/IC symptoms. The relaxation of the bladder detrusor muscle, improvement of microcirculation, and reabsorption of excessive potassium in the bladder (by inhibiting the cGMP-G-RhoA/Rho-kinase pathway and NO-cGMP pathway) can explain part of the BPS/IC symptoms, namely, pain and frequency.

Our decision to perform a subanalysis based on patients with HL and without was based on a previous Van Moh et al's²⁷ work. The group compared the characteristics between patients with and without HL, concluding that participants without HL reported more pain.²⁷ Contrarily, Killinger et al²⁸ conducted assessed pain characteristics in women grouped by IC/BPS subtype and no difference in pain distribution or characteristics in ulcerative and nonulcerative groups. Braunstein et al²⁹ also concluded no significant differences between symptom duration, history of gross hematuria, history of comorbid disease, or VAS score between the two groups.

The worst QoL of patients with HL observed in our study was previously described by El Khoudary et al³⁰ The higher rate of frequency and a lower bladder capacity were observed and seem to be the reason for the worst QoL baseline.

Although it is tempting to assume that probably there are significant overlaps in symptomatology between the two groups, the origin of both phenotypes could be different, and particular

treatments can be more effective in one group. That was not the case in our study, the results evidenced that there were no statistical differences between ulcer and no ulcer patients. This suggests that cystoscopy is needed to accurately identify patients with HL since the clinical evaluation is insufficient to distinguish the two groups. However, more research is needed in larger samples to determine whether differences exist.

The characteristics of our study (single-arm study, with low sample size, without a control group) make us impossible to take safe conclusions. Additionally, the elevated number of dropouts did not favor the paper. More clinical studies, namely, placebo controlled, should be promoted. The lack of patient compliance was also a limitation in our study.

In this context, and because tadalafil appears to have a quick effect on frequency and pain, it seems to be a drug with a possible role in a subset of refractory patients.

In conclusion, daily low-dose tadalafil could be an effective, simple, and well-tolerated therapy for BPS/IC patients' refractory for first-line therapies. Further randomized, controlled, multicenter trials with a larger population and a longer follow-up period are warranted to confirm these results and, if possible, to tailor this treatment for a specific phenotype of patients.

Ethics Committee Approval: Ethical committee approval was received from the local ethics committee (Protocol number 40-20: Papel do tadalafil na Cistite Intersticial).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - P.A.M., R.P.; Design - P.A.M., R.P.; Supervision - P.A.M., P.D., F.C., R.P.; Analysis and/or Interpretation - P.A.M., N.D., R.P.; Literature Search - P.A.M., N.D., F.C., R.P.; Writing Manuscript - P.A.M., R.P.; Critical Review - P.A.M., N.D., P.D., F.C., R.P.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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Publication 3

Effect of Intratrigoal Botulinum Toxin in Patients with Bladder Pain Syndrome/Interstitial Cystitis: A Long-Term, Single-Center Study in Real-Life Conditions

Abreu-Mendes P.; Ferrão-Mendes A.; Botelho F.; Cruz F.; Pinto R. *Toxins*

MDPI 2022, 14, 775. (DOI: 10.3390/toxins14110775)

Article

Effect of Intratrigenal Botulinum Toxin in Patients with Bladder Pain Syndrome/Interstitial Cystitis: A Long-Term, Single-Center Study in Real-Life Conditions

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Abstract: The high percentage of treatment failures seen in patients with bladder pain syndrome/interstitial cystitis (BPS/IC) managed conservatively frequently demands invasive treatment options. We aimed to evaluate the long-term efficacy and adverse events of intratrigenal botulinum toxin injection in such circumstances, as well as to determine possible predictors of response to toxin treatment. A retrospective cohort study included 47 female BPS/IC patients treated with onabotulinum toxin A (OnabotA) in a tertiary hospital between the years 2009 and 2022. All patients received 100 U of OnabotA in ten injections limited to the trigonal area. Patients were divided into three groups based on their treatment response as responders, non-responders and lost to follow-up due to non-medical reasons. The clinical and surgical records of the individuals were retrieved, including the 10-point visual analogue scale (VAS), the number of treatments, the time between injections, and the age at the first injection. A total of 25 patients (>50% of the cohort) were long-term responders, but none of the evaluated parameters was a predictor for this circumstance: age, pain intensity, or duration of improvement following the injection. The time between injections was stable (around 1 year). No severe adverse events were registered. The intratrigenal injection of botulinum toxin in patients with BPS/IC was an effective and safe long-term treatment for patients' refractory to conservative forms of treatment. Age, basal pain intensity, and time to injection request did not predict long-term response to OnaBotA.

Keywords: botulinum toxin A; onabotulinum toxin A; bladder pain syndrome/interstitial cystitis; long-term treatment

Key Contribution: Long-term intravesical injection of OnaBotA is effective and safe for BPS/IC patients refractory to conservative treatments.



Citation: Abreu-Mendes, P.; Ferrão-Mendes, A.; Botelho, F.; Cruz, F.; Pinto, R. Effect of Intratrigenal Botulinum Toxin in Patients with Bladder Pain Syndrome/Interstitial Cystitis: A Long-Term, Single-Center Study in Real-Life Conditions. *Toxins* **2022**, *14*, 775. <https://doi.org/10.3390/toxins14110775>

Received: 18 October 2022

Accepted: 5 November 2022

Published: 10 November 2022

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1. Introduction

The European Society for the Study of Interstitial Cystitis (ESSIC) defines bladder pain syndrome/interstitial cystitis (BPS/IC) as a persistent or recurrent chronic pelvic pain, pressure or discomfort perceived to be related to the urinary bladder in the absence of any identifiable pathology which could explain this symptom [1]. It must be accompanied by at least one other urinary symptom, such as an urgent need to void or urinary frequency [1]. Therefore, BPS/IC diagnosis is still largely one of exclusion.

BPS/IC has no curative treatment as of yet [2]. Thus, symptom control, with the main focus on pain, represents a key part of BPS/IC management. The first line of treatment is centered on patient education and stress control to inform the patient about the uncertain

evolution of the disease and to explain the importance of self-management by avoiding situations that may aggravate the symptoms [2]. When necessary, oral analgesic therapies and pharmacological agents intended to replenish the glycosaminoglycan layer and decrease urothelial permeability are used as the second-line treatment. When these measures are insufficient, surgical therapies may be introduced [3–5]. One option is the intratrigonal injection of onabotulinum toxin type A (OnaBotA) [6,7].

OnaBotA is a potent biological neurotoxin that accesses the neurons after binding the synaptic vesicle protein type 2C, a ubiquitous neuronal protein [6,8,9]. Once inside the neurons, the light chain of the toxin cleaves the proteins that are essential for the docking of the neuronal vesicles to the membrane in all types of neurons [9]. In sensory neurons, the trafficking of pain receptors from neuronal vesicles to the membrane of sensory neurons will be impaired and the release of CGRP and SP, two neuropeptides involved in neurogenic inflammation, will be substantially decreased [6,7,10]. In addition, a decrease of ATP release from urothelial cells occurs which may further impair bladder sensation [7,11,12]. The OnabotA-induced inhibition of the acetylcholine release from pre- and post-ganglionic parasympathetic neurons [13] is expected to have a limited role in BPS/IC, as this neurotransmitter is not involved in peripheral pain pathways and detrusor overactivity is a rare event associated with BPS/IC [9,14].

The application of OnabotA in the trigone is justified by the fact that this bladder region has the highest density of nociceptors [11,15,16]. Since 2004, multiple cohort studies and randomized placebo-controlled trials showed that OnaBotA was effective and safe in pain control in BPS/IC patients refractory to conservative treatment, in the dosage of 100 Units (U) [14,17–20]. In addition, trigonal injections of OnabotA also improve day and nighttime frequency, maximum functional bladder capacity, and overall quality of life, which are believed to result from less pain felt by patients during bladder filling [21].

One of the characteristics of OnabotA action is the limited duration of the effect, which, although variable, rarely extends for more than 12 months [7]. After this period of effect, BPS/IC symptoms tend to return, and repeated bladder injections are commonly requested by patients [21]. In previous prospective studies, overall, a statistically significant improvement in the pain as well as in other urinary symptoms has been reported, yet these results were mainly at short-term or after a few injection cycles [13,21–23]. Thus, information regarding the long-term effects and safety in real-life conditions is lacking.

We aim to report the results and safety of this intra-trigonal treatment in a real-life cohort of patients with a long-term follow-up. Differences between patients maintained in long-term therapy with intratrigonal OnaBotA treatment and those who stopped treatment, mainly for lack of response, were evaluated, looking for possible predictors of response. The rate of therapy maintenance was also assessed.

2. Results

2.1. Patients and Demographics

A total of 47 BPS/IC female patients available in the hospital records and that were refractory to lifestyle changes and oral/intravesical therapies (including non-opioid analgesics and anti-depressant drugs and anti-histaminics) were included. All patients received at least one intratrigonal injection of 100 U of OnaBotA. A total of 193 procedures were performed as depicted in Table 1, varying between 1 (10 patients) and 14 (one patient). The proportion of patients that received four or more treatments was 48% (see Table 1).

The mean age of the patients at the time of the first injection was 50.7 (± 14.5) years. The mean initial VAS score of the cohort was 5.7 (± 1.7), and the mean follow-up is 8.8 (± 4.2) years. The cohort demographics are presented in Table 2.

Table 1. Number of patients by the number of treatments.

Number of Treatments	N (Patients)	%
1	10	21.3
2	8	17.0
3	7	14.9
4	3	6.4
5	7	14.9
6	3	6.4
7	2	4.3
8	3	6.4
9	2	4.3
11	1	2.1
14	1	2.1
	47	100%

Table 2. Cohort demographics.

	Cohort
N (%)	47 (100%)
Age at first treatment	50.7 (± 14.5)
Initial VAS	5.7 (± 1.7)
Time between injection and request for another treatment (days)	500 (350–581)
Time between first injection and the request for the second treatment (days)	390 (287–590)
Number of Treatments	4.1 (± 2.9)
Follow-up (years)	8.8 (± 4.2)

2.2. Duration of Effect per Injection

The median interval between the injection and the patient's request for a new injection is graphically shown in Figure 1, and it was 500.5 days (P25: 350; P75: 581). The duration of the effect of each treatment seems to be relatively stable during the follow-up. The median time between the first injection and the request for the second injection was the shortest with a median duration of 390 days (P25: 287; P75: 590). The median intervals for patients' requests for another treatment increased afterwards, ranging between 414 days and 669 days.

To investigate possible predictors of response, three groups of patients were defined. Group A comprised all patients currently in treatment, which included patients with the disease controlled confirmed at a visit or patients who already asked for a new injection). Group B comprised patients who were non-responders to OnaBotA (they could have shown treatment response at an initial phase of the OnabotA program but meanwhile the treatment lost efficacy). Group C included patients lost to follow-up due to non-therapeutic causes.

When comparing the duration of effect between responders and non-responders, no differences were found, as shown in Table 3. We omitted group C in this comparison given the low number of patients and since the loss to follow-up was not related to the response to the toxin injections.

The 10th treatment onward medians were not represented in Figure 1 and Table 3 since only two patients reached that number of procedures.

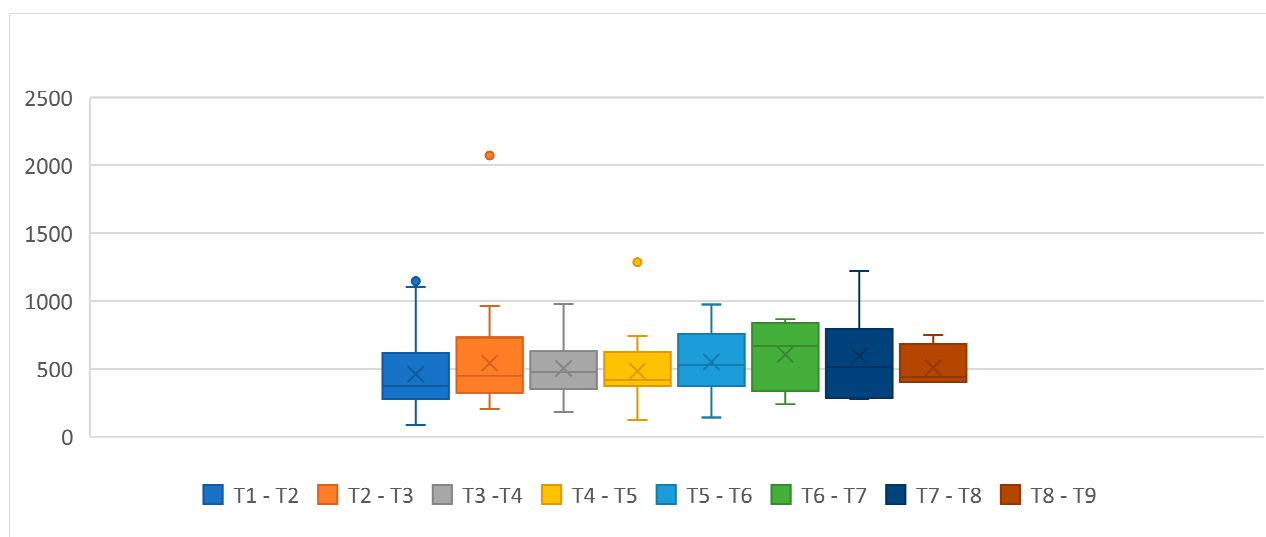


Figure 1. Median duration of effect between injections (Y-axis in days of effect duration counting time between an injection and the patient request for a new treatment).

Table 3. Median duration of the effect of each injection per injection, per group.

Treatment Response (in Days)	Group A	Group B	p-Value
After 1st treatment	447 (310–613)	337 (183–392)	0.57
After 2nd treatment	434 (308–553)	660 (327–1091)	0.64
After 3rd treatment	443 (343–622)	490 (279–673)	0.48
After 4th treatment	409 (377–647)	394 (184–571)	0.59
After 5th treatment	525 (405–845)	350 (140–562)	0.29
After 6th treatment	719 (399–848)		
After 7th treatment	543 (449–900)		
After 8th treatment	440 (403–681)		
After 9th treatment	450 (281–629)		

Interestingly, of the nine patients that had a response duration below the 25th percentile after the first injection, only two abandoned the OnabotA program due to a lack of response. The other seven are still in treatment, having more than five injections. Moreover, these patients had in the following injections a duration of effect within the median time. This suggests that the duration of the effect of the first injection should not be used as a predictor of long-term treatment success or failure.

2.3. Global Treatment Maintenance

Given that we could analyze the total number of patients treated with OnaBotA and identify which of them are still in treatment per each treatment, we were able to organize a Kaplan-Meier treatment maintenance graphic. The results are shown in Figure 2.

Notice that the graphic, which is not time-related, shows that more than half of the patients, 53%, had a favorable treatment response for a high number of treatments.

2.4. Possible Predictors

To evaluate the possible predictors, we compared the characteristics from group A and group B patients. The results are shown in Table 4.

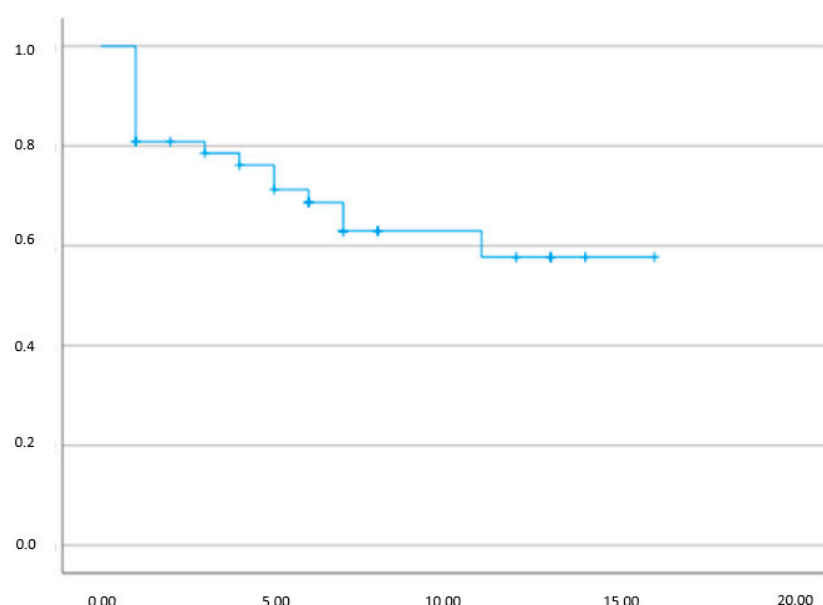


Figure 2. Kaplan-Meier curve of the cohort maintenance in OnaBotA treatment. The X-axis represents the number of injections, and the Y-axis represents the relative number of patients that were treated (100%). Each drop on the graphic line represents patients that lost treatment efficacy and the vertical dash along the graphic represents the moment patients were lost to follow-up. A total of 53% remained on treatment.

Table 4. Patients' features as possible predictors, per group.

	Groups A and B	Group A	Group B	<i>p</i> -Value
N (%)	42 (100%)	25 (59%)	17 (41%)	
Age of 1st treatment	49.7 (± 14.0)	51.8 (± 11.5)	46.6 (± 17.0)	0.29
Initial VAS	6.11 (± 1.2)	5.9 (± 1.3)	6.8 (± 0.5)	0.27
Time between treatments	432.0 (305–645)	337.0 (182–747)	447.5 (310–613)	0.72
Time between 1st and 2nd treatment	350.0 (287–598)	447.5 (310–613)	337.0 (183–392)	0.94
Number of Treatments	4.3 (± 3.0)	5.4 (± 3.1)	2.6 (± 2.1)	0.003
Follow-up (years)	6.9 (4.6)	9.4 (± 3.9)	3.3 (± 3.0)	0.001

As shown, 25 patients remain in treatment and 17 were non-responders to OnabotA. Notice that only one patient in group A had just one treatment (the patient has no symptoms after an injection carried out 15 months ago, with occasional flares easily managed with simple conservative measures).

The initial VAS score and the duration of the effect of the first treatment were not statistically different between responders (group A) and non-responders (Group B). The overall time between treatment and the subsequent request for reinjection was numerically inferior in the long-term responders in group A when compared to Group B, although the difference was not statistically significant ($p = 0.72$).

2.5. Adverse Events

In terms of adverse events, and after analyzing all of the procedures, three types of complications were reported: lower urinary tract infection, straining during micturition, and acute urinary retention with a high post-voiding residual with the need to initiate clean intermittent catheterization (CIC). The results are presented in Table 5.

In 71 procedures, there was no specific mention of adverse events, and thus the adverse events were classified as omitted. Of those procedures with information available, 58 procedures had no adverse event, simple urinary tract infection UTI was recorded in 36 cases, and UTI with symptoms of straining was reported in 13 post-op procedures. Straining without UTI occurred after 14 procedures and straining with incomplete voiding,

with the need for CIC, occurred in only one procedure (0.1%). No upper urinary tract infection was recorded.

Table 5. Total frequency of adverse events.

Adverse Events	Number	% (Total of 193)
Omitted	71	36.8%
None	58	30.1%
Urinary Tract Infection (UTI)	36	18.7%
Straining	14	7.2%
UTI + Straining	13	6.7%
Straining + incomplete voiding with the need for CIC	1	0.1%

3. Discussion

In the present paper, we describe our single-center experience with OnaBotA intra-trigonal injection in the treatment of BPS/IC patients. To the best of our knowledge, this is the first long-term evaluation of a BPS/IC cohort with this treatment in real-life conditions. Previously, short and mid-term evaluations done in our and other departments had contributed to the validation of OnaBotA treatment in this disease [14,21].

The great majority, 37 patients, corresponding to 78% of the total cohort, requested more than one treatment. In recent years, we changed our approach and started to re-treat the patients that presented an unsatisfactory duration response to the first treatment at least one more time. This change was based on the perception that even patients with a poor response duration after the first injection could have a good response to posterior treatments. Such subjective opinion was now undoubtedly confirmed by the analysis of the present cohort.

There were no significant differences in age, the time to reinjection request, and pain intensity before the first intervention between responders and non-responders. Caution should be taken when interpreting the variables of time length for treatment requests after the first treatment and overall treatments, since group B includes nine patients that only underwent one treatment and, consequently, fewer patients contributed to this variable.

Contrariwise, patients that maintain a positive response to the OnaBotA treatment and longer time in treatment (5.4 ± 3.1 vs. 2.6 ± 2.1 , $p = 0.03$), showed a longer period of follow-up in the urologic clinic (9.3 years vs. 3.4 years, $p < 0.001$). The reason is probably a selection bias because those that are more satisfied with the treatment results are more prone to continue in the program.

Interestingly, the intervals between injections were longer than expected, compared to the duration reported in well-controlled cohorts or randomized clinical trials, in which only OnabotA treatment was allowed [5,21]. On average, the duration of each injection among the responders exceeded one year. This may indicate that OnabotA injections eventually combined with simple conservative measures and eventually oral medication with which patients had previous experience and could be used at their own decision (essentially non-opioid analgesic drugs, amitriptyline, the leukotriene receptor antagonist montelukast and antihistaminics [2,24]) may eventually substantially reduce the necessity of toxin injections, decreasing the burden that repeated injections cause to patients and health facilities. The median time between injection and a new request seems independent of the number of total injections and independent of the duration of the previous treatment, with the possible exception of the interval between the first injection and the request for a second treatment.

The sustained duration of the effect, despite the increase in the number of procedures, suggests that intratrigonal sensory neurons do not develop tolerance to OnaBotA, even during long periods of administration. The use of botulinum toxin in other areas of bladder pathologies has shown the rare possibility of the development of antibodies as a cause of the appearance of resistance to the toxin [25,26]. It may be recalled that in a systematic review

evaluating long-term treatment with intravesical OnaBotA in another urinary condition, overactive bladder, the data regarding the time between reinjections was heterogeneous among the analysed studies [27]. In some, the interval between injections was stable, while in others it could become either longer or shorter.

As presented, seven of nine patients with a shorter time of effect after their first injection (lower than percentile 25) become good responders in subsequent treatments. As a matter of fact, they presented, in the following interventions, a time between injections and requests for retreatment similar to that found in the rest of the cohort. This could be justified by a cumulative therapeutic effect of the toxin, an adjustment on the parallel oral therapy, or by the stabilization of the disease. This finding could also be related to the inter-surgeon variation of the surgical technique and the process of reconstitution of OnaBotA. However, these two possibilities seem less probable, as if present they only occurred in the first treatment cycle.

Despite the significant number of patients with positive results that maintained the treatment in our cohort, approximately 53%, as seen in Figure 2, a large number of patients abandoned the long-term OnabotA program. One should remember that despite being effective in the long run, OnabotA injections can be rather unpleasant for BPS/IC patients. This had already been observed in overactive bladder and neurogenic detrusor overactivity cohorts, and reflects the low adherence to long-term reinjection programs [28]. Nevertheless, in our series almost half of the patients remained in the long-term program, exceeding the long-term adherence in OAB which may be as low as 10% [28]. Eventually, as the treatment for BPS/IC should be tailored-made for each patient, and since flares and remissions occur frequently, these different circumstances may introduce large variations in the time for patients to request another injection. [29].

The adverse effects observed in this group of patients are in line with other studies, with the risk of UTI and straining being the main concerns. This point should be fully discussed with the patients, since a UTI may markedly aggravate their symptomatology and the need for CIC will surely demand urethral manipulation.

Limitations

The main limitations of the study are the relatively small number of patients recruited, which were limited by the off-label nature of this treatment and the retrospective analysis of clinical records, and with the inherent risk of bias associated with the quality of the information in the files, including missing data. In addition, we could not provide reliable data on day and night time voiding frequency that we observed to decrease significantly in other studies [5]. However, from our perspective, it can be a more accurate representation of this treatment in real-world clinical practice. In this real-life scenario, the therapeutic effect of each treatment was determined by the time until a treatment request was made by the patients to avoid the bias of treatment delays due to fluctuations in the waiting list.

The readers should also be aware that different from evaluating oral pharmacological treatment effects, the OnaBotA treatment is a surgical procedure and there could be inter-surgeon variations of the surgical technique. We believe that this bias has a low impact as the procedure is well standardized.

Another limitation of this real-life study is relative to the use of other drugs other than OnaBotA by patients in this cohort. While patients could be medicated with a small number of pain-killers during the injections performed in a trial setting, these trial setting injections just represent a minor number of the treatments in the cohort. All of the other patients treated outside of a trial setting, and even trial patients once off the trial, were able to be medicated, or even automedicated, with non-opioid analgesics, anti-depressants and anti-histaminics to better control the symptomatology at their own discretion. Additional medication would probably be taken more often when the OnaBotA's effect begun to decrease. However, this is a real-life setting, and BPS/IC patients rarely achieve symptom control with monotherapy (especially the patients represented in this cohort that are refractory to oral and intravesical treatments). Despite the fact that other medical therapies

could have a role in explaining a more prolonged time of symptoms being under control and, consequently, a greater interval between OnaBotA injections, what is most important is that the BPS/IC condition was better controlled after OnaBotA treatments in responders.

The fact that this study reflects a real-life scenario could suggest that the mild adverse effects, being easily treated, could be underreported.

4. Conclusions

Intratrigoal injection of botulinum toxin A in patients with BPS/IC is an effective and safe long-term treatment option. Moreover, the duration of each treatment seems to be sustained along all of the treatments, even when the number of injections is high. Age, basal pain severity and short duration of the first treatment effect do not seem to predict whether a patient will be a long-term responder to OnaBotA. The main adverse effects are mild, simple UTIs and straining, occurring in a minority of procedures. A specific phenotype for OnaBotA responders is lacking, but given the widely positive effect of the treatment, it should be offered as a third line of therapy.

5. Material & Methods

This is a retrospective study, analyzing our cohort of patients diagnosed with BPS/IC and treated with intratrigoal botulinum toxin in a tertiary public university hospital in Porto (Porto, Portugal) between 2009 and 2022. The procedure and the OnaBotA preparation, in our institution, are carried out by different surgeons in the urology department and by different nurses in our surgical center.

BPS/IC is diagnosed in compliance with guidelines, following the exclusion of more common conditions through physical examination, patient history, uroculture, urinalysis, cystoscopy, urinary cytology, neurological examination and in some cases pelvic magnetic resonance. All patients performed bladder hydrodistension and urothelium biopsies, before initiating OnaBotA treatment, as part of the diagnostic workup. A total of 47 patients were identified.

The clinical and surgical records of the participants, available in our hospital database, were utilized for the analysis of data related to the interventions—every medical record such as written appointments, date of surgery and surgery details are available in the hospital database. The process of collecting the electronic records into files was conducted following the Ethics Committee's approval for the study. Patients were assessed for pain intensity using a 10-point visual analogue scale (VAS) (results were from 0 to 10; a higher number corresponded to higher pain). For the evaluation of each patient, the authors accessed the number of treatments and the treatment duration (the duration between an injection and the request for a new one). The overall time of disease follow-up was also accessed. Adverse effects of the procedure, such as urinary tract infection, straining and urinary retention were analyzed when recorded.

Three groups of patients were defined to identify possible predictors of outcome. Group A comprised all patients currently in treatment (with the disease controlled after an injection or patients that already requested a reinjection); group B comprised patients who were non-responders to OnaBotA (they could have already shown treatment response but now are non-responders to OnaBotA and abandoned the treatment but not the clinic); and group C are patients lost to follow-up but for non-therapeutic reasons.

A total of 25 patients met the criteria for group A, 17 met the group B criteria, and five met the criteria for group C. For the evaluation of possible predictors of long-term success, we compared the characteristics of group A with group B patients, such as age, the initial pain intensity evaluated by the VAS score, the overall treatment duration, and also the duration of the first treatment (excluding patients that only received one injection). Moreover, we evaluated whether a rapid loss of effect after the first treatment was predictive of a general treatment failure or the need for more frequent injections. The treatment maintenance was evaluated and displayed as a Kaplan-Meier graphic, providing information on the proportion of drop-outs and patients still in OnaBotA. The number

of injections performed before abandoning OnaBotA treatment, as well as the number of injections performed by patients still in treatment, are represented in the graphic.

Most treatments were performed outside of a trial, since from a total of 198 treatments, only 71 (35%) of them were in a clinical trial set EudraCT: 2014-001013-81, “Treatment of Bladder Pain Syndrome with Onabotulinum toxin A” (ProBaBle). This leads us to consider that these data reflect real-world practice.

5.1. Procedure Technique and Follow Up

In all our patients, the procedure, the drug and the dose administered were the same. The botulinum toxin A used was OnaBotA (Allergan, Irvine, CA, USA), and it was injected under light sedation through a 23-gauge needle (Coloplast A/S, Humlebaek, Denmark) inserted 3 mm into the trigonal wall with a 70°-lens cystoscopy control. A total of 100 Units were distributed throughout 10 sites (10 U per 1 mL saline)—Figure 3. A preoperative diagnostic workup was performed to guarantee that the individuals had a negative uric culture and no symptoms of cystitis. Patients were evaluated 2 to 3 weeks after the procedure to access early complications.

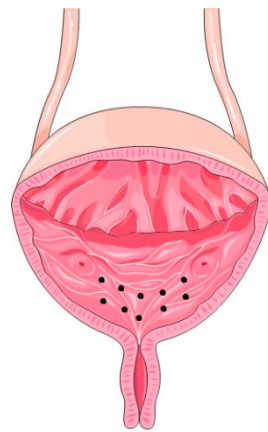


Figure 3. Schematic representation of the 10 bladder sites of OnaBotA injection.

5.2. Statistical Analysis

The statistical program IBM® SPSS® v.28.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. The Kolmogorov-Smirnov test was used to assess the normality of the distribution of continuous variables. Continuous variables with a normal distribution are presented as mean ($\pm$ standard deviation). Non-normally distributed variables are reported as the median (percentile 25; percentile 75) and a Student’s t-test was used to compare the variables with normal distribution.

The authors chose to compare groups A and B for predictor analysis. Group C includes five patients that abandoned OnaBotA treatment early for reasons unrelated to the treatment or their clinical situation.

Clearance from the hospital Ethics Committee (Protocol number 337-21: Effect of intratrigonal botulinum toxin in patients with BPS/IC in a single center) was obtained.

Author Contributions: Conceptualization, R.P. and P.A.-M.; methodology, F.B.; software, P.A.-M.; validation, F.C., R.P. and F.B.; formal analysis, A.F.-M. and F.B.; investigation, A.F.-M. and P.A.-M.; data curation, A.F.-M.; writing—original draft preparation, A.F.-M.; writing—review and editing, P.A.-M., R.P., F.B. and F.C.; visualization, F.B.; supervision, P.A.-M., R.P., F.B. and F.C.; project administration, R.P.; funding acquisition, All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional or Ethics Committee of Centro Hospitalar e Universitário de São João, Porto, Portugal (Protocol number 337-21: Effect of intratrigonal botulinum toxin in patients with BPS/IC in a single-centre, on 8 November 2021).

Informed Consent Statement: Patient consent was waived. Since the data is electronically recorded in the hospital system, after the Hospital's Ethics Committee study approval we could use those information without revealing patients' identity (which we did not).

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to Hospitals Ethics Committee policy.

Conflicts of Interest: The author Pedro Abreu-Mendes declares that only Francisco Cruz has the current conflict of interest: he is a consultant for Allergan, Astellas, Bayer, and Recordati; has received lecture fees from Allergan and Astellas; and is a trial investigator for Astellas, Bayer, Ipsen and Recordati.

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Publication 4

Comprehensive quantitative sensory testing shows altered sensory function in women with chronic pelvic pain: results from the Translational Research in Pelvic Pain (TRiPP) Study

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PAIN 164 (2023) 2528–2539 (DOI:10.1097/j.pain.0000000000002955)

Comprehensive quantitative sensory testing shows altered sensory function in women with chronic pelvic pain: results from the Translational Research in Pelvic Pain (TRiPP) Study

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Abstract

Chronic pelvic pain (CPP), despite its high prevalence, is still relatively poorly understood mechanistically. This study, as part of the Translational Research in Pelvic Pain (TRiPP) project, has used a full quantitative sensory testing (QST) paradigm to profile $n = 85$ women with and without CPP (endometriosis or bladder pain specifically). We used the foot as a control site and abdomen as the test site. Across 5 diagnostically determined subgroups, we found features which are common across different aetiologies, eg, gain of function in pressure pain threshold (PPT) when assessing responses from the lower abdomen or pelvis (referred pain site). However, disease-specific phenotypes were also identified, eg, greater mechanical allodynia in endometriosis, despite there being large heterogeneities within diagnostic groups. The most common QST sensory phenotype was mechanical hyperalgesia (>50% across all the groups). A “healthy” sensory phenotype was seen in <7% of CPP participants. Specific QST measures correlated with sensory symptoms assessed by the painDETECT questionnaire (pressure-evoked pain [painDETECT] and PPT [QST] [$r = 0.47$, $P < 0.001$]; mechanical hyperalgesia [painDETECT] and mechanical pain sensitivity [MPS from QST] [$r = 0.38$, $P = 0.009$]). The data suggest that participants with CPP are sensitive to both deep tissue and cutaneous inputs, suggesting that central mechanisms may be important in this cohort. We also see phenotypes such as thermal hyperalgesia, which may be the result of peripheral mechanisms, such as irritable nociceptors. This highlights the importance of stratifying patients into clinically meaningful phenotypes, which may have implications for the development of better therapeutic strategies for CPP.

Keywords: Quantitative sensory testing, Chronic pelvic pain, Interstitial cystitis, Bladder pain syndrome, Endometriosis

1. Introduction

Chronic pelvic pain (CPP) affects between 5% and 26.6% of women worldwide.^{1,19,77,78} Despite the high prevalence of CPP, there is still little understanding of the underlying mechanisms

giving rise to and maintaining pain in these women. The Translational Research in Pelvic Pain (TRiPP) study is a collaboration across sites in the United Kingdom, Europe, and United States which focusses specifically on endometriosis and

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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<http://dx.doi.org/10.1097/j.pain.0000000000002955>

interstitial cystitis or bladder pain syndrome (IC/BPS),¹⁵ with an overall aim of better understanding the mechanisms underlying CPP in women.¹⁶ Given the increasing evidence of similarities between CPP and other chronic pain conditions, we have focused on both local pelvic and systemic or central mechanisms.

Quantitative sensory testing (QST) is a psychophysical method of testing the function of the somatosensory nervous system.¹³ Individual somatosensory profiles including “sensory loss” and “sensory gain” have been shown to vary in different aetiologies and on an individual basis for the same diseases.^{6,13,41} To date, detailed QST profiling has not been performed in women with CPP, although many studies have used single sensory modalities.^{3,28,30,31,56,75} We used the QST paradigm developed by the German Research Network on Neuropathic Pain (DFNS),^{40,54} comprising a series of standardised tests to assess the detection and pain thresholds for different types of stimuli. These relate to specific neuroanatomical pathways with separate nerve fibre populations. In addition to characterising the sensory profiles associated with specific pathologies, it has also been shown that, across aetiologies, distinct clusters of sensory profiles can be found.^{8,70} Four subgroups can be defined using a published algorithm⁷⁰ representing (1) “sensory loss” (mechanical and thermal sensory loss), (2) “thermal hyperalgesia” (preserved sensory function with heat or cold hyperalgesia), (3) “mechanical hyperalgesia” (loss of thermal sensation with mechanical hyperalgesia or allodynia), and (4) “healthy” (sensation similar to the pain-free population).⁸ By profiling of human surrogate models, these profiles can be tentatively taken as evidence supporting denervation and peripheral and central sensitisation.⁶⁹

The aim of this study was to apply the DFNS QST profiling tool to assess sensory phenotypes in women with CPP. We hypothesise that many women with CPP have altered sensory profiles similar to other chronic pain conditions, whereas some features may be specific to their underlying diagnosis and aetiology. Given that the DFNS QST protocol is time consuming, we also aim to determine whether some of the QST measures correlate with scores from the painDETECT questionnaire,^{23,24} a brief self-report tool that could easily be integrated into clinical practice if shown to be of value in assessing underlying pain mechanisms.

2. Methods

2.1. Participant recruitment

Three sites participated in the study: University of Oxford, UK (OX), Boston's Children's Hospital, USA (BCH) & Instituto de Biologia Molecular e Celular, Portugal (IBMC). Participants from OX and BCH were selected based on criteria from an existing database of participants from parent studies. Participants from IBMC were recruited through urology clinics.¹⁵ All appropriate ethical approvals were secured before recruitment into TRiPP (ethics reference 19/YH/0030).

Recruitment was restricted to females aged 18 to 50 who were neither pregnant nor lactating. Participants were recruited into 1 of 5 groups: endometriosis-associated pain (EAP) who have previously received a surgical diagnosis of endometriosis and at least one type of pelvic pain >4/10; endometriosis-associated pain with comorbid bladder pain (EABP) who meet EAP criteria with additional pain experienced in the bladder and urinary symptoms (urinary frequency and/or urgency symptoms)⁴⁵; bladder pain syndrome (BPS) who fit the clinical presentation of pain perceived to arise from the bladder >4/10 and urinary

symptoms (urinary frequency and/or urgency), with no previous surgical diagnosis of endometriosis⁴⁵; pelvic pain without bladder pain or urinary symptoms and no previous surgical diagnosis of endometriosis (PP), and pain-free controls (CON). In summary, participants were either newly contacted and recruited (IBMC) or contacted after participation in a “parent” study (EndOX: A study to identify possible biomarkers in women with endometriosis, Oxford REC ref:09/H0604/58; Boston Center for Endometriosis [BCE]: A Cross-Institutional Biorepository and Database, IRB-P00004267) and then recruited into the current study (OX and BCH), more detailed information on participant criteria and study protocol can be seen in Demetriou et al.¹⁵ Participants had undergone surgery where this was a component of the standard of care. This includes all participants with an endometriosis diagnosis (EAP and EABP) and also all other participants from OX where recruitment was only from clinics. Most participants recruited from the community at BCH and from Urology clinics in IBMC had not had surgery; however, any potential participant in the BPS or PP groups who had had endometriosis identified previously was excluded. More details of the surgical history and where relevant endometriosis staging are given in **Table 2**. There were no recruitment criteria based on the duration of chronic pain or number of days participants experienced pain per month. **Table 2** shows the duration of pain in years for each of the study groups. Pain was not exclusive to the menstrual cycle, with participants reporting noncyclical pelvic pain, dyspareunia, dyschezia, and dysuria (as can be seen in another TRiPP manuscript illustrating the cohort).¹⁶

All participants gave informed consent. Data were collected between January 2020 and August 2021. All participants from IBMC had study visits and questionnaires completed in Portuguese. Validated Portuguese versions of study material was used where available, otherwise material was forward and backward translated. Unfortunately, all control participants were recruited at Boston; therefore, it is not possible to determine the effect of site in this cohort, although previous studies have shown little or no impact of the site of data collection on QST measures.⁶⁸

2.2. Study visit

After coordinated training of all experimenters, the DFNS QST protocol was performed on the dorsum of the right foot (control site) and the lower abdomen or pelvis (test site).

All QST sessions were performed in a temperature-controlled room at approximately 20°C. Participants were also asked to complete a battery of questionnaires, which included painDETECT^{23,24} as detailed in our protocol.¹⁵ Before the session, participants were also asked to complete a “How are you today?” questionnaire assessing factors that may impact on psychophysical measures: current pain intensity (Numerical Rating Scale [NRS] 0 to 10), state anxiety,⁵⁸ pain catastrophizing,⁶¹ medication and caffeine use that day, and day of menstrual cycle.

The QST script was translated into Portuguese for participants at IBMC. The QST script was forward and back translated before use in this study as no published script was available. A previous study has addressed issues of translating QST scripts into other European languages (not Portuguese, though) and found that it seems possible to produce highly reliable DFNS QST results across different research units, and, more importantly, also across countries and languages.⁶⁸

Quantitative sensory testing was performed by trained researchers who had undergone training with DFNS in Mannheim (September 2019) or attended a virtual training refresher session before recommencing data collection after the peak of the

COVID-19 pandemic halted clinical research. As a measure of outcome quality of this alignment, we averaged DFNS-standardized z-scores across all control subjects: the resulting mean was -0.03 (DFNS 95% CI 0.01 ± 0.25) and SD was 1.26 (DFNS 95% CI 0.99 ± 0.10). Thus, Boston (where CON were collected) had no systematic bias towards overestimating or underestimating QST parameters, but a larger variance than the original 10 sites of DFNS.⁴⁰

Although researchers tried to stay naïve to the participant group, many participants disclosed information about their pain during the study visit.

Quantitative sensory testing was applied to the lower abdomen or pelvis, below the umbilicus. Specifically, thermal, pinprick, and von Frey stimuli were delivered to the skin of the lower abdomen in varied locations to assess general sensation in the area and to avoid surgical scars, pressure pain threshold was measured on the muscles of the lower abdominal wall, and vibration detection was measured on the symphysis pubis (see **Table 1** for more information on QST measures).

2.3. Data analysis

All data were collected using the official QST form and manually uploaded to a secure database. Data inputting was independently verified. Data were analysed as per protocol,^{40,54} using MATLAB (R2021a) for data analysis and Prism9 to create figures. Published reference data were used to Z transform the data for the foot.⁵⁴ Published reference data are not available for the abdomen or pelvis, and therefore, after discussion with the DFNS (R.-D.T. and J.V.), published reference data for the back were used.⁵¹ A Z-score greater than 0 shows a gain of function, and a Z-score less than 0 shows a loss of function.

In addition, statistical comparisons were made between the pain groups (EAP, EABP, BPS, and PP) and our control group (CON). These comparisons were performed with Student *t* tests using the Z-transformed data. Multiple comparison correction was performed using Bonferroni correction, when looking at individual QST measures corrections were applied to QST “blocks” of thermal detection, thermal pain thresholds, mechanical pain thresholds, and mechanical detection.

When comparing painDETECT^{23,24} scores for individual questions, correlations with the relevant QST block were performed (ie, painDETECT question “is cold or heat (bath water) in this area occasionally painful?” correlated with thermal pain QST measures). Normality tests were performed on each painDETECT sensation variable (ie, those scored out of 5, excluding question on time course and spatial properties of the pain) and appropriate tests were used. Paraesthesia symptoms “burning” and “tingling” from painDETECT do not have comparators within QST so have been excluded from this analysis. In addition, the proportion of those reporting clinically significant painDETECT scores for sensations are reported (ie, are any responses >3 of 5, representing strongly or very strongly). To account for interindividual differences in pain sensitivity, painDETECT scores for each of these symptoms were recalculated by subtracting the mean across all 7 responses from each individual response⁹ when comparing scores between groups. Scores larger than zero thereby indicate a sensation that is more intense than the average individual symptom score.

Other variable scores such as pain intensity (Numerical Rating Scale [NRS] 0 to 10) and state anxiety (State-Trait Anxiety Inventory STAI-S⁵⁸) were normality tested and are reported appropriately.

For menstrual cycle stage, participants were asked to self-report whether they were taking any hormonal contraceptives,

the day of their last menstrual period, and typical length of their menstrual cycle. Those who were not currently taking any form of hormonal contraception, who indicated that they still had menstrual cycles, were categorised by menstrual phase according to the following protocol: based on a 28-day cycle, day 1 to 7 were classified as a menstrual phase, day 8 to 14 were classified as follicular or proliferative and day 15+ was the luteal or secretory phase. For participants whose cycle length deviated from 28 days, 14 days were subtracted from their reported cycle length, the secretory phase being held constant, and the remaining duration was assigned to the proliferative phase. For women who reported a variable cycle length, the min, mean, or max cycle length was determined, and stage was allocated accordingly. The menstrual phase was cross-checked by 2 researchers (L.C. and D.P.) to ensure consistency.

To determine whether individuals with CPP (EAP, BPS, EABP, and PP combined) could be categorised into the clusters previously described,⁸ a recently developed algorithm⁶⁴ was used. The clusters are as follows: ‘healthy,’ ‘sensory loss,’ ‘thermal hyperalgesia’ and ‘mechanical hyperalgesia.’ We used a deterministic approach such that participants were sorted into the cluster which they had the greatest score for, based on their abdominal sensory profiles. To assess differences between these clusters in questionnaire measures, analysis of variance or Kruskal–Wallis test was used according to normalcy.

3. Results

3.1. Demographics

Eighty-five women were recruited and underwent the full QST profiling (EAP $n = 25$, BPS $n = 13$, EABP $n = 15$, PP $n = 6$, and CON $n = 26$). Overall, most of the pain groups were well matched for age, menstrual cycle phase, and state anxiety scores (**Table 2**); however, the BPS group were significantly older than the CON and PP groups ($P = 0.010$ and $P = 0.090$, respectively) and significantly more anxious than the CON group ($P = 0.009$). The EAP, BPS, and EABP had significantly higher current pain intensity NRS scores than CON (all $P < 0.01$), see **Table 2**. 66.7% of participants were taking hormones, with the remainder being spread across menstrual cycle phases (**Table 2**) (for $n = 1$ no data on hormones or cycle were available and for $n = 5$ participants they were not on hormones, but it was not possible to determine the menstrual stage).

Table 3 shows the proportion of participants that reported clinically significant sensory symptoms on the painDETECT measure. For the endometriosis groups (EAP and EABP), pain attacks was the most common symptom, whereas for the BPS groups ongoing burning pain was the most common and this was also common in the comorbid group EABP. Patients with bladder pain (BPS and EABP) had more frequent pressure-evoked pain than EAP or PP.

One participant in the EAP group reported experiencing pain on the right foot on the day of testing; therefore, their control site data are excluded from all analysis.

3.2. Quantitative sensory testing profiles

Figure 1 shows the sensory profiles at the foot control site (**Fig. 1A**) and the lower abdomen test site (**Fig. 1B**) for the CON compared with those with CPP. There are significant differences between the CPP and CON groups for: thermal sensory limen at both the foot and abdomen ($t = -2.8$, $P = 0.032$ and $t = -3.5$, $P = 0.004$, respectively), suggesting loss of small fibre function in CPP, vibration detection at the foot ($t = -3.0$, $P = 0.017$)

Table 1**Quantitative sensory testing abbreviations and methods.**

Abbreviation	Full name	Method
CDT	Cold detection threshold	Cooled from baseline until participant senses “cooling.” Three repetitions are performed at each site. Arithmetic mean of change in temperature is used in analysis
WDT	Warm detection threshold	Warmed from baseline until participant senses “warming.” Three repetitions are performed at each site. Arithmetic mean of change in temperature is used in analysis
TSL	Thermal sensory limen	Warmed and cooled from baseline asking participant to indicate when they feel a change. Calculated by subtracting the arithmetic mean of the cool detections from the arithmetic mean of the warm detections during the alternations
PHS	Paradoxical heat sensation	During TSL, if participants report “warm” or “hot” sensations during cooling, this is a paradoxical heat sensation. These are counted, and there is a maximum of 3
CPT	Cold pain threshold	Cooled from baseline until participant feels pain sensation. Three repetitions are performed at each site. Arithmetic mean of absolute temperatures is used in analysis
HPT	Heat pain threshold	Heated from baseline until participant feels pain sensation. Three repetitions are performed at each site. Arithmetic mean of absolute temperatures is used in analysis
MDT	Mechanical detection threshold or tactile detection threshold	Using von Frey hairs the tactile detection threshold is determined by performing a modified method of limits. Five threshold determinations are made, each with a series of ascending and descending stimulus intensities. The final threshold is the geometric mean of these 5 series of suprathreshold and subthreshold stimuli intensities
MPT	Mechanical pain threshold	This test uses weighted pinprick stimuli. Five threshold determinations are made, each with a series of ascending and descending stimulus intensities. The final threshold is the geometric mean of the 5 suprathreshold and subthreshold readings (modified method of limits)
MPS	Mechanical pain sensitivity	To test for mechanical pain sensitivity, weighted pinprick stimuli of different stimulus intensities are used so that a stimulus–response function is obtained for pinprick-evoked pain (Numerical Rating Scale; range 0–100). Seven stimuli intensities are applied 5 times each at both test sites in a randomized order, during which the subject is asked to give a numerical pain rating immediately after each stimulus. The degree of pain sensitivity is calculated by the geometrical mean of the pain ratings given for pinprick stimuli (MPS)
DMA	Dynamic mechanical allodynia	Dynamic mechanical allodynia is tested by using the same test pattern as described for the MPS. Dynamic innocuous stimuli (Q-tip, cotton wisp, and soft brush) are applied in between the pinprick stimuli in a randomized order. Each of the 3 innocuous stimuli is tested 5 times on each test site. The degree of pain sensitivity is calculated by the geometrical mean of the pain ratings innocuous stimuli (DMA)
WUR	Wind-up ratio	The numerical pain rating (NRS; range 0–100) given for an applied series of repetitive pinprick stimuli of the same intensity (10 stimuli with a repetition rate of 1/s, 256 mN) is compared with the numerical pain rating of a single stimulus again of the same intensity. This procedure is repeated 5 times. A “wind-up” ratio is calculated by the arithmetic mean of the pain intensity rating for the series of stimuli divided by the arithmetic mean of the pain intensity rating for the single stimulus
VDT	Vibration detection threshold	This test is performed with a standardized tuning fork (64 Hz) that is placed over a bony prominence. The vibration detection threshold is determined by 3 series of descending stimulus intensities determined from the “wandering” tip of a triangle moved by means of the vibration and indicated on the tuning fork ²⁹ using the arithmetic mean of the values when the participant just stopped perceiving vibration (in x/8)
PPT	Pressure pain threshold	Using a pressure algometer (contact area 1 cm ²), the threshold for pressure induced pain is measured above a muscle in 3 series of slowly increasing stimulus intensities (0.5 kg/s, corresponding to ca. 50 kPa/s). The threshold is then determined as the arithmetic mean of the 3 series (in kPa).

Here are the abbreviations used for each QST measure in the DFNS protocol and the method by which they are collected. Methods are adapted from DFNS QST Investigator's Brochure Version 2.1.^{50,56}

indicating large fibre defect in CPP, and pressure pain threshold at the abdomen ($t = 3.0$, $P = 0.012$) showing large gain of function in CPP suggestive of central sensitization to deep tissue input.

Figure 2 shows the heterogeneity within subgroups showing the proportion with “normal” function, gain of function, and loss of function for each test variable, as well as illustrating differences within the CPP cohort divided into the TRiPP subgroups. At the test site (low abdomen), there was loss of function in thermal detection (indicating small fibre loss), as well as gain of function for

pressure pain thresholds, mechanical pain thresholds, mechanical pain sensitivity, or increased dynamic mechanical allodynia across groups.

At the test site, compared with our CON group, those with bladder pain (EABP and BPS combined) showed significant gain of function for pressure pain threshold ($t = 3.7$, $P = 0.003$), suggesting central sensitization to deep tissue input as well as loss of function for thermal sensory limen (TSL) ($t = -3.0$, $P = 0.02$), suggesting loss of small fibre function. However, those with endometriosis (EAP and EABP combined) had significant loss of

Table 2
Participant characteristics.

	EAP	BPS	EABP	PP	CON
No. of participants	25	13	15	6	26
Age	34 (22-50)	46 (27-51)	31 (20-51)	31 (25-34)	29.5 (21-45)
Current pain intensity	1 (0-6)	3 (0-8)	2 (0-7)	0 (0-2)	0 (0-2)
Duration of pain (y)	18 (7-33)	19 (0-37)	21 (6-35)	17 (11-22)	—
painDETECT score	9.2 (0-18)	14.6 (2-29)	14 (7-26)	9 (3-13)	N/A
painDETECT neuropathic, n (% of group)	0 (0)	5 (41.7)	3 (23.1)	0 (0)	N/A
painDETECT mixed n (% of group)	4 (23.5)	1 (8.3)	5 (38.5)	1 (16.7)	N/A
painDETECT nociceptive n (% of group)	13 (76.5)	6 (50)	5 (38.5)	5 (83.3)	N/A
State anxiety score	32.1 (20-49)	41 (20-67)	33 (22-56)	28.5 (20-43)	26 (20-41)
Menstrual phase n (% of group)					
Menstrual	2 (8)	0 (0)	0 (0)	1 (16.7)	4 (16)
Proliferative	1 (4)	0 (0)	1 (6.7)	0 (0)	4 (16)
Secretory	2 (8)	1 (8.3)	2 (13.3)	2 (33.3)	3 (12)
Taking steroid hormones n (% of group)	20 (80)	9 (75)	12 (80)	3 (50)	12 (48)
Medications n (% of group)					
NSAIDS	1 (4)	0 (0)	2 (13.3)	1 (16.7)	1 (3.8)
Other over-the-counter painkillers (eg, paracetamol)	3 (12)	1 (7.7)	3 (20)	0 (0)	0 (0)
Antidepressants/Anxiolytics	8 (32)	6 (46.2)	7 (46.7)	2 (33.3)	6 (23.1)
Medications for other health conditions (eg, allergy medication)	15 (60)	6 (46.2)	9 (60)	2 (33.3)	12 (46.2)
Vitamins/minerals/probiotics/herbal	5 (20)	1 (7.7)	2 (13.3)	0 (0)	7 (26.9)
Opioids	1 (4)	0 (0)	1 (6.7)	0 (0)	0 (0)
Muscle relaxants	1 (4)	2 (15.4)	0 (0)	0 (0)	0 (0)
Gabapentinoids	1 (4)	0 (0)	1 (6.7)	0 (0)	0 (0)
Surgery to diagnose/exclude endometriosis n (% of group)	25 (100)	0 (0)	14 (100)	2 (33.3)	0 (0)
Stage of endometriosis n (% within those who have had surgery to diagnose/exclude endometriosis)					
Stage I	9 (36)	0 (0)	7 (46.7)	0 (0)	—
Stage II	4 (16)	0 (0)	3 (20)	0 (0)	—
Stage III	2 (8)	0 (0)	1 (6.7)	0 (0)	—
Stage IV	6 (24)	0 (0)	2 (13.3)	0 (0)	—
Stage unknown	4 (16)	0 (0)	2 (13.3)	0 (0)	—
No endometriosis	0 (0)	0 (0)	0 (0)	0 (0)	—

Shown are participant demographics for each of the TriPP subgroups: EAP (endometriosis-associated pain), BPS (bladder pain and urinary symptoms), EABP (comorbid endometriosis and bladder symptoms), PP (pelvic pain without endometriosis or bladder symptoms), and CON (controls without pelvic pain or endometriosis). Age is given in years with mean and range. Pain intensity is given as median score on 0 to 10 NRS scale with range. Duration of pain is given in years as a median score with range; for the CON group, this is not applicable as they are pain free. Current state anxiety score is given as a mean and range based on State-Trait Anxiety Inventory—State Questionnaire.⁶⁰ Scores of 20 to 39, 40 to 59, and 60 to 80 indicate low, moderate, and high anxiety, respectively. painDETECT²⁵ score is given as mean and range. Medications taken in the 24 hours before the study visit, as broken down to medication groups are shown as counts. Surgery to diagnose or exclude endometriosis is given as counts and percentages per group. The stage of endometriosis shown as count and percentage of those who had received surgery to diagnose or exclude endometriosis.

function for TSL at both the test ($t = -3.5$, $P = 0.003$) and control site ($t = -3.2$, $P = 0.008$) (ie, small fibre loss). In the PP compared with CON groups, there was greater dynamic mechanical allodynia ($t = -10.7$, $P < 0.001$). However, it should be remembered that this was a small group ($n = 6$). All other measures were within the 95% confidence intervals from the reference data (shown by grey box).

3.3. Quantitative sensory testing clusters

All 4 previously identified QST clusters were present in the cohort of women with CPP. Profiles were consistent with those of

healthy subjects in only a small number (6.8%) of women with CPP; thus, more than 93% had QST sensory profiles suggesting some altered somatosensory processing. Cluster allocation for the TriPP subgroups (EAP, BPS, EABP, and PP) are shown in **Figure 3**. For all TriPP subgroups, the most common cluster allocation was “mechanical hyperalgesia” with at least 50% of participants in each group fitting this cluster, which suggests some central sensitization.

There was no significant difference between the clusters for age ($P = 0.52$), pain intensity rating (NRS 0-10 at the time of testing) ($P = 0.72$), state anxiety ($P = 0.40$), or painDETECT score ($P = 0.23$).

Table 3**Reported symptoms from painDETECT.**

Sensory symptom	EAP (n = 24)	EABP (n = 15)	BPS (n = 13)	PP (n = 6)
Burning	8.3%	33.3%	69.2%	0%
Prickling	0%	6.7%	30.8%	16.7%
Mechanical allodynia	4.2%	0%	7.7%	0%
Painful attacks	37.5%	53.3%	23.1%	16.7%
Thermal hyperalgesia	4.2%	0%	0%	0%
Numbness	4.2%	6.7%	0%	0%
Pressure-evoked pain	12.5%	33.3%	23.1%	0%

Proportion of participants in each pain group reporting clinically significant symptoms (ie, a score >3, strongly or very strongly) in the painDETECT questionnaire.²⁵
BPS, bladder pain syndrome; EABP, endometriosis-associated pain with comorbid bladder pain; EAP, endometriosis-associated pain; PP, pelvic pain.

3.4. Comparison with painDETECT scores

Correlations between sensory descriptors from painDETECT and relevant QST measures are shown in **Table 4**. Significant correlations were found between the descriptor “does slight pressure in this area, eg, with a finger, trigger pain?” (pressure-evoked pain) and the pressure pain threshold ($r = 0.47$, $P < 0.001$) and “is cold or heat (bath water) in this area occasionally painful?” (thermal hyperalgesia) and the heat pain threshold ($r = 0.32$, $P = 0.032$). The item “is light touching (clothing, a blanket) in this area painful?” (mechanical allodynia) was strongly correlated with mechanical pain sensitivity ($r = 0.38$, $P = 0.009$) but not with dynamic mechanical allodynia ($r = 0.12$, $P = 0.378$ uncorrected); this item may reflect skin hypersensitivity but not necessarily to light touch.

In addition, there was a significant difference between the QST clusters described above and individually mean adjusted scores for painDETECT variables: numbness ($F = 3.71$, $P = 0.017$) and mechanical allodynia ($F = 3.15$, $P = 0.032$). Post hoc tests showed for numbness a significant difference between sensory loss and thermal hyperalgesia clusters ($t = 2.93$, $P = 0.018$, uncorrected) as well as between thermal hyperalgesia and healthy ($t = -2.255$, $P = 0.041$, uncorrected), although these did not withstand multiple comparison correction. For mechanical allodynia, there were significant differences between sensory loss and thermal hyperalgesia clusters ($t = -4.10$, $P = 0.006$), sensory loss and mechanical hyperalgesia ($t = -5.88$, $P = 0.0006$), and sensory loss and healthy ($t = -2.50$, $P = 0.032$, uncorrected).

4. Discussion

This is the first study using the comprehensive DFNS QST protocol for profiling and phenotyping women with CPP.^{40,54} When looking at the abdominal site, we see gain of function in pressure pain thresholds suggesting changes in pain processing pathways to deep tissue input. We also see loss of function in thermal detection thresholds, suggesting loss of small fibre function. However, when divided into clinically defined subgroups (ie, by the presence of endometriosis or bladder pain), more nuanced differences are seen, specifically a gain of function for pressure pain thresholds and a loss of function for heat pain thresholds for those with bladder pain and a gain of function for dynamic mechanical allodynia for those with endometriosis. Both those with endometriosis or bladder pain showed loss of function for thermal sensory limen (detecting changes from warm to cool). There is, however, marked heterogeneity in the sensory profiles seen even within clinically defined subgroups, and this is illustrated by the observation that all 4 sensory phenotypes are

represented. Mechanical hyperalgesia seems to be the most common sensory phenotype across all clinical subgroups. Notably, only 6.8% of CPP have “healthy” sensory function.

4.1. Mechanisms of pain

Traditionally, pelvic pain has been considered predominantly a visceral pain condition⁵ although increasing evidence suggests that there is frequently a central component.³³ Given alterations in sensory function found at the abdomen or pelvis, our data suggest that some women with CPP (regardless of underlying pathology) have changes to peripheral nerve function. These changes could be the result of neuropathic-like pain, which the painDETECT scores suggest could be present in this cohort.^{12,23,24} Alternatively, these could be due to nociplastic or central mechanisms.^{4,21,36,37,71}

The most striking observation is gain of function in PPT. Although there was only a statistically significant difference between CON and those in EABP and BPS groups, **Figure 2** illustrates that across all the CPP groups there is a large proportion demonstrating a gain of function in PPT. Although it is not possible to determine from our data how this change is generated, the association with those with bladder pain and urinary symptoms particularly suggests that there may be a potential role for referred hyperalgesia secondary to viscerosomatic communication.^{74,76}

The data also showed significant gain of function in DMA in 22.5% of the participants with endometriosis (EAP and EABP) (as seen in **Fig. 2**) which is consistent with studies describing allodynia on the abdomen.^{48,60} We can only speculate about factors generating this observation; however, it is important to remember the role of laparoscopy in both the diagnosis and treatment of endometriosis and the investigation of CPP more broadly.^{17,34,49} All participants in EAP and EABP groups, and some of the BPS group, were initially recruited into parent studies at the time of surgery, and many will have had multiple surgeries both for their pelvic pain and for other indications (eg, acute appendicitis or caesarean sections). These surgeries carry the risk of postoperative pain, localised hyperexcitability, and numbness.^{22,52,57,62}

The gain of function in pressure pain thresholds suggest peripheral or central sensitization to deep input, whereas mechanical pain measures (MPT, MPS, and DMA) suggest central sensitization to cutaneous input.

Importantly, we also see loss of function in response to stimuli across all subgroups of CPP. Although hyperalgesia and allodynia have been reported in CPP,^{3,28,30,31,56} such loss of function markers may suggest different mechanisms which could play an

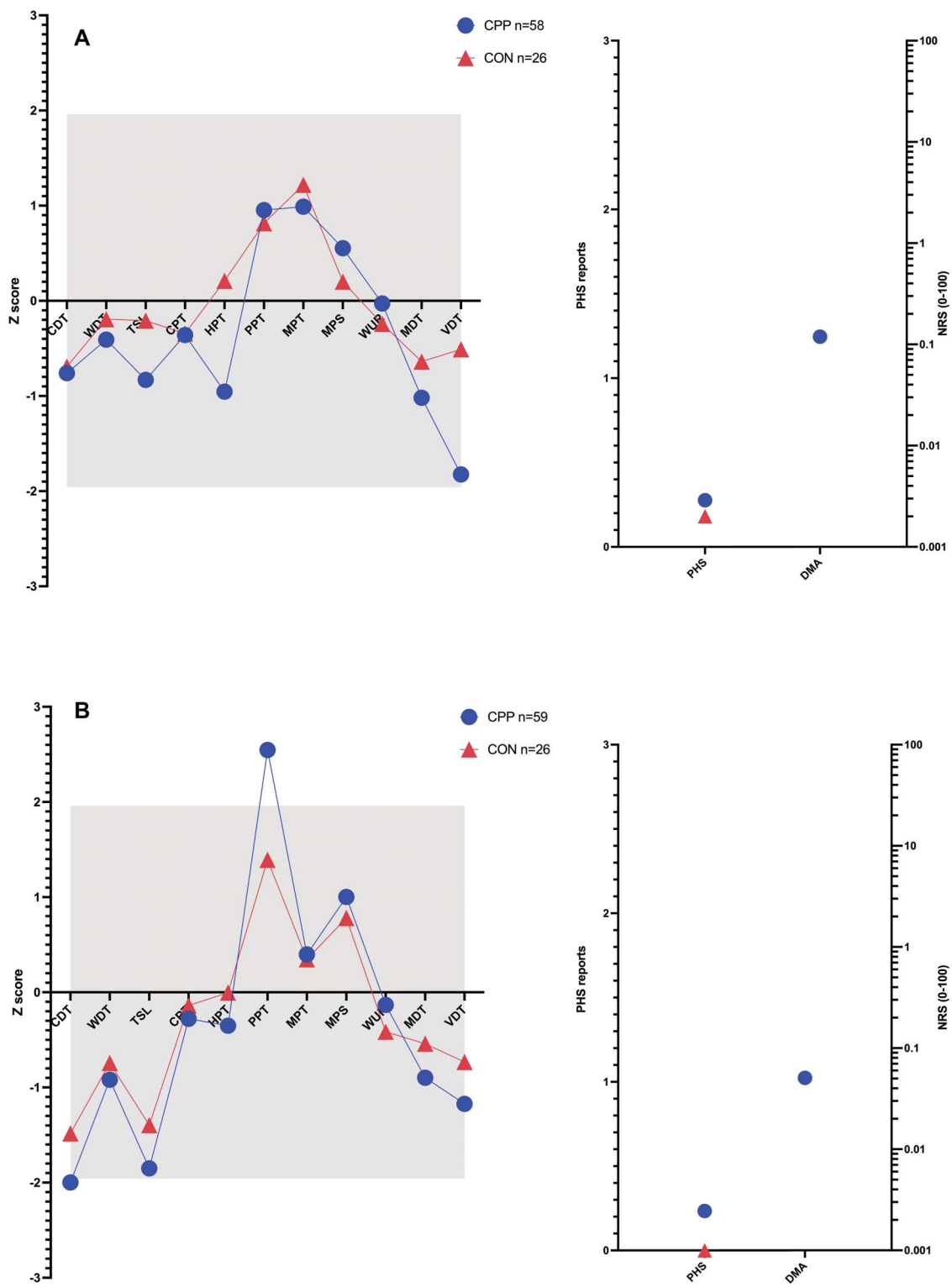


Figure 1. (A) Sensory profiles at the foot control site and (B) sensory profiles at the abdomen. CDT, cold detection threshold; CON group, controls without pain; CPP, participants with chronic pelvic pain; CPT, cold pain threshold; DMA, dynamic mechanical allodynia (DMA ratings do not occur in healthy people (published reference data) or in our CON group); HPT, hot pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PHS, paradoxical heat sensation; PPT, pressure pain threshold; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind up ratio.

important role in understanding CPP. This loss of function could be due to “deafferentation” as has been seen in other conditions.^{8,29,65} Alternatively, it could be due to “descending defunctionalization” of nonnociceptive somatosensory processing, as has

been suggested in neuropathic pain conditions where sensory loss is seen on ipsilateral and contralateral areas.¹⁸

The sensory manifestations are not consistent across women, either when considering the cohort as a whole or dividing into

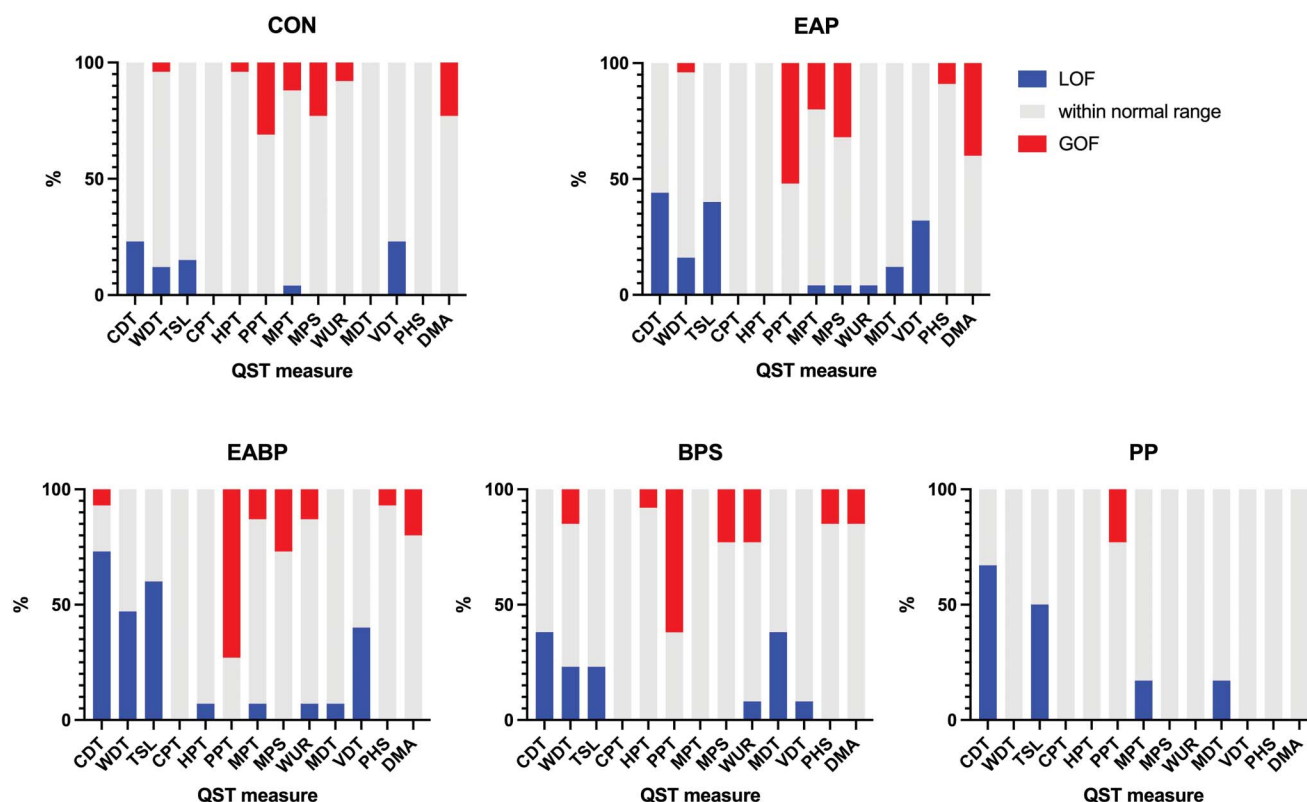


Figure 2. Heterogeneity within and between TRiPP subgroups as shown by the distribution of loss of function and gain of function to QST measures at the abdomen test site. Shown in red are the proportion of the group which have gain of function relative to the normal range from reference data. Shown in grey are those within the normal range. Shown in blue is the proportion showing loss of function. BPS, bladder pain; CDT, cold detection threshold; CON, pain-free controls; CPT, cold pain threshold; DMA, dynamic mechanical allodynia; EABP, comorbid endometriosis and bladder pain; EAP, endometriosis-associated pain; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PHS, paradoxical heat sensation; PP, pelvic pain without endometriosis or bladder symptoms; PPT, pressure pain threshold; QST, quantitative sensory testing; TSL, thermal sensory limen; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind up ratio.

clinically determined subgroups. This is, however, consistent with the known heterogeneity of CPP^{2,11,77} and may contribute to the variation seen in the efficacy of standard treatments.^{25,77} Strategies to subgroup women based on their somatosensory profiles (and possible underlying mechanisms) could be of enormous benefit in this context.

We explored whether mechanistically relevant clusters could be identified in the present cohort as in other patient groups.^{8,64,70} The strategy applied⁷⁰ identifies 4 subgroups in patients with neuropathic pain, believed to represent (1) those with irritable nociceptors^{14,20} (thermal hyperalgesia), (2) deafferentation^{10,20,65} (sensory loss), (3) central sensitization^{10,20} (mechanical hyperalgesia), and (4) those with normal peripheral nerve function. Although it remains to be seen if these clusters respond differently to treatment,^{8,69,70} there is preliminary evidence suggesting they will⁷ and differences in pain interference between these clusters have been shown.²⁷ We were able to identify women fitting each of these clusters, suggesting that the 3 mechanistically different sensory abnormal groups are relevant to CPP.

The commonest sensory phenotype allocation was “mechanical hyperalgesia.” Human surrogate models of known central sensitization in the spinal cord had this pattern, in particular mechanical pain measures (MPT, MPS, and DMA); some here may, therefore, have spinal long-term potentiation.⁵⁵ On the other hand, gain of function for pressure pain threshold has been seen in fibromyalgia,^{43,46} complex regional pain syndrome,^{42,66} and after sleep deprivation⁵⁹ or other stressors^{44,67}; although still central sensitization, the neuronal populations involved may be different. The

“thermal hyperalgesia” phenotype seen in the BPS group particularly may represent the concept of irritable nociceptors.⁸

4.2. Clinical relevance

Chronic pelvic pain is challenging to treat; therefore, it is important to better understand the underlying pain mechanisms and to identify

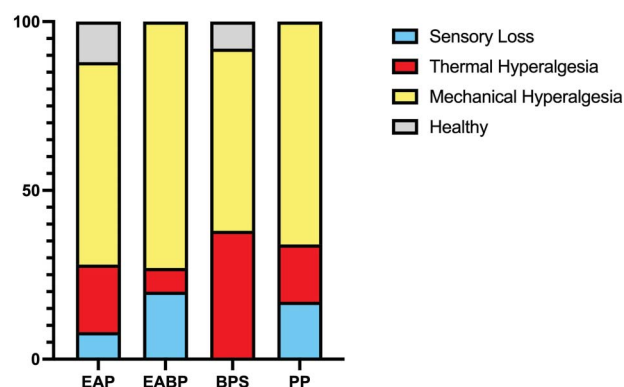


Figure 3. Cluster allocation by group. Sensory phenotypes of sensory loss, thermal hyperalgesia, mechanical hyperalgesia, and healthy determined by published algorithm⁷⁰ based on individual QST profiles. BPS, bladder pain group; EABP, comorbid endometriosis and bladder pain; EAP, endometriosis-associated pain group; PP, pelvic pain without endometriosis or bladder symptoms; QST, quantitative sensory testing.

Table 4
Correlations between relevant painDETECT measures and quantitative sensory testing measures.

QST measure	painDETECT question	<i>r</i>	<i>P</i>
CPT	Is cold or heat (bath water) in this area occasionally painful?	0.25	0.12
HPT	Is cold or heat (bath water) in this area occasionally painful?	0.32*	0.032*
VDT	Do you suffer from a sensation of numbness in the area?	−0.28	0.036 (uncorrected)
MDT	Do you suffer from a sensation of numbness in the area?	−0.20	0.28
PPT	Does slight pressure in this area, eg, with a finger, trigger pain?	0.47*	<0.001*
MPT	Is light touching (clothing, a blanket) in this area painful?	0.03	0.85 (uncorrected)
MPS	Is light touching (clothing, a blanket) in this area painful?	0.38*	0.009*
DMA	Is light touching (clothing, a blanket) in this area painful?	0.12	0.38 (uncorrected)

Shown are *r* values from Pearson correlation and *P* values which are corrected for multiple comparisons (using Bonferroni correction) unless otherwise stated. painDETECT questions relate to clinical symptoms: “Is cold or heat (bath water) in this area occasionally painful?” relates to thermal hyperalgesia; “do you suffer from a sensation of numbness in the area?” relates to numbness; “does slight pressure in this area, eg, with a finger, trigger pain?” relates to pressure evoked pain; “Is light touching (clothing, a blanket) in this area painful?” relates to mechanical allodynia.
* Significance *P* < 0.05.
CPT, cold pain threshold; DMA, dynamic mechanical allodynia; HPT, hot pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; VDT, vibration detection threshold.

strategies to determine who might respond to specific treatments. The present findings highlight that dysfunction in somatosensory processing pathways is present for many women with CPP no matter the underlying cause. Importantly, we found that >93% of our cohort are classified as having altered somatosensory nervous system function, yet clinically medications targeting peripheral or central somatosensory signalling are not routinely used. This disconnect needs to be addressed to improve patient care.

The sensory phenotypes from QST used in this study represent one potential stratifier; however, the utility of QST clinically is currently limited due to time, equipment and/or training requirements. Although there are ongoing efforts to create simple, clinically accessible, bedside QST tools,^{38,53,72} the use of patient-completed questionnaires would be even cheaper and more efficient. Although studies have had mixed results when trying to find tight parallels between QST and painDETECT previously,^{26,32,47,63,73} this study shows correlations between factors assessing particular sensory phenomena. Notably PPT assessed using QST, which clearly showed a gain of function in many participants with CPP, showed strong correlation with the response to the relevant painDETECT question (“does slight pressure in this area, eg, with a finger, trigger pain?”). Interestingly, however, numbness was not correlated with loss of function measures, and this may be an important limitation of this approach. Further work is needed to determine both the utility and acceptability of using painDETECT as a component of clinical and research assessments of CPP in situations where QST is not feasible.

4.3. Limitations

Attempts were made to ensure consistency of data by using the same equipment at all 3 sites and training all personnel together in data collection methods. Although the overall sample size is comparable with other QST studies,^{35,39,50} when divided into clinical subgroups particularly the PP group is too small to meaningfully interpret results. Where appropriate, comparisons were made between those with and without endometriosis and those with and without bladder pain.
In addition, we cannot exclude that some of the differences we found were based in site differences of this multisite study. This is based in the respective centre’s expertise and clinical focus. A future study should aim to include recruiting at centres covering all groups equally.
The lower abdomen or pelvis test site was selected as the most clinically meaningful area as this is often the referred pain site in

CPP patients. However, there is no published reference QST data from this site; we, therefore, used published QST data from the trunk area.⁵¹ The study compared patient data with a control group (CON), and data were z-transformed using the trunk reference data. We did not consider it appropriate to use the control group as reference data for z-transformation as the sample size is not large enough and the spread of ages is relatively limited.

5. Conclusions

The present multicentre study showed significant changes of somatosensory function in women with CPP, with 6.8% showing a “healthy” sensory profile. Specific sensory alterations are present across different underlying pathologies, whereas others are disease specific. Our findings suggest that there is central sensitization to deep and cutaneous inputs in women with CPP, in addition to a variety of alterations in peripheral nerve function. The data showed a moderate correlation between QST measures and relevant descriptors from painDETECT, suggesting painDETECT may have utility for phenotyping CPP patients in specific settings. Understanding somatosensory processing may be helpful for phenotyping CPP patients. Stratification methods such as these may in future guide personalised pain management and thus should be considered when designing clinical trials.

Conflict of interest statement

L. Coxon: no competing interests. J. Vollert received consulting fees from Vertex Pharmaceuticals, Embody Orthopaedic, and Casquar. D. Perro receives financial support from the Canadian Institute of Health Research Doctoral Foreign Study Award. C. E. Lunde: no competing interests. J. Ferreira-Gomes: no competing interests. A. Charrua: no competing interests. P. Abreu-Mendes: no competing interests. J. Birch: no competing interests. J. Meijlink: member of scientific advisory board for Glycologix. L. Hummelshoj: no competing interests. A. Hoffmann: employee of Bayer AG, Germany. Q. Aziz: no competing interests. L. Arendt-Nielsen: no competing interests. E. Pogatzki-Zahn received financial support from Grunenthal and Mundipharma for research activities and advisory and lecture fees from Grünenthal, Novartis, and Mundipharma. In addition, she receives scientific support from the German Research Foundation (DFG), the Federal Ministry of Education and Research (BMBF), the German Federal Joint Committee (G-

BA), and the Innovative Medicines Initiative (IMI) 2 Joint Undertaking under grant agreement No 777500. This joint undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. All money went to the institution E. Pogatzki-Zahn is working for. M. Krassowski: no competing interests. E. Evans: no competing interests. L. Demetriou: no competing interests. S. A. Missmer has been an advisory board member for AbbVie and Roche and receives research funding from the National Institutes of Health, the US Department of Defense, the J. Willard and Alice S. Marriott Foundation, and AbbVie; none are related to the presented work. The J. Willard and Alice S. Marriott Foundation supported enrollment of and data collection from the A2A cohort in Boston from which TriPP data were sampled. C. M. Becker: Research Grants from Bayer Healthcare, MDNA Life Sciences, Roche Diagnostics, European Commission, and NIH. His employer has received consultancy fees from Myovant and ObsEva for work outside of this project. K. T. Zondervan: reports grant funding from EU Horizon 2020, NIH US, Wellbeing of Women, Bayer AG, Roche Diagnostics, Evotec-Lab282, and MDNA Life Sciences, outside the submitted work. A. W. Horne reports grant funding from the MRC, NIHR, CSO, Wellbeing of Women, Roche Diagnostics, Astra Zeneca, Ferring, Charles Wolfson Charitable Trust, and Standard Life. His employer has received consultancy fees from Roche Diagnostics, AbbVie, Nordic Pharma, and Ferring, outside the submitted work. In addition, A. W. Horne has a patent for a serum biomarker for endometriosis pending. C. B. Sieberg: no competing interests. F. Cruz: consultant, speaker, or investigator for Allergan (Abbvie), Astellas, Bayer, Ipsen, and Recordati. R.-D. Treede: Ad board for BAYER, IASP task force on chronic pain classification. J. Nagel: employee and shareholder of Bayer AG, Germany. K. Vincent declares research funding from Bayer Healthcare and honoraria for consultancy and talks and associated travel expenses from Bayer Healthcare, Grunenthal GmBH, AbbVie, and Eli Lilly.

Acknowledgements

Authors thank Natalie Cuccia (for help collecting data), Miriam Szabo (for data entry), Emily Tan (for data entry), and Lewis Carney (for data entry). This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 777500. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA Companies. This project was preregistered on clinicaltrials.gov: NCT04001244.

Center for Neuroplasticity and Pain (CNAP) is supported by the Danish National Research Foundation (DNRF121). Financial support was provided by the J. Willard and Alice S. Marriott Foundation for establishment of and baseline data collection within the A2A cohort—from which the Boston-based TriPP population was sampled.

Article history:

Received 21 October 2022

Received in revised form 26 April 2023

Accepted 27 April 2023

Available online 7 June 2023

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Publication 5

The Role of Urinary VEGF in Observational Studies of BPS/IC Patients: A Systematic Review

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Diagnostics 2022, 12, 1037.; (DOI:10.3390/diagnostics12051037)

The Role of Urinary VEGF in Observational Studies of BPS/IC Patients: A Systematic Review

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Abstract: Background: Bladder pain syndrome/interstitial cystitis (BPS/IC) is a chronic pain condition, often underdiagnosed, with an important impact on patient quality of life. More recently, an association between VEGF and its receptors has been suggested in BPS/IC pathophysiology, due to their role in promoting angiogenesis and inflammation, which can enhance bladder pain. Eventually, VEGF may be used as a biomarker for the diagnosis and prognostication of BPS/IC. To further clarify this issue, this review aims to critically summarize the available information, giving rise to a solid starting point for future studies. Methods: We systematically searched PubMed and Embase, using the queries “urinary VEGF”, “urinary VEGF” AND “pain”, “urinary VEGF” AND “lower urinary tract symptoms” and “urinary VEGF” AND “LUTS” from January 2016 to February 2022. Results: A total of 1026 papers were identified from which 7 articles were included in this study, which assessed 1036 participants. Regarding VEGF levels, overactive bladder (OAB) and healthy patients were used for comparison with BPS/IC patients. VEGF concentration seems to be higher when compared to healthy patients and overactive bladder (OAB) patients. Higher levels of VEGF were associated with pain severity, while a decrease in VEGF concentration was associated with pain and symptom improvement in women. However, these findings were not constant in all studies. Conclusions: There is a trend toward a relevant association between increased VEGF levels and pain or symptom severity in BPS/IC patients. Although there are some discrepancies among the studies and the number of patients included is small, VEGF and its receptors should be considered for future studies regarding its use in BPS/IC pathophysiology, diagnosis and prognostication.

Keywords: interstitial cystitis; bladder pain syndrome; urinary VEGF; VEGF receptors



Citation: Abreu-Mendes, P.; Costa, A.; Charrua, A.; Pinto, R.A.; Cruz, F. The Role of Urinary VEGF in Observational Studies of BPS/IC Patients: A Systematic Review. *Diagnostics* **2022**, *12*, 1037. <https://doi.org/10.3390/diagnostics12051037>

Academic Editor: Henning Reis

Received: 29 March 2022

Accepted: 19 April 2022

Published: 20 April 2022

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1. Introduction

Bladder pain syndrome/interstitial cystitis (BPS/IC) is a chronic pain condition, often underdiagnosed, that affects primarily women [1]. It is a symptomatic-based syndrome in which the main complaint is bladder-related pain or discomfort, accompanied by other storage lower urinary tract symptoms such as polyuria and nocturia [2]. The pain is usually relieved after emptying the bladder. Since BPS/IC is an exclusion diagnostic [3], it can only be performed after ruling out other causes such as infection, cancer and gynecological conditions, among other pelvic and neurological painful conditions [4–6]. This entity has an important impact on the quality of life of these patients, since it may lead to a decrease in work productivity, sleep quality and sexual dysfunction, among other factors [7]. Considering the current evidence, this condition is phenotyped in two subgroups that are defined by the presence or absence of Hunner's lesions [8]. In the classic definition

provided by the European Society for Study of Interstitial Cystitis (ESSIC), the cystoscopy findings are divided into three groups, and only when Hunner's lesions are present, it could be defined as IC, and the category in these criteria is 3X. The other two types of findings, 1X and 2X, correspond to normal urothelium or glomerulations, respectively, and define the disease as BPS [9,10]. Of note, in some guidelines, patients with normal cystoscopy findings and normal biopsies (ESSIC 1A) are considered to have "hypersensitive bladder", as it will appear later in the paper [3]. The Hunner's lesions are characterized by a reddened concentric vascularized area that upon distension of the bladder cause urothelium split and bleeding [11]. Bleeding can be explained by angiogenesis that leads to glomerulation (factor required for the diagnosis of IC), which is evidence of blood vessel fragility [12]. Moreover, this condition is associated with urothelial hyperpermeability, allowing small ions passage to the bladder wall where they can activate nociceptors already sensitized by inflammatory agents [13]. As a matter of fact, inflammation in BPS/IC patients is associated with an increased tissue concentration of inflammatory cytokines [14,15].

Previous studies showed that bladder changes could have the participation of vascular endothelial growth factor (VEGF) and its receptors (VEGF-R) [16]. The presence of VEGF may contribute to hyperalgesia experienced by the patients. VEGF seems to be associated not only with the vascular genesis but also with neuronal control and nerve repair [17,18]. It has been also reported that there is an up-regulation of HIF-1 α (Hypoxia-inducible factor 1-alpha) and, consequently, overexpression of VEGF, since HIF-1 α acts as a transcriptional mediator to VEGF in the presence of hypoxia. This may be more relevant in umbrella apical cells where hypoxia may occur, related to lower bladder perfusion during the filling phase [19].

A previous study demonstrated that the intravesical instillation of VEGF in the bladder could cause changes similar to Bacillus Calmette–Guerin bladder instillations, with reduced time between micturitions and lower voiding pressures [20]. Additionally, an up-regulation of sodium channels at the level of bladder dorsal root ganglia neurons was noted, associated with augmented abdominal sensitivity to mechanical stimulation [20].

Besides the VEGF molecule and its isoforms, a total of three membrane VEGF receptors were identified [21]. While the role of VEGFR1 in angiogenesis is uncertain, other VEGF receptors such as VEGFR2 and VEGFR3 were proven to have an important part in angiogenesis [22,23]. The VEGFR1 is expressed in sensory neurons and monocyte/macrophages, promoting inflammation and pain, although some evidence seems to point toward this receptor being a "decoy", blocking the VEGF ligation to other subtypes related to angiogenesis [17]. The second subtype is the main one responsible for promoting angiogenesis and increasing vascular permeability, and it is produced in endothelial cells. Finally, VEGFR3 is responsible for generating the main signal for lymphangiogenesis [24]. Both VEGFR1 and VEGFR2 may be found in urothelial cells. More importantly, there is a soluble isoform of the VEGFR1 (sVEGF-R1), which is responsible for trapping VEGF, preventing it from activating the membrane receptors [21,25]. These facts support that this pathway, involving VEGF and its receptors, could be relevant as a possible target in the management of this disease [24].

Considering everything mentioned above concerning urinary VEGF and receptors and its potential use in BPS/IC disease management (a subject on which there is a lack of recent studies), this review aims at critically summarizing the available information, giving rise to a solid starting point for future studies.

More precisely, we focused our search on patients diagnosed with bladder pain syndrome, comparing them to healthy patients or patients with other urinary conditions reported, regarding urinary VEGF and its receptors levels. We anticipate the existence of clinical evidence on a statistically significant difference in urinary VEGF and its receptors levels between the compared groups.

2. Materials and Methods

This study took into account the preferred items for systematic reviews and meta-analyses (PRISMA) statements [26].

Until 28 February 2022, we systematically searched PubMed and Embase, using the queries “urinary VEGF”, “urinary VEGF” AND “pain”, “urinary VEGF” AND “lower urinary tract symptoms” and “urinary VEGF” AND “LUTS” (no Mesh Terms were available), regarding English articles and the time frame of January 2016 to February 2022. Once the searches were combined, titles and abstracts were retained. In the identification phase, a total of 1026 papers were obtained. Then each investigator independently proceeded to the screening phase as a way of minimizing selections influenced by a single-person bias [27]. Every duplicate and every study that was not related to pain (e.g., related to diabetes, hypertension or bladder cancer) or that was in the pre-clinical stage was excluded, for a total of 1014 papers (393 for the pre-clinical stage, 299 for non-pain related studies, 322 for duplicates). Twelve (12) eligible articles remained. The inclusion criteria were aimed at studies whose results were published not only as full articles but as abstracts as well; given the low number of publications in this area, the authors preferred to use all the possible information. In fact, studies in which full articles were not available were considered for further analysis as well, knowing the impossibility to correctly evaluate them. We considered studies that involved patients diagnosed with bladder pain syndrome without any restriction at the moment of the diagnosis. The comparing group could be healthy patients or patients with other urinary conditions. The objective was to search for studies regarding urinary VEGF and, when present, its receptor level changes between groups, since it was expected that there could be a significant difference between them. Due to the lack of studies in this specific subject (especially in the clinical stage), only studies that followed case-control, cohort or cross-sectional study design were found and analyzed. Finally, we had a total of 7 included articles for thorough analysis: six full articles and one abstract for which the integral article was not accessible for complete analysis. Those articles were, once again, analyzed independently by the two investigators (P.A.M and A.C.) as a way of minimizing bias induced by a single-person analysis [27]. Each of them collected data regarding the studies’ characteristics such as title, first author name, publication year, study design, quality assessment (using quality assessment tools later mentioned), sample size and characteristics, and study aim. They also collected important findings and limitations for each study analyzed. If there was a discrepancy between the two investigators’ collected information, a third one would intervene.

When analyzing the studies, once more, we confirmed whether it involved patients with bladder pain diagnosis. The articles which subdivided these patients into different groups, such as patients with Hunner’s lesions (IC) and without Hunner’s lesions (BPS), were considered. We simplified and considered them both as patients with BPS/IC. The idea was to measure urinary VEGF and its receptor levels to verify if there were any changes in them as an outcome when comparing different groups. Once more, BPS/IC patients could be compared to healthy patients or patients diagnosed with another pathology.

For quality and risk of bias assessment for each type of study, we used previously published checklists. More specifically, for case-control and cohort studies, we used the Newcastle Ottawa Scale (NOS) [28], since it is the most known scale for assessing bias and quality in observational studies; it is easy to use and can be applied to cohort and case-control studies [29]. While reading the papers, we were able to confirm whether the item was met in the article. Regarding cross-sectional studies, we used JBI’S checklist [30]. These quality assessments are stated in Appendix A (Tables A1–A3, respectively).

When considering the abstract also included in this review, several of the quality assessment parameters were not available for analysis. However, considering its relevance for the present review, we chose to include it.

3. Results

3.1. Study Selection

A total of 1026 papers were obtained (652 from PubMed and 374 from Embase). Of these, we proceeded to the screening in which we excluded duplicates ($n = 322$) and any study regarding non-pain-related issues ($n = 299$; e.g., issues related to diabetes, bladder cancer and hypertension) or in the pre-clinical stage ($n = 393$; involving in vitro and animal studies). In the next phase, we had 12 eligible articles. Upon the full reading of the papers taken into account, we considered 7 to be included in this systematic review: six (6) full articles [31–36] and one (1) abstract [37] of which the integral article was not accessible for complete analysis. The article selection process is stated in the flow diagram shown in Figure 1.

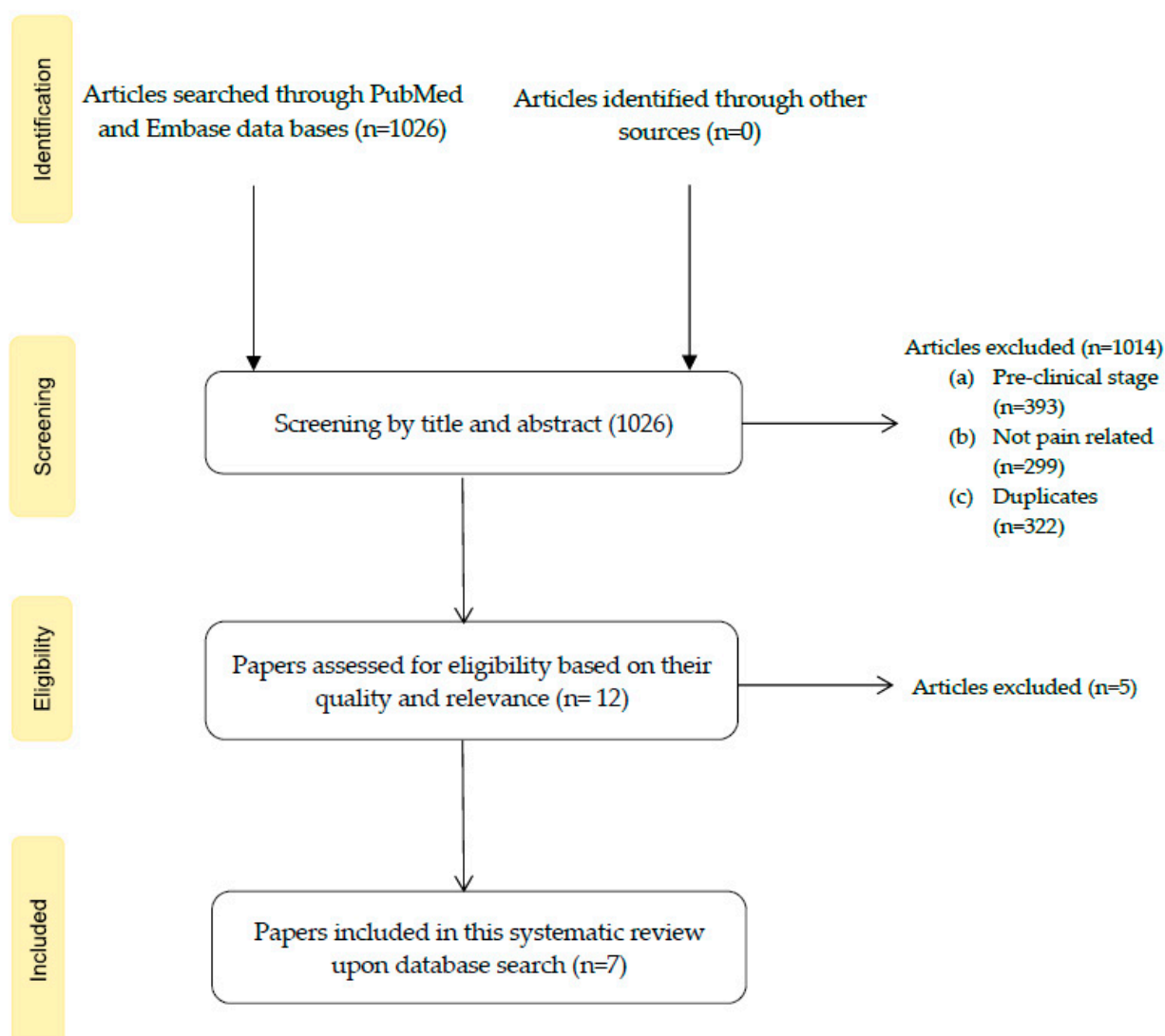


Figure 1. Flow diagram showing the study selection process.

The articles included in this review were submitted to a quality assessment as already stated (see Tables A1–A3 from Appendix A).

3.2. Studies' Overall Characteristics

As for study characteristics, the data regarding title, first author, publication year, sample size, sample characteristics, study aim and quality assessment were also collected and gathered in a table for easier access (see Table A4). We included mostly papers

with good quality (we were not able to check for quality parameters for only one of the studies [37]). Moreover, five papers followed a case-control design [31–34,37], while one was a cohort study [35], and the other one left was a cross-sectional one [36]. One of the studies involved a big sample size ($n = 491$) [31]. However, the others had smaller sample sizes (maximum $n = 216$ [35] and minimum $n = 36$ [33]). A total of 1036 participants were involved. Each study aim is also reported in Table A4 (Appendix B).

Caution must be taken in the nomenclature used. Some studies use the term Urological Chronic Pelvic Pain Syndrome (UCPPS) to encompass both males with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) and female patients with BPS/IC. Furthermore, in some studies, IC patients are called HIC, and BPS patients are named as NHIC. The last nomenclature (HIC and NHIC) is not used in the manuscript.

3.3. Individual Characteristics, Findings and Limitations

The information regarding each study's characteristics is shown in Table A4. As for the relevant findings regarding urinary VEGF and its receptors, other findings (such as questionnaires provided during the study) and limitations can be stated in Table A5 (Appendix B).

The first study analyzed was “Identification of novel non-invasive biomarkers of urinary chronic pelvic pain syndrome: findings from the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network”, a case-control study by Dagher et al. published in 2017 [31]. We classified it as having a quality of 8/9. This article addresses the identification of urinary biomarkers, such as VEGF and VEGF-R1, NGAL (Neutrophil gelatinase-associated lipocalin), and MMP-9/NGAL (Matrix metalloproteinase 9/Neutrophil gelatinase-associated lipocalin) complex in UCPPS, including both female and male patients. Here, they considered in three groups (UCPPS patients for patients who reported a non-zero score on pelvic pain scale; Positive Controls defined for the presence of non-urological associated syndrome; Healthy Controls for patients without any pathology), with a total of 491 patients. A clean-catch midstream urine was collected. The authors reported that although urinary VEGF concentration in females was significantly higher in UCPPS patients than in HC (mean UCPPS = 3.72, mean HC = 3.46; $p = 0.0076$), it did not distinguish female UCPPS from HC overall (AUC = 0.58). Males with UCPPS reported higher VEGF (mean UCPPS = 1.78, mean HC = 0.98, $p < 0.0001$) and VEGF-R1 (mean UCPPS = -0.33 , mean HC = -1.25 , $p = 0.004$) levels compared to healthy controls. Moreover, in univariable analysis, significance for a relation between increased urinary normalized VEGF concentration and pain severity in women was found ($\beta = 1.280$, $p = 0.0127$). No association was found after adjustment for other biomarkers ($p = 0.2502$). As a limitation in this study, only UCPPS with more severe urologic pain were included to maximize the potential to find significance.

“Comparison of inflammatory urine markers in patients with interstitial cystitis and overactive bladder” is a case-control study published in 2018 by Furuta et al. (with a quality rating of 7/9) [32]. In this paper, the authors analyzed the results based on the following conditions: 30 patients with BPS, 30 patients with IC and 28 patients with OAB syndrome. In this case, they obtained the levels of 40 urinary biomarkers. Mid-stream urine was collected. Two questionnaires were handed out to patients: the O’Leary and Sant Symptom and Problem Index (divided into two parts, OSSSI and OSPI) and the Visual Analogue Scale (VAS). Both assessed pain and bothersome levels associated with BPS/IC. In all the questionnaires, higher score values were indicative of more severe symptoms: VAS from 0 to 10, OSPI from 0 to 16 and OSSSI from 0 to 20. Regarding urinary VEGF levels, they were significantly increased in both IC (mean 48.7 ± 28.1) and BPS patients (mean 69.7 ± 40.1). When compared to urinary levels of VEGF in OAB patients (mean 19.5 ± 7.5), both showed a statistically significant difference ($p < 0.05$), suggesting that BPS/IC patients present a severe chronic inflammatory bladder state. For that reason, an increased level of VEGF was considered BPS/IC-specific. There was no significant difference between the IC and BPS groups. Regarding the scores of the questionnaires,

when compared to the OAB group (OSSI: 1.1 ± 1.1 ; OSPI: 0.6 ± 1.1 ; VAS: 0.4 ± 0.9), both groups (IC and BPS) had higher scores for OSSI and OSPI, while also reporting higher VAS scores for pain. More precisely, the results were IC (OSSI: 10.3 ± 5.5 ; OSPI: 9.0 ± 4.8 ; VAS: 5.5 ± 3.3) and BPS (OSSI: 8.1 ± 4.4 ; OSPI: 7.6 ± 4.9 ; VAS: 4.8 ± 3.3), with all the parameters showing a statistically significant difference ($p < 0.01$) when compared to OAB patients. As a limitation, this study was performed in a small sample and, since they were already diagnosed, they had already received some treatment for the respective disease, which could also have influenced the results. These facts were not considered when the statistical analysis was performed. Furthermore, previous episodes of hydrodistension could have interfered with the results, even though investigators tried to minimize this limitation by waiting 3 months after the last hydrodistension episode before collecting the samples and the data.

As for “Angiogenesis in bladder tissues is strongly correlated with urinary frequency and bladder pain in patients with interstitial cystitis/bladder pain syndrome” by Furuta et al., it is a case-control study, published in 2019, classified as 6/9 [33]. The study aimed to determine whether there was an association between bladder inflammation, angiogenesis, fibrosis, and urothelial denudation in biopsied bladder specimens when comparing BPS/IC patients with controls. VEGF was measured directly from bladder specimens. The authors considered three groups with twelve female patients each: one with BPS patients, the second with IC and the third one with healthy controls. Bladder specimens were collected from each patient and underwent an immunohistochemical analysis for the expression of VEGF; other biomarkers, such as TNF- α (Tumor necrosis factor alfa), mast cell tryptase, CD31 (Cluster of differentiation 31), TGF- β (Transforming growth factor-beta) and E-cadherin, were also assessed. Then, two questionnaires regarding IC symptoms and problems were given to the patients (OSSI and OSPI, respectively) while the VAS (Visual Analogue Scale) score was used for assessing bladder pain. As for the results, VEGF levels were statistically significantly higher in IC and BPS patients when compared to the control group ($p < 0.01$ for both cases). Additionally, BPS and IC patients had higher OSSI, OSPI and VAS mean scores. More precisely, the results for IC were (OSSI: 15.9 ± 2.6 ; OSPI: 15.0 ± 1.2 ; VAS: 9.4 ± 0.9) and for BPS were (OSSI: 9.3 ± 4.7 ; OSPI: 8.5 ± 4.7 ; VAS: 5.9 ± 2.1), all of them with a significance of $p < 0.01$ when compared to the respective questionnaires of the control group. Therefore, these results lead to new insights on basic pathophysiology in BPS/IC. As the main limitation, this study presented a small sample size for each group.

Regarding “Molecular Taxonomy of Interstitial Cystitis/Bladder Pain Syndrome Based on Whole Transcriptome Profiling by Next-Generation RNA Sequencing of Bladder Mucosal Biopsies”, it is a case-control study, published in 2019 by Akiyama et al. (with 7/9 in quality assessment) [34]. In this study, a total of 42 women were assessed and divided into four groups: IC, BPS (in this study, it was needed for the patient to have inflammation in bladder biopsies to be considered BPS), hypersensitive bladder (HSB) (a term usually used in Asian guidelines for BPS patients with normal cystoscopy and normal bladder biopsy) and controls. The groups enrolled 12, 11, 10 and 9 patients, respectively. Bladder mucosae were biopsied from consecutive patients. As for VEGF relative expression, the following results were found: IC (Median 0.412; 99% CI 0.035–0.048), BPS (Median 0.014; 99% CI 0.013–0.018), HSB (Median 0.016; 99% CI 0.012–0.024) and controls (Median 0.014; 99% CI 0.014–0.018). The authors report that the expression of VEGF was higher in IC patients when compared to BPS ($p = 0.002$), HSB ($p = 0.004$) and controls ($p = 0.007$). There was no significant difference when comparison was carried out between BPS, hypersensitive bladder and controls. Another statistical analysis was performed with the intent to verify whether there was a relation between VEGF levels in IC patients and symptom severity. Higher VEGF expression was significantly associated with symptom severity, revealing a positive correlation with storage symptoms ($\rho = 0.47$; $p < 0.01$), voiding symptoms ($\rho = 0.40$; $p = 0.02$), daytime frequency ($\rho = 0.35$; $p = 0.03$) and nocturia ($\rho = 0.40$; $p = 0.01$). Lower bladder capacity was correlated with higher VEGF urinary levels ($\rho = -0.46$; $p < 0.01$). The authors also stated a significant association between higher VEGF levels and higher

OSSI ($p = 0.44$; $p < 0.01$), OSPI ($p = 0.46$; $p < 0.01$) and VAS pain ($p = 0.49$; $p = 0.001$) scores, indicating a positive correlation. To evaluate if there were significant differences between the groups in the questionnaires scores, the authors performed a pairwise comparison between IC, BPS, HSB and control groups. The results for each questionnaire were OSSI (IC: 15.8 ± 3.7 ; BPS: 12.3 ± 3.0 ; HSB: 9.8 ± 4.8 ; control: 3.3 ± 1.4), OSPI (IC: 13.3 ± 2.1 ; BPS: 12.4 ± 2.3 ; HSB: 10.7 ± 2.6 ; control: 1.8 ± 2.0) and VAS pain score (IC: 8.2 ± 2.3 ; BPS: 5.7 ± 3.3 ; HSB: 4.9 ± 2.4 ; control: 0); they all had a $p < 0.01$ significance. These results point out a significant difference in each questionnaire assessment between all four groups, with the IC group presenting the highest scores. The relation between microvessel density and VEGF expression was also assessed, revealing a positive and significant association between them ($p < 0.0001$). There were some limitations in this study, such as the small sample involved and the fact that some samples involved an admixture of urothelium and lamina propria, which could mean that there was an underestimation of the results regarding markers expression due to inappropriate samples.

The fifth paper included was “Association of Longitudinal Changes in Symptoms and Urinary Biomarkers in Patients with Urological Chronic Pelvic Pain Syndrome: A MAPP Research Network Study”, which was published in 2021; it is a prospective cohort study by Roy et al. [35] with a quality rating of 7/9. The authors randomly selected 126 UCPPS patients from a previously defined cohort: 116 were female and 100 were male patients [38]. In this study, the aim was to analyze a series of non-invasive biomarkers for their ability to objectively monitor the longitudinal clinical status of UCPPS patients. A urine sample was collected non-invasive way from a group of 216 UCPPS patients at three time points: at baseline (0), 6 months later and then at the end of 12 months. The samples were analyzed in duplicate and in a double-blinded manner as a way of minimizing bias. An odds ratio (OR) > 1 meant a greater likelihood of symptom improvement with an increase in a certain protein, whereas an odds ratio (OR) < 1 meant a greater likelihood of symptom improvement with a decrease in its concentration. It was shown that most regression or progression occurs within the first 6 months. More specifically, the results indicate that a decrease in the urinary VEGF was associated with a greater likelihood of urologic pain improvement (OR = 0.78; $p = 0.03$). On the other hand, there was not a significant association between VEGF-R1 and overall urologic pain symptoms (OR = 0.88; $p = 0.06$). For every finding mentioned above, it was proposed in this study that the measurement of both urinary VEGF and VEGF-R1 (in a non-invasive and inexpensive way) could be used to monitor progression or treatment efficacy. Besides, since it may play an important role in UCPPS pathophysiology, it was suggested as a possible target for future treatments.

A possible limitation for this study is suboptimal reliability in data concerning some of the biomarkers, such as MMP biomarkers.

Finally, as for the article “Relationship of Bladder Pain With Clinical and Urinary Markers of Neuroinflammation in Women With Urinary Urgency Without Urinary Incontinence”, a cross-sectional study by A. Soriano et al., published in 2021 [36], it involved 101 women (with age superior to 18 years) with mild urinary urgency without incontinence [38]; 62 were considered to present bladder pain. The aim was to assess if bladder pain is associated with the presence of neurogenic inflammation in the bladder wall and neuroinflammatory biomarkers in the urine in women with urinary urgency without incontinence. They submitted one questionnaire for the determination of urinary symptoms (F-GUPI), one for the determination of interstitial cystitis problems (OSPI), one for assessment of the interstitial cystitis symptoms (OSSI) and one questionnaire concerning neuropathic pain (PainDETECT). Midstream urine was collected. They also provided a sample of urine for urinary neuropeptides, including VEGF and NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor) and Opn (urinary osteopontin) measurement. Only 84 patients provided urine samples. After applying a Visual Analogue Scale for pain, the women included in this study were divided into two groups (BP—bladder pain; NBP—non-bladder pain); those groups were later compared. As for the results, the group

classified as BP reported higher scores (and therefore worse symptomatology and quality of life) in all the questionnaires. More specifically, the reported results were (mean BP = 28.1, mean NBP = 12.3, $p < 0.001$) for F-GUPI, (mean BP = 9.8, mean NBP = 6.7, $p < 0.001$) for OSSl, (mean BP = 8.7, mean NBP = 4.9, $p < 0.001$) for OSPI and (mean BP = 13.3, mean NBP = 5.1, $p < 0.001$) for PainDETECT. Considering the measurement of urinary biomarkers, elevated normalized VEGF levels were significantly associated with bladder pain ($\beta = 0.04$ pg/mg Cr, 95% CI 0.001–0.07, $p = 0.04$). In this study, urinary VEGF measurements were considered as a possibility for phenotyping or a way of following patients' responses to treatments. Possible confounders were taken into account while performing statistical analysis. However, as a limitation, no healthy control group was present, and the total cohort was small.

Even though we were not able to access the complete article "Relationship of Pain Catastrophizing With Urinary Biomarkers in Women With Bladder Pain Syndrome" (just the abstract), we decided to include this abstract due to its significant relevance in the subject approached in this review [37]. This study, written by Soriano et al., followed a case-control study design and was published in 2021. No quality assessment was possible, since there was no access to the full article. However, the results indicate a higher pain catastrophizing score associated with lower VEGF levels ($p = 0.03$), while bladder pain was associated with higher VEGF levels ($p = 0.01$). Moreover, higher catastrophizing scores were significantly associated with worse urinary symptoms, greater pelvic and neuropathic pain and worse quality of life scores (all $p < 0.01$). There was no possibility to assess this study's specific limitations since we did not access the rest of the article.

Overall, in all the studies analyzed, VEGF and VEGF-R1 (in some studies) had significantly elevated or higher levels (if compared to healthy controls or patients diagnosed with other pathologies) in BPS/IC patients. Furthermore, it was stated that a decrease in urinary VEGF concentration could lead to symptom improvement in women. On the other hand, in some of the studies, we were able to see a positive association between BPS/IC groups and OSSl, OSPI and VAS scores. Finally, lower levels of VEGF were significantly associated with higher pain catastrophizing personality. However, it is important to remember the impossibility to access the full article regarding the later results. The small samples used in most of the studies are a limitation across most studies.

4. Discussion

There is a lack of understanding of BPS/IC pathophysiology. As previously stated, it encompasses bladder-related pain or discomfort, and it is frequently accompanied by other storage lower urinary tract symptoms [2,4]. Even though it affects primarily women, and contrary to previous beliefs, pain severity was reported to be similar in both genders, while bladder-focused symptoms were more frequently reported by women [39]. On the other hand, there is an increasing disease approach based on biomarkers, as they could be truly valuable in a condition with a symptom-based diagnosis, that is, lacking robust phenotyping and having multiple confounding diseases. According to our review, VEGF and its receptors may have an important pathophysiological role. As reported, high levels of VEGF are associated with pain severity, while a decrease in VEGF concentration is associated with pain and symptom improvement in women [31]. In addition, its concentration seems to be higher when compared to healthy patients [33] and OAB patients [36]. An association between increased VEGF levels and higher OSS scores and VAS pain scores was also seen. Since VEGF urinary levels would allow a frequent and economical assessment, its use in BPS/IC diagnostics, relapse prediction and management should be considered in the future. As previously stated, VEGF is associated with angiogenesis [22], and it is proven that its inhibition leads to a decrease in inflammation [40]. Therefore, it would be potentially beneficial to consider anti-angiogenic therapy as a method of disease management. This strong VEGF expression in IC patients could be caused by the hypoxia in bladder tissues during the filling phase, leading to upregulation of urinary VEGF expression, causing bladder fibrosis and a decrease in capacity.

All the papers present in this review took into account the association between increased levels of VEGF and BPS/IC symptom severity, while only two showed an association between increased levels of VEGF-R1 [31,35] and BPS/IC symptoms. Indeed, it is important to consider its role in pain regulation, since VEGF-R1 contributes to VEGF-R2 response modulation, thereby influencing pain [17]. Moreover, as previously reported, intravesical instillation of VEGF leads to an increase in bladder sensory fiber density and the development of pelvic hypersensitivity in mice. Furthermore, its inhibition through a systemic treatment with VEGF-neutralizing antibodies in an animal model reduced pelvic hypersensitivity [41].

It is important, however, to highlight the lack of studies regarding urinary VEGF receptors. One of the two studies here presented had some limitations regarding the cases' representativeness and data reliability [31]. For that reason, further studies involving bigger sample sizes would be necessary to verify the results here presented.

In this review, we faced some limitations. Indeed, some of the studies comprised a small sample [32–34,36]. Overall, even though we were only able to include seven articles, the majority (6) were considered to have good quality when the quality assessment was performed. In one case, we could only have access to the article's abstract, missing potentially important information.

When there was a subdivision of patients between IC and BPS, we opted for a simplification by consider them all as BPS/IC patients when discussing the results. However, the fact that these two groups could potentially represent different entities should be mentioned [42] since some studies reported a tendency to present pancystitis, frequent clonal B-cell expansion and epithelial denudation [43] in patients with Hunner's lesions present. For that reason, it would be beneficial to keep that in mind when creating future studies regarding this subject.

5. Conclusions

There was a tendency toward a significant association between increased VEGF levels and pain or symptom severity, even though it was not equal in every study. Although there is a small amount of data here analyzed regarding this subject among the results presented, VEGF and its receptors should still be considered for future studies regarding its use in BPS/IC diagnostics and management. However, these studies must involve bigger samples and follow a study design with better quality of clinical evidence as a way of minimizing limitations.

Author Contributions: Conceptualization, P.A.-M. and F.C.; methodology, P.A.-M. and A.C. (Aurora Costa); software, A.C. (Aurora Costa); validation, A.C. (Ana Charrua), R.A.P. and F.C.; formal analysis, A.C. (Aurora Costa); investigation, P.A.-M. and A.C. (Aurora Costa); resources, P.A.-M.; data curation, P.A.-M. and A.C. (Aurora Costa); writing—original draft preparation, A.C. (Aurora Costa); writing—review and editing, P.A.-M., R.A.P. and F.C.; visualization, A.C. (Aurora Costa); supervision, R.A.P. and F.C.; project administration, F.C.; funding acquisition, none. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

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

Appendix A

Table A1. Quality assessment of cohort studies included in systematic review (Newcastle–Ottawa Scale (NOS) for case control studies [29]).

Title	Selection			Comparability		Results			Final Score
	Representativeness of the Exposed Cohort	Selection of the Non-Exposed Cohort	Ascertainment of Exposure	Outcome of Interest Was Not Present at Start of Study	Comparability of Cohorts Based on the Design or Analysis	Assessment of Outcome	Follow-Up Long Enough for Outcomes to Occur	Adequacy of Follow-Up of Cohorts	
<i>Association of Longitudinal Changes in Symptoms and Urinary Biomarkers in Patients with Urological Chronic Pelvic Pain Syndrome: A MAPP Research Network Study</i>	(1)	-	(1)	-	(2)	(1)	(1)	(1)	7/9
<i>Relationship of Pain Catastrophizing With Urinary Biomarkers in Women With Bladder Pain Syndrome</i>	Not enough data in study ´s abstract for quality assessment								

Table A2. Quality assessment of case-control studies included in systematic review (Newcastle–Ottawa Scale (NOS) for case control studies [29]).

Title	Selection				Comparability		Results		Final Score
	Case Definition Adequate	Representativeness of the Cases	Selection of Controls	Definition of Controls	Comparability of Cases and Controls Based on the Design or Analysis	Ascertainment for Exposure	Same Method of Ascertainment in Cases/Controls	Non-Response Rate	
<i>Identification of novel non-invasive biomarkers of urinary chronic pelvic pain syndrome: findings from the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network</i>	(1)	-	(1)	(1)	(2)	(1)	(1)	(1)	8/9
<i>Comparison of inflammatory urine markers in patients with interstitial cystitis and overactive bladder</i>	(1)	(1)	-	-	(2)	(1)	(1)	(1)	7/9
<i>Angiogenesis in bladder tissues is strongly correlated with urinary frequency and bladder pain in patients with interstitial cystitis/bladder pain syndrome</i>	(1)	(1)	-	-	(1)	(1)	(1)	(1)	6/9
<i>Molecular Taxonomy of Interstitial Cystitis/Bladder Pain Syndrome Based on Whole Transcriptome Profiling by Next-Generation RNA Sequencing of Bladder Mucosal Biopsies</i>	(1)	(1)	(1)	(1)	(1)	(1)	-	(1)	7/9

Table A3. Quality assessment for cross-sectional studies (JBI critical appraisal checklist for cross sectional studies [31]).

Title	Relationship of Bladder Pain with Clinical and Urinary Markers of Neuroinflammation in Women with Urinary Urgency without Urinary Incontinence		
	Yes	No	Not Applicable
Were the criteria for inclusion in the sample clearly defined?	(1)		
Were the study subjects and the setting described in detail?	(1)		
Was the exposure measured in a valid and reliable way?	(1)		
Were objective, standard criteria used for measurement of the condition?	(1)		
Were any confounding factors identified?	(1)		
Were strategies to deal with confounding factors stated?	(1)		
Were the outcomes measured in a valid and reliable way?	(1)		
Was appropriate statistical analysis used?	(1)		
Total		8/8	
Overall Appraisal	Included ☒	Excluded ☐	Seek further information ☐

Appendix B

Table A4. Table with studies' characteristics (title, first author, publication year, sample size, sample characteristics, study aim and quality assessment).

Title	Author Year	Study Design	Sample Size	Sample Characteristics	Study Aim	Quality Assessment
Identification of novel non-invasive biomarkers of urinary chronic pelvic pain syndrome: findings from the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network	A. Dagher 2017	Case-control	491	UCPPS: 120 male and 139 female participants (Mean age = 43.3 y); Race: 229 white and 10 black; Ethnicity: 19 Hispanic and 239 Non-Hispanic HC group: 50 male and 75 female participants (Mean age = 42.5 y); Race: 90 white and 16 black; Ethnicity: 8 Hispanic and 117 Non-Hispanic PC group: 32 male and 75 female participants (Mean age = 41.6 y); Race: 76 white and 12 black; Ethnicity: 10 Hispanic and 96 Non-Hispanic	To compare urinary levels of VEGF/VEGF-R1 and NGAL, MMP-9/NGAL complex between UCPPS, patients and HC and PC patients while correlating them with symptom severity	8/9

Table A4. Cont.

Title	Author Year	Study Design	Sample Size	Sample Characteristics	Study Aim	Quality Assessment
<i>Comparison of inflammatory urine markers in patients with interstitial cystitis and overactive bladder</i>	A. Furuta 2018	Case-control	88	OAB: 28 patients (7 male; 21 female); Mean age (57.7 y); BPS: 30 patients (9 male; 21 female); Mean age (60.2 y); IC: 30 patients (9 male; 21 female); Mean age (63.5 y)	To investigate the contributing factors of chronic inflammation in IC patients	7/9
<i>Angiogenesis in bladder tissues is strongly correlated with urinary frequency and bladder pain in patients with interstitial cystitis/bladder pain</i>	A. Furuta 2019	Case-control	36	Control: 12 females (Mean age 56.7 y); BPS: 12 females (Mean age 53.3 y); IC: 12 females (Mean age 57.0 y)	To clarify the correlation among bladder inflammation, angiogenesis, fibrosis and urothelial denudation using immunochemistry, OSS, OSPI and VAS pain scores	6/9
<i>Molecular Taxonomy of Interstitial Cystitis/Bladder Pain Syndrome Based on Whole Transcriptome Profiling by Next-Generation RNA Sequencing of Bladder Mucosal Biopsies</i>	Y. Akiyama 2019	Case-control	42	IC: 12 patients (Mean age: 70.5 y) BPS: 11 patients (Mean age: 58.8 y) Hypersensitive bladder: 10 patients (Mean age: 63.5 y) Control group: 9 patients (Mean age: 64.6 y)	To perform transcriptome of BPS/IC subtypes and non-BPS/IC subtypes controls, providing the basis of a molecular taxonomy of BPS/IC, and explore biological pathways specifically affected in each subtype	7/9
<i>Association of Longitudinal Changes in Symptoms and Urinary Biomarkers in Patients with Urological Chronic Pelvic Pain Syndrome: A MAPP Research Network Study</i>	R. Roy 2021	Prospective cohort	216	UCPPS patients: 116 female and 100 male patients	To analyze a series of non-invasive biomarkers for their ability to objectively monitor the longitudinal clinical status of UCPPS patients	7/9

Table A4. Cont.

Title	Author Year	Study Design	Sample Size	Sample Characteristics	Study Aim	Quality Assessment
<i>Relationship of Bladder Pain with Clinical and Urinary Markers of Neuroinflammation in Women with Urinary Urgency Without Urinary Incontinence</i>	A. Soriano 2021	Cross-sectional	101	NBP (Mean age = 48.9): Caucasian: 32 patients; African American: 4 patients; Other: 2 patients BP (Mean age = 44.6): Caucasian: 55 patients; African American: 5 patients; Other: 2 patients	To assess if bladder pain is associated with presence of neurogenic inflammation in the bladder wall and neuroinflammatory biomarkers in the urine in women with urinary urgency without incontinence	8/8
<i>Relationship of Pain Catastrophizing With Urinary Biomarkers in Women With Bladder Pain Syndrome</i>	A. Soriano 2021	Cohort	62	Women who met AUA criteria of bladder pain syndrome	To assess greater pain catastrophizing with higher urinary biomarkers (VEGF and BDNF) levels in women with bladder pain syndrome	No quality assessment possible
Abbreviations: BP—Bladder Pain group; BPS—Bladder Pain Syndrome; F-GUPI—Female Genitourinary Pain Index; HC—Healthy Controls; HSB—Hypersensitive Bladder; IC—Interstitial Cystitis; NBP—Non-Bladder Pain group; OAB—Overactive Bladder; OSPI—O’Leary–Sant Problem Indexes; OSSI—O’Leary–Sant Symptom Indexes; PC—Positive Control (non-urological associated syndrome); UCPPS—Urological Chronic Pelvic Pain Syndrome; VAS—Visual Analogue Scale; VEGF—Vascular Endothelial Growth Factor; VEGF-R1—Vascular Endothelial Growth Factor-receptor.						

Table A5. Table with studies’ findings and limitations.

Title	VEGF/VEGF-R1 Levels in Groups	VEGF/VEGF-R1 Levels’ Relations with Symptoms	Questionnaire Scores	Limitations
<i>Identification of novel non-invasive biomarkers of urinary chronic pelvic pain syndrome: findings from the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network</i>	VEGF concentration: Females: (mean UCPPS = 3.72, mean HC = 3.46; $p = 0.0076$) Males: (mean UCPPS = 1.78, mean HC = 0.98, $p < 0.0001$) VEGF-R1 concentration: Males: (mean UCPPS = -0.33 , mean HC = -1.25 , $p = 0.004$)	Relation between VEGF concentrations and Pain severity: Females: $\beta = 1.280$, $p = 0.0127$; Males: $\beta = -0.692$, $p = 0.0627$ Urinary severity: Females: $\beta = 1.125$, $p = 0.0378$; Males: $\beta = -1.070$, $p = 0.0126$ Relation between VEGF-R1 concentrations and Pain severity: Females: $\beta = 0.366$, $p = 0.2198$; Males: $\beta = -0.257$, $p = 0.2638$ Urinary severity: Females: $\beta = 0.117$, $p = 0.7092$; Males: $\beta = 0.038$, $p = 0.8851$	-	Only UCPPS with more severe urologic pain were included

Table A5. Cont.

Title	VEGF/VEGF-R1 Levels in Groups	VEGF/VEGF-R1 Levels' Relations with Symptoms	Questionnaire Scores	Limitations
<i>Comparison of inflammatory urine markers in patients with interstitial cystitis and overactive bladder</i>	VEGF levels: OAB (Mean 19.5 ± 7.5) BPS (Mean 69.7 ± 40.1) * IC (Mean 48.7 ± 28.1) *	-	OAB (OSSI- 1.1 ± 1.1 ; OSPI: 0.6 ± 1.1 ; VAS: 0.4 ± 0.9) IC (OSSI: 10.3 ± 5.5 *; OSPI: 9.0 ± 4.8 *; VAS: 5.5 ± 3.3 *) BPS (OSSI: 8.1 ± 4.4 *; OSPI: 7.6 ± 4.9 *; VAS: 4.8 ± 3.3 *)	Small sample size Previous bladder hydrodistension history might influence results
<i>Angiogenesis in bladder tissues is strongly correlated with urinary frequency and bladder pain in patients with interstitial cystitis/bladder pain</i>	VEGF expression levels in immunohistochemical staining: Increase in IC ($p < 0.01$) and BPS ($p < 0.01$) vs. controls	-	Control (OSSI- 0.9 ± 0.9 ; OSPI: 0.0 ± 0.0 ; VAS: 0.1 ± 0.2) IC (OSSI: 15.9 ± 2.6 **; OSPI: 15.0 ± 1.2 **; VAS: 9.4 ± 0.9 **); BPS (OSSI: 9.3 ± 4.7 **; OSPI: 8.5 ± 4.7 **; VAS: 5.9 ± 2.1 **)	Small sample size
<i>Molecular Taxonomy of Interstitial Cystitis/Bladder Pain Syndrome Based on Whole Transcriptome Profiling by Next-Generation RNA Sequencing of Bladder Mucosal Biopsies</i>	VEGF expression in bladder biopsies: BPS: Median 0.014; 99% CI 0.013–0.018 HSB: Median 0.016; 99% CI 0.012–0.024 Controls: Median 0.014; 99% CI 0.014–0.018 IC: Median 0.412; 99% CI 0.035–0.048, $p = 0.002$ vs. BPS; $p = 0.004$ vs. HSB; $p = 0.007$ vs. controls	VEGF expression in IC patients correlated with: - Storage symptoms ($\rho = 0.47$; $p < 0.01$); - Voiding symptoms ($\rho = 0.40$; $p = 0.02$) - Daytime frequency ($\rho = 0.35$; $p = 0.03$) - Nocturia ($\rho = 0.40$; $p = 0.01$) - Decrease in hydrodistension ($\rho = -0.46$; $p < 0.01$)	OSSI: (IC: 15.8 ± 3.7 ; BPS: 12.3 ± 3.0 ; HSB: 9.8 ± 4.8 ; control: 3.3 ± 1.4) OSPI: (IC: 13.3 ± 2.1 ; BPS: 12.4 ± 2.3 ; HSB: 10.7 ± 2.6 ; control: 1.8 ± 2.0) VAS pain score: (IC: 8.2 ± 2.3 ; BPS: 5.7 ± 3.3 ; HSB: 4.9 ± 2.4 ; control: 0) Significant differences ($p < 0.01$) between all groups within each questionnaire	Small sample size Use of some inadequate biopsy samples

Table A5. Cont.

Title	VEGF/VEGF-R1 Levels in Groups	VEGF/VEGF-R1 Levels' Relations with Symptoms	Questionnaire Scores	Limitations
<i>Association of Longitudinal Changes in Symptoms and Urinary Biomarkers in Patients with Urological Chronic Pelvic Pain Syndrome: A MAPP Research Network Study</i>		Relation between VEGF levels and symptom improvement: Pain severity: OR = 0.78 ($p = 0.03$) Urinary severity: OR = 1.06 ($p = 0.62$) Relation between VEGF-R1 levels and symptom improvement: Pain severity: OR = 0.88 ($p = 0.06$) Urinary severity: OR = 0.92 ($p = 0.23$)		Suboptimal reliability data concerning some of the biomarkers
<i>Relationship of Bladder Pain with Clinical and Urinary Markers of Neuroinflammation in Women with Urinary Urgency Without Urinary Incontinence</i>		VEGF levels association with: - Urgency: β 0.09, 95% CI -0.12 – 0.29 ($p = 0.39$) - Bladder pain: β 0.04, 95% CI 0.001 – 0.07 ($p = 0.04$) - Neuropathic pain: β 0.10 95% CI -0.15 – 0.35 ($p = 0.42$)	NBP: (F-GUPI: 12.3 ± 6.1 ; OSSI: 6.7 ± 3.4 ; OSPI: 4.9 ± 3.2 ; PainDETECT: 5.1 ± 4.8) BP: (F-GUPI: 28.1 ± 6.9 ***; OSSI: 9.8 ± 4.9 ***; OSPI: 8.7 ± 4.2 ***; PainDETECT: 13.3 ± 8.6 ***)	Small sample size No healthy patients group control
<i>Relationship of Pain Catastrophizing with Urinary Biomarkers in Women with Bladder Pain Syndrome</i>		Lower VEGF levels: Higher pain catastrophizing ($p = 0.03$) Higher VEGF levels: Bladder pain ($p = 0.01$)	Higher catastrophizing scores: worse urinary symptoms and quality of life, greater pelvic and neuropathic pain scores (all $p < 0.01$)	Not reported in abstract

Abbreviations: BP—Bladder Pain group; BPS—Bladder Pain Syndrome; F-GUPI—Female Genitourinary Pain Index; HC—Healthy Controls; HSB—Hypersensitive Bladder; IC—Interstitial Cystitis; NBP—Non-Bladder Pain group; OAB—Overactive Bladder; OSPI—O’Leary – Sant Problem Indexes; OSSI—O’Leary Sant Symptom Indexes; PC—Positive Control (non-urological associated syndrome); UCPPS—Urological Chronic Pelvic Pain Syndrome; VAS—Visual Analogue Scale; VEGF—Vascular Endothelial Growth Factor; VEGF-R1—Vascular Endothelial Growth Factor-receptor. * $p < 0.01$ vs. OAB; ** $p < 0.01$ vs. controls; *** $p < 0.001$ vs. NBP.

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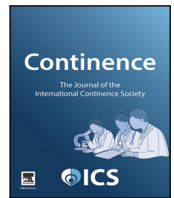
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Publication 6

Soluble Vascular Endothelial Growth Factor Receptor 1 (VEGFR1): A Possible Biomarker in BPS/IC?

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Continence 2025 (DOI:10.1016/j.cont.2025.101898.)



Soluble vascular endothelial growth factor receptor 1 (VEGFR1): A possible biomarker in BPS/IC?

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ARTICLE INFO

Keywords:

Bladder Pain Syndrome
Interstitial Cystitis
Biomarker
VEGF
Serotonin

ABSTRACT

Introduction: Interstitial cystitis/bladder pain syndrome (IC/BPS) pathophysiology is not fully understood. Vascular endothelial growth factor (VEGF) has been implicated in bladder vasculature alterations and nociception, but the role of its soluble receptor, VEGFR1, remains poorly explored. This study aimed to determine whether urinary VEGF and VEGFR1 levels could be biomarkers for IC/BPS. As a post hoc analysis, we also examined VEGFR1 variation and its relationship with the clinical characteristics of IC/BPS patients and urinary serotonin levels.

Material and Methods: An observational cross-sectional pilot study was conducted after clearance from the ethics committee. Eighteen female IC/BPS patients and 19 healthy age-matched females without past urological conditions were included. ESSIC criteria were used to phenotype. The patient's symptoms evaluated were bladder pain, urinary frequency, and life quality. Patients during flare episodes or taking antidepressant drugs were excluded. Urine samples from patients and healthy subjects were used to measure VEGF, VEGFR1, and serotonin urinary levels. The relation of VEGFR1 with serotonin and clinical parameters was assessed.

Results: Patients and healthy volunteers had a similar median age. No statistical differences in VEGF were detected. Urinary VEGFR1 was significantly lower in patients. Soluble VEGFR1 did not correlate to serotonin levels or clinical parameters. The urinary serotonin levels were similar between groups.

Conclusion: Urinary VEGFR1, but not urinary VEGF, might be a useful IC/BPS biomarker. Further investigation is necessary to confirm the present observation and validate the relationship of VEGFR1 with clinical parameters and serotonin.

1. Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic urological disease more common in women that is characterized by pelvic pain or discomfort perceived to the bladder, accompanied by urinary symptoms like urgency or frequency [1]. The exact causes or pathophysiology of the disease are yet to be clarified [2]. Still today, the diagnosis is established after excluding a long list of confoundable diseases that can cause bladder and pelvic pain and urological symptoms. Phenotyping IC/BPS patients could be relevant in identifying homogeneous groups of patients with similar pathophysiology and potentially with a good response to a specific treatment [1].

The Vascular Endothelial Growth Factor (VEGF) is among the molecules with possible pathophysiologic interest. This molecule has

emerged as a potential player related to the fragility of the bladder blood vessels in the mucosa, manifested in many patients by vascular glomerulations and the intense pain reported during bladder filling [3]. Cystoscopy with hydrodistension is the gold standard (and only) method to phenotype the patients according to these bladder changes [1]. As this is an invasive and painful method for patients, identifying reliable urinary biomarkers such as VEGF represents a promising strategy to overcome these limitations and advance non-invasive diagnostic approaches.

Previous observations reported that VEGF acts as an angiogenesis promoter in both physiological and pathological settings and that VEGF receptors play a role in inflammation-induced lymphangiogenesis, angiogenesis, and vessel permeability [2,4]. Moreover, these studies point to VEGF as an agent responsible for modulating the urinary bladder

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<https://doi.org/10.1016/j.j.cont.2025.101898>

vascular stability. This information aligns with existing knowledge of its involvement in vascular glomerulations and Hunners' lesions in IC/BPS patients [5]. Furthermore, high levels of VEGF lead to neuroplasticity and sprouting of the nerve fibers in models of neuropathic pain [5], raising the hypothesis that this might also be true in visceral pain. The indirect observation that IC/BPS patients express high levels of urinary VEGF, which seem to correlate with pain severity, further supports this hypothesis [6]. However, not all data in the literature describes the same variation of urinary VEGF levels in IC/BPS patients, which may difficult the patients' phenotyping [6].

VEGF promotes the above-mentioned effects by modulating its three known receptors: VEGFR1, VEGFR2, and VEGFR3. Although the three receptors have a soluble form, soluble VEGFR1 has the highest affinity for VEGF [7,8], and its presence in the urine has been shown to exhibit biomarker potential in the early stages of diabetic nephropathy [9]. Circulating VEGFR1 is thought to sequester VEGF, reducing free VEGF available to bind membrane-bound VEGF receptors [10]. Hence, evaluating urinary VEGFR1 could serve as an alternative biomarker for phenotyping patients according to their bladder characteristics.

In this exploratory study, we aimed to understand if our sample of IC/BPS patients presented changes in their urinary levels of VEGF and VEGFR1. As a post hoc analysis, we have correlated the urinary levels of VEGFR1 with patients' characteristics. Also, as a post hoc analysis, we have compared the levels of urinary serotonin between healthy subjects and IC/BPS patients since this molecule might have a role in IC/BPS [11–13].

2. Materials and methods

2.1. Patients

This work is an exploratory study that followed an observational cross-sectional study design. As this is a side study using samples from another study, the number of samples to be used was limited to the samples available from the principal study.

Eighteen female IC/BPS patients, previously diagnosed according to ESSIC criteria, and 19 age-matched healthy female volunteers were enrolled after signing an informed consent [1].

Patients were excluded if experiencing a flare period (worsening of the condition in the last 3 months) and if medicated with amitriptyline or other antidepressant at the time of the study.

Volunteers were enrolled if they presented a clear history of urological condition, without urinary symptoms, and had an innocent urinalysis.

2.2. Determination of the VEGF, VEGF R1, and serotonin levels in the urine

Patients and volunteers signed a consent form to have their urine and clinical data collected. On the scheduled appointment day (8 am to 12 pm), patients were asked to urinate into a collection flask, and the urine samples were aliquoted and stored at -80°C .

We determined the levels of VEGF, VEGFR1, and Serotonin by ELISA. The quantification of urinary VEGF levels was carried out using the VEGF (human) ELISA Kit (Enzo, ENZ-KIT156-0001), following the producer protocol (detection limit 4.712 pg/ml). The quantification of VEGF R1 levels was carried out using VEGF R1 (FLT1) Human SimpleStep ELISA[®] kit (Abcam, ab195210), following the producer protocol (detection limit: 0.391 ng/ml). The Serotonin ELISA Kit (Abcam, ab133053) was used to quantify the urinary serotonin levels. The producer protocol was followed (detection limit 0.49 ng/ml).

2.3. Patients characterization

During the same visit as the urine collection, the investigators gathered clinical and demographic data from the patients (quantitative data are presented as median (Q1, Q3)). Disease severity was assessed using different scales. Bladder pain was measured with a visual analogue scale (VAS) ranging from 0 to 10, where higher scores indicate greater pain. The O'Leary-Sant Score (OSS) (ranging from 0 to 37 points, with higher scores indicating more severe complaints), being the OSS Symptom index (score ranging from 0 to 20, where 20 represents the most severe symptoms) a specific part of the OSS that we evaluated individually. Quality of life (QoL) was evaluated using the final question of the International Prostate Symptom Score: "If you were to spend the rest of your life with your urinary condition just the way it is now, how would you feel about that?" (ranging from 0 to 6, with 6 indicating the worst possible QoL).

2.4. Statistical analysis

The primary outcomes were the urinary levels of VEGF and VEGFR1. The comparison of VEGF and VEGFR1 urinary levels was performed using InVivoStat. The urinary levels of VEGF and VEGFR1 of control and IC/BPS groups were compared using a two-tailed unpaired t-test. Data was transformed to meet the test assumptions. Data is presented as least square means (first quartile, third quartile). We considered an alpha of 0.05 to test the hypothesis "The urinary levels of VEGF or VEGFR1 are different in both groups".

As a posthoc analysis, a simple regression analysis was performed using GraphPad Prism 10.4.1, and the coefficient of determination was used to measure the proportion of the variance of the clinical parameters that explained the variation of the urinary VEGFR1 levels. To control the false discovery rate (FDR), the adjusted p-values were calculated using the Benjamini–Hochberg correction method. We assumed that a coefficient of determination [0.00–0.19] was very weak, [0.20–0.39] weak, [0.40–0.59] moderate, [0.60–0.79] strong, and [0.80–1.00] very strong. To provide information for future studies, the different IC/BPS patient phenotypes were evidenced in Figs. 3 and 5, although they were not considered a covariant nor a blocking factor in the analysis.

Also as a post hoc analysis, we compared the urinary levels of serotonin in both the control and IC/BPS groups, using InVivoStat. The Mann–Whitney test was used because even after data transformation, the assumptions for using a parametric test were not achieved. Data is presented as median (first quartile, third quartile). We considered an alpha of 0.05 to test the hypothesis "The urinary levels of serotonin are different in both groups".

As serotonin has cross-linked functions with VEGF-related molecules, although the exact mechanisms remain unclear, a simple regression analysis was performed. The coefficient of determination (R^2) was used to assess how much of the variance in urinary serotonin levels or clinical parameters could be explained by changes in urinary VEGFR1 levels.

3. Results

3.1. Determination of the VEGF and soluble VEGFR-1 in the urine of healthy subjects and IC/BPS patients

Urinary VEGF levels from the control and IC/BPS groups did not follow a normal distribution (see raw data in the supplementary file 1). After performing the RANK transformation of the data (Fig. 1), we have compared the urinary levels of VEGF in the control (16.13 (11.22, 21.04)) and the IC/BPS (22.03 (16.98, 27.08)) groups using a two-tailed unpaired t-test and obtained a $p = 0.0982$. There was

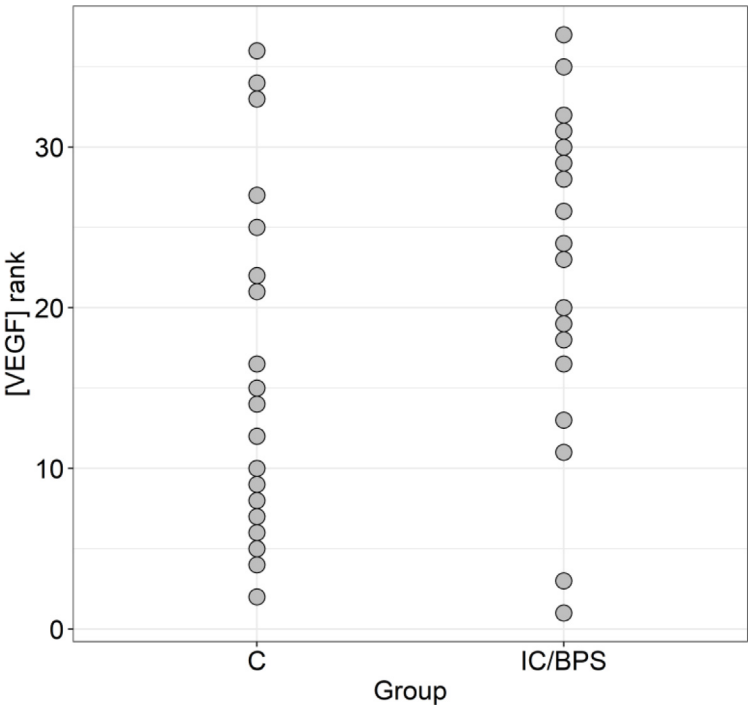


Fig. 1. Graph of ranks for the control and IC/BPS groups. The data are presented as rank values for each group, with individual points representing the data for each subject. No statistically significant difference was observed between the groups ($p = 0.0982$) using a two-tailed unpaired t-test).

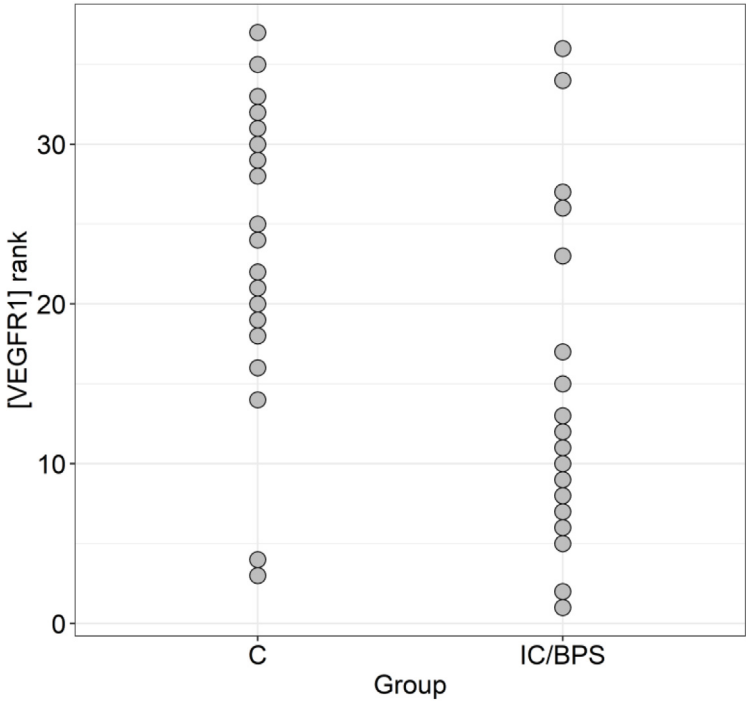


Fig. 2. Graph of ranks for the control and IC/BPS groups. The data are presented as rank values for each group, with individual points representing the data for each subject. A statistically significant difference was observed between the groups ($p = 0.0129$) using a two-tailed unpaired t-test).

insufficient evidence to support the rejection of the null hypothesis; therefore, we considered no significant differences in the urinary levels of VEGF among both groups. After performing the RANK transformation of the urinary levels of VEGFR1 (Fig. 2), we have compared the mean values in the control (23.21 (18.54, 27.88)) and in the IC/BPS (14.56 (9.75, 19.36)) groups using a two-tailed unpaired t-test and obtained a $p = 0.0129$ (see raw data in the supplementary file 2). The

analysis provided evidence to reject the null hypothesis, suggesting a statistically significant difference in VEGFR1 urinary levels between the two groups.

3.2. Demography

The trial included 18 female patients, with a median age of 43 (40, 54). The control group consisted of 19 age-matched female healthy

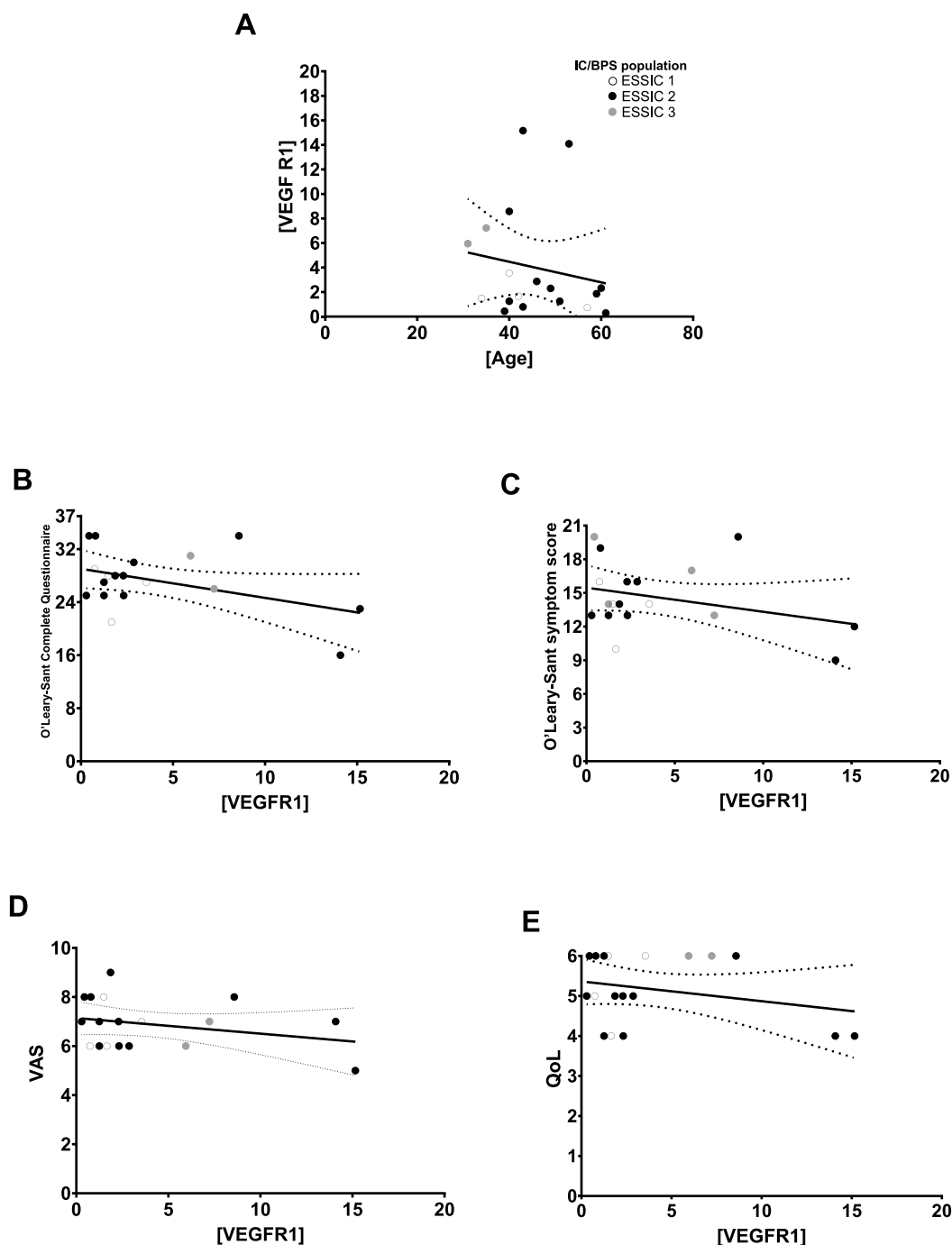


Fig. 3. XY graphs showing the relation between soluble VEGF R1 (ng/ml) in the urine of IC/BPS patients with (A) the patient's age, (B) the O'Leary-Sant Interstitial Cystitis Complete questionnaire (Symptom and Problem Index), (C) the O'Leary-Sant Interstitial Cystitis Symptom Score, (D) the visual analog scale for pain, and (e) the quality-of-life score. Each dot represents an individual. Data was compared using a simple linear regression.

volunteers with a median age of 44 (39, 56). Both groups had a similar median age ($p > 0.05$).

Patients were phenotyped according to the ESSIC criteria based on their cystoscopic findings: 22% (4 out of 18) were type 1 (normal bladder at cystoscopy), 55% (10 out of 18) were type 2 (glomerulations with bladder distention), and 22% (4 out of 18) were type 3 (Hunner's lesions were present).

Patients presented a median VAS score of 7 (6, 8) and a median score of 28 (25, 30) points in OSS. In the symptoms questionnaire of OSS, the median score was 14 (13, 18). The quality of life had a median score of 5 (4, 6).

3.3. Linear regression analysis of VEGFR1 in relation to pain and urinary symptoms

The regression model did not show statistical significance in any analyses, specifically in the associations between VEGFR1 levels and pain and urinary symptoms (Fig. 3). Only 3% of the changes in the urinary VEGFR1 levels could be explained by the increase in the age of the patients (corrected $p = 0.49$). VEGFR1 variations only explained 18% of the score of the O'Leary-Sant Interstitial Cystitis Complete questionnaire (corrected $p = 0.46$), 10% of the Symptom Index isolated questionnaire of the O'Leary Sant score (corrected $p = 0.60$), 8% of the

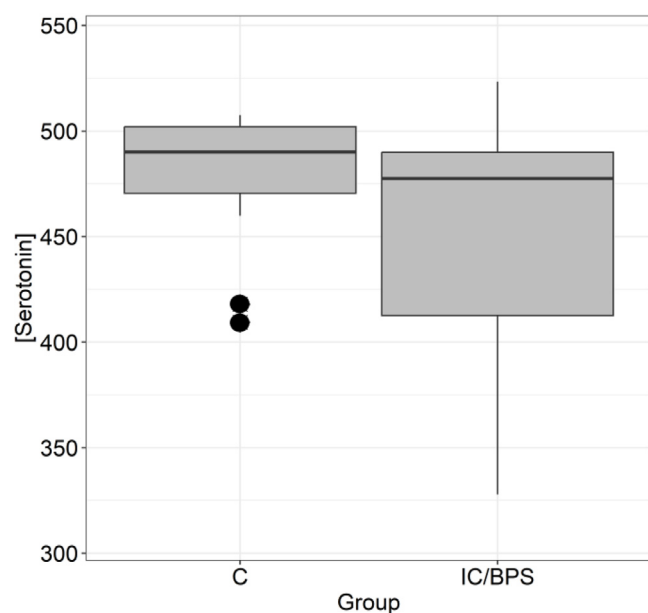


Fig. 4. Boxplot showing the median and the interquartile range of the serotonin values (ng/ml) in the urine of healthy subjects (C; $n = 18$) and of IC/BPS patients ($n = 18$). The whiskers extend to the most extreme data values. The black dots represent individual observations that lie outside the outlier range.

patient's subjective experience of pain (VAS) score (corrected $p = 0.52$), and 7% of the patient's overall well-being (QoL) score (corrected $p = 0.45$).

3.4. Determination of the serotonin in the urine of healthy subjects and of IC/BPS patients and its relation with urinary VEGFR1 levels

The healthy volunteers and IC/BPS patients seem to present similar urinary levels of serotonin ($p = 0.2230$, Mann-Whitney test; Fig. 4; (see raw data in the supplementary file 3)). The median level of urinary serotonin in controls was 490.1 (470.5, 502.1) mg/ml, while the IC/BPS population presented median levels of 477.6 mg/ml (412.7, 490.0) (Fig. 4).

The regression model did not show statistical significance (corrected $p = 0.56$). Only 3% of the variation in the urinary levels of serotonin was explained by the changes in VEGFR1. However, the highest serotonin levels were associated with the lowest levels of soluble VEGFR1 (Fig. 5).

4. Discussion

In our IC/BPS patients, urinary VEGF levels were similar to those of healthy subjects. These results contrast with previous findings showing that IC/BPS patients exhibit higher urinary VEGF levels than OAB patients [14], who, in turn, are known to have urinary VEGF levels similar to or higher than those of healthy controls [15]. Interestingly, that study found no differences between IC/BPS patients with and without Hunner's lesions, suggesting that even when patients present high urinary VEGF levels, these levels do not differentiate between the two phenotypes [15]. Thus, urinary VEGF levels do not seem to demonstrate face validity as a biomarker for identifying IC/BPS patients with vascular impairment in the bladder wall. However, our study did not assess the relationship between urinary and bladder wall VEGF levels. To our knowledge, no study has yet performed such a comparison. Hence, future research that involves bladder biopsies should include this analysis to ensure the construct validity of VEGF as a biomarker for IC/BPS. VEGF as a follow-up biomarker for IC/BPS (external validation)

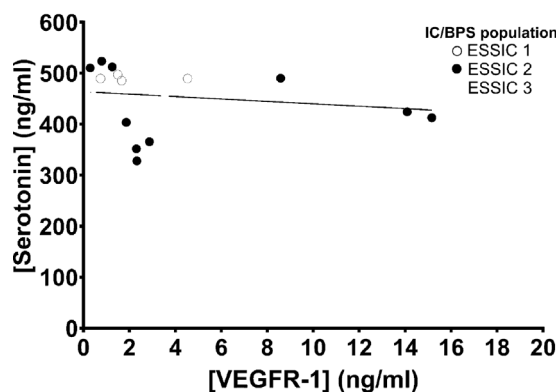


Fig. 5. XY graph showing the relation between the serotonin levels (ng/ml) in the urine of IC/BPS patients with the levels of soluble VEGFR1 (ng/ml).

is also controversial. A longitudinal study showed that women with IC/BPS maintained their urinary VEGF levels within a 6-month interval [16]. Nonetheless, regardless of the signal-to-noise ratio presented by IC/BPS patients, the intra-individual response to treatment may allow urinary VEGF levels to be used as a follow-up biomarker. Urinary VEGF levels have decreased in IC/BPS patients after intravesical OnabotulinumtoxinA injections combined with hydrodistention [2].

In the present work, urinary soluble VEGFR1 levels were significantly lower in IC/BPS patients. Immunostaining studies revealed that urinary VEGF can bind to membrane VEGFR in the apical cells of the urothelium [17]. Since urinary VEGFR1 may act as a decoy for the urinary VEGF [18], its reduced levels in the urine of IC/BPS patients could allow more free urinary VEGF to interact with the apical urothelial cells and exert its effects. Given that VEGF exerts its effect primarily through binding to membrane VEGFR and that VEGFR2 expression in umbrella cells remains unchanged in IC/BPS patients [17], this may support the use of the urinary VEGFR1 levels as a potential biomarker for IC/BPS.

In the present study, we did not evaluate if the decreased levels of urinary VEGFR1 reflected the expression of the receptor in the bladder wall. Previous studies have described that the expression of membrane VEGFR1 was lower in IC/BPS when compared to the bladder expression in stress urinary incontinence [17], suggesting a possible correlation between the membrane VEGFR1 and the urinary VEGFR1. However, the same previous study showed that IC/BPS patients had a similar expression of membrane VEGFR2 when compared to the bladder expression in stress urinary incontinence [17], and in our study, the levels of urinary VEGFR2 were under the limit of detection both in IC/BPS patients and controls (data not shown).

There is limited data from previous studies on the role of soluble and membrane-bounded VEGFR1 in pain and, consequently, on the face and construct validity of the urinary levels of VEGFR1 as a biomarker for IC/BPS. In chronic pelvic pain syndrome, for instance, urinary levels of VEGFR1 in women were similar to the levels of healthy subjects [19], showing that the VEGFR1 found in the urine did not correlate with the pain felt by the patients. Our post hoc analysis also found no relationship between the urinary levels of VEGFR1 and the O'Leary Saint Symptom or VAS scores.

The lack of a relationship between the VEGFR1 and the clinical parameters evaluated may reflect the limitations imposed by the patient inclusion criteria. For instance, our patients had a VAS score greater than 4, which may have led to a clustering of patients with a similar clinical phenotype. As a result, the variation in VEGFR1 levels may reflect variability within this homogeneous group rather than differences among distinct clinical clusters. On the other hand, patients had a heterogeneous profile of urinary complaints that could have significantly affected the analysis of VEGFR1 and its relationship with the O'Leary-Sant questionnaire. Hence, future study designs should consider these characteristics.

VEGF and serotonin have crosslinked functions, although the mechanism that entangles these molecules' pathways is still unclear. In IC/BPS, the modulation of the serotonin pathway by amitriptyline suggests that the serotonergic pathway is imbalanced in this pathology. Antidepressants such as amitriptyline promote an increase in VEGF levels, leading to neurogenesis [20–23], which suggests that, in the hippocampus, both VEGF and serotonin have a protective effect. In the periphery, serotonin exhibits angiokine properties [24], promoting the release of VEGF [25] and supporting angiogenesis. In IC/BPS, the effect of VEGF in the periphery seems deleterious. VEGF is one of the molecules responsible for the glomerulation formation in the bladder wall of patients with IC/BPS [26]. Also, VEGF is associated with bladder inflammation in IC/BPS [2]. The serotonin pathway is also hyperactive in animal models of bladder inflammation [27].

Additionally, cats with feline IC presented higher urinary serotonin levels than healthy cats [13]. Considering all this, we conducted a post hoc analysis to study the urinary serotonin levels in IC/BPS patients and their relationship with the urinary levels of VEGFR1. Although the urinary serotonin levels between IC/BPS patients and volunteers were not different, the former had a more extensive distribution range of urinary serotonin levels. This increased variability may indicate underlying heterogeneity in serotonergic regulation among IC/BPS patients, suggesting that diverse pathophysiological mechanisms could be at play. The heterogeneity in urinary serotonin could influence pain perception [27] and vascular responses [25], highlighting the multifactorial nature of IC/BPS pathophysiology and the need for biomarkers that capture these complex interactions. The observation that patients with the highest levels of urinary serotonin presented the lowest levels of VEGFR1 is particularly intriguing. One hypothesis raised by these results is that higher serotonin levels may influence the regulation or shedding of VEGFR1, resulting in reduced concentrations of its soluble form. These variations can potentially cause an imbalance of angiogenic signals, as lower levels of the decoy receptor might allow for increased availability of VEGF to interact with its functional receptors. However, given the exploratory nature of this post hoc analysis and the lack of statistical significance, these observations should be interpreted with caution. Future studies with larger sample sizes and targeted experimental designs must clarify whether this relationship is consistent and elucidate the underlying biological pathways.

In fact, one clear drawback of the present study is the low number of patients and volunteers enrolled, which is related to the study's exploratory nature. Although our IC/BPS patient cohort was phenotyped according to the ESSIC criteria, the analysis was conducted on the groups as a whole. Another limitation of the study was the lack of normalization of the urinary levels of VEGF and VEGFR1, which should be considered in future studies. The MAPP Research Network has validated a methodology based on the measurement of total protein concentration in urine that, on top of allowing an accurate normalization, also overcomes the problems associated with urine dilution and the freeze/thaw of the samples [28].

5. Conclusion

In conclusion, urinary VEGF levels do not appear to be a good biomarker for IC/BPS, whereas urinary VEGFR1 levels may be a promising candidate. Nonetheless, more robust proof-of-concept experiments are needed. The usefulness of urinary VEGFR1 as a phenotyping biomarker should also be confirmed, considering the need to include different patient subgroups in the validation experiments. Despite the lack of statistical significance as a biomarker in our study design, serotonin might play a role in IC/BPS, and its cross-talk with VEGF pathways should be further investigated.

Ethics approval

Ethical approval from the hospital ethical committee was obtained (Hospital de Sao Joao #061114 (Probable)). Patients and volunteers provided consent.

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This work does not require a trial registration since it compares urinary levels of molecules without any known clinical significance (so far) between patients and healthy subjects. Furthermore, it was approved by our hospital ethics committee. Written informed consent was obtained from the participants for publication.

Funding

No type of funding was used.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.cont.2025.101898>.

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Discussion

As observed in a PubMed literature search, the number of studies published on BPS/IC has consistently grown throughout the last few years. Despite all the investigations done worldwide, a lot is yet to unravel. The fact that diagnosis is based on excluding other conditions leads to a delayed diagnosis. In some cases, it can be even worse with misdiagnosed or undiagnosed patients. Despite multiple theories about the origin, we cannot identify the cause. In the same way, we could not identify a biomarker or set of them for the disease. Phenotyping has evolved in the last decades, particularly phenotyping, with a direct positive consequence in disease management. The non-ulcerative and ulcerative classification leads to specific treatment options in the latter (lesions-directed surgical treatment), while clinical phenotyping using questionnaires such as UPOINT is indicated to provide some orientation regarding management priorities. A more fine phenotyping, or cluster identification, that could guide the etiology and effective, curative treatments is still lacking. Of the treatments successively introduced in the last decades, even the FDA-approved ones have fragile evidence to support their use. Analgesics assume the cornerstone of the treatment, with good evidence in improving pain and life quality. However, even when prescribed correctly and according to the pain ladder, the chronic use of high doses of potent analgesics, such as opioids, can lead to addiction, aggravating a public health problem in many countries. The positive results observed with PPS were undermined by the macular adverse events identified in some patients using high doses. It is under discussion if BPS/IC patients are more prone to maculopathy and if an ophthalmologic evaluation should be performed

before the introduction of PPS therapy or even as part of the standard evaluation of BPS/IC patients (171). Another approved drug for BPS/IC, amitriptyline, seems to be grounded in conflicting results, as the effects can only be detected when high doses are used (172). OnabotA is one particular treatment that is effective and safe, but the evidence is based on small single-center pilot studies. All in all, effective and longstanding treatment options are lacking, ultimately leading to patients with unresolved chronic pain and a general loss of life quality.

The scarcity of knowledge about this condition nowadays demonstrates the relevance of this thesis. The lack of valid treatments, despite the multiple drugs and existent treatment options, led us to investigate potential new treatments, namely the oral use of silodosin and tadalafil, as well as investigate the real-life data related to the OnaBotA use. Moreover, we evaluated the potential role of urinary VEGF and the soluble VEGFR1 as potential biomarkers of BPS/IC and the use of QST to identify sensory changes in somatic sensations and contribute to defining clinical clusters.

Therapeutical response to the investigated treatments

Silodosin

In our clinical work with silodosin treatment, we have observed that 60% of patients had a simultaneous improvement of bladder pain and urinary frequency above the scores defined a minimal important difference (MID). When parameters were evaluated separately, 11 of the 17 patients improved urinary frequency, and 14 out of 17 improved bladder pain scores. All the secondary endpoints, OSS, and life quality also showed statistically significant improvements. We evaluated a specific psychological trait of our cohort related to stress level and ability to cope with stress with two simple questions. We observed that most of the group (13 out of 17, approximately 76%) aggravated the BPS/IC complaints when under stress, and they recognized themselves as having a low capacity to cope with stress. Since silodosin is an alpha-blocker of the sympathetic system, and the latter is closely related to stress and anxiety (173), we could speculate that the present cohort and its vulnerability to stress with low coping abilities could have impacted the study results. Moreover, in a previous study conducted by the IMI consortium in which we took part, one-third of the patients with BPS/IC identified stress as an inducer or worsening trigger for pelvic pain (174), further reinforcing the importance of adrenergic blockade.

The performed exploratory subanalysis did not show any demographic or clinical characteristics that could be used to identify a potential phenotype more prone to treatment response. Of relevance, the absence of adverse events, as expected, underlines the safety of this drug.

The results observed in our study align with previous findings from our group related to the role of the adrenergic system in the BPS/IC, both in clinical

and pre-clinical studies. A previous clinical study showed an overactivity of the sympathetic nervous system in BPS/IC patients (53). Patients were submitted to the Tilt test, followed by measurements of cardiovascular parameters and NE urinary and plasmatic levels, indicating a strong resting-state sympathetic overactivity. A parallel pre-clinical evaluation published in the same paper (54) showed that subcutaneous phenylephrine, an α 1-adrenergic receptor agonist, could elicit a BPS-IC-like behavior in female rodents. The administration of capsaicin, a desensitizing agent of TRPV1, blocked this pain behavior. These observations and the demonstration of a co-localization of α 1 adrenoreceptors and TRPV1 in bladder sensory nerves reinforce the idea of a link between the noradrenergic system and pain pathways.

Following the line of investigation linking the sympathetic nervous system and the pain pathways, an additional pre-clinical study was conducted (69). A cohort of female Wistar rats was subcutaneously injected with phenylephrine to induce chronic pain and bladder hyperactivity. The BPS/IC-like behavior was accessed through mechanical cutaneous tests and bladder cystometry, which permitted the evaluation of both pain and urinary symptoms. It was, once again, stressed that chronic adrenergic stimulations lead to BPS/IC-like behavior, with lowered abdominal pain threshold and higher voiding frequencies. The novelty of this study was the use of different α -blockers to identify which receptor subtype was involved in this pathway, namely α 1 type A or type D (175). The results have shown that silodosin (specific α 1A antagonist) and prazosin (non-subtype specific α 1 antagonist) were able to reverse the BPS/IC behavior developed after phenylephrine chronic stimulation. However, these same changes were not reversed by administering naftopidil, the α 1D-

specific antagonist. Most importantly, the results showed that the subtype alpha 1A antagonist silodosin could potentially be used to treat BPS/IC patients.

The possible relation between chronic pain, such as BPS/IC, and the sympathetic nervous system imbalance is long known and has been investigated in recent years. Furthermore, a positive correlation between higher stress levels and BPS/IC symptoms aggravation, namely pain and urinary frequency, has been shown (176,177). The relation observed in patients between stress and clinical aggravation and the known fact that direct chronic adrenergic stimulation caused BPS/IC-like behavior led to a pre-clinical evaluation using animal stress models to evaluate if early-life chronic stress could induce later-in-life BPS/IC behavior.

Findings in the stress models reproduced the data from the phenylephrine experiments. Two different models, the water avoidance stress test and the maternal deprivation model, imposed early-life stress on animal models to induce later in life a BPS/IC-like behavior (55,56). We have observed an imbalance in the adrenergic system in female rodents, as well as a pro-nociception behavior observed in both studies, suggesting once again a cross-talk between these systems. Most importantly, in both models, the rodents exposed to silodosin, compared to placebo, improved both abdominal pain and bladder frequency (55,56).

The data built from all the previous clinical and pre-clinical studies led our group to conduct an open clinical trial of this drug in patients with refractory cases of BPS/IC, and the results are part of this thesis. Silodosin is highly selective for alpha1A-adrenoceptors, with a safe cardiovascular profile, as shown in benign prostate hyperplasia/benign prostate enlargement (BPH/BPE) studies (178,179).

Moreover, the lack of semen emission that affects men under silodosin treatment is not relevant in women.

One could hypothesize that early-in-life insults or stressful events and the consequent elevated systemic NE levels could be responsible for long-term dysregulations on the pain pathways, eventually involving the hypothalamic-pituitary-adrenal axis (180). Nevertheless, caution should be taken when translating the early-in-life insult in pre-clinical models to female patients, even knowing that a significant proportion of BPS/IC patients lived an episode of child trauma. The IMI consortium recently evaluated the occurrence of childhood and adulthood trauma in our cohort of BPS/IC patients, with the results being presented in the IASP 2024 World Congress of Pain (181). Both childhood and adulthood trauma were more common in patients than in controls, meaning that in patients, the existence of traumatic events, including sexual abuse, and the associated adrenergic stimulation could leave a permanent imprint in the pain pathways. Furthermore, although assuming that the cause for elevated urinary and plasmatic NE levels is long-term exposure to stress and not specific to bladder pain, the relation between NE levels and treatment response can be used for potential phenotyping (173).

These results can be used as a push to keep evaluating the relationship between BPS/IC and the adrenergic system. In the future, patient characteristics should be evaluated more thoroughly, with a pre-planned analysis of predictors of treatment response. A randomized double-blinded process to compare silodosin with other commonly used treatments should be put in motion with previous characterization of stress events in the past and initiation of the flares. Furthermore, silodosin's effect on flares' frequency and severity should be

explored. A complete evaluation of the patient profile before and after treatment may shed light on the condition mechanisms, using the QST and other urinary and plasmatic molecules, such as NE and NGF, should be used. Patient trauma history could be used to clarify if the silodosin effect differs between these two potential clusters. Despite all the flaws of our study, we believe this pilot study has shown the clinical relevance of alpha-blockers in BPS/IC patients and the safety of their use.

Tadalafil

In another investigation, we conducted a pilot study evaluating the therapeutical effect of tadalafil 5mg in refractory BPS/IC female patients. Primary endpoints were pain intensity and urinary frequency, with a relative reduction of 48% and 51%, respectively. The bladder pain, evaluated by visual analog scale (VAS), improved by 3 or more points, the MID, in 8 of the 10 patients. All 10 patients that completed the trial presented a reduction in the total number of micturitions superior to 2 per day. Statistically significant improvements were also seen in the secondary endpoints: life quality, functional bladder capacity, and OSS score. When patients were asked for their subjective opinions, the parameters in which they felt a more significant improvement by the 4th week of the trial was urinary frequency, while by the 12th week of the trial, the most important was pain.

Tadalafil had a similar effect on the non-ulcerative and ulcerative phenotypes despite a minor improvement in bladder capacity of the ulcerative phenotype, contrary to the improvement observed in non-ulcerative patients. These findings align with the expected capacity of ulcerative bladders, with some degree of fibrosis (101). When comparing the two phenotypes, it is also worth

mentioning that urinary frequency reduced significantly by the 4th week in the BPS patients, while in IC patients, it was seen by the 12th week. Improvements in pain intensity and OSS were significant only at the 12th week in both phenotypes. At the end of the trial, none of the phenotypes seemed to benefit more than the other, leading us to assume both can benefit from a tadalafil trial when other treatment options have failed.

The exact mechanism or mechanisms behind the PED5i therapy are still unknown. However, improvements were seen both in our pilot trial and the randomized control trial published by Chen *et al.* (84). The underlying rationale suggested by Chen's group was that increasing NO through the inhibition of phosphodiesterase-5 would alleviate symptoms of bladder pain by promoting detrusor relaxation and enhancing microcirculation. Several theories have been proposed to explain the improvements observed in BPS/IC patients: smooth muscle relaxation, cell proliferation, neuronal activity, and tissue perfusion (182). At pre-clinical levels, the NO pathway has the so-called dual effect; in some cases, an analgesic effect is demonstrated, while in other cases, a pro-nociceptive effect was found (81,183). A pre-clinical study with cyclophosphamide-induced cystitis in rats observed a decrease in afferent activity of both A delta and C-fibres elicited by bladder distention (184).

All these explanations do not fit well with the observations from Logadottir Y. *et al.*, which found an increased NO concentration in bladder air samples, specifically with IC phenotype (185). Considering these findings, we would expect that a drug that prolongs the availability of NO would aggravate the symptoms. However, in this paper, Logadottir Y *et al.* discuss the fact that the findings in their cohort were not in line with those of other investigators. In fact, this same

investigation group had previously failed (186) to replicate the positive results with L-arginine (187,188), the NO precursor. Moreover, another hypothesis should be considered. The NO overproduction could be, in fact, a mechanism used by the bladder to compensate for changes caused by the disease. If this was the case, the positive effect of tadalafil administration could be explained.

We think that tadalafil, in the future, can have a role in the treatment of BPS/IC patients even though we cannot predict which subgroups could benefit more. However, given the drug's safety, a trial with tadalafil can be offered to refractory patients with the ulcerative and non-ulcerative phenotype, knowing that urinary frequency improvements are more likely to appear in the first weeks while pain improves later. More complex and robust studies should be performed to confirm this pilot study and determine which patients may benefit the most from this class of drugs.

Botulinum Toxin Type A

Intravesical injections of OnaBotA in BPS/IC have been extensively studied, although not in the same proportion of muscular and neurological dysfunctions such as dystonias, hyperhidrosis and migraine, detrusor overactivity, and overactive bladder (189). The subtype A of botulinum toxin has a more prolonged action duration and, for that reason, is the one used in clinical practice. The injection of the toxin in the bladder is expected to cause an inhibition of detrusor muscle activity, modulate the bladder sensory input, and reduce suburothelial inflammation (190). In previous studies, OnabotA injection reduces the bladder expression and potentially the spinal cord release of pain-related neuropeptides like Substance P and Calcitonin-Gen Related Peptide (CGRP) (191). Moreover, it reduces ATP release from the urothelium and urinary NGF,

brain-derived neurotrophic factor (BDNF), and VEGF (66,189,192). A significant reduction in TRPV1 trafficking from transport vesicles into the membrane of nociceptive afferent neurons causes a significant decrease of these receptors in the human bladder after OnabotA injection (193). A decrease in expression in the purinergic receptor subtype P2X3 was also observed in the human bladder following botulinum treatment (193). Simultaneously, a decrease in urothelial ATP release from the bladder wall may contribute to suppressing bladder sensory activity of P2X3 expressing small-diameter bladder fibers. Despite being more known for its blocking action of neurotransmitters, it has been observed that OnaBotA injection is also responsible for the reduction of urothelial inflammation, with a reduction of the number of active mast cells, as well as a reduction in VEGF expression (192,194).

Initially, the injection technique was pan-vesically until our group introduced the intratrigoal injection of 100 U of OnaBotA divided by 10 injections (36,162). This localization for the injections was decided due to the high abundance of nociceptors in this part of the human bladder and their scarcity in lateral walls and dome (195). Furthermore, the intratrigoal injection technique is easy to learn, and the adverse events are usually minor and self-limited (160).

Across different studies and cohorts, the tendency has been for positive results with an improvement in both bladder pain and urinary symptoms (160). These results have consolidated OnaBotA as a surgical therapeutical option in BPS/IC, recommended across different guidelines worldwide (6). Different randomized placebo-controlled small studies suggested a short-term efficacy of OnaBotA in this condition. Pinto R. *et al.* showed that repeated injections

following symptoms relapse, until a total of 4 repetitions, maintained efficacy and safety (162). However, real data are lacking.

The retrospective study comprehended in this thesis includes 47 female patients, all treated in our department, from 2009 to 2022 (196). We aimed to evaluate the long-term treatment adherence and efficacy, the safety, and the treatment duration along the multiple treatments and identify possible predictors to identify responders. A total of 193 treatments were performed. Most patients, 78%, requested more than one treatment, and almost half of the cohort (47%, 22 patients) received at least 4 treatments.

The median interval per injection showed surprising results, with a median time between injections of 500 days. This interval was relatively stable until the eighth injection, with the range of medians between 414 and 669 days. The injection interval with the lower duration of effect was between the first and second, presenting a median of 390 (287; 590) days. However, despite a lower treatment duration in the first injection, additional treatments had a longer duration, as already referred (164). The duration of the effect of the first injection was evaluated as a predictor of a patient becoming a long-term responder, but this was not the case. Of the 9 patients who were in the lower percentile of treatment response duration of the first injection, seven became long-term responders. The shorter duration of the first injection could be related to technical issues, both the injection technique or the drug preparation, or to the lack of self-awareness of the real duration and effect of the treatment by the patients who were informed from data obtained in controlled studies. The fact that patients initially non-responsive to OnaBotA could become responsive was already demonstrated in patients with neurogenic detrusor overactivity (197), which was

further confirmed in this study. Also, in another cohort of neurogenic patients with detrusor overactivity, it was observed that injection failure can occur in up to one-third of patients (198). Our understanding of this finding is that the first injection should not be used as a predictor of response, and even non-responders should try a second treatment.

We also admit that another cause for the longer intervals between injections in our cohort might have been the possibility of (self) combination with other drugs during the tail effect of OnabotA. It is known that BPS/IC is rarely controlled in a monotherapy set, and this can be more evident than previously reckoned (199). In the case of BPS/IC, the typical fluctuation of symptoms between remission and flares can also explain the long intervals between injections.

This new data on real-life treatment duration is relevant not only to inform the patients, since a more prolonged period between injections makes the treatment more attractive but also to estimate the burden of the treatment to the health system.

Despite observing that the majority of the patients were responders, only 53% of patients maintained regular reinjections for symptom control. Although OnaBotA injections are performed in the operation room, free of discomfort and with a short time of hospitalization, when performed repeatedly, the procedure can become very bothersome to the patients and can be seen as a downside of the treatment. Nevertheless, the maintenance rate in our cohort compares favorably with the use of OnaBotA in other conditions. In OAB cohorts under long-term OnaBotA treatment, adherence can be as low as 10% (200).

Somatosensorial system evaluation in BPS/IC patients

As previously explained, the QST is a set of cutaneous tests used to evaluate the central and peripheral nervous systems through somatosensorial system changes (201). They represent an opportunity to assess the nervous system in an accessible and non-invasive way.

In the study that is part of this thesis, patients with pelvic pain of different origins were submitted to QST. The objective was to compare patients with different conditions causing pelvic pain and their respective QST results, trying to understand the mechanisms behind chronic pelvic pain. Of notice, the QST test was performed on the foot and the abdomen, the first as a control and the latter as a test site. Although the abdomen is not the usual site and previous data are lacking, it was decided to perform the tests in this location since it is the most common site of referred pain in patients with pelvic pain (202).

The pattern of changes observed was different in each group of patients, evidencing the heterogeneity that can be behind a common symptom, pelvic pain. Up to 93% of patients presented QST changes, indicating profound modifications in the somatosensitive system.

From the five possible groups in which patients could be classified based on their abnormalities, BPS/IC patients presented mostly mechanical and thermal hyperalgesia in the abdomen. The majority presented mechanical hyperalgesia, observed through a “gain of function” (lowered threshold) in the parameters of pressure pain threshold (PPT), mechanical pain sensitivity (MPS), and dynamic mechanical allodynia (DMA). These specific findings of gain of function are in favor of the central sensitization phenomenon. While the lower PPT is associated with central sensitization to the deep tissue input, changes seen in MPS and DMA

are in favor of central sensitization to the cutaneous input. Furthermore, these changes in PPT have been seen in cases of fibromyalgia, sleep deprivation, and stress. Of relevance, most of the patients enrolled in the study fit in the mechanical hyperalgesia cluster regardless of the cause for pelvic pain and even in controls. This finding suggests that central sensitization may be a transverse mechanism underlying various chronic pain conditions (203) but can also mean that this specific test or measure of the QST is too sensitive or even that the abdomen region is rather sensible to pressure. Furthermore, an association was observed between PPT and bladder pain with urinary symptoms, demonstrating a probably referred visceros-somatic communication (204–206).

Thermal hyperalgesia was also observed in BPS/IC patients, and it is assumed to be related to sensitized nociceptors (207). These nociceptors have lower thresholds for activation when exposed to thermal and even mechanical stimuli. This hyperactivity and gain of function can explain the allodynia present in these patients. Furthermore, one can argue that these hyperactive nociceptors can be the genesis for central sensitization, denoting that these clusters can be related and not exclusively exist, as was seen in our cohorts. Some BPS/IC patients also presented QST results compatible with the sensory loss cluster, with loss of function in vibration and temperature sensation. This cluster occurs with peripheral nervous system changes with loss of function of small fibers (temperature) but also of large fibers (vibration) and can be related to a phenomenon of “painful hypoesthesia” (207).

One should remember that this study demonstrates that each condition present in this study, despite having pelvic pain as a common feature, can have different changes at the neuronal level. Moreover, it can indicate that somatic

changes are present in these patients besides the bladder pain. One possible cause for these findings could be the NGF molecule. It has been previously shown in pre-clinical studies that bladder instillation of NGF and consequent binding to the bladder receptor Tropomyosin Receptor Kinase A (TrkA) leads to bladder pain and thermal hypersensitivity, mediated by TRPV1 (65). Furthermore, the levels of NGF at the urinary and serum levels are significantly elevated when compared to healthy controls (67). A future research project with simultaneous profiling of the somatosensorial nervous system, with QST and NGF levels, could potentially help to define more precise types of clinical and biological clusters.

The clinical relevance of profiling QST will be proportional to the capacity to improve treatment efficacy by addressing the different components altered in the QST. It is known that different clusters respond differently to treatment options, meaning that the same treatment can have different efficacy in patients with the same disease, as observed in BPS/IC (207). In practice, these QST results could be used to differentiate and treat a specific nociceptive mechanism rather than the chronic pain condition. The next step could be to test this hypothesis by designing a clinical trial where BPS/IC patients were grouped by QST cluster, as tried before, with different clinical phenotyping systems (208,209).

One should not forget that in our paper, BPS/IC patients were not phenotyped according to the ESSIC criteria, which could have been necessary since doubt about the relationship between BPS and IC still exists (101).

A secondary objective of the paper was to correlate the findings of QST with the questionnaire PAINdetect, used to evaluate neuropathic pain, which is

more accessible than performing the QST (210). The only parameter that showed a strong correlation between the QST and the questionnaire was PPT with the question, "*Does slight pressure in this area, e.g., with a finger, trigger pain?*". Another possible confounding element can be, once again, that this correlation used the results obtained in the abdomen, which is not the usual location. For now, it does not seem that PAINdetect can be used instead of QST.

VEGF and its receptors in BPS/IC patients

The VEGF pathway has been identified as a potential actor in BPS/IC for quite a long time. This molecule could explain bladder changes and symptoms to some degree, particularly when vascular disruption and bladder pain exist. Furthermore, different mechanisms could explain the involvement. In animal models, the intravesical instillation of VEGF was responsible for increased urinary frequency and lower abdominal mechanical pain threshold (211). Another group repeated this pre-clinical experiment, and besides the pelvic hypersensitivity, an increase in the density of bladder sensory fibers was observed (212). Elevated levels of urinary VEGF were interpreted as a survival mechanism in response to the insult that causes IC (213). The VEGF elevation would activate a positive feedback mechanism with urothelium VEGFR, and a snowball of urothelial permeability, edema, pain, and vascular permeability would explain the condition at the bladder level. Furthermore, higher levels of VEGF were associated with bladder changes, namely vascular and neuronal (70,213,214).

A pre-clinical study showed that the subcutaneous injection of VEGF leads to a lower abdominal pain threshold and that the pain behavior was reversed by antagonists to VEGFR1, but not by antagonists to VEGFR2, which confirms the idea that membrane VEGFR1 is related to nociception (75). To further elucidate the complexity, additional studies indicate that VEGFR1 may exhibit distinct functions depending on its transmembrane or soluble form. A hypothesis postulates that the transmembrane receptor functions as a facilitator of nociception, whereas the soluble form serves as a VEGF decoy by sequestering circulating VEGF, thereby inhibiting the activation of pain pathway (215,216). Furthermore, a study employing immunostaining techniques observed that

urinary VEGF binds to membrane-bound VEGFR in the apical cells of the urothelium, and noted that the levels of transmembrane VEGF receptors, specifically VEGFR2, remained unchanged. Assuming the decoy function of soluble VEGFR1, lower levels of soluble VEGFR1 would allow more free VEGF to bind to transmembrane receptors expressed in urothelium umbrella cells, activating this pathway (217).

Following evidence of a possible link between the VEGF pathway and BPS/IC, we decided firstly to investigate the existing data in clinical studies, which led to a systematic review included in this thesis (218). The previous clinical studies in BPS/IC included in the systematic review have shown, with some degree of unanimity, higher levels of urinary VEGF and VEGFR1 in BPS/IC patients when compared to healthy controls or OAB patients (23,70,219–222). A correlation was found between the elevated levels of urinary VEGF and higher pain intensity and more severe urinary symptoms (219). In a longitudinal study with chronic pelvic pain patients (222), lower urinary VEGFR1 at baseline was associated with a higher probability of resolving pain at mid-term. However, a decrease of VEGFR1 at 6 months was not associated with an improvement in pain, specifically in BPS female patients.

The systematic review showed an absence of studies evaluating simultaneously urinary VEGF and soluble VEGFR1. The correlation between the latter and clinical parameters had not been the object of evaluation. This led us to investigate the urinary levels of VEGF and the soluble VEGFR1 in our cohort of BPS/IC female patients. A cohort of 18 BPS/IC patients and 19 healthy volunteers was used.

The median level of urinary VEGF of the volunteers was 16.13 (11.2; 21.04), while the median level in the urine of BPS/IC patients was 22.03 (16.98; 27.08) pg/ml, –without statistic significance. The levels of soluble VEGFR1 in the urine of volunteers was 23.21 (18.54; 27.88) ng/ml, while the median level of VEGFR1 in patients was 14.56 (9.75; 19.36) ng/ml, which translated to a statistically significant difference between the two groups, with a *p*-value of 0.0129. We also evaluated a possible relation between the levels of soluble VEGFR1 and the clinical characteristics of our cohort of patients, such as age, OSS, pain, and quality of life. However, none of these parameters correlated with VEGFR1 urinary level.

The VEGFR1 results observed in our cohort differed from those reported in our systematic review. In a study present in our systematic review, authors observed that chronic pelvic pain syndrome female patients, for instance, presented urinary levels of VEGFR1 in women were similar to the levels of healthy subjects and that, when comparing the levels between VEGFR1 patients, higher levels of VEGFR1 were associated with more pronounced pain and urinary symptoms (219). On the other hand, in our cross-sectional study, urinary levels of soluble VEGFR1 were significantly lower in patients compared to healthy subjects, which can favor the decoy theory. The variability observed in the results among studies may reflect the challenges inherent in accurately distinguishing between the membrane-bound and soluble forms of VEGFR1, and consequently, in measuring them with precision.

Furthermore, in our study, the variation of the soluble VEGFR1 levels did not correlate with the clinical severity scores between patients, which is a

downside when thinking of soluble VEGFR1 as a disease biomarker during follow-up.

All in all, the VEGF pathway, considering its soluble VEGFR1, can be a promising biomarker. Although VEGFR1 urinary levels do not correlate to the clinical characteristics of this patient cohort, the statistical difference observed between patients and healthy controls may indicate a role of soluble VEGFR1 in the BPS/IC pathophysiology. In the future, it might be helpful to investigate if low levels of VEGFR1 identify a specific BPS/IC phenotype, a study that will require a much larger number of patients.

Conclusions

Our work was focused on the assessment of new treatment options for BPS and the real-life data of OnabotA in the long-term; the evaluation of the somatosensory system of BPS/IC patients with QST as a possible cluster definer; and the potential role of the urinary VEGF molecule and its receptors in BPS/IC condition:

- Silodosin was an effective and safe treatment in refractory BPS/IC patients, improving pain and urinary symptoms. These results emphasize the role of the adrenergic system in this condition and substantiate a future placebo RCT.
- Tadalafil seems safe and effective in refractory female patients. However, the clinical profile of patients who might benefit the most from tadalafil was not disclosed. This trial justifies a future RCT with better patient phenotyping.
- Repeated intratrigonal injection of 100 U of OnabotA shows a safe and persistent effect through multiple injections with a duration of effect longer than one year.
- Using QST to evaluate the somatosensorial system in BPS/IC patients can be a promising tool for phenotyping. Most BPS/IC patients have characteristic somatosensory changes compatible with “gain of function”. However, some patients presented other profiles, indicating the presence of different clusters in BPS/IC patients. Further studies should also evaluate its relationship with biomarkers and clinical clusters.

- The VEGF pathway does not seem useful as a biomarker, but the role of soluble VEGF receptor 1 as a potential future biomarker should be clarified.

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