BLADDER PAIN SYNDROME/ INTERSTITIAL CYSTITIS

Studies on classic BPS/IC, ESSIC type 3C,

with special reference to the role of nitric oxide

Ýr Logadóttir

Institute of Clinical Sciences at Sahlgrenska Academy

University of Gothenburg

Sweden

2012

Sólarljóð

81 kvæði þetta,
   er þér kennt hefig,
   skaltu fyr kvikum kveða,
   Sólarljóð,
   er sýnast munu
   minnst at mörgu login.

82 Hér við skiljumst
   og hittast munum
   á feginsdegi fira;
   drottinn minn
   gefi dauðum ró,
   en hinum líkn, er lifa.

   Úr útgáfu Ólafs Briem.

TABLE OF CONTENTS

LIST OF PAPERS

This thesis is based on the works contained in the following papers, which are referred to in the text by their Roman numerals:

I.   Yr Logadottir, Magnus Fall, Christina Kåbjörn-Gustafsson, Ralph Peeker. **Clinical characteristics differ considerably between phenotypes of bladder pain syndrome/interstitial cystitis.** *Scand J Urol Nephrol, 2012;46: 365-70.*

II.   Yr Logadottir, Ingrid Ehrén, Magnus Fall, N. Peter Wiklund, Ralph Peeker. **Intravesical nitric oxide production discriminates between classic and nonulcer interstitial cystitis.** *J Urol, 2004;171; 1148-1151.*

III.   Yr Logadottir, Lena Hallsberg, Magnus Fall, Ralph Peeker, Dick Delbro. **Bladder pain syndrome/interstitial cystitis ESSIC type 3C: High expression of inducible nitric oxide synthase in inflammatory cells.** *Scand J Urol Nephrol, 2012; early online.*

IV.   Yr Logadottir, Catharina Lindholm, Pernilla Jirholt, Inger Gjertsson, Magnus Fall, Dick Delbro, Ralph Peeker. **Cytokine responses in BPS/IC Type 3C.** *Manuscript.*

ABSTRACT

Patients presenting with symptoms of Bladder Pain Syndrome (BPS) are challenging to the urologist. Previously known as Interstitial Cystitis (IC), this syndrome has been extensively debated and investigated. IC mainly affects women, only 1 or 2 out of 10 patients are males. There is a need of consensus on classification since there are quite diverging opinions. BPS/IC is divided in two main subgroups, classic ulcerative and non-ulcerative forms, which have different histopathological, immunological and neurobiological features and respond differently to a variety of treatments. The symptoms are similar, with chronic pain related to bladder filling and urinary frequency. The International Society for the Study of BPS (ESSIC) has proposed diagnostic criteria, classification and nomenclature based on how the diagnosis was established. Classic IC is now referred to as BPS Type 3C, which indicates that the patient has a Hunner lesion, is diagnosed with cystoscopy, bladder hydrodistention under general anaesthesia, and histopathological examination of bladder tissue sample. For simplicity, the remaining group will here be referred to as non-Hunner BPS patients.

The aims of this thesis were; to further describe a patient population with the diagnosis of BPS/IC, to investigate the nitric oxide (NO) production in the BPS/IC urinary bladder, to analyse the source of NO production, to localise the presence of the iso-enzyme, inducible Nitric Oxide Synthase (iNOS), and to survey inflammatory mediators in the bladder tissue of BPS ESSIC Type 3C/classic IC patients compared to healthy controls.

The hallmark of BPS Type 3C compared to non-Hunner BPS patients is the ulceration in the mucosa and the inflammation in the bladder wall, including increased mast cell count. This is not found in the non-Hunner group of patients. The Hunner BPS Type 3C patients are older by 10 to 20 years at diagnosis and have markedly smaller bladder capacity when measured under general anaesthesia. The end stage is a fibrotic small bladder. This does not happen in non-Hunner BPS.

We have shown that the BPS Type 3C bladder produces large quantities of NO, which is not the case in non-Hunner BPS patients or healthy controls. The iso-enzyme iNOS, believed to be the catalyst in NO production, is found in large amounts within the inflammatory infiltrate of the BPS Type 3C bladder, as well as in the urothelial cells, in both BPS groups and healthy controls. The spectrum of inflammatory markers in the bladder wall of BPS Type 3C patients indicates that the inflammation is similar to what is seen in certain diseases believed to be of autoimmune origin. The paper on cytokine responses in BPS/IC Type 3C, with increase in mRNA expression of interleukin-17, opens up novel research avenues with expectations for new pharmacological targets for the treatment of this condition.

Key words: Bladder Pain Syndrome (BPS), Interstitial Cystitis (IC), Nitric Oxide (NO), Nitric Oxide Synthase (NOS), Inflammatory mediators, Interleukin-17, Mast cells.

ABBREVIATIONS

BCG	Bacillus Calmette-Guèrin
BPS	Bladder Pain Syndrome
BTX-A	Botulinum toxin A
CD	Cluster designation
DMSO	Dimethyl Sulfoxide
EDRF	Endothelium-derived relaxing factor
ESSIC	European Society for the Study of Interstitial Cystitis, now the International Society for the Study of BPS
GAG-layer	Glycosaminoglycan layer
HBO	Hyperbaric oxygen
IC	Interstitial Cystitis
ICS	International Continence Society
IFN-γ	Interferon gamma
IL-	Interleukin-(number)
KCl	Potassium chloride
MMS	Mucosal Mast Cell
MS	Mast Cell
NGF	Nerve Growth factor
NIDDK	National Institute of Diabetes, Digestive and Kidney Diseases
NIH	National Institute of Health
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
eNOS/NOS3	endothelial-Nitric Oxide Synthase
iNOS/NOS2	inducible-Nitric Oxide Synthase
nNOS/NOS1	neuronal-Nitric Oxide Synthase
PBS	Painful Bladder Syndrome
PPS	Pentosan polysulphate sodium
TGF-β	Transforming Growth factor beta
TNF-α	Tumor Necrosis factor alpha
UTI	Urinary tract infection

INTRODUCTION

BLADDER PAIN SYNDROME/INTERSTITIAL CYSTITIS

HISTORICAL PERSPECTIVES

Interstitial cystitis (IC) was first described in 1887 by A.J.C. Skene and the aetiology and pathophysiology is still a mystery [1]. Guy L. Hunner, some 30 years later, described the ulcerations of the urothelium in IC patients, called "submucous ulcer" or Hunner´s ulcer [2, 3]. In 1949, a large series of patients with IC was presented by John Hand [4]. He described different endoscopic and histopathological findings and discussed various treatment options, many of which are still used [4]. At this time, IC was described as a true inflammatory disorder.

As time passed, patients lacking the typical bladder inflammation but presenting with similar symptoms were included in the IC diagnosis. In 1987, the National Institute of Health - National Institute of Diabetes, Digestive and Kidney Diseases in USA (NIH-NIDDK), presented a consensus statement on inclusion as well as exclusion criteria for the diagnosis of IC, to ensure that comparable patient populations were used for research [5]. These criteria included some findings, such as glomerulations submucousally after bladder distention and Hunner's lesions, as well as a long list of exclusion criteria. The NIDDK criteria were revised in 1990. The inadequate clinical definition of IC resulted in the use of the NIDDK criteria also in clinical settings [6]. Using urgency in the NIDDK inclusion criteria led to some confusion and the possible inclusion of patients without pain. It is, however, clear that the reason for urinary frequency in BPS is pain emerging when the bladder is filling and not urge to avoid leakage of urine [7, 8]. The definitions were expanded by the International Continence Society (ICS). In 2002, the ICS defined the term Painful Bladder Syndrome (PBS) as "the complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased day- and night-time frequency, in the absence of proved urinary infection or other obvious pathology" [7, 9]. Patients with urgency were assigned overactive bladder syndrome. The diagnosis of IC was reserved to patients with typical cystoscopic and

histological features, without other features, while the term PBS included all cases of pain in the bladder region [7].

Several European physicians interested in IC met in Copenhagen in May 2003 in an attempt to reach a consensus on how to perform the evaluation of patients with suspected IC. This group formed the European Society for the Study of IC/PBS (ESSIC). A year later, ESSIC published recommendations regarding the diagnosis and standard investigations for Bladder pain syndrome/Interstitial cystitis (BPS/IC) [10]. The society formed a consensus regarding standardisation of investigational procedures, which focuses on positive findings and excludes confusable diseases. The umbrella term was changed from Painful Bladder Syndrome to Bladder Pain Syndrome to fit the taxonomy of pain syndromes [11-13]. In a transition period, the name Bladder pain syndrome/Interstitial cystitis (BPS/IC) can be used in parallel with BPS. Last year, 2011, this society changed its name to the International Society for the Study of BPS, but the abbreviation, ESSIC, has not changed.

Thus, the concepts have changed dramatically and this is a continuous, evolving process. One entity has not changed, although the understanding of its signification and frequency has varied quite markedly, namely the classic Hunner type of disease. This thesis will mainly deal with this entity, now referred to as BPS ESSIC type 3C (classic IC) and its association to nitric oxide.

THE URINARY BLADDER

The urinary bladder collects urine produced by the kidneys and empties regularly when filled. Embryologically it is derived from the urogenital sinus. The motor innervation includes sympathetic nerves and parasympathetic nerves. The sensory input from the bladder is transmitted to the central nervous system via general visceral afferent fibres that follow the course of the sympathetic efferent nerves, except from the inferior part of the bladder, where they follow the course of the parasympathetic efferent nerves [14].
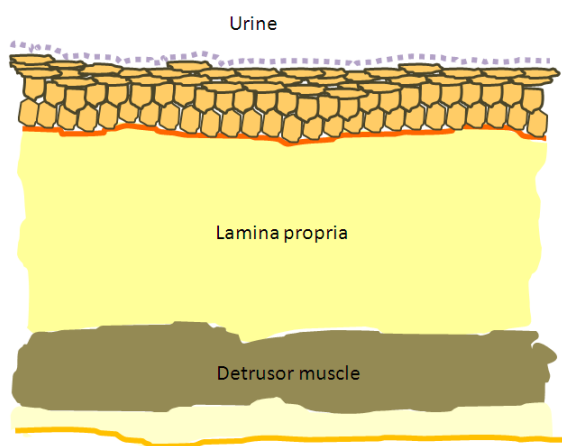
Cross section of the bladder wall.

The mucousal layer consists of the urothelium covered by the glycosaminoglycan layer and the suburothelium/lamina propria.
Between is the basement membrane.
The muscle layer consists of the detrusor muscle.
The serosal layer covers the bladder. The peritoneum covers the abdominal part of the bladder.

Figure 1. Cross section of the bladder wall

The bladder wall is composed of an inner mucousal layer, the detrusor muscle and the outer serosal layer (Figure 1). The mucosa is composed of the urothelium and the sub-urothelium or lamina propria, divided by the basement membrane which can be seen in the microscope. The urothelium consists of a multilayered structure of epithelial cells, transitional epithelium, with tight junctions between the cells. The deepest cells are cuboidal in their form and the most superficial apical cells are flattened like an umbrella. This composition is beneficial with regard to the storage function of the bladder [15, 16]. The urothelium is not just a barrier between the contents of the lower urinary tract and the underlying tissues but also has a sensory function by transducing information to the afferent nervous system in the sub-urothelium and underlying muscle [17, 18]. The barrier function of the urothelium is maintained by a glycosaminoglycan layer on the surface (GAG-layer), membrane lipids, tight junction proteins and uroplakins [19].

Both the urothelium and especially the sub-urothelium have various and multiple sensory receptors [20, 21]. Bladder afferent and efferent nerve endings/receptors are located in close proximity to the urothelium. Disturbances of the urothelial barrier function can influence these receptors and alter the signalling from the bladder mucosa [22].

DIAGNOSTIC CRITERIA

In the early days of Skene and Hunner, the definition of IC was a true inflammatory disorder. As the criteria for IC widened and included fairly normal-appearing bladders with the clinical symptoms of pain related to bladder filling, it was clear that the term Interstitial Cystitis is a heterogeneous syndrome, with two subtypes, the "classic" ulcerous form and the so-called "early" or non-ulcerous form [23-26]. There are no reports, however, that the non-ulcerous form progresses to classic IC [27]. In fact, the non-ulcer and classic forms of IC are characterised by different histopathological, immunological and neurobiological features as well as by different responses to a variety of treatment modalities. The clinical symptoms are however similar, with chronic pain related to bladder filling and urinary frequency [24, 28].

Biopsy	Cystoscopy with hydrodistention			
	Not done	Normal	Glomerulations[1]	Hunner´s lesion[2]
Not done	XX	1X	2X	3X
Normal	XA	1A	2A	3A
Inconclusive	XB	1B	2B	3B
Positive[3]	XC	1C	2C	3C

[1]cystoscopy; glomerulations grade 2-3
[2]with or without glomerulations
[3]histology showing inflammatory infiltrates and/or detrusor mastocytosis and/or intra-fascicular fibrosis
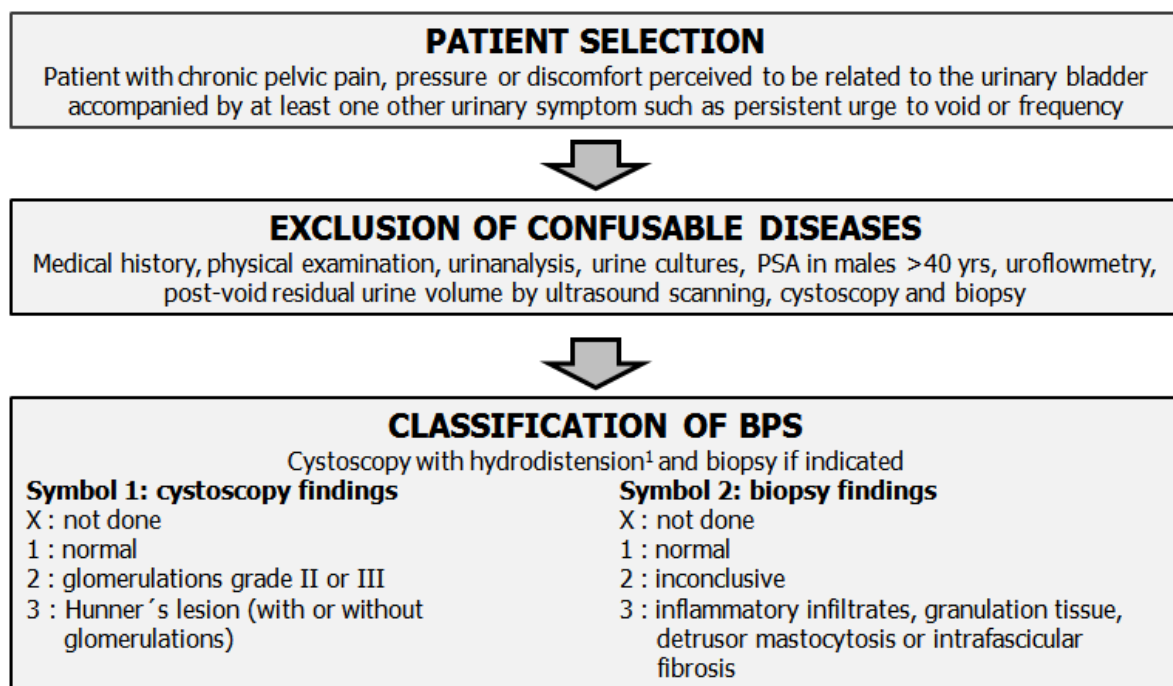
Van de Merwe et al. Eur Urol 2008;53:60-67.

Figure 2.

Diagnostic criteria, classification, and nomenclature for bladder pain syndrome/interstitial cystitis; an ESSIC proposal.

According to ESSIC, the diagnosis of bladder pain syndrome (BPS/IC) should be made on the basis of the symptom of chronic pain related to the urinary bladder, accompanied by at least one other urinary symptom such as day- and night-time frequency, exclusion of confusable diseases as the cause of the symptoms and cystoscopy with hydrodistention and biopsy, if indicated (Figure 2) [11, 29]. Using this system for classification allows for comparison of subgroups in clinical materials according to which diagnostic procedures were used and their outcome.

The cystoscopic requirement for diagnosis of BPS ESSIC type 3C is the finding of at least one Hunner´s lesion, typically presented as a reddened mucousal area with small vessels radiating towards a central scar, fibrin deposit or coagulum. This site ruptures with increasing bladder distention, with petechial oozing of blood from the lesion and the mucousal margins. A typical, slightly bullous oedema develops around the lesion after distention is relieved. The histopathological requirement is a typical inflammatory infiltrate, interfascicular fibrosis and a characteristic mast cell distribution [4, 15, 24, 30-37]. The non-Hunner BPS/non-ulcer IC has an unspecific appearance when performing cystoscopy. During bladder hydrodistention one can observe development of shallow cracks in the urothelium and multiple superficial petechial bleedings, so-called glomerulations. The histopathological appearance shows a fairly normal bladder mucosa, even though some small sub-urothelial bleedings and tiny cracks in the mucosa may be seen in accordance with the cystoscopic findings [15, 24].

PATIENT SELECTION
Patient with chronic pelvic pain, pressure or discomfort perceived to be related to the urinary bladder accompanied by at least one other urinary symptom such as persistent urge to void or frequency

⬇

EXCLUSION OF CONFUSABLE DISEASES
Medical history, physical examination, urinanalysis, urine cultures, PSA in males >40 yrs, uroflowmetry, post-void residual urine volume by ultrasound scanning, cystoscopy and biopsy

⬇

CLASSIFICATION OF BPS
Cystoscopy with hydrodistension[1] and biopsy if indicated

Symbol 1: cystoscopy findings
X : not done
1 : normal
2 : glomerulations grade II or III
3 : Hunner´s lesion (with or without glomerulations)

Symbol 2: biopsy findings
X : not done
1 : normal
2 : inconclusive
3 : inflammatory infiltrates, granulation tissue, detrusor mastocytosis or intrafascicular fibrosis

[1] in the same session as the cystoscopy above if possible

Van de Merwe et al. Eur Urol 2008;53:60-67.

Figure 3.

Diagnostic criteria, classification, and nomenclature for bladder pain syndrome/interstitial cystitis; an ESSIC proposal.

Hereafter, the term BPS ESSIC type 3C will be used instead of the previous denomination IC classic type and non-Hunner BPS will be used instead of the previous denomination IC non-ulcer type.

A standardised evaluation should be used in patients presenting with symptoms suspected to be BPS/IC, in order to confirm the diagnosis and exclude other diseases or confusable diseases, figure 3 [38]. Following is a short description of the ESSIC recommendations, with slight modification. This has even been the praxis, since the seventies, at the Department of Urology, Sahlgrenska University Hospital (except the modified KCl test and a regular use of the symptom scoring tests under nr 5) [38].

In office evaluation:

1. A thorough medical history should be obtained with emphasis to previous diseases in the pelvic region and previous therapies. Recurrent urinary tract infections (UTI), previous operations or radiation treatment are excluding factors. Allergies and autoimmune diseases should be noted.

2. A physical examination is mandatory. In females, the examination should include vaginal examination with pain mapping of the vulvar and vaginal region, palpation over the bladder, urethra, and inner genitalia as well as the pelvic floor. This is important in order to exclude e.g. endometriosis, vulvodynia and urethritis. In males, it is mandatory to perform a digital rectal examination with pain mapping of the scrotal-anal area, to exclude e.g. prostatitis or anal pathology.

3. No specific laboratory test for diagnosis is readily available. When appropriate, urine should be cultured to exclude urinary tract infection (UTI) or tuberculosis (if sterile pyuria is found). Urine cytology should be taken in risk groups and cultures for Ureaplasma, Chlamydia and other sexually transmitted diseases taken as well, when appropriate.

4. Symptom evaluation is mandatory. Therefore, a voiding diary should be used, first in the initial evaluation of the patients and thereafter when following changes of symptoms and various treatment modalities. The voiding diary should be recorded during 24 hours for 2 to 3 days, with time, volume and bladder sensation as well as marking of night voiding. The O´Leary-Sant Symptom Score can be used to further

clarify the impact of symptoms [39] and complemented with the Quality of Life Score from the International Prostate Symptom Score [40].

5. Pain evaluation is easily recorded using the Visual Analoge Scale (VAS) for pain, e.g. when filling out the voiding diary and when following symptom progress and various treatment modalities.

6. Urodynamics is helpful when excluding detrusor overactivity with urge symptoms mimicking BPS/IC. Bladder outlet obstruction can be a differential diagnosis in males.

7. Modified KCl test: The empty bladder is filled, using a Foley balloon catheter, with 500 ml saline (0.9%) at a rate of 50 ml/min until the maximum volume is reached. The bladder is then drained and the filling volume noted. Repeated instillation, now with 500 ml 0.2 M potassium chloride (KCl) at a rate of 50 ml/min is used to calculate the filling volume difference. A difference in bladder volume > 30% is considered positive. Besides reduction of bladder volume with o.2 M KCl there is a stronger feeling of urgency in BPS/IC patients compared to saline filling [41, 42].

8. Cystoscopy under local anaesthesia should be done if other causes of symptoms are suspected, such as urethritis, bladder abnormalities or bladder malignancy.

In hospital evaluation:

Cystoscopy under general anaesthesia using a rigid cystoscope, with distention of the bladder, and, when appropriate, biopsy from the bladder, is mandatory in patients with suspected BPS/IC. A thorough inspection of the bladder mucosa is essential describing any changes during filling and refilling of the bladder. Mapping of bladder changes by drawings or photographs is helpful, if available. Special attention should be taken to observe if radiating vessels, fibrin deposits, hyperaemia, oedema, cracks, scars or any mucousal changes are present. Bladder distention is then performed using Glycine or corresponding filling fluid to allow for coagulation after biopsies. Infusion pressure of at least 80 cm water above the symphysis pubis is used to fill the bladder. Inspection of the bladder is performed under the distention process and changes in the bladder mucosa are noted until the maximum capacity is reached and held for about 3 minutes. The fluid is inspected for bleeding and volume is measured. The bladder is filled again to 1/3 to 2/3 of maximum capacity and inspected for mucousal changes that are graded (Figure 4).

Classification:	
Grade O	normal mucosa
Grade I	petechiae in at least two quadrants
Grade II	large submucousal bleeding (ecchymosis)
Grade III	diffuse global mucousal bleeding
Grade IV	mucousal disruption, with or without bleeding/oedema

Figure 4.

Classification of mucousal changes following bladder distention.

In cases where lesions are found in the bladder, biopsies, including detrusor muscle, are taken under good visibility without distending the bladder to more than half of its capacity, to avoid bladder perforation and further trauma to the bladder wall. If no mucousal changes, e.g. Hunner´s lesions, are found, a minimum of three biopsies are taken using a large forceps to include the detrusor muscle, two from the side walls and one from the bladder dome. If ulceration is noted, the recommendation is to use electroresection in order to completely resect all mucousal changes including the typical oedema that appears around the Hunner lesion. Biopsies are fixed in neutral buffered 4% formalin to prepare for morphological evaluation.

Morphological evaluation:

The pathology report should include: Description of the mucosa, both the urothelium and the suburothelium/lamina propria, as well as the detrusor muscle. It should reveal whether the epithelium is intact, detached, or if there is any finding of dysplasia. It should also say if there is any inflammation in the subepithelium and if so, give a description with regard to the appearance of inflammatory cells and their location. The report should also comprise information about the possible occurrence of intra- and inter-fascicular fibrosis in the detrusor muscle. Eventually, it should state if there are any findings of mast cells in the bladder wall, including location, and number per mm^2 in the detrusor.

Definition of bladder mastocytosis	
< 20	mast cells/mm2 : no detrusor mastocytosis
20–28	mast cells/mm2 : grey zone
> 28	mast cells/mm2 : detrusor mastocytosis

ESSIC Copenhagen 2003
Nordling et al, Eur Urol 2004;45:662-9

Figure 5.

Definition of bladder mastocytosis.

A mast cell density of more than $20/mm^2$ has been shown to have 88% diagnostic specificity and 95% sensitivity for BPS/IC, according to Kastrup et al [43]. The same authors even showed that the amount of collagen staining material was significantly increased in the intra- and inter-fascicular muscle tissue of the bladder in these patients [43]. The methodology for quantifying mast cells is well described by Larsen et al [44].

EPIDEMIOLOGY

The prevalence of BPS/IC has been reported in several studies and varies dramatically, mainly because of differences in definitions of the syndrome. In questionnaire-based studies, taking USA and Finland as examples, reports of prevalence from 1.5 per 100,000 to 20,000 per 100,000 have been reported [45-47]. This illustrates the remarkable lack of uniformity in the definition of BPS/IC, a problem that has to be resolved. However, numerous reports agree that there is a female predominance with five to ten times more women than men [35, 45, 46, 48, 49].

AETIOLOGY

The aetiology of BPS/IC is still unknown and several hypotheses have been put forward since the work of Skene and Hunner. Without subdividing this syndrome in its different categories, the task of searching for the aetiologies is probably unattainable as the situation may be more complex than previously envisioned [50]. Some of the main theories involve infection, inflammation, urothelial dysfunction, GAG-layer dysfunction (glycosaminoglycan), autoimmune mechanisms and a genetic predisposition.

Mast cells, inflammation and autoimmunity:

Mast cells have been a focus of interest for several decades and are believed to play a central role in the pathogenesis and pathophysiology of BPS/IC. They are multifunctional immune cells and develop from a specific bone marrow progenitor cell and migrate into perivascular tissue spaces [51]. Besides being involved in allergic and late-phase reaction they are also involved in innate immunity and autoimmunity and in disorders such as asthma, rheumatoid arthritis and BPS/IC [52, 53]. Mast cell activation can be triggered by numerous non-immunologic stimuli such as bacteria, chemicals, neuropeptides and acetylcholine [54]. They are found in increased numbers in the detrusor both in Hunner´s BPS as well as in non-Hunner´s BPS, 6- to 8-fold higher compared with controls and 2- to 3-fold higher, respectively [55]. They are also found in increased quantity in both the Lamina propria and the detrusor in BPS type 3C/classic IC compared with non-Hunner BPS/non-ulcer IC [15, 56]. Mast cells are among other sites, found in the connective tissue of mucousal membranes and in non-mucousal sites such as skin. Enerbäck showed that there is strong evidence of the existence of a distinctive mucousal mast cell (MMC) phenotype in man as well as in murine species (rats). These human MMC contain a heparin proteoglycan that is highly susceptible to blocking by formaldehyde and exhibit a lower critical electrolyte concentration of dye-binding than mast cells of other connective tissue sites. The connective tissue mast cells are located in typical connective tissues such as skin [57]. Irani et al identified two distinctive mast cell proteinase phenotypes, MC_T (tryptase) and MC_{TC} (containing both tryptase and chymase) [58, 59]. Others have, however, suggested that these differences rather reflect maturation or functional activity related to tissue site rather than being an indication of fixed phenotypic properties [60].

Mast cells are like multifunctional chemical factories. Their cytoplasm is filled with granules storing molecules of varying nature. Some of these are pre-synthesised, e.g. heparin, histamine, proteases, phospholipases, chemotactic substances and cytokines, others are synthesised de novo, e.g. cytokines (especially IL-6), leukotrienes, prostaglandins, nitric oxide and tumor necrosis factor-α (TNF- α) [61, 62]. The enzymes tryptase and chymase have been implicated in promoting matrix degradation [63], but on the other hand mast cells have been documented in fibrotic processes in vivo, too. Certain mast cell mediators (i.e. chymase, tryptase, TNF- α, TGF-β) have been found to exert fibrogenic effects by causing fibroblast proliferation and/or increased collagen synthesis [64, 65]. Mast cells in the detrusor muscle synthesise the pro-fibrotic cytokine transforming growth factor beta (TGF-β) and can therefore play a role in detrusor fibrosis [66].

Rothrock et al have reported stress-related exacerbation of BPS/IC symptoms in patients compared with controls and the relationship of stress was stronger among patients with more severe disease [67]. Family genetic linkage studies have also shown that panic disorder is associated with BPS/IC, possibly through neurogenic inflammation involving mast cells [68, 69]. Corticotropin-releasing hormone (CRH) regulates the hypothalamic-pituitary-adrenal axis and is expressed peripherally in the spinal cord, dorsal root ganglia, sympathetic ganglia and mast cells [70]. As mast cells also express CRH-receptors, they can be activated by CRH secreted from these non-CNS sites and release cytokines and other proinflammatory mediators [71]. This can explain the exacerbation of stress on many conditions other than BPS/IC, such as atopic dermatitis, psoriasis, eczema and urticaria.

Urothelial dysfunction and glycosaminoglycan (GAG) layer:

Parsons et al emphasised the importance of the glycosaminoglycan surface layer on the bladder epithelium to maintain its impermeability [72]. They demonstrated that patients with IC had epithelial dysfunction, so-called leaky epithelium, when installing a solution of concentrated urea into the bladder and measuring its absorption 45 minutes later. The normal subjects absorbed 4.3% while the IC patients absorbed 25%. Interestingly, the patients with Hunner´s lesion had the highest absorption rate, 34.5%, while the non-ulcer IC patients absorbed 22.8% [72]. Impairment in GAG-layer could render the urothelial cells defenceless to the high potassium concentration in the urine (mean concentration of 63mEq/L). Potassium concentration >8mEq/L can damage cells [73].

Genetic predisposition:

There are several recent reports demonstrating that the gene expression profile of the bladder tissue in patients with ulcerative IC (BPS type 3C) differs from the non-ulcerative form, mainly as expression of genes related to immune and inflammatory responses [74, 75]. Other authors have found the same expression for increased proinflammatory genes in urine sediment in patients with Hunner´s lesion but not in non-Hunner patients [76]. The gene expression in non-Hunner BPS/non-ulcer IC has revealed urothelial abnormalities, including tight junction proteins, neurokinin receptors and acid-sensing channels [77, 78]. In a self-reported symptom study from a nationwide screening for complex diseases in The Swedish Twin Registry, Altman et al found that the genetic influences were only modest for the possibility of developing BPS, whereas the environmental factors were substantial [79]. The prevalence was 1.1% and 2.4% for males and females, respectively [79]. It has to be noted, though, that the subjects were born between 1959 and 1985, i.e. they were 25 to 50 years of age. The median age at diagnosis of classic BPS/IC type 3C is in a higher age interval, which means that this category may have been underrepresented [28].

CLINICAL SYMPTOMS AND PROGRESSION OF THE DISEASE

The cardinal symptom of BPS/IC is pain or discomfort perceived to be related to the bladder and the filling of the bladder, accompanied by at least one other urinary symptom; persistent urge to void or urinary frequency [11, 38]. Classification of BPS/IC types is fundamental when advising the patient in order to recommend treatment and in evaluation of the possibility of progressive tissue/bladder damage in the future [23, 28, 80]. It is worth noting that patients with non-Hunner BPS/non-ulcer IC are 10 to 20 years younger than BPS ESSIC type 3C/classic IC patients when diagnosed and have significantly larger bladder capacity, both concerning functional capacities during day- and night-time as well as during general anaesthesia, thus objective recording has to be included in the evaluation [28].

As the treatment and progression of BPS ESSIC type 3C/classic IC is different from non-Hunner BPS/non-ulcer IC, the two entities will be discussed separately. The diagnostic procedure with bladder hydrodistention and resection of ulcers and inflammation of the bladder mucosa in general anaesthesia often gives excellent clinical response in BPS type 3C/classic IC [81]. In most cases, however, the symptoms recur and even though more than 40% of patients have a lasting effect of this treatment for more than 3 years, it can and should be repeated, when needed [81]. In cases when performing repeated hydrodistention of the bladder with resection of recurrent Hunner´s lesions, it is noted that the maximum volume under anaesthesia diminishes, so do symptoms of pain (unpublished data). In these patients histopathology shows fibrosis, both in the mucousal layer and the detrusor. When these patients progress to small capacity fibrotic bladders, urinary diversion or urinary bladder reconstruction with supratrigonal resection of the bladder and ileocystoplasty usually relieves all symptoms and most of these patients become free of symptoms for their remaining lifetime [80, 82]. The pain in patients with non-Hunner BPS/non-ulcer IC is usually not relieved by surgical intervention [80-82].

In non-Hunner BPS/non-ulcer IC patients, the symptoms are often varying over time, with more intense periods in-between. Macroscopic bladder changes with the development of bladder fibrosis are not noted in this group of patients. Treatment should therefore be directed to relieve pain with various conservative methods [23, 24].

TREATMENT

Selecting treatment for patients with BPS/IC is difficult, and empirical, as the aetiologies are unknown. Some kind of algorithm has to be used. It is essential to have a reliable diagnosis with regard to subtype. The treatment recommendations and reports in the literature are difficult to interpret because this aspect has not been acknowledged [83, 84]. Thus, reliable conclusions of the following account of more popular alternatives are in many ways limited by the lack of subtyping of the studied patient groups.

Per-oral medical treatment:

There are numerous substances that have been tried for patients with BPS/IC. Some of these have been studied in a prospective way. There are some drugs that are believed to have a stabilising effect on the urothelium or the GAG-layer.

Pentosan polysulphate sodium (PPS; ELMIRON®) has been shown, in a multicentre double-blind and placebo-controlled study (150-200 mg twice daily), to improve symptoms of pain, urgency and frequency, but not nocturia, compared with placebo [85]. Another study with higher dosages (300mg compared to 600 and 900 mg) showed improvement in symptom scores, however related to treatment duration rather than dosage of the drug [86]. Fritjofsson et al, the only study considering subtyping, demonstrated that PPS had more favourable effect in classic than in nonulcer IC regarding pain, but the increase in volume and the decrease in frequency was more pronounced in the nonulcer IC patients [87].

Hydroxyzine is a histamine₁-receptor antagonist that can block neuronal activation of mast cells by inhibition of serotonin secretion from thalamic mast cells and neurons. Hydroxyzine hydrochloride (Atarax) is used with a starting dose of 25 mg at bedtime, increasing to 50 mg or even 75 mg, if tolerated. In one preliminary study, more than 90% of patients responded favourably [88], but another prospective, randomised, placebo-controlled, seven-centre study failed to show a statistically significant effect of combinations of PPS and hydroxyzine compared with placebo [89].

Amitriptyline is a tricyclic antidepressant that has been reported to provide symptomatic relief (symptom score, pain and urgency intensity) in one randomised placebo-controlled, double-blind study [90]. Long-term use of 25 to 150 mg (mean dose 55 mg) taken at dinner time showed overall patient satisfaction with excellent or good therapeutic results in 46% of the patients but the dropout rate was 31% due to nonresponse to treatment (all cases) and side effects (86%) [91].

In recent studies, performed by Sairanen et al [92, 93], the use of the immunosuppressant *cyclosporine A* was investigated. In the first study, the patients improved significantly during one year of treatment and the effect was maintained throughout the following five years. If the treatment was discontinued the symptoms recurred within months, but if treatment was restarted the symptoms disappeared again [92]. In the later study, the results favoured cyclosporine A when compared with PPS, response rates 75% and 19%, respectively, however at the cost of more adverse events in the cyclosporine A treated group [93].

Intravesical treatment:

This treatment regimen is based on the application of the active medication directly into the target organ. Thus, there is a need for repeated intermittent catheterisation, preferably performed by the patient. This can be painful in patients with BPS/IC and there is risk of infections. Various medications have been tried and used.

Dimethyl sulfoxide (DMSO) is a colourless chemical solvent and water-soluble liquid with a special garlic odour. It penetrates the skin and other membranes very readily and without damaging them. It has been used as a carrier for other compounds such as topical analgesics and anti-inflammatory drugs. Installation of a 50% DMSO solution has been studied and compared to placebo in BPS/IC patients. One trial demonstrated 53% subjective improvement compared to 18% in placebo-treated patients and 93% versus 35% objective improvement, respectively [94]. Another trial on 28 patients showed that DMSO was well tolerated and side-effects were not more common or pronounced in patients with classic (BPS/IC type 3C) compared to non-ulcer disease (non-Hunner BPS/IC). The treatment effect lasted 16-72 months [95].

Bacillus Calmette-Guérin (BCG) as an intravesical immunomodulatory treatment was studied in a small prospective, double-blind pilot study and demonstrated response rates of 60% versus 27% for IC patients and placebo, respectively [96]. A crossover trial of BCG vs. DMSO did not show any improvement in the BCG-treated patients which was the case with DMSO, both as the first treatment as well as at crossover following BCG failure [97].

Pentosan polysulphate (PPS) has also been studied as an intravesical treatment and found superior to placebo, but the study population was small, 20 active and 10 placebo subjects [98].

Hyaluronic acid and *chondroitin sulphate* are interesting being naturally occurring glycosaminoglycans (GAG). Positive responses using intravesical instillations have been reported to be about 50% in clinical trials, including some long-term benefits [99-101].

Miscellaneous treatments:

One of the early treatment reports, in 100 patients, by Bumpus claims that *hydrodistention* achieved symptom improvement over several months [102]. There are no controlled studies showing any direct benefits in symptom relief in BPS/IC patients and bladder distention is therefore to be regarded mainly as a diagnostic tool.

Botulinum toxin A (BTX-A) is mainly used in treatment of neurogenic/overactive bladder disorders, but has been tried for treatment of BPS/IC, too. There is no conclusive evidence that BTX-A has any benefit in BPS patients. Studies by Smith [103] and Giannantoni [104] reported improvement in BPS patients, at variance with Kuo [105].

Hyperbaric oxygen (HBO) treatment is interesting as it has been used with great benefits in patients suffering from irradiation cystitis [106, 107]. Van Ophoven et al found increase in baseline functional bladder capacity, decreased voiding frequency and pain scale improvement at 12 months follow-up in 4 out of 6 patients treated [108]. In their later study (double blind, sham controlled) with a total of 21 patients, 3 of the 14 treated patients, but none of the controls, responded to treatment, with 12 months lasting effect [109]. The patients in both studies were diagnosed according to the NIDDH criteria, but not subdivided into classic BPS/IC type 3C and non-Hunner BPS/non-ulcer IC.

Surgical treatment:

Conservative surgery with complete transurethral resection or coagulation of Hunner´s lesion relieves symptoms for a varying time period [81, 110]. An alternative method is the transurethral application of the neodymium:yttrium-aluminum-garnet (Nd:YAG) laser [111]. These treatments provide relief of pain and increase bladder capacity, but not forever. Sooner or later the lesions recur since the ablation of lesions is not a cure of the disease. Treatment of recurrence of lesions is to repeat ablation. This treatment is, however, not applicable to non-Hunner BPS patients.

Major surgery should be reserved for patients with contracted fibrotic end stage Hunner´s bladders. Reports have shown that those patients are very good responders especially to subtotal cystectomy and cystoplasty [80, 82, 112]. Other major surgical procedures are the construction of a conduit urinary diversion or, more seldom, construction of a continent pouch, with or without cystectomy. These procedures are the last resort and should only be contemplated with great caution in patients other than end stage BPS/IC type 3C [80, 113].

NITRIC OXIDE AND NITRIC OXIDE SYNTHASE

In 1998, the Nobel Prize in Physiology or Medicine was awarded to Drs Robert Furchgott, Ferid Murad and Louis Ignarro, for their discovery of nitric oxide (NO) as a biological mediator. The vasodilatory effects of NO, first noted, was previously termed "endothelial-derived relaxing factor" (EDRF) [114, 115].

Nitric oxide, a gas at temperatures down to -152°C, is slightly soluble in many solvents and can diffuse relatively easily across biological membranes. NO is a signalling molecule that regulates various physiological and pathophysiological responses in biological systems. In low concentrations, NO participates in many physiological processes and has cytotoxic and cytostatic defensive mechanisms against tumours and pathogens in high concentrations [116].
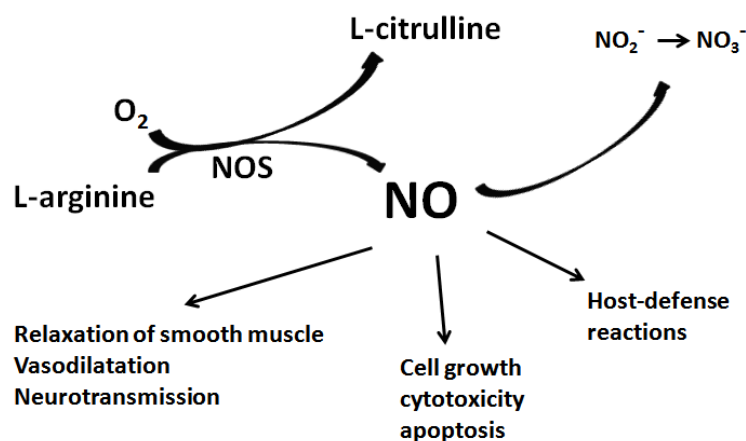
Figure 6.

The formation of NO by the enzymatic action of NOS, converting L-arginine to L-citrulline. NO reacts rapidly with haemoglobin to form nitrate (NO_3^-) and methemoglobin which is the major endpoint of NO metabolism *in vivo* [117]. The major breakdown product of NO in aqueous solutions is nitrite (NO_2^-) by reaction with oxygen (O_2).

Nitric oxide synthase (NOS) generates nitric oxide (by-product L-citrulline) via a catalytic combination of L-arginine and molecular oxygen and reduces nicotinamide-adenine-dinucleotide phosphate (NADPH) as co-substrates (Figure 6). Flavin-adenine-dinucleotide

(FAD), flavin-mononucleoride (FMN), tetrahydrobiopterin (BH_4) and haem are cofactors of all NOS isoenzymes, and the haem center has a sequence similarity to cytochrome P-450 reductase [118, 119]. The L-arginine/NO pathway can be inhibited by several analogues of L-arginine, of which the first to be identified was N^{ω}-monomethyl-L-arginine [116]. This substance had been found to prevent L-arginine-dependent cytotoxicity in murine macrophages [120]. Citrulline that is formed as a by-product of the NOS reaction can be recycled to arginine by successive actions of argininosuccinate synthetase and argininosuccinate lyase, forming the citrulline-NO cycle [121].

Table I.   **Nitric oxide synthases**

Isoform	Expression	Acitvity
nNOS/NOS1	Constitutive	Ca^{2+} dependent
iNOS/NOS2	Inducible	Ca^{2+} independent
eNOS/NOS3	Constitutive	Ca^{2+} dependent

There are three genes coding for the enzyme NOS; neural-NOS (n-NOS/NOS-1), inducible-NOS (i-NOS/NOS-2) and endothelial-NOS (e-NOS/NOS-3), Table I. Two of these enzymes are constitutive, calcium, and calmodulin-dependent and release, within seconds, femtomolar or picomolar concentrations of NO upon e.g. receptor stimulation by selective agonists. Calmodulin is tightly bound to the iNOS enzyme at all times and independent of variations in calcium concentration [118]. The inducible isoforme is regulated at a pretranslational level and its synthesis can be induced by proinflammatory cytokines like TNF-α, IFN-γ or IL-1β [122]. This causes the release of large quantities (nanomolar concentrations) of NO several hours after exposure to the respective stimulus, which process may continue for a sustained manner (hours, days). The NO thus formed by the three isoenzymes can act on a number of target enzymes and other proteins, but the most important physiological signalling pathway is the activation of soluble guanylyl cyclase and the generation of cyclic GMP [123, 124].

Inducible NOS is not usually expressed in cells, but can be induced transcriptionally by various proinflammatory stimuli, e.g. bacterial lipopolysaccharide and cytokines, including IL-1β, TNF-α and IFN-γ [125]. Although primarily identified in macrophages, the expression

of the enzyme can be induced in virtually any cell or tissue [126, 127]. The high level of NO released by iNOS has an effect as immune effector molecule in killing tumour cells, in halting viral replication and in eliminating various pathogens [123]. The high levels of NO produced by activated inflammatory cells may be harmful not just to pathogens, but can also harm healthy or normal cells. The NO radical can interact with superoxide (O_2^{-*}) leading to formation of peroxynitrite ($ONOO^-$) which can induce nitrosative and oxidative stress [128, 129]. The biological half-life of NO is approximately 5 sec and *in vitro* the half-life of NO may be several minutes [117].

To summarise, the effects of NO depend on its concentration. Increased NO production may play an important role in the pathogenesis of various diseases both by direct effects of NO and its breakdown products, or its effect on immune cell functions [130-133].

INFLAMMATORY MEDIATORS IN BPS/IC

According to the early definition of Skene, Hunner and Hand, interstitial cystitis is a true inflammatory disorder [1-4]. Later, the concept was changed but, regarding phenotyping of BPS/IC we are dealing with a heterogeneous syndrome and the BPS ESSIC type 3C or classic IC is the entity characterised by inflammation. Non-Hunner BPS/non-ulcer IC is probably not a "true" inflammatory disorder as the histopathology does not show infiltration of inflammatory cells [24]. Histopathologically, BPS/IC type 3C has several characteristics. However, obtaining biopsies to support the diagnosis has by some been regarded to be unnecessarily traumatic. Research has been done with the hope to identify a marker of BPS/IC, preferably to be found in the urine and avoid invasive procedures. The abundance of candidates tested so far has to be regarded as experimental [134]. The lack of a rational subtyping of the syndrome makes many of these efforts in vain.

Interleukin-6 (IL-6) has been shown to correlate positively with pain scores and is elevated in urine samples from the bladder of BPS/IC patients [135]. Erickson et al analysed the bladder inflammation in association with the clinical features and urinary markers. They showed that patients with severe inflammation responded significantly better to bladder distention, and were older and had smaller bladder capacity than those with mild inflammation. The severity of inflammation was significantly associated with urinary IL-6 levels. The presence of T and B cells was strongly associated with overall severity of inflammation [25]. Some years later the same group presented results from analyses of several urine markers and did not find any robust association between biopsy findings and urine markers. The strongest association was a positive association between urine interleukin-8 (IL-8) levels and bladder mast cell count within the lamina propria [134]. Gene expression analyses of urine sediment did not discriminate non-Hunner patients from controls, but did show that patients with Hunner´s lesion had increased proinflammatory gene expression in the urine sediment similar to previous microarray studies of bladder biopsies [76, 136]. Other authors have attempted to use IL-6 as a diagnostic marker and a predictor of response to therapies, but failed to find a statistical significance, which might be because of lack of subgrouping, or, as the authors suggest, because of subsets of patients with diseases of different aetiologies [137].

Chemokine upregulation in response to external or internal danger signals will recruit leukocytes into the tissue [138]. Tyagi et al investigated several chemokines (C-X-C modified chemokine) in urine from both subgroups of BPS/IC patients and controls and found that levels of 5 of 8 tested chemokines in urine were ten to 100 times higher in BPS/IC patients than in asymptomatic controls. In Hunner BPS/ulcerative IC patients the urinary CXCL-1, CXCL-10, Nerve Growth factor (NGF) and IL-6 was five to twenty times increased compared to non-Hunner BPS/non-ulcer IC patients [139].

IL-10 is a cytokine with anti-inflammatory properties that plays a decisive role for preventing excessive damage to host tissues during an immune response and maintaining normal tissue homeostasis. Dysregulation of IL-10 is associated with enhanced immunopathology in response to infection as well as an increased risk for development of autoimmunity [140]. IL-10 can be produced by a wide range of immune cells including monocytes, T helper 2 cells, dendritic cells, regulatory T cells, and B cells and has been shown to be important for maintenance of tissue and immune homeostasis in mucosal surfaces, e.g. intestines, colon, mouth, nasal passages and lungs where the body is in constant contact with the microbial flora [141].

The IL-17 family cytokines, consisting of IL-17 A to F, are important players in regulating innate epithelial immune responses against microbial and fungal pathogens. In addition, recently described T helper cell subset, Th17, has been shown to be important in the pathogenesis of several chronic autoimmune diseases such as rheumatoid arthritis and multiple sclerosis, and in allergies [142]. The role of IL-17A in BPS/IC is unknown.

As previously pointed out, NO appears to be an important player in the pathogenesis of BPS type 3C/classic IC. Moreover, it is an interesting inflammatory marker and easily measured although it requires a special device [143-146]. NO is produced by the catalytic action of iNOS on L-arginine and molecular oxygen [116]. The iNOS is regulated at a pretranslational level and can be induced by proinflammatory cytokines, TNF-α, IFN-γ and IL-1β [122].

AIMS OF THE THESIS

1. To describe a patient population with the diagnosis of BPS/IC according to age at diagnosis, clinical symptoms and findings, and the difference between the main BPS subtypes, non-Hunner BPS/non-ulcer IC versus BPS ESSIC type3C/classic IC.
   (Paper I)

2. To investigate the nitric oxide production in the urinary bladder in patients with BPS/IC compared to controls.
   (Paper II)

3. To analyse the source of intraluminal bladder nitric oxide production, by using histopathological methods and immunohistochemistry to localise the presence of inducible Nitric Oxide Synthase (iNOS).
   (Paper III)

4. To analyse a number of inflammatory mediators in bladder tissue from patients with BPS ESSIC type 3C/classic IC as compared to healthy controls.
   (Paper IV)

MATERIALS AND METHODS

PATIENTS

Paper I:

The presented series comprises the 379 consecutive patients in our database, diagnosed at Sahlgrenska University Hospital between 1976 and 2011. All patients conformed to the NIH-NIDDK criteria [5] and the ESSIC criteria [11]. All patients were subjected to cystoscopy with hydrodistention, performed as previously described [147, 148] and biopsy retrieval followed by histopathological examination. Based upon these findings the subjects were either referred to the ESSIC Type 3C or a group comprising the other possible phenotypes proposed by ESSIC, for simplicity herein referred to as non-Hunner patients. The requirement for the BPS type 3C diagnosis was the cystoscopic finding of at least one Hunner´s lesion and the histopathological finding of the typical inflammatory infiltrates and characteristic mast cell distribution [4, 15, 30-37]. For example, the database included age at diagnosis and symptom initiation, functional bladder volume in 2x24-hour voiding diaries and bladder capacity under general anaesthesia. Maximum voided volume was defined as the largest reported volume during the 24-hour period. All information was reviewed once again going back to the clinical records.

Paper II:

The patients in paper II were recruited in 2003. All of them had a well established diagnosis prior to the study. Of the 17 patients, 10 had BPS type 3C/classic IC and 7 non-Hunner BPS/non-ulcer IC. In the BPS type 3C group, 3 of the patients were in remission and thus without symptoms. Some patients were studied twice with an interval of 6 to 9 months. Controls were patients with other urological complaints including overactive bladder but excluding infections and malignant diseases. All patients were free from bacteruria (defined as >1.000 bacteria/ml). The sex ratio was similar in all groups. The average patient age was 59 years in the BPS type 3C/classic IC group, 45 years in the non-Hunner BPS/non-ulcer IC group, and 46 years in controls.

Paper III:

Biopsies from the patients in Paper II were retrieved from the archives at the Department of Pathology and used for investigation of the source of NO production in this specific group of patients. Four patients not conforming to the type 3C criteria, i.e. non-Hunner BPS/IC patients, were used for comparison.

Paper IV:

Twelve patients (medium age 63.4 years), previously diagnosed with BPS/IC Type 3C [38, 149], were recruited during the period June 2009 to November 2011. All subjects had symptom recurrence and were scheduled for repeated cystoscopy, hydrodistention and transurethral resection (TUR) under general anaesthesia, being our routine treatment for this entity. Samples for analyses were taken from the bladder mucosa by cold biopsy forceps. Of all specimens we were able to use samples obtained at 7 occasions, in 6 patients. Six controls (medium age 55.2 years) were recruited from healthy women undergoing TVT operation for stress urinary incontinence. All patients and controls were free from infection. The reason for not using samples from all 12 BPS/IC type 3C patients was the following; in the first 2 patients (one of which was the only male patient) the samples were fresh frozen but the preparation for RT-PCR was technically unsuccessful, samples from one patient were obtained by electroresection but the tissue was found to be too damaged for RT-PCR analysis. In one case the amount of tissue was not sufficient and one case (referral case) the diagnosis was found to be incorrect.

METHODS

Cystoscopy, bladder distention and biopsy retrieval:
(Papers I and III)

The bladder was distended at a pressure of 70-80 cm H_2O under general anaesthesia. Specimens were obtained by electroresection with the equipment set at as low intensity as possible. The tissue was fixed either in formaldehyde (4%) or in IFAA (0.6% formaldehyde, 0.5% acetic acid in distilled water (4 h) followed by 70% ethanol (12 h)).

Biopsy retrieval:
(Paper IV)

Ten biopsies were retrieved from the bladder mucosa with a 5 mm biopsy cup forceps, with the intention not to include the detrusor muscle. These biopsies, both from patients and controls, were put in cold RNA-later and frozen at minus 70ºC 1-2 days later. Two additional biopsies from controls were fixated in phosphate buffered formaldehyde (4%) and biopsies from the TUR operation on BPS/IC patients were fixated in formaldehyde (4%). The specimens were embedded in paraffin and then subsequently processed for routine histopathology and immunohistochemistry, respectively.

Routine histopathology and mast cell detection:
(Papers I, III and IV)

Consecutive serial 5µ sections of paraffin-embedded tissue were cut using mirror technique and stained with haematoxylin-eosin and van Gieson and subjected to immune staining. Until 2007, mast cells were metachromatically detected using Toluidine blue staining. Thereafter, mast cell tryptase labelling has been used as previously described [44, 56, 150]. The Dept. of Pathology at Sahlgrenska University Hospital performed the standard staining with HE and van Gieson as well as the mast cell tryptase labelling, according to the protocol as previously described [44, 56, 150]. The number of mast cells in the detrusor, being regarded as a cardinal characteristic of BPS/IC was counted at 100 x magnification using a grid [43, 44].

Measuring NO in the urinary bladder:

(Paper II)

A catheter balloon (100% silicon) was filled with 25 ml air and incubated for 5 min, after having been introduced into the empty urinary bladder. The air was aspirated by a 30 ml Terumo syringe and immediately examined in a chemiluminescence NO ananlyser (NIOX, Aerocrine, Stockholm, Sweden), a method previously described [143]. As reference, the same volume of air from the surroundings was examined at the same time. The NIOX software automatically calculates the peak plateau of NO value and automatically adjusts for recovery rate. The detection level for NO was 1.5 ppb and the NIOX device was calibrated with 10,000 ppb NO in N2 calibration gas (AGA Linde, Stockholm, Sweden). The NIOX software runs a self-test between each patient to check the validity of the calibration and to detect any zero-point drift. The chemiluminescence assay is highly specific for NO and there is no interference from other nitrogen oxides [151].

Immunohistochemistry and methodological considerations:

(Papers III and IV)

Histology is essential for understanding and visualising detail in tissue samples and understanding of disease processes. Immunohistochemistry is based on detecting antigens in tissue section by binding an antibody to a specific antigen, some inherent cell component inside a cell or in the cell membranes. To visualise the antibody-antigen interaction the antibody is often conjugated to an enzyme (peroxidase is often used) that catalyses a reaction producing a change in colour that can be visualised. Antibodies are mainly γ globulins raised by immunising rabbits, mice, pigs or goats with a certain antigen. Monoclonal antibodies have absolute specificity for a single antigen molecule, whereas polyclonal antiserum contains antibodies to different epitopes on the immunogen and with a capacity of a strong detection of the antigen. Strict controls are necessary to increase specificity of the assumed positively noted immunoreactions. First, experiments are repeated with exclusion of the primary antibody. Second, the antibody is applied after it has been absorbed by pre-incubation with its immunogenic peptide, when such is commercially available.

In Paper III, two different antibodies against iNOS were used, both being rabbit polyclonal anti-iNOS, as well as two different antibodies to nitrotyrosine, namely rabbit polyclonal and mouse monoclonal anti-nitrotyrosine, respectively. A mouse monoclonal anti-CD68 was used

as a macrophage-monocyte marker [152]. The reason for using two different antibodies to iNOS as well as anti-nitrotyrosine was to maximise the specificity of the detection of the iNOS antigen. In Paper IV, the avidin-biotin-complex (ABC) method was applied and the primary antibody was a goat polyclonal anti-IL-17A antibody. In Paper III, for negative controls, the horse serum or anti-iNOS antibody was used after pre-absorption with an iNOS peptide. In both papers the DAB solution was used as substrate, producing a brown staining.

In Paper III the Envision method was used. It is quicker, more sensitive and easier than the ABC-method. The ABC-method uses the high affinity of avidin for biotin, avidin has four binding sites for biotin, which amplifies the signal. It is most often used as a three-step indirect method, where the primary antibody binds specifically to the antigen, and then a secondary, biotinylated antibody specific for the primary antibody. The third step consists of adding biotin-avidin complexes binding to the secondary antibody. The Envision method is biotin-free and therefore significantly reducing non-specific staining resulting from endogenous avidin-biotin activity. The secondary polymer can be conjugated with horse radish peroxidise, as was used in Paper III. Both the ABC-method and Envision methods are sensitive and provide good signals.

Real time Polymerase Chain Reaction (RT-PCR):

(Paper IV)

Real-time PCR is a quantitative way to analyse transcript levels in a RNA sample. RNA is converted to cDNA before the amplification with specific primers for the target gene. The reaction mixture also includes a fluorescently labelled probe. The method uses the exponential growth of the amplified specific fragments and the subsequent increase in fluorescence in the reaction to quantify the concentration of transcripts in the sample.

RNA was prepared from the biopsies using RNeasy® mini kit (Qiagen, Solna, Sweden) and converted to cDNA using High Capacity cDNA kit with RNase inhibitor (Applied Biosystems, Foster City, CA, USA). The expression levels of the different genes in each sample were analysed in duplicate in a reaction consisting of 10 ng cDNA in a 20 μl total volume on a Viia7 Real Time PCR System (Applied Biosystems, Foster City, CA, USA). The gene expression levels were analysed using inventoried assays from Applied Biosystems,

(Foster City, CA, USA); TNF-α, IL-6, IL-10, INF-γ, iNOS/NOS2, TGF-1β, IL-4 and IL-17A. All Expression data for the genes analysed were related to the reference gene human beta actin.

Statistical analyses:

(Papers I and II)

Data were analysed on a desktop computer with a statistical software package (StatView 4.5 Abacus Concepts, California). For significance analyses, the Mann-Whitney test for unpaired comparisons was used.

(Papers III and IV)

For significance analyses, a non-parametrical test, the Mann-Whitney U test was applied throughout, using IBM SPSS statistics 20 software. The non-parametrical test was used because of the skewed distribution of the sample.

RESULTS

Paper I:

The majority, 217 of the 379 patients (318 women and 61 men) were diagnosed with BPS/IC ESSIC type 3C (175 women and 42 men), and the remaining 162 patients with BPS non-Hunner disease. The average age was 62 and 42 years respectively at the time of diagnosis, the difference being statistically significant (p<0.001) (Fig 4, Paper I). The average maximum voided volume was 206 ml and 289 ml respectively (p<0.001). The average bladder distention capacity under general anaesthesia was also significantly different between the two groups, 459 ml for BPS type 3C and 743 ml for BPS non-Hunner disease (p<0.001).
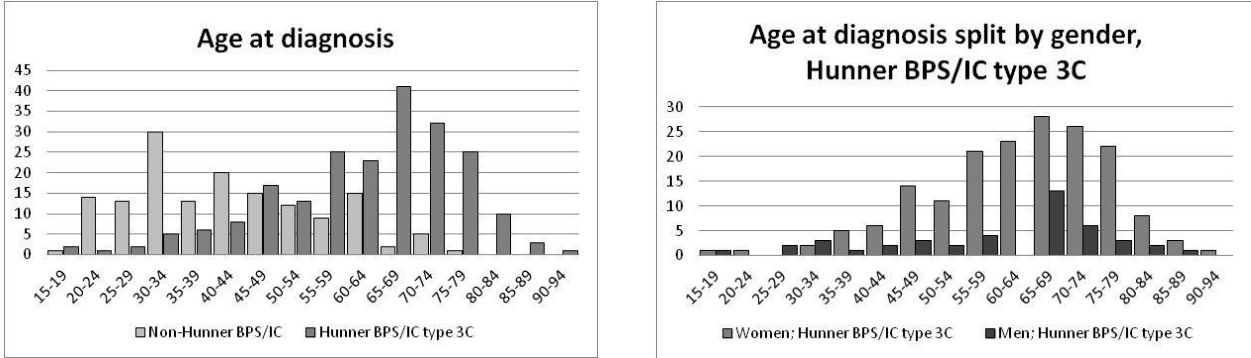
Figure 7.
Age at diagnosis in Hunner BPS/IC type 3C and non-Hunner BPS/IC. The figure on the right shows the same patients split by gender for the Hunner PBS/IC type 3C.
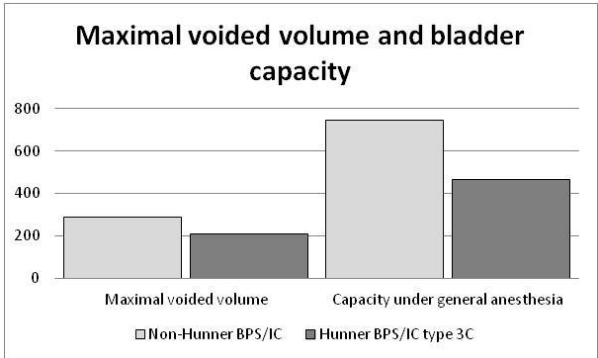
Figure 8.
Maximum voided volume and bladder capacity in non-Hunner BPS/IC versus Hunner BPS/IC patients.

Paper II:

All patients with BPS type 3C/classic IC showed high or very high levels of NO. None of the other patients, BPS non-Hunner type/non-ulcer IC or controls had any significant increase in NO in the bladder. The NO level in patients with BPS type 3C/classic IC was not related to symptoms, but rather to the assignment to this specific subgroup of BPS/IC. However, disease stage seemed to influence NO levels. Thus, in patients free from symptoms the NO levels were increased and the highest levels of NO were found in patients in the initial phase of BPS type 3C/classic IC.
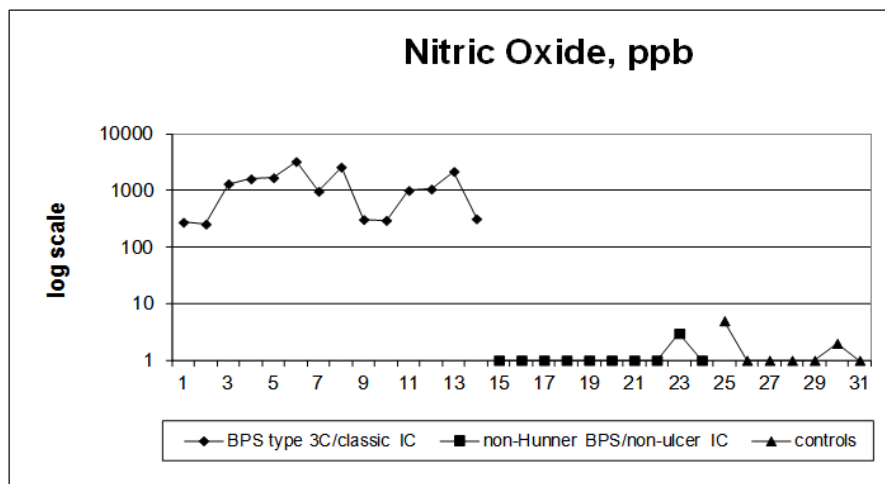
Figure 11.

Nitric Oxide level in parts per billion (ppb) from BPS type 3C/classic IC patients, non-Hunner BPS/non-ulcer IC patients and controls.

Table II.

Subtype, age and sex in 17 patients with BPS/IC and 6 controls.
Results of NO measurements (ppb).

Age	Sex	Diagnosis	First measurement		Second measurement	
			NO	Symptoms	NO	Symptoms
77	F	Classic IC	275	Yes		
73	M	Classic IC	255	Yes	1000	Yes
65	F	Classic IC	1313	Yes		
65	F	Classic IC	1604	Yes		
62	F	Classic IC	1679	No	1058	Yes
61	F	Classic IC	3253	No		
60	F	Classic IC	967	Yes		
54	F	Classic IC	2596	No	2175	No
41	M	Classic IC	307	Yes	316	Yes
35	F	Classic IC	297	Yes		
60	F	Nonulcer IC	1	Yes		
56	M	Nonulcer IC	1	Yes	1	Yes
53	F	Nonulcer IC	1	Yes		
46	F	Nonulcer IC	1	Yes	3	No
44	F	Nonulcer IC	1	Yes	1	Yes
38	F	Nonulcer IC	1	Yes		
20	F	Nonulcer IC	1	Yes		
66	F	Control	5			
56	F	Control	1			
54	F	Control	1			
36	F	Control	1			
35	M	Control	1			
30	F	Control	2		1	

Paper III:

In routine histopathology, the tissues exhibited transmural inflammatory infiltrates of varying intensity with a composition of various cell types, e.g. lymphocyte-like cells, neutrophil and eosinophil granulocytes, mast cells, plasma cells and macrophages. In many cases, the urothelium was detached. In non-Hunner subjects such inflammatory infiltrates were absent and the urothelium was intact.

Immunoreactive cells, positive for CD68, were few and were noted within the inflammatory infiltrates, as characteristically demonstrated in the figure in Paper III. CD68 is a glycoprotein which binds to low density lipoprotein found in cytoplasmic granules and is expressed in macrophages and monocytes [152, 153].

In the BPS type 3C subjects, strong immunoreactivity to inducible nitric oxide synthas, was noted within the urothelium, when present, (figure C, D, Paper III), but in particular within the inflammatory infiltrates, seemingly confined to lymphocytes and macrophages (figure E, Paper III). In non-Hunner subjects iNOS immunoreactivity was noted only in the urothelium, not in the suburothelium/lamina propria and to some lesser degree than in the Hunner subjects. Nitrotyrosine immunoreactivity (iNOS) was demonstrated in the urothelium, which was considerably detached, and in the inflammatory cells, (figure, Paper III).

Paper IV:

The mRNA expression levels of IL-10 (p = .005), IL-6 (p = .010), IL-17A (p = .046) and iNOS (p = .032) were significantly increased in patients with BPS/IC Type 3C compared to the healthy control group. TNF-α, TGF-β, and IFN-γ levels did not differ between patients and controls (Table III). IL-4 was not detectable, neither in the patients, nor in the controls (Figure 13). IL-4 was nighter detectable in patients nor controls.

Mast cells tryptase histochemistry was performed to localise and quantify mast cells in the biopsies. Increased number of mast cells was found in all layers of the bladder wall in patients with BPS/IC Type 3C, with median counts of 52/ mm^2 (range 42 to 95 mast/mm^2) in the detrusor muscle, compared to almost no mast cells seen in healthy controls (Figure 14). Immunohistochemistry was focused on Il-17A. In the controls, there was moderate to strong immunoreactivity (IR) in the urothelium, alone. Conversely, in the BPS/IC Type 3C

specimens, in addition to the urothelium (when present), moderate to strong IR were noted in a fraction of the infiltrate of the lamina propria, (figure 3, Paper IV).
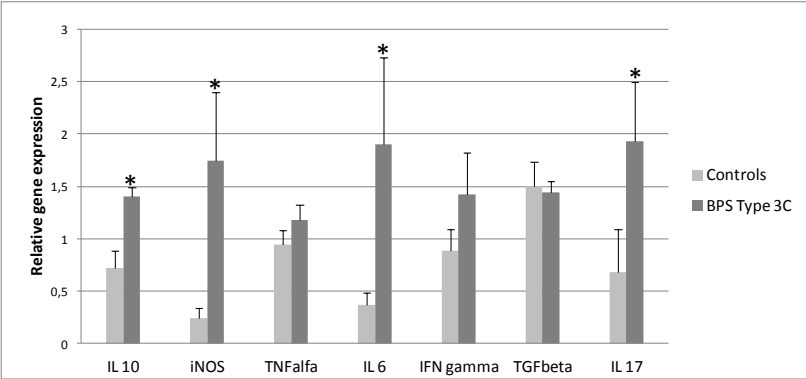
Figure 13.

Gene expression in bladder biopsies from patients with BPS type 3C/classic IC and healthy controls. Gene expression was analysed by real time PCR and the results were related to the reference gene Beta-actin. Results are presented as mean ± SEM. *=p-value less than .05.
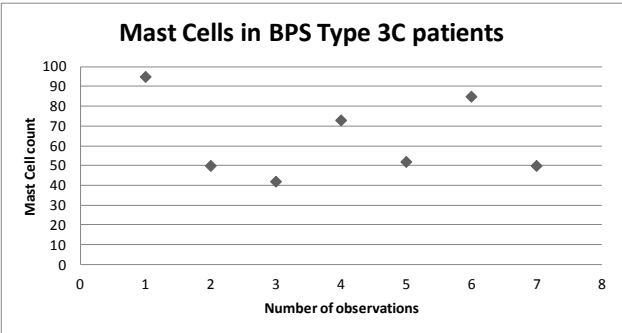
Figure 14.

Mast cell counts in BPS type 3C/classic IC patients.

Table III.

Comparison of the inflammatory markers tested with Real time Polymerase Chain Reaction of biopsies from patients diagnosed with BPS Type 3C and healthy controls.

Inflammatory marker	Controls (n = 6) Mean	Controls SEM	Patients (n = 7) Mean	Patients SEM		ρ value (unpaired Mann-Whitney U test)
IL-4					Undetectable	
IL-6	0.3717	0.11929	1.9043	0.82456		0.010*
IL-10	0.7233	0.16572	1.4057	0.09005		0.005*
IL-17A	0.6783	0.41607	1.9271	0.57245		0.046*
iNOS	0.2417	0.09711	1.7486	0.65283		0.032*
TNF alpha	0.9483	0.13345	1.1829	0.14247		NS
TGF beta	1.5000	0.23341	1.4400	0.10834		NS
INF gamma	0.8850	0.20573	1.4229	0.39634		NS

* Significant difference between controls and BPS patients, $\rho < 0.05$

ETHICAL CONSIDERATIONS

The studies were approved by the Ethics Committee of Sahlgrenska University Hospital, which also serves as the Internal Review Board for clinical studies. The Ethical Committee is now denominated the Regional Ethical Review Board, University of Gothenburg, Sweden (Papers III and IV).

GENERAL DISCUSSION

The understanding of the peculiar inflammatory disease of the urinary bladder has changed since the term Interstitial Cystitis was first introduced by Skene in 1887. About thirty years later it was given the name "elusive ulcer" by Hunner [1, 3]. The cardinal symptom of pain related to filling of the bladder includes many patients without any visible inflammation of the bladder [4]. This fact led to a shift in the meaning of the term Interstitial Cystitis. Subsequently, this name comprised a heterogeneous syndrome [24-26, 28, 154]. Subtyping classic ulcerative IC versus non-ulcerous IC was not used consequently, though. This has led to confusion as research results could not be interpreted or compared; the composition and findings in patients included in different studies were not specified. To include all patients with bladder pain, the "umbrella term" Bladder Pain Syndrome (BPS) was proposed. Classification, diagnostic steps and nomenclature for BPS/IC have been suggested [11, 13]. The term BPS ESSIC type 3C is now equivalent to classic IC and non-Hunner BPS to non-ulcerous IC.

Symptoms and clinical findings:

The key to the diagnosis is the early recognition of symptoms to avoid delay in initiating treatment [155]. There are several studies indicating clinical differences between BPS type 3C and non-Hunner BPS, including a number of studies of our own. Age at diagnosis differs, where the non-Hunner patients are younger by 10 to 20 years. Maximum voided volume and bladder capacity measured under general anaesthesia is significantly greater in non-Hunner BPS. Response to various treatment regimes differs, while the female predominance is comparable between the BPS subtypes [4, 23, 25, 28, 34, 48, 49, 156, 157]. Histopathology also demonstrates the heterogenic features. BPS type 3C/classic IC has distinct features and should be dealt with as a specific entity [15, 25, 28, 48, 147, 154, 156]. To further analyse BPS it is imperative to create a number of databases including information on patient characteristics, voiding diaries, and the result of diagnostic procedures and treatments. Then, comparing results between different research groups would give prerequisites for a deeper analysis of phenotypes.

Biological markers:

The search for a biological marker for the diagnosis of BPS/IC has not been successful, partly because it requires detailed knowledge of the disease subtypes [134], since it is unlikely that a single biomarker could cover the entire spectrum. Many biomarkers, both in urine and blood, have been investigated so far without any general useful one being identified. In our study on NO vaporisation, we found a striking difference between NO levels in the bladder of patients with BPS ESSIC type 3C/classic IC compared to those with non-Hunner BPS/non-ulcer IC and controls [158]. This simple method is feasible for discrimination between the two main BPS subtypes but does not serve as a marker for the diagnosis [143, 158]. The therapeutic implications are conflicting, though. Smith and co-workers reported a decrease in IC-related symptoms with oral administration of L-arginine [159]. This result was not confirmed by Ehrén and co-workers [160], a finding that tallies with reports from other authors [161]. This is explainable, as L-arginine is a substrate for nitric oxide production.

Nitric oxide and inflammation:

Our results show that NO most likely originates from the inflammatory infiltrate in the bladder mucosa as well as from the urothelium, which is in agreement with other authors [162, 163]. The mechanisms of action behind the induction of NO production and the promotion of underlying immunoregulatoring events remain obscure. Nevertheless, our results clearly indicate that the inflammation and NO production go hand in hand (Paper II). The enzyme iNOS is the most likely NOS isoenzyme to produce such high concentration of NO and that might explain the destruction of the urothelium seen in BPS type 3C cases, as NO has been shown to have cytostatic and cytotoxic tissue damaging properties [116, 164]. The iNOS is found in the urothelium; both in patients and controls but seems to be inactive in those without elevated NO production (Paper III) [145, 146, 162]. The rich infiltration of inflammatory cells in the bladder mucosa is probably the main origin of the extreme NO production [162]. This is in fact in line with some results from other authors who have found a connection between elevated NO and certain diseases, some of which are regarded as autoimmune diseases, e.g. asthma, rheumatoid arthritis, systemic lupus erythematosus and ulcerative colitis [130, 132, 165].

Inflammation and inflammatory markers:

What initiates and maintains the inflammation seen in BPS type 3C/classic IC is still a mystery. We found a significantly higher mRNA expression of the proinflammatory cytokines IL-6 and IL-17 and of the anti-inflammatory IL-10, but not TNF-α and IFN-γ, in the BPS/IC Type 3C bladder biopsies compared to healthy controls. There was also increased mRNA expression of iNOS in concordance with previous work [158, 163]. It has been shown, that IL-17A can induce iNOS expression in chondrocytes and murine splenocytes [166], but it remains to be determined if IL-17A also induces iNOS in human epithelial or urothelial cells. The inflammatory response in BPS/IC has been suggested to be of a Th1 type [74]. Our findings suggest that the inflammation in BPS/IC in fact might be of a Th17 type as has been shown in other chronic inflammatory diseases, e.g. rheumatoid arthritis, initially held to be of a Th1 type. The increased expression of IL-10 is likely to reflect the host attempt to control excessive tissue damage of the chronic inflammation and it seems that the IL-10 response is inadequate to control the tissue inflammation.

Future studies are needed to reveal the cellular source of IL-17A as well as IL-10, and IL-6 in the urinary bladder tissue in BPS/IC type 3C.

Mast cells:

The role of mast cells has been debated. Irani et al suggested the denomination of two distinctive proteinase phenotypes, one containing only tryptase and the other containing both tryptase and chymase [58]. However, other authors have suggested that variable proteinase composition may reflect the stage of maturation and/or functional activity [60] rather than being an indicator of fixed phenotypic properties related to tissue site [59].

The explanation behind the activation of the immune system in BPS type 3C/classic IC is probably complex. In recent years, the role of dendritic cells (DCs) as key players in modifying immune responses has interested researchers. Dendritic cells not only activate lymphocytes, but also tolerise T-cells to antigens that are innate to the body (self-antigens), thereby minimising autoimmune reactions [167]. Recently, Engel et al concluded that some of the abundant DCs recruited in urinary tract infection contributed to innate immune effector functions [168]. What triggers the proposed aberrant immune response in BPS type3C/classic IC is not known. It is also unknown whether DCs subsets play a role in this context as they might have lost their tolerogenic potential or have been imprinted with an erroneous function by the local microenvironment, a suggestion recently put forward as an explanation behind

the inflammatory response in Crohn´s disease [169], a condition previously shown to have certain histopathological similarities with BPS type 3C/classic IC [15].

Summary:

At this time, there are no simple biomarkers for the diagnosis of BPS available, but this field is an important research area for the future. NO can be used to subdivide BSP type 3C/classic IC from the non-Hunner´s BPS/non-ulcer IC. On the other hand, biomarkers might be more available when analysing biopsies from the bladder, an important argument for obtaining biopsy material. This requires invasive methods when harvesting samples from the bladder, which is in line with the ESSIC recommendations, to include cystoscopy with hydrodistention and bladder biopsies for diagnosis and subtyping of patients suspected of having BPS [11, 38]. This practice is also, since the seventies, consistent with the routines at the Department of Urology, Sahlgrenska University Hospital.

CONCLUSIONS AND FUTURE PERSPECTIVES

Collecting and entering clinical and research data continuously over a long time in databases for each patient diagnosed with BPS is important for analysing the syndrome of Bladder Pain. Then, comparing results between different research groups would give prerequisites for a deeper analysis of phenotypes.

We have shown that nitric oxide has an important role in BPS ESSIC type 3C/classic IC. The specific inflammatory cascade that results in this explicit stimulation of iNOS, leading to this extreme production of NO, remains to be further analysed.

We have shown that the enzyme iNOS is present in the healthy urothelium, but it seems not to be active without stimulation (e.g. infectious agents, BCG). The most likely origin of the extreme NO production in BPS ESSIC type 3C/classic IC, is the inflammatory infiltrate in the bladder wall, with its highest concentration adjacent to the basement membrane of the mucosa. Whether NO is a direct causative agent for the tissue degradation and destruction in BPS type3C/classic IC, or acts indirectly through the immune system, or if it is an innocent bystander, remains to be determined.

We have found increase in mRNA expression of proinflammatory cytokines (IL-6 and 17) as well as of the anti-inflammatory IL-10, but not TNF-α or IFN-γ, which might indicate a Th17 type of inflammation, comparable to some diseases believed to be of autoimmune genesis. This might open up novel research avenues with expectations for new pharmacological targets for the treatment of this so bothering condition, BPS ESSIC Type 3C.

ACKNOWLEDGEMENTS

Elisabeth Ståhlgren, for being a friend and for excellent tutoring through the English language. For travelling by car with me, through the night, without fear, in the steep coastal mountains of south Italy, on our tour to Mauro´s wedding.

Catharina Lindholm and Inger Gjertsson for opening the mystical doors to immunology and giving me insight into a world that is fascinating and mysterious, and for being co-authors of Paper IV.

Inga, Yrsa, Cattis and Auður, my true heroines. PA with his tasty kitchen gourmet solutions. You have made my life better. Statistics, graphs, lab-methods and other mysterious things are so easy for you all.

My parents, Logi and Oddný, who emphasised the value of education and ethics in order to be an independent and self-governing individual able to choose one´s own way of life.
My sisters, Hrund and Sigga; without you, life would have been lonely, meaningless and uneventful. As our father said, sisters should keep together, support and take care of each other.

SUMMARY IN SWEDISH - SAMMANFATTNING PÅ SVENSKA

Patienter som är drabbade av syndromet smärtsam blåsa, på engelska Bladder Pain Syndrome (BPS), utgör en utmaning inom urologin. Tillståndet drabbar huvudsakligen kvinnor, bara 1 eller 2 av 10 patienter är av manligt kön. Behovet av en allmänt accepterad klassifikation är uppenbart, eftersom detta är ett heterogent syndrom. BPS indelas huvudsakligen i två undergrupper, klassisk sårbildande typ och icke-sårbildande typ, vilka karaktäriseras av olika histopatologiska, immunologiska och neurobiologiska kännetecken liksom av olika svar på olika behandlingsmetoder. De kliniska symptomen är likartade, med kronisk smärta relaterad till blåsfyllnad samt mycket tätt återkommande trängningar till vattenkastning. The International Society for the Study of BPS (ESSIC) har föreslagit diagnostiska kriterier, klassifikation och nomenklatur för BPS, vilka är grundade på en strikt indelning i enlighet med hur diagnosen fastställes. Den tidigare klassiska sårbildande typen kallas nu BPS Type 3C, vilket innebär att patienten har ett så kallat Hunner´s sår i blåsan, diagnostiseras med cystoskopi, utspänning (i narkos) av blåsan och histopatologisk undersökning av ett vävnadsprov från blåsan. För enkelhetens skull kommer de återstående patienter i denna avhandling att refereras till som non-Hunner BPS patienter.

Syftet med denna avhandling var följande; att noggrannare beskriva patientgruppen med diagnosen BPS, att undersöka produktionen av kväveoxid, på engelska nitric oxide (NO), i urinblåsa med BPS, att analysera källan till NO-produktionen, att lokalisera närvaron av iso-enzymet, inducible Nitric Oxide Synthase (iNOS), och att analysera en panel av inflammatoriska mediatorer i blåsvävnad från patienter med BPS ESSIC Type 3C.

Det som skiljer patienter med BPS Type 3C från patienter med non-Hunner BPS är sårbildningen i slemhinnan och inflammationen i blåsväggen. Detta hittar man inte hos patienter med non-Hunner sjukdom. Vi visar nu att patienter med Hunner BPS Type 3C är 10 till 20 år äldre vid diagnos och har betydligt mindre blåskapacitet vid mätning i narkos. Inflammationen i blåsan kan i värsta fall utvecklas till en fibrotisk liten skrumpblåsa. Detta är inte fallet hos non-Hunner BPS.

Vi har också visat att blåsor av typ BPS Type 3C producerar en enorm mängd NO, vilket inte är fallet hos patienter med non-Hunner BPS eller hos friska kontroller. Iso-enzymet iNOS, som tros vara det begränsande enzymet i NO produktionen, finns i höga kvantiteter i inflammatoriska infiltrat från BPS Type 3C blåsor, liksom även i celler från urotelet. Urotelet visar även uttryck av iNOS hos non-Hunner patienter och friska kontroller. Vidare visade vi att mRNA-värdena för pre-inflammatoriska interleukiner, IL-6 och IL-17, samt iNOS och anti-inflammations IL-10, var förhöjda hos BPS Type 3C. Proteinuttrycket av IL-17 var uppreglerat och lokaliserat till inflammatoriska celler samt fanns även i urotelet.

Våra resultat bekräftar de slående skillnaderna mellan huvudformerna av BPS och understryker nödvändigheten av adekvat klassificering i kliniska studier. Panoramat av inflammatoriska markörer i blåsväggen hos patienter med BPS Type 3C visar på en likhet med den typ av inflammation som ses i vissa sjukdomar som man tror är av autoimmunt ursprung. Fynden av ökat uttryck av iNOS, liksom en uppreglering av IL-17, öppnar vägen för ny forskning med förväntningar på att finna nya farmakologiska angreppssätt för behandling av detta så plågsamma tillstånd.

REFERENCES

1.	Skene, A.J.C., *Diseases of Bladder and Urethra in Women. .* New York: Wm Wood, 1887. **167**.
2.	Hunner, G.L., *A rare type of bladder ulcer in women; report of cases.* Boston Med Surg J, 1915. **172**: p. 660-664.
3.	Hunner, G.L., *A rare type of bladder ulcer in women; further notes, with a report of eighteen cases.* J.A.M.A., 1918. **70**: p. 203-212.
4.	Hand, J.R., *Interstitial cystitis; report of 223 cases (204 women and 19 men).* J Urol, 1949. **61**(2): p. 291-310.
5.	Gillenwater, J.Y. and A.J. Wein, *Summary of the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases Workshop on Interstitial Cystitis, National Institutes of Health, Bethesda, Maryland, August 28-29, 1987.* J Urol, 1988. **140**(1): p. 203-6.
6.	Hanno, P.M., et al., *The diagnosis of interstitial cystitis revisited: lessons learned from the National Institutes of Health Interstitial Cystitis Database study.* J Urol, 1999. **161**(2): p. 553-7.
7.	Abrams, P., et al., *The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society.* Neurourol Urodyn, 2002. **21**(2): p. 167-78.
8.	Hanno, P.M., C.R. Chapple, and L.D. Cardozo, *Bladder pain syndrome/interstitial cystitis: a sense of urgency.* World J Urol, 2009. **27**(6): p. 717-21.
9.	Warren, J.W., et al., *Using the International Continence Society's definition of painful bladder syndrome.* Urology, 2006. **67**(6): p. 1138-42; discussion 1142-3.
10.	van de Merwe, J.P., *Interstitial cystitis; definitions and confusable diseases. ESSIC Meeting 2005 Baden.* J Eur Urol Today, 2006: p. 6, 7, 16 and 17.
11.	van de Merwe, J.P., et al., *Diagnostic criteria, classification, and nomenclature for painful bladder syndrome/interstitial cystitis: an ESSIC proposal.* Eur Urol, 2008. **53**(1): p. 60-7.
12.	Baranowski, A.P., et al., *Urogenital pain--time to accept a new approach to phenotyping and, as a consequence, management.* Eur Urol, 2008. **53**(1): p. 33-6.
13.	Fall, M., et al., *EAU guidelines on chronic pelvic pain.* Eur Urol, 2010. **57**(1): p. 35-48.
14.	Hallén, K. and N. Wiklund, *Urinvägarnas embryologi, anatomi och fysiologi.* Urologi. Eds Damber J-E, Peeker R, 2nd ed. Lund. Studentlitteratur, 2012.
15.	Johansson, S.L. and M. Fall, *Clinical features and spectrum of light microscopic changes in interstitial cystitis.* J Urol, 1990. **143**(6): p. 1118-24.
16.	Foster, C.S., Dodson, A., Desmond, A.D., Fordham, M, *Pathology of the Urinary Bladder, major problems in pathology. Ed: Foster, C.S., Ross, J.S.,Saunders. Philadelphia.* 2004.
17.	Birder, L.A. and W.C. de Groat, *Mechanisms of disease: involvement of the urothelium in bladder dysfunction.* Nat Clin Pract Urol, 2007. **4**(1): p. 46-54.
18.	Khandelwal, P., S.N. Abraham, and G. Apodaca, *Cell biology and physiology of the uroepithelium.* Am J Physiol Renal Physiol, 2009. **297**(6): p. F1477-501.
19.	Wu, X.R., et al., *Uroplakins in urothelial biology, function, and disease.* Kidney Int, 2009. **75**(11): p. 1153-65.

20.   Apostolidis, A., et al., *Decreased sensory receptors P2X3 and TRPV1 in suburothelial nerve fibers following intradetrusor injections of botulinum toxin for human detrusor overactivity.* J Urol, 2005. **174**(3): p. 977-82; discussion 982-3.

21.   Apostolidis, A., P. Dasgupta, and C.J. Fowler, *Proposed mechanism for the efficacy of injected botulinum toxin in the treatment of human detrusor overactivity.* Eur Urol, 2006. **49**(4): p. 644-50.

22.   Birder, L.A., et al., *How does the urothelium affect bladder function in health and disease? ICI-RS 2011.* Neurourol Urodyn, 2012. **31**(3): p. 293-9.

23.   Koziol, J.A., H.P. Adams, and A. Frutos, *Discrimination between the ulcerous and the nonulcerous forms of interstitial cystitis by noninvasive findings.* J Urol, 1996. **155**(1): p. 87-90.

24.   Fall, M., S.L. Johansson, and F. Aldenborg, *Chronic interstitial cystitis: a heterogeneous syndrome.* J Urol, 1987. **137**(1): p. 35-8.

25.   Erickson, D.R., D.A. Belchis, and D.J. Dabbs, *Inflammatory cell types and clinical features of interstitial cystitis.* J Urol, 1997. **158**(3 Pt 1): p. 790-3.

26.   Messing, E.M. and T.A. Stamey, *Interstitial cystitis: early diagnosis, pathology, and treatment.* Urology, 1978. **12**(4): p. 381-92.

27.   Peters, K.M., et al., *Are ulcerative and nonulcerative interstitial cystitis/painful bladder syndrome 2 distinct diseases? A study of coexisting conditions.* Urology, 2011. **78**(2): p. 301-8.

28.   Peeker, R. and M. Fall, *Toward a precise definition of interstitial cystitis: further evidence of differences in classic and nonulcer disease.* J Urol, 2002. **167**(6): p. 2470-2.

29.   Homma, Y., et al., *Clinical guidelines for interstitial cystitis and hypersensitive bladder syndrome.* Int J Urol, 2009. **16**(7): p. 597-615.

30.   Peeker, R., L. Atanasiu, and Y. Logadottir, *Intercurrent autoimmune conditions in classic and non-ulcer interstitial cystitis.* Scand J Urol Nephrol, 2003. **37**(1): p. 60-3.

31.   Parsons, C.L., *Interstitial cystitis; clinical manifestations and diagnostic criteria in over 200 cases.* Neurourol Urodyn, 1990. **9**: p. 241-50.

32.   Wyndaele, J.J., J. Van Dyck, and N. Toussaint, *Cystoscopy and bladder biopsies in patients with bladder pain syndrome carried out following ESSIC guidelines.* Scand J Urol Nephrol, 2009. **43**(6): p. 471-5.

33.   Frankson, C., *Interstitial cystitis; a clinical study of 59 cases.* Acta Chir Scand, 1957. **113**: p. 51-62.

34.   Hanno, P., J. Nordling, and M. Fall, *Bladder pain syndrome.* Med Clin North Am, 2011. **95**(1): p. 55-73.

35.   Koziol, J.A., *Epidemiology of interstitial cystitis.* Urol Clin North Am, 1994. **21**(1): p. 7-20.

36.   Irani, A.M., et al., *Detection of MCT and MCTC types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies.* J Histochem Cytochem, 1989. **37**(10): p. 1509-15.

37.   Enerbäck L., M.H., Mayrhofer G., *Methods for the identification and characterisation of mast cells by light microscopy.* In: Befus AD, Bienenstock J, Denburg JA, editors. Mast cell differentiation and heterogeneity. New York: Taven Press;, 1986: p. 405-17.

38.   Nordling, J., et al., *Primary evaluation of patients suspected of having interstitial cystitis (IC).* Eur Urol, 2004. **45**(5): p. 662-9.

39.   O´Leary MP, S.G., *The Interstitial Cystitis Symptom and Problem Indices; rationale, development and application.* In: Sant GR, editor. Interstitial Cystitis. Philadelphia: Lippinocott-Raven, 1997: p. p. 271-6.

40.   Barry MJ, B.J., Donovan J, Jonas U, Kurth KH, Likacas B, et al., *Measuring the symptoms and health impact of benign prostatic hyperplasia and its treatments.* In: Benign Prostatic Hyperplasia. Plymouth: Health Publications Ltd.;, 2001: p. p. 201-25.

41.   Riedl CR, D.K., Pflüger H, Hohlbrugger G., *Intravesical hyaloronic acid for the treatment of interstitial cystitis.* J Urol 2003; 169(Suppl.), 2003.

42.   Daha, L.K., et al., *Comparative assessment of maximal bladder capacity, 0.9% NaCl versus 0.2 M Kcl, for the diagnosis of interstitial cystitis: a prospective controlled study.* J Urol, 2003. **170**(3): p. 807-9.

43.   Kastrup, J., et al., *Histamine content and mast cell count of detrusor muscle in patients with interstitial cystitis and other types of chronic cystitis.* Br J Urol, 1983. **55**(5): p. 495-500.

44.   Larsen, M.S., et al., *Quantifying mast cells in bladder pain syndrome by immunohistochemical analysis.* BJU Int, 2008. **102**(2): p. 204-7; discussion 207.

45.   Parsons, C.L. and V. Tatsis, *Prevalence of interstitial cystitis in young women.* Urology, 2004. **64**(5): p. 866-70.

46.   Oravisto, K.J., *Epidemiology of interstitial cystitis.* Ann Chir Gynaecol Fenn, 1975. **64**(2): p. 75-7.

47.   Leppilahti, M., et al., *Prevalence of clinically confirmed interstitial cystitis in women: a population based study in Finland.* J Urol, 2005. **174**(2): p. 581-3.

48.   Logadottir, Y., et al., *Clinical characteristics differ considerably between phenotypes of bladder pain syndrome/interstitial cystitis.* Scand J Urol Nephrol, 2012. **46**(5): p. 365-70.

49.   Richter, B., et al., *Bladder pain syndrome/interstitial cystitis in a Danish population: a study using the 2008 criteria of the European Society for the Study of Interstitial Cystitis.* BJU Int, 2010. **105**(5): p. 660-7.

50.   Hanno, P., et al., *Bladder Pain Syndrome Committee of the International Consultation on Incontinence.* Neurourol Urodyn. **29**(1): p. 191-8.

51.   Sant, G.R., et al., *The mast cell in interstitial cystitis: role in pathophysiology and pathogenesis.* Urology, 2007. **69**(4 Suppl): p. 34-40.

52.   Rottem, M. and Y.A. Mekori, *Mast cells and autoimmunity.* Autoimmun Rev, 2005. **4**(1): p. 21-7.

53.   Sant, G.R. and T.C. Theoharides, *The role of the mast cell in interstitial cystitis.* Urol Clin North Am, 1994. **21**(1): p. 41-53.

54.   Theoharides, T.C., D. Kempuraj, and G.R. Sant, *Mast cell involvement in interstitial cystitis: a review of human and experimental evidence.* Urology, 2001. **57**(6 Suppl 1): p. 47-55.

55.   Larsen, S., et al., *Mast cells in interstitial cystitis.* Br J Urol, 1982. **54**(3): p. 283-6.

56.   Peeker, R., et al., *Recruitment, distribution and phenotypes of mast cells in interstitial cystitis.* J Urol, 2000. **163**(3): p. 1009-15.

57.   Enerback, L., *Mucosal mast cells in the rat and in man.* Int Arch Allergy Appl Immunol, 1987. **82**(3-4): p. 249-55.

58.   Irani, A.A., et al., *Two types of human mast cells that have distinct neutral protease compositions.* Proc Natl Acad Sci U S A, 1986. **83**(12): p. 4464-8.

59.   Irani, A.M. and L.B. Schwartz, *Neutral proteases as indicators of human mast cell heterogeneity.* Monogr Allergy, 1990. **27**: p. 146-62.

60.   Aldenborg, F. and L. Enerback, *The immunohistochemical demonstration of chymase and tryptase in human intestinal mast cells.* Histochem J, 1994. **26**(7): p. 587-96.

61.   Galli, S.J., *New concepts about the mast cell.* N Engl J Med, 1993. **328**(4): p. 257-65.

62. Batler, R.A., et al., *Mast cell activation triggers a urothelial inflammatory response mediated by tumor necrosis factor-alpha.* J Urol, 2002. **168**(2): p. 819-25.

63. Chen, R., et al., *Macrophages, but not T and B lymphocytes, are critical for subepidermal blister formation in experimental bullous pemphigoid: macrophage-mediated neutrophil infiltration depends on mast cell activation.* J Immunol, 2002. **169**(7): p. 3987-92.

64. Mekori, Y.A. and D.D. Metcalfe, *Mast cells in innate immunity.* Immunol Rev, 2000. **173**: p. 131-40.

65. Howard, P.S., et al., *Mast cell chymase is a possible mediator of neurogenic bladder fibrosis.* Neurourol Urodyn, 2004. **23**(4): p. 374-82.

66. Vitsky, A., et al., *Homeostatic role of transforming growth factor-beta in the oral cavity and esophagus of mice and its expression by mast cells in these tissues.* Am J Pathol, 2009. **174**(6): p. 2137-49.

67. Rothrock, N.E., et al., *Stress and symptoms in patients with interstitial cystitis: a life stress model.* Urology, 2001. **57**(3): p. 422-7.

68. Weissman, M.M., et al., *Interstitial cystitis and panic disorder: a potential genetic syndrome.* Arch Gen Psychiatry, 2004. **61**(3): p. 273-9.

69. Theoharides, T.C., *Panic disorder, interstitial cystitis, and mast cells.* J Clin Psychopharmacol, 2004. **24**(4): p. 361-4.

70. Theoharides, T.C., et al., *Mast cells as targets of corticotropin-releasing factor and related peptides.* Trends Pharmacol Sci, 2004. **25**(11): p. 563-8.

71. Theoharides, T.C., et al., *Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its proinflammatory effects.* Endocrinology, 1998. **139**(1): p. 403-13.

72. Parsons, C.L., J.D. Lilly, and P. Stein, *Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis).* J Urol, 1991. **145**(4): p. 732-5.

73. Parsons, C.L., et al., *Abnormal urinary potassium metabolism in patients with interstitial cystitis.* J Urol, 2005. **173**(4): p. 1182-5.

74. Ogawa, T., et al., *CXCR3 binding chemokine and TNFSF14 over expression in bladder urothelium of patients with ulcerative interstitial cystitis.* J Urol, 2010. **183**(3): p. 1206-12.

75. Gamper, M., et al., *Gene expression profile of bladder tissue of patients with ulcerative interstitial cystitis.* BMC Genomics, 2009. **10**: p. 199.

76. Blalock, E.M., et al., *Gene expression analysis of urine sediment: evaluation for potential noninvasive markers of interstitial cystitis/bladder pain syndrome.* J Urol, 2012. **187**(2): p. 725-32.

77. Sanchez Freire, V., et al., *MicroRNAs may mediate the down-regulation of neurokinin-1 receptor in chronic bladder pain syndrome.* Am J Pathol, 2010. **176**(1): p. 288-303.

78. Sanchez-Freire, V., et al., *Acid-sensing channels in human bladder: expression, function and alterations during bladder pain syndrome.* J Urol, 2011. **186**(4): p. 1509-16.

79. Altman, D., et al., *The genetic and environmental contribution to the occurrence of bladder pain syndrome: an empirical approach in a nationwide population sample.* Eur Urol, 2011. **59**(2): p. 280-5.

80. Rossberger, J., et al., *Long-term results of reconstructive surgery in patients with bladder pain syndrome/interstitial cystitis: subtyping is imperative.* Urology, 2007. **70**(4): p. 638-42.

81. Peeker, R., F. Aldenborg, and M. Fall, *Complete transurethral resection of ulcers in classic interstitial cystitis.* Int Urogynecol J Pelvic Floor Dysfunct, 2000. **11**(5): p. 290-5.

82. Peeker, R., F. Aldenborg, and M. Fall, *The treatment of interstitial cystitis with supratrigonal cystectomy and ileocystoplasty: difference in outcome between classic and nonulcer disease.* J Urol, 1998. **159**(5): p. 1479-82.

83. Fall, M., F. Oberpenning, and R. Peeker, *Treatment of bladder pain syndrome/interstitial cystitis 2008: can we make evidence-based decisions?* Eur Urol, 2008. **54**(1): p. 65-75.

84. Dimitrakov, J., et al., *Pharmacologic management of painful bladder syndrome/interstitial cystitis: a systematic review.* Arch Intern Med, 2007. **167**(18): p. 1922-9.

85. Mulholland, S.G., et al., *Pentosan polysulfate sodium for therapy of interstitial cystitis. A double-blind placebo-controlled clinical study.* Urology, 1990. **35**(6): p. 552-8.

86. Nickel, J.C., et al., *Randomized, double-blind, dose-ranging study of pentosan polysulfate sodium for interstitial cystitis.* Urology, 2005. **65**(4): p. 654-8.

87. Fritjofsson, A., et al., *Treatment of ulcer and nonulcer interstitial cystitis with sodium pentosanpolysulfate: a multicenter trial.* J Urol, 1987. **138**(3): p. 508-12.

88. Theoharides, T.C., *Hydroxyzine in the treatment of interstitial cystitis.* Urol Clin North Am, 1994. **21**(1): p. 113-9.

89. Sant, G.R., et al., *A pilot clinical trial of oral pentosan polysulfate and oral hydroxyzine in patients with interstitial cystitis.* J Urol, 2003. **170**(3): p. 810-5.

90. van Ophoven, A., et al., *A prospective, randomized, placebo controlled, double-blind study of amitriptyline for the treatment of interstitial cystitis.* J Urol, 2004. **172**(2): p. 533-6.

91. van Ophoven, A. and L. Hertle, *Long-term results of amitriptyline treatment for interstitial cystitis.* J Urol, 2005. **174**(5): p. 1837-40.

92. Sairanen, J., T. Forsell, and M. Ruutu, *Long-term outcome of patients with interstitial cystitis treated with low dose cyclosporine A.* J Urol, 2004. **171**(6 Pt 1): p. 2138-41.

93. Sairanen, J., et al., *Cyclosporine A and pentosan polysulfate sodium for the treatment of interstitial cystitis: a randomized comparative study.* J Urol, 2005. **174**(6): p. 2235-8.

94. Perez-Marrero, R., L.E. Emerson, and J.T. Feltis, *A controlled study of dimethyl sulfoxide in interstitial cystitis.* J Urol, 1988. **140**(1): p. 36-9.

95. Rossberger, J., M. Fall, and R. Peeker, *Critical appraisal of dimethyl sulfoxide treatment for interstitial cystitis: discomfort, side-effects and treatment outcome.* Scand J Urol Nephrol, 2005. **39**(1): p. 73-7.

96. Peters, K., et al., *The efficacy of intravesical Tice strain bacillus Calmette-Guerin in the treatment of interstitial cystitis: a double-blind, prospective, placebo controlled trial.* J Urol, 1997. **157**(6): p. 2090-4.

97. Peeker, R., et al., *Intravesical bacillus Calmette-Guerin and dimethyl sulfoxide for treatment of classic and nonulcer interstitial cystitis: a prospective, randomized double-blind study.* J Urol, 2000. **164**(6): p. 1912-5; discussion 1915-6.

98. Bade, J.J., et al., *A placebo-controlled study of intravesical pentosanpolysulphate for the treatment of interstitial cystitis.* Br J Urol, 1997. **79**(2): p. 168-71.

99. Morales, A., et al., *Intravesical hyaluronic acid in the treatment of refractory interstitial cystitis.* J Urol, 1996. **156**(1): p. 45-8.

100. Kallestrup, E.B., et al., *Treatment of interstitial cystitis with Cystistat: a hyaluronic acid product.* Scand J Urol Nephrol, 2005. **39**(2): p. 143-7.

101. Sorensen, R., *Chondroitin sulphate in the treatment of interstitial cystitis and chronic inflammatory disease of the urinary bladder.* Eur Urol Suppl, 2003. **2(4)**: p. 16-8.

102. Bumpus, H., *Interstitial cystitis; its treatment by overdistension of the bladder.* Med Clin North Am, 1930. **13**: p. 1495-8.

103. Smith, C.P., et al., *Botulinum toxin A has antinociceptive effects in treating interstitial cystitis.* Urology, 2004. **64**(5): p. 871-5; discussion 875.

104. Giannantoni, A., et al., *New frontiers in intravesical therapies and drug delivery.* Eur Urol, 2006. **50**(6): p. 1183-93; discussion 1193.

105. Kuo, H.C., *Preliminary results of suburothelial injection of botulinum a toxin in the treatment of chronic interstitial cystitis.* Urol Int, 2005. **75**(2): p. 170-4.

106. Capelli-Schellpfeffer, M. and G.S. Gerber, *The use of hyperbaric oxygen in urology.* J Urol, 1999. **162**(3 Pt 1): p. 647-54.

107. Crew, J.P., C.R. Jephcott, and J.M. Reynard, *Radiation-induced haemorrhagic cystitis.* Eur Urol, 2001. **40**(2): p. 111-23.

108. van Ophoven, A., et al., *Hyperbaric oxygen for the treatment of interstitial cystitis: long-term results of a prospective pilot study.* Eur Urol, 2004. **46**(1): p. 108-13.

109. van Ophoven, A., et al., *Safety and efficacy of hyperbaric oxygen therapy for the treatment of interstitial cystitis: a randomized, sham controlled, double-blind trial.* J Urol, 2006. **176**(4 Pt 1): p. 1442-6.

110. Fall, M., *Conservative management of chronic interstitial cystitis: transcutaneous electrical nerve stimulation and transurethral resection.* J Urol, 1985. **133**(5): p. 774-8.

111. Rofeim, O., et al., *Use of the neodymium: YAG laser for interstitial cystitis: a prospective study.* J Urol, 2001. **166**(1): p. 134-6.

112. Nielsen, K.K., et al., *Failure of combined supratrigonal cystectomy and Mainz ileocecocystoplasty in intractable interstitial cystitis: is histology and mast cell count a reliable predictor for the outcome of surgery?* J Urol, 1990. **144**(2 Pt 1): p. 255-8; discussion 258-9.

113. Baskin, L.S. and E.A. Tanagho, *Pelvic pain without pelvic organs.* J Urol, 1992. **147**(3): p. 683-6.

114. Moncada, S., R.M. Palmer, and E.A. Higgs, *The discovery of nitric oxide as the endogenous nitrovasodilator.* Hypertension, 1988. **12**(4): p. 365-72.

115. Furchgott, R.F. and J.V. Zawadzki, *The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine.* Nature, 1980. **288**(5789): p. 373-6.

116. Moncada, S. and A. Higgs, *The L-arginine-nitric oxide pathway.* N Engl J Med, 1993. **329**(27): p. 2002-12.

117. Beckman, J.S. and W.H. Koppenol, *Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly.* Am J Physiol, 1996. **271**(5 Pt 1): p. C1424-37.

118. Knowles, R.G. and S. Moncada, *Nitric oxide synthases in mammals.* Biochem J, 1994. **298 (Pt 2)**: p. 249-58.

119. Alderton, W.K., C.E. Cooper, and R.G. Knowles, *Nitric oxide synthases: structure, function and inhibition.* Biochem J, 2001. **357**(Pt 3): p. 593-615.

120. James, S.L. and J.B. Hibbs, Jr., *The role of nitrogen oxides as effector molecules of parasite killing.* Parasitol Today, 1990. **6**(9): p. 303-5.

121. Mori, M., et al., *Regulation of the urea cycle enzyme genes in nitric oxide synthesis.* J Inherit Metab Dis, 1998. **21 Suppl 1**: p. 59-71.

122. Morris, S.M., Jr. and T.R. Billiar, *New insights into the regulation of inducible nitric oxide synthesis.* Am J Physiol, 1994. **266**(6 Pt 1): p. E829-39.

123. Forstermann, U. and W.C. Sessa, *Nitric oxide synthases: regulation and function.* Eur Heart J, 2012. **33**(7): p. 829-37, 837a-837d.

124. Ignarro, L.J., *Haem-dependent activation of guanylate cyclase and cyclic GMP formation by endogenous nitric oxide: a unique transduction mechanism for transcellular signaling.* Pharmacol Toxicol, 1990. **67**(1): p. 1-7.

125. Nussler, A.K., et al., *Stimulation of the nitric oxide synthase pathway in human hepatocytes by cytokines and endotoxin.* J Exp Med, 1992. **176**(1): p. 261-4.

126. Nussler, A.K. and T.R. Billiar, *Inflammation, immunoregulation, and inducible nitric oxide synthase.* J Leukoc Biol, 1993. **54**(2): p. 171-8.

127. Forstermann, U., et al., *Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions.* Hypertension, 1994. **23**(6 Pt 2): p. 1121-31.

128. Huie, R.E. and S. Padmaja, *The reaction of no with superoxide.* Free Radic Res Commun, 1993. **18**(4): p. 195-9.

129. Wink, D.A. and J.B. Mitchell, *Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide.* Free Radic Biol Med, 1998. **25**(4-5): p. 434-56.

130. Nagy, G., et al., *Central role of nitric oxide in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus.* Arthritis Res Ther, 2010. **12**(3): p. 210.

131. Petros, A., D. Bennett, and P. Vallance, *Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock.* Lancet, 1991. **338**(8782-8783): p. 1557-8.

132. Middleton, S.J., M. Shorthouse, and J.O. Hunter, *Increased nitric oxide synthesis in ulcerative colitis.* Lancet, 1993. **341**(8843): p. 465-6.

133. McCartney-Francis, N., et al., *Suppression of arthritis by an inhibitor of nitric oxide synthase.* J Exp Med, 1993. **178**(2): p. 749-54.

134. Erickson, D.R., et al., *Urine markers do not predict biopsy findings or presence of bladder ulcers in interstitial cystitis/painful bladder syndrome.* J Urol, 2008. **179**(5): p. 1850-6.

135. Lotz, M., et al., *Interleukin-6 and interstitial cystitis.* J Urol, 1994. **152**(3): p. 869-73.

136. Erickson, D.R., et al., *Differentiation associated changes in gene expression profiles of interstitial cystitis and control urothelial cells.* J Urol, 2008. **180**(6): p. 2681-7.

137. Daha, L.K., et al., *Is there a relation between urinary interleukin-6 levels and symptoms before and after intra-vesical glycosaminoglycan substitution therapy in patients with bladder pain syndrome/interstitial cystitis?* Int Urogynecol J Pelvic Floor Dysfunct, 2007. **18**(12): p. 1449-52.

138. Perez de Lema, G., et al., *Chemokine expression precedes inflammatory cell infiltration and chemokine receptor and cytokine expression during the initiation of murine lupus nephritis.* J Am Soc Nephrol, 2001. **12**(7): p. 1369-82.

139. Tyagi, P., et al., *Urinary chemokines as noninvasive predictors of ulcerative interstitial cystitis.* J Urol, 2012. **187**(6): p. 2243-8.

140. Rutz, S. and W. Ouyang, *Regulation of interleukin-10 and interleukin-22 expression in T helper cells.* Curr Opin Immunol, 2011. **23**(5): p. 605-12.

141. Pestka, S., et al., *Interleukin-10 and related cytokines and receptors.* Annu Rev Immunol, 2004. **22**: p. 929-79.

142. Wilke, C.M., et al., *Deciphering the role of Th17 cells in human disease.* Trends Immunol, 2011. **32**(12): p. 603-11.

143. Ehren, I., et al., *Measurement of luminal nitric oxide in bladder inflammation using a silicon balloon catheter: a novel minimally invasive method.* Urology, 1999. **54**(2): p. 264-7.

144. Ehren, I., et al., *Nitric oxide: a useful gas in the detection of lower urinary tract inflammation.* J Urol, 1999. **162**(2): p. 327-9.

145. Morcos, E., et al., *Endogenously formed nitric oxide modulates cell growth in bladder cancer cell lines.* Urology, 1999. **53**(6): p. 1252-7.

146. Morcos, E., et al., *Bacillus Calmette-Guerin induces long-term local formation of nitric oxide in the bladder via the induction of nitric oxide synthase activity in urothelial cells.* J Urol, 2001. **165**(2): p. 678-82.

147. Fall, M., S.L. Johansson, and A. Vahlne, *A clinicopathological and virological study of interstitial cystitis.* J Urol, 1985. **133**(5): p. 771-3.

148. Fall, M., *Transcutaneous electrical nerve stimulation in interstitial cystitis. Update on clinical experience.* Urology, 1987. **29**(4 Suppl): p. 40-2.

149. Gillenwater, J.Y., *National institute of kidney and urologic diseases.* J Urol, 1994. **152**(2 Pt 1): p. 491.

150. Aldenborg, F., et al., *Metaplastic transformation of urinary bladder epithelium: effect on mast cell recruitment, distribution, and phenotype expression.* Am J Pathol, 1998. **153**(1): p. 149-57.

151. Archer, S., *Measurement of nitric oxide in biological models.* FASEB J, 1993. **7**(2): p. 349-60.

152. Kunisch, E., et al., *Macrophage specificity of three anti-CD68 monoclonal antibodies (KP1, EBM11, and PGM1) widely used for immunohistochemistry and flow cytometry.* Ann Rheum Dis, 2004. **63**(7): p. 774-84.

153. Holness, C.L. and D.L. Simmons, *Molecular cloning of CD68, a human macrophage marker related to lysosomal glycoproteins.* Blood, 1993. **81**(6): p. 1607-13.

154. Peeker, R., et al., *Immunologic and neurobiologic characteristics support that interstitial cystitis is a heterogeneous syndrome.* Urology, 2001. **57**(6 Suppl 1): p. 130.

155. Rosenberg, M.T., D.K. Newman, and S.A. Page, *Interstitial cystitis/painful bladder syndrome: symptom recognition is key to early identification, treatment.* Cleve Clin J Med, 2007. **74 Suppl 3**: p. S54-62.

156. Fall, M., et al., *Clinical characteristics support that interstitial cystitis is a heterogeneous syndrome.* Urology, 2001. **57**(6 Suppl 1): p. 129-30.

157. Holm-Bentzen, M., et al., *Painful bladder disease: clinical and pathoanatomical differences in 115 patients.* J Urol, 1987. **138**(3): p. 500-2.

158. Logadottir, Y., et al., *Intravesical nitric oxide production discriminates between classic and nonulcer interstitial cystitis.* J Urol, 2004. **171**(3): p. 1148-50; discussion 50-1.

159. Smith, S.D., et al., *Improvement in interstitial cystitis symptom scores during treatment with oral L-arginine.* J Urol, 1997. **158**(3 Pt 1): p. 703-8.

160. Ehren, I., et al., *Effects of L-arginine treatment on symptoms and bladder nitric oxide levels in patients with interstitial cystitis.* Urology, 1998. **52**(6): p. 1026-9.

161. Cartledge, J.J., A.M. Davies, and I. Eardley, *A randomized double-blind placebo-controlled crossover trial of the efficacy of L-arginine in the treatment of interstitial cystitis.* BJU Int, 2000. **85**(4): p. 421-6.

162. Logadottir, Y., et al., *Bladder pain syndrome/interstitial cystitis ESSIC type 3C: High expression of inducible nitric oxide synthase in inflammatory cells.* Scand J Urol Nephrol, 2012.

163. Koskela, L.R., et al., *Localization and expression of inducible nitric oxide synthase in biopsies from patients with interstitial cystitis.* J Urol, 2008. **180**(2): p. 737-41.

164. Moncada, S., *The 1991 Ulf von Euler Lecture. The L-arginine: nitric oxide pathway.* Acta Physiol Scand, 1992. **145**(3): p. 201-27.

165. Ghosh, S. and S.C. Erzurum, *Nitric oxide metabolism in asthma pathophysiology.* Biochim Biophys Acta, 2011. **1810**(11): p. 1008-16.

166. Muhl, H., M. Bachmann, and J. Pfeilschifter, *Inducible NO synthase and antibacterial host defence in times of Th17/Th22/T22 immunity.* Cell Microbiol, 2011. **13**(3): p. 340-8.

167. Banchereau, J. and R.M. Steinman, *Dendritic cells and the control of immunity.* Nature, 1998. **392**(6673): p. 245-52.

168. Engel, D., et al., *Tumor necrosis factor alpha- and inducible nitric oxide synthase-producing dendritic cells are rapidly recruited to the bladder in urinary tract infection but are dispensable for bacterial clearance.* Infect Immun, 2006. **74**(11): p. 6100-7.

169. Magnusson, M.K. and M.J. Wick, *Intestinal Dendritic cell and macrophage subsets: Tipping the balance to Crohn´s disease?* Eur J Microbiol Immunol, 2011. **1**: p. 19-24.