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# Cyclosporine A in the Treatment of Interstitial Cystitis

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ACADEMIC DISSERTATION

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# ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals.

I Sairanen J, Forsell T, Ruutu M: Long term outcome of patients with interstitial cystitis treated with low dose cyclosporine A. J Urol 171: 2138-2141, 2004

II Sairanen J, Tammela TLJ , Leppilahti M, Multanen M, Paananen I, Lehtoranta K, Ruutu M: Cyclosporine A and pentosan polysulfate sodium in the treatment of interstitial cystitis: A randomized comparative study. J Urol 174: 2235-2238, 2005

III Sairanen J, Tammela TLJ , Leppilahti M, Onali M, Forsell T, Ruutu M: Potassium sensitivity test (PST) as a measurement of treatment efficacy of painful bladder syndrome / interstitial cystitis. A prospective study with cyclosporine A and pentosan polysulfate sodium. Neurourol Urodyn 26: 267-270, 2007

IV Sairanen J, Hotakainen K, Tammela TLJ, Stenman U-H, Ruutu M: Urinary epidermal growth factor and interleukin-6 levels in patients with painful bladder syndrome / interstitial cystitis treated with cyclosporine or pentosan polysulfate sodium. Urology (In press).

V Sairanen J, Leppilahti M, Tammela TLJ, Paananen I, Aaltomaa S, Taari K, Ruutu M: Evaluation of a quality of life questionnaire in patients with PBS/IC and the impact of four different treatments on it. Submitted.

The thesis also contains some unpublished data.
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# ABBREVIATIONS

| | |
|---|---|
| AUA | American Urological Association |
| APF | antiproliferative factor |
| BCG | bacillus Calmette-Guerin |
| CP/CPPS | chronic prostatitis / chronic pelvic pain syndrome |
| CyA | cyclosporine A |
| DMSO | dimethyl sulfoxide |
| EGF | epidermal growth factor |
| ESSIC | European Society for the Study of Interstitial Cystitis |
| FDA | Food and Drug Administration |
| GAG | glycosaminoglycan |
| GRA | global response assessment |
| HB-EGF | heparin binding epidermal growth factor |
| HRQOL | health-related quality of life |
| IC | interstitial cystitis |
| ICDB | interstitial cystitis database |
| IL-6 | interleukin-6 |
| MC | mast cell |
| NIDDK | National Institute of Diabetes and Digestive and Kidney Diseases |
| NSAID | non-steroidal anti-inflammatory drug |
| PBS/IC | painful bladder syndrome/interstitial cystitis |
| PPS | pentosan polysulfate sodium |
| PST | potassium sensitivity test |
| THP | Tamm-Horsfall protein |
| QoL | quality of life |
| VAS | visual analogue scale |

# ABSTRACT

Painful bladder syndrome/interstitial cystitis (PBS/IC) is a debilitating inflammatory bladder disease of unknown etiology. The symptoms include suprapubic pain related to bladder filling, accompanied by other symptoms such as daytime or night-time frequency. While the pathophysiology of the disease remains a matter of investigation and it probably differs from patient to another, no major breakthrough in the field of medical therapies has been achieved. Inflammatory changes in the bladder biopsies are observed in at least some patients with a clinical picture of PBS/IC. These include infiltration of lymphocytes throughout the bladder wall, as well as the appearance of mast cells (MC), and denudation of normal urothelium. A higher incidence of autoimmune diseases is seen among PBS/IC patients compared with an asymptomatic population. There is a need for effective oral therapy for PBS/IC, and for this reason, we decided to try a new compound for the treatment of this disease which has proved so unresponsive to previous therapeutic efforts.

Cyclosporine A (CyA) is a calcineurine inhibitor with anti-inflammatory effects which has the capability to block pro-inflammatory genes, and it has been hypothesized that this effect could benefit PBS/IC patients with regard to their symptoms.

A previous empiric pilot study of CyA treatment had shown promising short term results in PBS/IC. Our aim was to evaluate if CyA therapy is effective in a longer follow-up, which would provide an ideal basis for running a prospective, randomized trial. We decided to compare CyA with pentosan polysulfate sodium (PPS), which is a United States Food and Drug Administration (FDA) approved drug for PBS/IC.

In the first retrospective study we observed that the good clinical effect of CyA is sustained in long-term use and the subjective symptoms are alleviated over the course of time. In the following prospective trial the use of CyA resulted in a greater decrease in points in the validated, subjective symptom questionnaire than PPS ($p<0.001$), and the global response assessment (GRA) showed superior results in the CyA group ($p<0.001$). The parameters in voiding diaries also improved significantly greater extent after CyA therapy ($p<0.001$), and this also applied to the pain score. CyA side effects of were common, but serious adverse events were rare. Patient's overall tolerance to PPS was identical. A greater number of patients wanted to continue CyA treatment at the end of the study.

To be able to evaluate the effect of CyA and PPS more thoroughly, during this prospective trial we also tested the treatment effect on the potassium sensitivity test (PST) in all 64 patients and the urinary markers epidermal growth factor (EGF) and interleukin-6 (IL-6) in 37 patients, which served as objective markers for treatment response and the state of current disease. An impact of CyA on pre-treatment positive PST test was observed, while PPS had no effect on this parameter.

The urinary EGF levels decreased after CyA therapy, while the urinary IL-6 levels decreased only in older patients. PPS had no effect on these urinary markers.

Overall, CyA is a viable treatment option in patients with PBS/IC who fulfil the NIDDK criteria and have serious symptoms and in whom previous attempts to alleviate symptoms have failed. Our results support the need for future clinical studies with drug compounds that modulate inflammation in PBS/IC bladder.

# INTRODUCTION

The clinical picture of bladder dysfunction, painful bladder syndrome/interstitial cystitis (PBS/IC) was recognized 100 years ago by one of the pioneers of modern urology, Maximilian Nitze (1907). The condition of PBS/IC was later popularized by Hunner, who described the lesions seen in bladder cystosopy, as elusive ulcers (Hunner 1915). The symptoms associated with probable PBS/IC had already been recognized before Nitze and Hunner, as the clinical condition called "tic douloureux of the bladder" was described in 19$^{th}$ century (Parrish 1836). The term "interstitial cystitis" in association with urinary symptoms and bladder ulceration was first introduced by the gynaecologist AJC Skene in 1878 (Skene 1878). Since those days great progress has been made in characterizing the disease, but there is still no reason to withdraw the adjective "elusive" preceding the term ulcer.

The unknown etiology of PBS/IC has led to difficulties in finding effective treatments for the disease symptoms, which are typically, pain related to bladder filling and frequency during the day and at night.

Those patients with refractory disease may be helped efficiently only with major surgery, which means removal of the diseased bladder sub- or supratrigonally (Irwin and Galloway 1994, Webster and Galloway 1987), or even total cystectomy. Even after major surgery the outcome is reported to be poor in 8 to 17% of cases (Kontturi et al. 1991, Linn et al. 1998, van Ophoven et al. 2002). The frustration of both the patients and physicians after failed surgical intervention is justified.

The basic idea to use immunosuppressive agents in the treatment of PBS/IC lies in their effectiveness in inflammatory and various autoimmune diseases. In PBS/IC, inflammatory cells are seen in abundance in bladder wall (Christmas 1994, Peeker et al. 2000a).

The first report on immunosuppressive treatment of PBS/IC with corticosteroids was promising, but it could not be repeated later (Dees 1953, Pool 1967). Studies with corticotherapy are, however, occasionally still reported (Soucy and Gregoire 2005).

The Finnish urologists Oravisto and Alfthan decided to try immunosuppressant azathioprine in the treatment of refractory PBS/IC in the 70s. It was administered to 38 patients. Pain disappeared completely in 22 patients and frequency in 20 patients, including two very severe cases with contracted bladder (Oravisto and Alfthan 1976). Despite the promising results, azathioprine was never used widely, mainly due its toxicity.

Cyclosporine (CyA) has been reported to be an effective treatment in multiple chronic inflammatory diseases (Faulds et al. 1993). It was these findings that provided the impulse to also try it in PBS/IC patients. CyA was effective in an open, small series with 11 patients (Forsell et al. 1996), and the results warranted further studies on this promising treatment.

The aim of this thesis was to show if CyA is a viable option in PBS/IC patients and whether the potential effect is maintained over a longer period of time. In a prospective, randomized study we compared CyA with an approved drug therapy, pentosan polysulfate sodium (PPS).

We also wanted to test whether either of these drug therapies has any effect on objective biochemical markers, which included urinary epidermal growth factor (EGF) and interleukin-6 (IL-6). The concentration of these markers has been shown to be higher in PBS/IC patients than in controls (Keay et al. 1997, Lotz et al. 1994).

Furthermore, we used potassium sensitivity test (PST) as a tool to evaluate the treatment effect and tested at the same time the usefulness of this somewhat controversial test.

The health-related quality of life was assessed with a generic questionnaire before and after CyA and PPS treatment. We also included in the quality of life study the patients (n=87) who participated to a previous prospective, randomized study comparing the efficacy of intravesical dimethyl sulfoxide (DMSO) and bacillus Calmette-Guerin (BCG) to PBS/IC symptoms. In that study the same questionnaire was used in the follow-up as well. The cohort of these two independent studies was used in evaluation of the feasibility of the questionnaire.

# REVIEW OF THE LITERATURE

## Painful bladder syndrome / interstitial cystitis (PBS/IC)

### Definition / Name of the disease

There is currently ongoing lively discussion on the nomenclature and classification of PBS/IC. Traditionally, the condition of suprapubic or pelvic pain related to bladder filling with possible relief of pain after voiding and which causes day- and night time frequency, is called interstitial cystitis (IC) (Hanno 1994). In the case of IC, no other evident cause for the symptoms is present. In most cases, the differential diagnosis is bacterial urinary infection. The term IC suggests that the patient has inflammatory changes in deeper layers of the bladder wall. This finding is not consistent in all patients, while the phenotype of the disease has similarities despite different pathology in bladder biopsies.

International continence society (ICS) has written reports on the standardisation of terminology of lower urinary tract function since 1976. In the most recent report, ICS prefers the term painful bladder syndrome (PBS) to IC (Abrams et al. 2002). In the same context, ICS defines PBS as the complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased day-time and night-time frequency, in the absence of proven urinary infection or other obvious pathology. The ICS definition is suggested to be insufficiently sensitive as it identified only 66% of the 138 patients having a diagnosed by experts (Warren et al. 2006).

The term painful bladder was introduced to literature in 1951 (Bourque 1951). Later, the term was modified to painful bladder disease by Holm-Bentzen, who divided the patients into two groups according to detrusor mast cell density. Patients with more than 28 mast cells per $mm^2$ were named IC patients and those with less mast cells were named painful bladder disease patients. The clinical pictures of IC or painful bladder disease do not differ from each other (Holm-Bentzen et al. 1987a). Later, other reports using the term painful bladder syndrome appeared (Ramahi and Richardson 1990, Witherow et al. 1989). The necessity of keeping the term IC alive has been stressed in various expert meetings. The importance of term IC is based on the historical background; it is widely known by physicians and patient associations and the term IC has possible implication of inflammatory changes in bladder biopsies which might influence the selection of treatment. As a result of the expert meetings, use of a combination of both IC and PBS is recommended, leading to abbreviated terms PBS/IC or IC/PBS. Recently European Society for the Study of Interstitial Cystitis (ESSIC) suggested converting the term PBS/IC to bladder pain syndrome (BPS) (van de Merwe et al. 2008). In that classification all patients with typical clinical symptoms of PBS/IC would have BPS, despite what is seen at cystoscopy. The cystoscopic changes after hydrodistension or histological changes in biopsies would form subtypes of BPS. Patients presenting interstitial inflammation

would fulfil the requirements of the original term of IC. As IC is well known among urologists, patients and patient associations, including IC in the overall term (BPS/IC) could be used in parallel to BPS during a transition period. Time will tell whether the urological community will accept this latter suggestion.

PBS/IC is traditionally divided into two entities, classic and non-ulcer PBS/IC. Patients that have classic PBS/IC have an ulcerous lesion called Hunner's ulcer in cystoscopic examination. Ulcer in this context means that it is recognizable by eye. A more proper word would be Hunner's lesion, as it is a distinctive reddish area of inflammation with small vessels radiating towards a central scar which ruptures and bleeds after hydrodistension (Peeker and Fall 2000b). Classic PBS/IC is uncommon comprising 5 to 20% of PBS/IC patients (Koziol et al. 1996, Parsons 1990). In non-ulcerous form of PBS/IC examination no ulcer is seen at cystoscopy. In the course of or after bladder filling, development of multiple glomerulations and superficial mucosal bleeding are seen. They vary in location and magnitude. The abundance of glomerulations is associated with reduced bladder capacity under anaesthesia (Nigro et al. 1997).

## NIDDK criteria

The National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) made an effort to improve the description of IC at workshops in 1987 and 1988, when the criteria were introduced and reviewed, respectively (**Table 1**) (Gillenwater and Wein 1988, Wein et al. 1990). The resulting strict criteria were meant for research purposes. When using the criteria in inclusion of patients to study arms, the study population would be homogenous and the results would be more comparable between studies. At the time the criteria were introduced, the authors assumed that they would not be used as diagnostic definitions of PBS/IC. Despite this the criteria were quickly adopted in everyday practise. In a study with 379 patients clinically diagnosed with PBS/IC by experienced clinicians, only 32% of patients met all NIDDK criteria (Hanno et al. 1999). Two-thirds of the patients would not have been included on the basis of NIDDK criteria, which make these unsuitable for defining the clinical syndrome of PBS/IC.

**Table 1**. *NIDDK diagnostic criteria for interstitial cystitis (Wein et al. 1990).*

| INCLUSION CRITERIA (MUST BE PRESENT) | EXCLUSION CRITERIA (ANY OF THE FOLLOWING AUTOMATICALLY EXCLUDES THE PATIENT) |
|---|---|
| pain associated with the bladder or urinary urgency | Bladder capacity grater than 350 ml on awake cystometry using either a gas or liquid filling medium |
| Hunner`s ulcer or Glomerulations on cystoscopic evaluation after hydrodistension of the bladder (80 to 100 cm water pressure for 1 to 2 minutes) | Absence of an intense urge to void with the bladder filled to 100 ml gas or 150 ml water during cystometry, using a fill rate of 30 to 100 ml per minute |
| | The demonstration of phasic involuntary bladder contractions on cystometry using the fill rate described previously |
| | Duration of symptoms less than 9 months |
| | Absence of nocturia |
| | Symptoms relieved by antimicrobials, urinary antiseptics, anticholinergics or antispasmodics |
| | A frequency of urination, while awake, of less than 8 times a day |
| | A diagnosis of bacterial cystitis or prostatitis within a 3-month period |
| | Bladder or ureteral calculi |
| | Active genital herpes |
| | Uterine, cervical, vaginal or urethral cancer |
| | Urethral diverticulum |
| | Cyclophosphamide or any other type of chemical cystitis |
| | Tuberculous cystitis |
| | Radiation cystitis |
| | Benign or malignant bladder tumours |
| | Vaginitis |
| | Age less than 18 years |

## Epidemiology

Epidemiological studies of PBS/IC are difficult to perform due to inconsistency of the diagnostic criteria and the rarity of the disease. Different approaches have been used in efforts to estimate the prevalence of PBS/IC.

In 1975, Oravisto published his findings of the prevalence of PBS/IC in Finland. He believed that almost all patients with PBS/IC in the surrounding of Uusimaa were diagnosed by physicians and known in his urological unit. Having calculated the number of patients and the population of Uusimaa area, an estimate of 18.1 cases per 100 000 women of all ages and 10.6 cases for both genders was given (Oravisto 1975). A similar estimate of prevalence (8–16/100 000) was published later in a Dutch study based also on physician-assigned diagnostics (Bade et al. 1995).

A more precise estimate of 67/100 000 women was given by Curham, based on the diagnosis of PBS/IC in medical records of all participants of Nurses Health Study who reported having PBS/IC (Curhan et al. 1999).

A postal survey among a randomly selected female population using a validated symptom questionnaire (O`Leary-Sant symptom and problem scores) on PBS/IC resulted in a prevalence estimation of severe IC-like symptoms of 450/100 000 women (Leppilahti et al. 2002). In a further clinical evaluation of 21 of the 32 patients scoring more that 7 points in the O`Leary-Sant symptom score, the prevalence of clinically confirmed probable PBS/IC was 300/100 000 women (Leppilahti et al. 2005).

The male to female ratio in patients with PBS/IC is traditionally considered to be 1:10, but recently a 1:5 ratio was calculated (Clemens et al. 2005).

The great variability between the estimates in the prevalence of PBS/IC is due to the inconsistency of diagnostic methods and criteria used.

## Etiology

The etiology of PBS/IC has remained elusive since the first days when attempts were made to define it. Numerous factors that have been suggested may play a role in the development of the clinical syndrome. The theories that have gained most interest are presented below.

### Epithelial dysfunction

The bladder has a capability to maintain large gradients for water, ions, protons and ammonium between the urine and blood for prolonged periods (Hicks 1975). In humans, the urine osmolality varies between 50 and 1000 mosmol/kg$H_2O$, while blood osmolality remains constant between 280 and 290 mosmol/kg$H_20$. The urinary pH also varies between 4.5 and 8 while blood pH is stable at 7.4. The urothelial barrier function is therefore important in maintaining homeostasis. The main sites responsible for the unpermeability are the tight junctions of the uppermost urothelial cells (so called umbrella cells) in combination with apical membranes (Negrete et al. 1996). The apical membranes are covered with rigid plaques which contain five major integral membrane proteins (uroplakins) important in maintaining urothelial unpermeability (Jenkins and Woolf 2007). The outer surfaces of urothelial cells are covered with a layer of diverse proteoglycans. Proteoglycans are complex macromolecules with a central protein and at least one glycosaminoglycan (GAG) chain attached to them through a serine residue (Hurst et al. 2007). There are four main families in the structure of GAG: heparins and heparin sulphates, chondroitin and dermatan sulphates, hyaluronate, and keratan sulphates (Hurst et al. 2007). In bladder epithelium decorin, perlecan and syndecan-1 are proteclycans that are studied the most (Hurst et al. 2007). Hyaluronate is the only unsulfated proteoglycan and it is not bound covalently to the core protein. Heparin-like molecules are covering most cell surfaces and are acting as receptors and modulators to proteins. The GAG layer was shown to play a key role in the antibacterial defence mechanisms of the bladder (Parsons et al. 1975), but proteoglycans can serve also as receptor for bacterial attachment (Rostand and Esko 1997). Fimbriated Escherichia Coli

attach to mannose residues of uroplakins. Glycoprotein-51 and Tamm-Horsfall protein may act as competitive inhibitors of binding to uroplakin receptor (Byrne et al. 1999, Pak et al. 2001). Destroying the GAG layer with protamine, the uptake of intravesical urea was increased (Parsons et al. 1990). This finding has been argued against, as protamine also destroys the umbrella cells and not only the GAG layer (Davis and Avots-Avotins 1982). Thus the reason for increased permeability is suggested to be due to larger defects in the urothelium (Elbadawi 1997). There are no clinical studies showing that the permeability is enhanced in PBS/IC. Radiolabeled diethylenetriaminepentaacetis-acid (DTPA) did not penetrate the urothelial barrier differently in PBS/IC patients and controls (Chelsky et al. 1994). After hydrodistension, the permeability of the PBS/IC bladder measured by absorption of intravesical rhamnose-lactulose solution is increased (Erickson et al. 2000). But according to an animal model, this may be caused by distension trauma (Leppilahti et al. 1999).

It is suggested that movement of urinary potassium into the bladder interstitium is important in causing PBS/IC symptoms (Parsons 2007). This theory is based on two animal models where isolated pelvic nerves could be stimulated by potassium and marked hypersensitivity to potassium was seen after injury of bladder epithelium with protamine sulphate (Chuang et al. 2003, Moss et al. 1997). Further evidence is provided by the finding of abnormal urinary metabolism of potassium in PBS/IC patients. Urine potassium levels were lower in PBS/IC patients than in controls (Parsons et al. 2005). This was thought to be due to migration of potassium ions into the bladder wall.

It is suggested that normal urine may contain a certain cationic cytotoxic factor increasing urothelial permeability, allowing potassium to penetrate the urothelium for depolarizing the underlying nerves and muscles. Pentosan polysulfate could neutralize the toxic factor and suppress potassium-mediated bladder hyperactivity (Rajasekaran et al. 2006). This toxic factor, which remains still undefined, can be neutralized by a kidney derived glycoprotein, Tamm-Horsfall protein (THP) (Stein et al. 2005). It was recently suggested that the THP in PBS/IC patients might be structurally deficient compared with controls, having importance in pathogenesis of PBS/IC (Parsons et al. 2007).

Overall, the hypothesis of epithelial dysfunction as an etiological factor behind PBS/IC is based on the following ideas: the mucus of the bladder wall is responsible for the permeability of the bladder and it is dysfunctional in most patients with PBS/IC, while the PST test is positive in PBS/IC and mucus repair with exogenous GAG (like PPS) heals patients symptoms (Parsons 2007). However, the literature does not support these speculations and it is possible that epithelial dysfunction is a secondary phenomenon following inflammation of the bladder wall (Elbadawi 1997).

### Mast cell activation

Mast cells (MC) are derived from hematopoietic stem cells. They do not usually circulate in mature form, but instead their differentiation occurs locally in the microenvironment in which they ultimately reside (Galli et al. 2005). In mammals, mast cells are widely distributed throughout vascularised tissues beneath and within epithelia and in close

proximity to blood vessels, nerves and smooth muscle cells. Mast cells are involved in allergic reactions, such as anaphylaxis and asthma, in which they are stimulated by immunoglobulin E (IgE) bound to surface receptors and by specific antigens (Kawakami and Galli 2002). Mast cells can also be activated by non-immunological stimulants such as bacteria, chemicals, kinins, neuropeptides and acetylcholine (Galli et al. 2005). Mast cell mediators are granule-stored, presynthesized molecules or are synthesized de novo (especially IL-6, leukotrienes, prostaglandins, nitric oxide and tumour necrosis factor-α) (Sant et al. 2007). Activated mast cells produce many types of cytokines which in turn recruit and activate leukocytes. Activated leukocytes produce further cytokines, which have prolonged effects on resident target cells. Persistent allergen exposure leads to chronic inflammation with long-term changes in structure and function of underlying tissues (Paul 1999).

The role of increased number of mast cells in patients with PBS/IC has been of interest for 50 years. A mast cell count in the detrusor muscle of >20 MC/mm$^2$ was even promoted to be of diagnostic value for PBS/IC (Kastrup et al. 1983). Increased mast cell counts are more consistently prevalent in classic, ulcerous form of PBS/IC, in which they are abundantly present in the epithelia (Peeker et al. 2000a).

Activated mast cells in bladder tissue are best identified by transmission electron microscopy (TEM) or by assessment of their secretes (Sant et al. 2007). Typical ultrastructural signs of mast cell activation in patients with PBS/IC are heterogeneous morphology with intragranular secretion without compound exocytosis (Letourneau et al. 1996). Histamine contents that are produced by mast cells are increased in PBS/IC in the bladder wall (Kastrup et al. 1983) and urinary content of histamine metabolites and mast cell proteinase tryptase are increased in the urine of PBS/IC patients (Boucher et al. 1995, el-Mansoury et al. 1994).

Vasoactive and inflammatory mediators secreted by mast cells may be involved in development of PBS/IC symptoms (Theoharides et al. 2001). Tryptase is shown to provoke microvascular leakage and stimulation of protease-activated receptors (PARs) causing inflammation and neuronal hyperexcitability (Boucher et al. 1995). Vascular endothelial growth factor (VEGF) is a mast cell mediator, and glomerulations during hydrodistention are highly associated with the overexpression of angiogenic growth factors in the bladder (Tamaki et al. 2004). IL-6 is can be induced from mast cells by bacterial lipopolysaccharides. IL-6 is elevated in urine in PBS/IC and IL-6 positive cells are present in both mucosal and detrusor layers of PBS/IC bladders (Peeker et al. 2000a). Immobilization stress induced bladder mast cell activation in rats and the secretion of IL-6 in them was inhibited by intravesical sodium hyaluronate (Boucher et al. 2002).

It has been suggested that mast cells interact with the nerve endings present in the close proximity (Elbadawi 1997). Neuropeptides, for example substance-P and neurokinin A, are released by sensory nerves when triggered by a stimulus such as pain (Foreman 1987a). Expression of neurokinin-1 receptors and substance-P positive nerves has been demonstrated in PBS/IC (Marchand et al. 1998, Pang et al. 1995). These neuropeptides activate mast cells and have been shown to cause injury with increased permeability of epithelial surfaces of the airway mucosa (McDonald 1987). Mast cells have been shown to mediate the severity of experimental cystitis in animal models (Ahluwalia et al. 1998,

Bjorling et al. 1999). Treatments inhibiting mast cells have been tried with varying success and new treatments are under development for PBS/IC (Theoharides and Sant 2005).

## Neural upregulation

Sensory nervous system, especially when stimulated chronically, can generate inflammation (Foreman 1987b). Chronic inflammation or nerve injury may lead to alteration of peptides and peptide receptors leading to functional consequences (Wiesenfeld-Hallin and Xu 2001). Nerve fiber density is increased in ulcerative PBS/IC and the amount of histamine is related to it (Lundeberg et al. 1993). Harrison and co-workers (1990) proposed that small diameter sensory nerves in the bladder wall may have a role in the transmission of the sensation of pain and in the triggering of inflammatory reactions. Purinergic ATP signalling is upregulated in the PBS/IC bladder. Also P2X3-receptors, through which ATP acts and evokes neural discharge, are upregulated during in vitro bladder smooth muscle stretch. It is suggested that urothelial cells can phenotypically mimic sensory neurons (Sun and Chai 2004). In PBS/IC bladder biopsies the level of nerve growth factor (NGF) is increased and this may enhance sensitization of nociceptor fibres and also increase numbers of mast cells (Lowe et al. 1997).

Nervous system itself contributes to the chronic nature of PBS/IC despite the etiology. PBS/IC patients may have upregulation of the limbic responses involved in anxiety and in stress leading to altered pain perception and abnormal modulation of afferent pain signals. This was suggested as Twiss and his study group showed amygdala modulated startle blink reflex (defensive involuntary eye blink in response to sudden stimuli) to be greater in PBS/IC patients than in controls (Twiss et al. 2007).

The proposal of neurogenic inflammation as an etiological factor is like an umbrella under which theories of epithelial dysfunction, mast cell activation, infection, immunological and autoimmunological mechanism fit well.

## Infection

The history of PBS/IC patient's symptoms is easily mixed with that of bacterial cystitis, and therefore often antibiotics are prescribed. A prospective, randomized, placebo-controlled study showed that combinations of antibiotics may sometimes be associated with decreased symptoms in some patients, but they do not represent a major advance in the therapy for PBS/IC (Warren et al. 2000). There are studies that suggest infection as an etiological factor behind PBS/IC, but they are outnumbered by other studies which are negative to bacterial findings. Wilkins and associates (1989) suggested PBS/IC to be of infectious origin, as 12 of their 20 patients had positive bacterial culture in urine or in urethral swab. Their study lacked a control group and the bacteria cultured were in eight cases Gardnerella vaginalis and Lactobacillus saphrophyticus. Ureaplasma urealyticum and Mycoplasma hominis have also been cultured in urine in some patients with PBS/IC

like symptoms (Hedelin et al. 1983). An important report was that by Haarala and co-workers (1996) as they could not find bacterial DNA in bladder biopsies nor in urine of PBS/IC patients - an ongoing bacterial infection was excluded to be the cause of PBS/IC. There is no evidence of Helicobacter pylori or Borrelia burgdorferi infections preceding PBS/IC (Agarwal and Dixon 2003, Haarala et al. 2000).
Infection as a sole etiological factor behind PBS/IC is not widely supported. However, it has been speculated that the chronically damaged epithelium is prone to colonization with various micro-organisms, and the resulting exposure to micro-organisms or other urinary antigens prompts the inflammatory response commonly seen in this disorder (Keay and Warren 1998).

## Autoimmunity

PBS/IC has many features of an autoimmune disease; chronic character, waxing and waning of the symptoms, overlap with other systemic autoimmune diseases, and higher female incidence and prevalence. Systemic autoimmune diseases have no clear definition. The statement that a certain disease is autoimmune is made on the basis of the presence of autoantibodies and localization of the antibody, complement and T-lymphocytes in the diseased tissue (Paul 1999). The overlap between PBS/IC and known autoimmune diseases is clear. Patients with PBS/IC were 40 times more likely to have systemic lupus erythematosus, 100 times more likely to have inflammatory bowel disease and 40.6% of PBS/IC patients have allergies compared with 22.5% in general population (Alagiri et al. 1997). Rheumatoid arthritis is also more prevalent among PBS/IC patients (Peeker et al. 2003). A population-based study in Finland showed that among patients with Sjören's syndrome, the prevalence of probable PBS/IC is 15 to 20 times higher than it is in general population (Leppilahti et al. 2003).

Autoantibodies are found in the serum in 36–94% of the patients with PBS/IC (Ochs et al. 1994, Oravisto 1980). The autoantobody level has been shown to diminish after cystectomy (Jokinen et al. 1973). The autoantibodies are not bladder specific. It is believed that they arise in response to injury and might not be directly involved in triggering PBS/IC (Ochs et al. 1994).

Complement systems include plasma proteins which enhance phagocytosis and the function of agents that increase vascular permeability. They serve as chemoattractants for inflammatory cells and create lytic multiprotein complexes (Paul 1999 . Complement pathway is classically activated by antigen bound antibodies of the IgM or IgG class. Immunoglobulines together with C3 class complement are seen in the bladder ultrastructure in PBS/IC (Helin et al. 1987). It has been suggested that following the binding of autoantibodies to antigens in the bladder mucosa, activation of complement is involved in tissue injury and in the chronic self-perpetuating inflammation typical of this disease. To date this study is the only one showing complement activation in bladder biopsies in PBS/IC. In one study, C3 class complement was found in the urine of PBS/IC patients (Frandsen et al. 1988), but the results were not reproducible in another study (Steinert et al. 1994).

The presence of lymphocytic infiltrate is a common feature in biopsies of PBS/IC bladders (Christmas 1994). The number of T cells is not pathognomonic to PBS/IC as the number of T cells is not higher than in bacterial cystitis (Christmas 1994). No differences are seen in T cell numbers n circulating blood between PBS/IC patients and normal controls (Miller et al. 1992).

PBS/IC patients express more HLA class II molecules in the urothelium, HLA-DR, which is a feature common to a number of autoimmune conditions (Christmas and Bottazzo 1992). The expression of HLA class I molecules was also higher in PBS/IC patients, and this might reflect direct CD $8^+$ cytotoxicity as the class I molecules bind to CD $8^+$ T cell receptors (Christmas and Bottazzo 1992). The HLA-DR molecule respectively binds to the CD 4 receptors of T cells, which are then stimulated to secrete cytokines and are able to proliferate (Paul 1999). CD $8^+$ cells are predominant in the urothelium and CD $4^+$ in the lamina propria of the PBS/IC bladder (MacDermott et al. 1991).

## Urinary markers

Diagnosis of painful bladder syndrome/interstitial cystitis (PBS/IC) is based on clinical symptoms and exclusion of any confusable disease. Objective markers for PBS/IC would be of benefit in diagnosing PBS/IC, predicting the treatment response, and as an indicator of outcome. Many urinary markers have been studied in PBS/IC (**Table 2**). Beside those listed in **Table 2**, some other markers are also reported, but the data on these have not proved to be reproducible in later studies. A valid urinary marker should clearly distinguish PBS/IC from healthy controls with minimal overlap in these groups (Erickson 2001). Unfortunately, most of the markers are not specific for PBS/IC.

**Table 2**. *Urinary markers studied in PBS/IC.*

| Urinary marker | Expression in PBS/IC | references |
|---|---|---|
| MUC-1 glycoprotein | decreased | (Erickson et al. 1996) |
| Heparin binding epidermal growth factor | decreased | (Keay et al. 1997) |
| Glycoprotein-51 | decreased | (Byrne et al. 1999) |
| Nitric oxide synthase | decreased | (Smith et al. 1996) |
| Hyaluronic acid | increased | (Erickson et al. 1998, Wei et al. 2000) |
| Histamine | increased | (el-Mansoury et al. 1994, Yun et al. 1992) |
| Methylhistamine | increased | (el-Mansoury et al. 1994) |
| 1,4-methyl-imidazole acetid acid | increased | (Holm-Bentzen et al. 1987c) |
| Eosinophilic cationic protein | increased | (Lose et al. 1987) |
| Antiproliferative factor | increased | (Keay et al. 1996, Keay et al. 2000) |
| Epidermal growth factor | increased | (Keay et al. 1997) |
| Insulin-like growth factor | increased | (Keay et al. 1997) |
| Insulin-like growth factor-binding protein | increased | (Keay et al. 1997) |
| Kallikrein | increased | (Zuraw et al. 1994) |
| Autoantibodies | increased | (Keay et al. 1997) |
| Nitric oxide | increased | (Lundberg et al. 1996) |
| Nerve growth factor | increased | (Okragly et al. 1999) |
| Norepinephrine | increased | (Stein et al. 1999) |
| Interleukin-2 | increased | (Peters et al. 1999) |
| Interleukin-6 | increased | (Lamale et al. 2006, Peters et al. 1999) |
| Interleukin-8 | increased | (Peters et al. 1999) |
| Tryptase | increased | (Okragly et al. 1999) |
| Leukotriene E4 | increased | (Bouchelouche et al. 2001) |
| Eosinophil protein x | increased | (Bouchelouche et al. 2001) |

## Epidermal growth factor (EGF)

Epidermal growth factor (EGF) was found in urine as early as 1939, but not identified. It was noted, that extracts of human urine inhibited gastric acid secretion (Gray et al. 1939). This antisecretory factor was called urogastrone. EGF was discovered in 1962 in salivary glands of mice (Cohen 1962). Animal derived EGF was later observed to stimulate DNA synthesis not only in mouse fibroblasts but also in human cells (Hollenberg and Cuatrecasas 1973).

EGF is a 6 kDa molecule consisting of 53 amino acids. It is a heat-stable, single-chain polypeptide which is found in almost all body fluids in humans (Mattila 1989). EGF in blood was found in platelets which release the substance during coagulation or tissue trauma (Oka and Orth 1983).

The origin of urinary EGF is thought to be renal (Mattila 1989). High concentrations of prepro-EGF mRNA have been detected in the kidney, localized in the thick ascending limb of Henle (TALH) and the distal convoluted tubule (Harris 1991). However, it was shown later that bladder epithelial cells are also capable of expressing EGF (Keay et al. 2003).

EGF is known to promote wound healing (Laato 1988). This can be observed in vivo in animals when they lick their wounds. In this way, high concentration of salivary EGF is transported into the injured site and this promoted healing.

Between the ages 20 and 70 years, females express more EGF in urine than males (Mattila 1989). There is no significant difference between the urinary EGF levels of non-pregnant women and those of pregnant women (Watanabe 1990). Urinary EGF levels are reduced as a function of age, and normal values of urinary EGF should therefore take this parameter into account (Chou et al. 1997).

In PBS/IC, EGF in the urine may be elevated because of abnormalities in the growth factor it self or in its respective receptor. EGF may be a marker for physiological abnormalities that are, as yet, unknown in PBS/IC. APF is shown to upregulate the expression of EGF in bladder urothelial cells (Keay et al. 2000). Perhaps most likely, EGF may be elevated in response to epithelial damage and the need for reepithelialisation, but are not causally related to PBS/IC (Keay et al. 1997).

### *Urinary EGF in PBS/IC*

Elevated EGF concentrations have been detected in the urine in PBS/IC patients. EGF can be measured with routine, commercial available enzyme-linked immunosorbent assays. However, detection of urinary EGF is not currently used in diagnosing PBS/IC in the clinical setting.

The concentration of urinary EGF in PBS/IC patients was compared with that measured in bacterial cystitis patients and asymptomatic patients by Keay and collaborators (Keay et al. 1997). The mean concentration of immunoreactive EGF (22.9±3.2 ng/mg creatinine) was higher in PBS/IC-patients than in patients with bacterial cystitis (6.0±1.9 ng/mg creatinine) or asymptomatic controls (9.7±1.7 ng/mg creatinine). The urinary EGF levels reported in previous publications are listed in **Table 3**.

**Table 3**.

| Reference | Patients | EGF |
|---|---|---|
| (Keay et al. 1997) | Female IC/PBS patients n=50 (age not reported) | 22.9±3.2 ng/mg creatinine |
| | Female bacterial cystitis patients n =15 | 6.0±1.9 ng/mg creatinine |
| | Female asymptomatic controls n=31 | 9.7±1.7 ng/mg creatinine |
| (Zhang et al. 2005a) | PBS/IC patients with ulcer n=38 (Mean age 41±11.1 years, 36 females and two males) | 21.90±1.19 ng/ml |
| | PBS/IC patients without an ulcer n=26 (mean age 46±10.6 years, 23 females and 3 males) | 16.32±1.44 ng/ml |
| | Age and gender matched asymptomatic controls n=30 | 6.49±0.57 ng/ml |
| | Bacterial cystitis n=10 (mean age 36.4±8.8 years, 8 females and 2 males) | 6.32±1.26 ng/ml |
| (Keay et al. 2004) | IC/PBS men n=24 (mean age 52.8 ± 19.4 years) | 20.2±2.9 ng/ml |
| | Asymptomatic control men n=36 (mean age 47.0±11.1 years) | 15.3±1.6 ng ml |
| | Chronic CP/CPPS n=41 (mean age 45.7±13 years) | 13.9±1.4 ng/ml |

The mean urinary level of EGF is increased in patients with PBS/IC, but in individual cases there is overlapping compared with the concentrations in asymptomatic controls (Keay et al. 1997). So EGF measurement is therefore not an excluding tool in diagnosing PBS/IC. Measurement of urinary EGF levels has been used in the follow up of treatment response (Keay et al. 2007). 230 patients were enrolled in a prospective study comparing the clinical efficacy of six BCG instillations with placebo instillations given over a period of 10 weeks. Measurement of urinary EGF was repeated 34 weeks after of the first instillation. No changes were detected in the concentration of urinary EGF in the placebo group nor the BCG treated patients nor in subgroups of patients who had benefited from placebo or BCG therapy (Keay et al. 2007).

## Interleukin-6 (IL-6)

Interleukin-6 is a pleiotropic inflammatory cytokine that was cloned in 1986 (Hirano et al. 1986). At that time it was named B-cell stimulatory factor–2 (BSF-2) since it was capable of inducing final maturation of B cells to plasma cells. As independent groups described the same cytokine, different names were first assigned. Because they all were identified as the same molecule, the use of IL-6 was proposed and accepted.
IL-6 is produced by T cells, monocytes, macrophages and synovial fibroblasts (Van Snick 1990). IL-6 is involved in many biologic processes, such as T cell activation, induction of the acute phase responses, stimulation of the growth of haematopoietic precursor cells and in the proliferation of synovial fibroblasts (Van Snick 1990).
In many inflammatory diseases such as asthma, rheumatoid arthritis, psoriasis and inflammatory bowel diseases, IL-6 production is increased (Barnes and Karin 1997).

### *Urinary IL-6 in PBS/IC*

Urinary IL-6 has been studied as a biological marker for inflammation in the bladder. Lotz and co-workers showed that urinary IL-6 was detectable at higher concentrations (169.28 ± 90.91 pg/ml) in 71 patients with PBS/IC than in 20 asymptomatic controls (34.8 ± 6.35 pg/ml) (Lotz et al. 1994). It was also shown that IL-6 levels were lower in the urine collected from the ureters than in normally voided urine (Lotz et al. 1994). This supports the hypothesis that urinary IL-6 mainly originates from the bladder.
The existence of high IL-6 in urine was also demonstrated later. Erickson and co-workers showed that the more severe the inflammation detected in the bladder biopsies, the higher the probability of increased IL-6 concentration in the urine (Erickson et al. 1997). Peters and co-workers compared urinary cytokines of symptomatic PBS/IC patients with BCG treated patients and asymptomatic controls. They found out that, IL-2, IL-6 and IL-8 concentrations were higher in patients with active PBS/IC (Peters et al. 1999). Lamale and his study group (2006) reported the mean concentration of IL-6 to be higher in patients with PBS/IC (n=40) than in controls (n=29) (2.26±1.72 pg/ml versus 0.63±0.26 pg/ml). Human detrusor smooth muscle cells express and release monocyte chemoattractant

protein-1 (MCP-1) and IL-6 in vitro when stimulated with mast cell activators IL-4 and IL-13 separately or in combination with IL-1β and TNF-α (Bouchelouche et al. 2006).

Other urinary markers that are expressed differently in PBS/IC than in asymptomatic controls may also affect urinary IL-6 concentrations. HB-EGF concentrations are reduced in PBS/IC and HB-EGF has been shown to inhibit IL-6 expression in vivo (Rocourt et al. 2007). The increased histamine concentrations may also increase IL-6, as shown in synovial sarcoma cell lines (Wang et al. 2006).

The simultaneous changes in multiple urinary markers in PBS/IC are still probably independent findings without a causal relationship.

## Antiproliferative factor (APF)

First indirect evidence of an existing toxic factor in the urine of PBS/IC patients was gained when PBS/IC patients were observed to be more sensitive than normal controls to autologous or foreign urine from other PBS/IC patients in the skin patch test (Clemmensen et al. 1988). When human bladder cells were exposed to PBS/IC urine, in vitro inhibition of 3H-thymidine incorporation was seen as a sign of decreased cell proliferation (Keay et al. 1996). An antiproliferative factor (APF) was suggested to be responsible for this action. APF was found in 90% of patients with PBS/IC and in 9% of controls and it originated from bladder cells and not the kidneys (Keay et al. 1998, Keay et al. 1999, Keay et al. 2001). The APF is purified to be a frizzled 8 protein-related sialoglycopeptide growth factor inhibitor. Its bioactivities include suppression of urothelial cell proliferation, reduction of the production of HB-EGF in the urothelial cells and increase in transcellular permeability (Keay et al. 2003, Zhang et al. 2005b). The action of APF is postulated to be mediated by stimulation cell cycle regulatory protein p 53 (Kim et al. 2007). APF receptor is also detected in the cytoskeleton of the membrane of the urothelial cells (Conrads et al. 2006). APF levels decreased, as a marker of treatment response, in patients after percutaneous sacral third nerve neurostimulation (Chai et al. 2000).

# Diagnosing PBS/IC

The diagnosis of PBS/IC as a clinical syndrome requires that other etiological diseases be ruled out.
A complete history of the patient is the cornerstone of diagnosis, with special interest paid to previous pelvic diseases. Clinical examination of these patients includes inspection and palpation of the lower abdomen and the pelvic area, vaginal and rectal examination. A urine dipstick test should be carried out in order to rule out urinary tract infection, and urine cytology to rule out urothelial carcinoma (Nordling et al. 2004).
Cystoscopy is a strightforward examination to exclude diagnosis other than PBS/IC. Cystoscopy provides important information on the anatomy and volume of the bladder. When cystoscopy is carried out under anaesthesia, hydrodistension can be performed, and this may also have at least a temporary therapeutic effect on symptoms in half of the patients (Ottem and Teichman 2005). After hydrodistension, pathognomonic glomerulations in bladder wall can be seen, as required in the NIDDK criteria (Gillenwater and Wein 1988, Wein et al. 1990). Only with cystoscopy it is possible to differentiate classic PBS/IC from non-ulcerous form (Peeker and Fall 2002).
Bladder biopsies are included in a thorough evaluation, thus excluding carcinoma in situ, and these should be deep enough to be able to count detrusor mast cells (Nordling et al. 2004). The biopsy information is important in classifying patients into the various subgroups of PBS/IC. A classification which might have an impact on the choice of therapy (Leiby et al. 2007). Urodynamic tests are performed to find out if there is coexistent detrusor overactivity with PBS/IC. At least in the case of screening patients for studies where NIDDK criteria are obligatory, the urodynamic study cannot be omitted.

## Urinary diaries

Urinary diaries contain basic information on voiding patterns, and no diagnosis of PBS/IC can be made without them.
This tool is essential in defining the severity of symptoms. It contains data on urinary frequency at day-time or night-time, 24-hour urine production, maximum and minimum single voided urine volume, by calculation, mean voided volume, and intake of fluids (Abrams et al. 2002).
Patients should be properly instructed on how to keep diaries. They are usually kept for two or three days, but longer recording periods are also proposed. A three-day diary proved to be superior to seven-day diary, due to lack of compliance in making diary entries for a longer period (Tincello et al. 2007). Furthermore, a retrospective in 305 patients showed that three-day urinary diaries can be reduced to a single day (Mazurick and Landis 2000).

## Symptom questionnaires

Successful treatment of PBS/IC is defined as objective benefit from given therapy. This can be measured by simple global response assessment (GRA) or by more profound questionnaires of different symptoms in which the severity of symptoms is quantified.
Different questionnaires are used in reporting the treatment effect. Holm-Bentzen and co-workers asked the patients to express a numerical value from 1 to 5 for pain, frequency, nocturia, and dysuria. A mean of the total sum constituted the symptom score (Holm-Bentzen et al. 1987b). In another study, the score for urgency, frequency, nocturia, and pain was defined such that the patients graded their symptom severity freely at the beginning of the study and then expressed the symptom improvement as 0%, 25%, 50%, 75% or 100% at the control visits (Parsons and Mulholland 1987).

The first validated symptom questionnaire for PBS/IC was introduced in 1994 (Keller et al. 1994). In this pilot study, questions were asked on seven different symptoms including burning sensation in the bladder, urgency to urinate, going to the bathroom frequently during the day, bladder discomfort, bladder pain, getting up at night to go to bathroom, and difficulty in sleeping because of bladder problems. The response options were graded (from 0 = not at all to 6 = a lot). Only 10 patients with PBS/IC and 7 asymptomatic controls participated in the validation procedure, which greatly decreases its value.

### The O`Leary-Sant questionnaire (Interstitial Cystitis Symptom and Problem Indices)

As none of the previously presented symptom questionnaires had gained popularity, guidelines for validating symptom indices in urology were presented and it was concluded that these questionnaire instruments must be subjected to scientific testing before taken into practice (O'Leary et al. 1992). An adequately validated questionnaire was introduced in 1997 (appendix) (O'Leary et al. 1997). The symptom questionnaire was not designed to act as a screening tool for patients, however, it appears to function in discriminating patients with PBS/IC and those without it. Only 10% of PBS/IC patients have symptoms so mild symptoms that they are expected to score 6 points or less out of maximum points 20, and 16 points in the symptom and problem indices (O'Leary et al. 1997). The O`Leary-Sant questionnaire was intended to be particularly useful in clinical trials of new therapies for PBS/IC and is recommended for use in such trials (Lubeck et al. 2001). Ever since, the O`Leary-Sant questionnaire has been used in the majority of prospective intervention studies. It has been is suggested that the questionnaire should be revised, as it underestimates the prevalence and degree of urgency in PBS/IC patients (Diggs et al. 2007).
The original and the Finnish version of the O`Leary-Sant questionnaire are shown in the appendix.

## Visual analogue scale (VAS) for pain

A VAS scale is a simple tool to define and quantify patient's pain. The VAS scale has been validated for chronic and in acute thermic pain (Price et al. 1983) and in different medical conditions. However, it has not been validated in PBS/IC.

The patient is asked to express the worst pain experienced from a scale from 0 to 10 cm. The worst pain can be assessed since yesterday, since last week or recently. The patient can also mark the least pain experienced. That is suitable in situations when the pain is consistent.

Questions on pain are also included in the O`Leary-Sant questionnaire, but they make up only one fourth of questions. Pain is a major, and the most prevalent, complaint in the symptoms of PBS/IC (Bogart et al. 2007, O'Leary et al. 1997) and the evaluation of treatment effect should include specific assessment of pain.

## Potassium sensitivity test (PST) / KCL-test

A previous study showed that the bladder capacity in rats was decreased by higher urinary concentrations of potassium ions (Hohlbrugger and Lentsch 1985). It was postulated that the altered permeability to intravesical cations triggers the symptoms in patients with PBS/IC, but not in controls who exhibit normal permeability. The increased permeability could permit the depolarisation of sensoric nerve endings leading to decreased bladder capacity and pain related to bladder filling. The increased bladder permeability in PBS/IC is suggested (Parsons et al. 1991), but this theory is also challenged (Chelsky et al. 1994). Abnormal sensitivity to intravesical potassium was shown in 70% of 33 patients with PBS/IC, in 100% of four radiation cystitis patient, in 18% of 11 PBS/IC patients treated successfully with pentosan polysulfate and in 9% of 22 asymptomatic controls (Parsons et al. 1994). Abnormal sensitivity was defined as a volume of 45 ml of 0.4 M KCL which, when instilled intravesically, provoked more pain or urgency compared with the same volume of water. For the details in conducting of this test see Methods.

Retrospective analysis showed that patients with positive PST were more likely to respond to treatment with a tricyclic antidepressant combined with intravesical heparin or oral pentosan polysulfate (Teichman and Nielsen-Omeis 1999). In this particular study, only 61% of patients were PST positive. Gregoire and co-workers (2002) reported a positive PST rate in 83%, negative PST in 13% and an equivocal test in 4% of 189 patients with PBS/IC.

The PST test provokes intensive pain in some patients, which can make it poorly tolerable. A modified test with 0.2 M KCL enhances the tolerability of PST (Daha et al. 2003). Both concentrations test the same phenomenon and they are not specific for PBS/IC. The test does not help to differentiate PBS/IC from detrusor overactivity (Philip et al. 2006) and the test is positive in bacterial cystitis or radiation cystitis. In addition, men with prostatitis often react with a positive PST (Hassan et al. 2007). It has been proposed that intravesical potassium may not simply act on urothelial sensory nerve endings; it may also stimulate detrusor muscle contraction (Philip et al. 2007).

Overall, the rational of PST and its usefulness in diagnosing PBS/IC or predicting treatment outcome has not yet been demonstrated, and it is considered an optional tool in the assessment of PBS/IC (Nordling et al. 2004).

## Global response assessment (GRA)

Global response assessment (GRA) was published as a treatment outcome indicator in a controlled trial in patients with PBS/IC in 1993 (Parsons et al. 1993). In this study, patients were allowed to choose one category of symptom change which best reflected their subjective opinion. Categories included symptoms getting worse, no change (0%), slightly better (25%), moderately better (50%), greatly better (75%), or symptoms are gone (100%). Successful therapy was defined as at least a 50% improvement in the symptoms. Since the report, GRA has been used as a primary end point of treatment efficacy in many trials (Propert et al. 2002). In some trials a centered 7-point scale is used which include three categories for symptom worsening, one neutral and three categories symptom improvement (Sant et al. 2003).

The symptom questionnaires are responsive to change overtime as measured by GRA in patients with PBS/IC. A 1.2 point change in the O`Leary-Sant index corresponded to a one-category change in the five category GRA (Propert et al. 2006). It is recommended that in future GRA should also be used as a primary outcome parameter in clinical trials and a validated symptom questionnaire should be used as a secondary outcome parameter to evaluate individual symptoms (Propert et al. 2002, Propert et al. 2006).

## Quality of life of patients with PBS/IC

The quality of life (QoL) in PBS/IC patients is usually measured by common validated questionnaires which cover the most important and easily evaluated aspects of life. There is no specific questionnaire for PBS/IC and therefore generic forms like SF-36, Rand-36 or MOS-36 are mainly used (Michael et al. 2000, Peters et al. 1997, Rothrock et al. 2003, Simon et al. 1997). According to Simon and co-workers (1997), PBS/IC patients with more severe symptoms had impaired capacity to cope with basic daily routines compared with patients with less severe symptoms. Women with PBS/IC have greater differences in vitality and mental health than women with rheumatoid arthritis or hypertension (Michael et al. 2000). The impaired QoL is due to pain, which is the most prevalent symptom (Simon et al. 1997). As expected, pain has an impact on the QoL despite its localisation (Bech 1999, Buskila 2001, Solomon 1997).

Patients with PBS/IC have more depressive symptoms compared with healthy controls (Rothrock et al. 2002), and sexual problems are common (Keltikangas-Järvinen et al. 1988, Nickel et al. 2007). After a thorough psychometric analysis of 31 PBS/IC patients, it was concluded that psychotic or neurotic symptoms were not important in the course of PBS/IC, but the personality of PBS/IC patients differs from non-PBS/IC patients (Keltikangas-Järvinen et al. 1988). Female PBS/IC patients who cope with PBS/IC related

symptoms by catastrophizing have impairments in various aspects of QoL (Rothrock et al. 2003). It is reported that females and males have different strategies for coping with different diseases and the treatment outcome differs between the genders (Jensen et al. 2001, Moons et al. 2003, O'Dea et al. 1999); this calls for separate questionnaire for both genders. The validated generic questionnaires have been shown to work well in different pain syndromes. However, there is concern that issues of importance to patients are missed and a disease specific questionnaire would be of great value in evaluating patients with pelvic pain (Neelakantan et al. 2004).

# Treatment of PBS/IC

PBS/IC is a chronic disease with unpredictable treatment response. Curative treatment is not available, if cystectomy is not regarded as such. As the etiology of the disease is elusive and probably multifactorial, treatment guidelines are of limited benefit only.
In literature, a great number of different treatment strategies are proposed. The treatments recommended to patients depend on the individual physician. The treatment is usually initiated with easily available treatments which are then upgraded into more complex treatments if the disease is found to be refractory. In Interstitial Cystitis Database Study (ICDB) a total of 183 different treatments were reported to be prescribed (Rovner et al. 2000). The guidelines on chronic pelvic pain from the European Association of Urology (EAU) include treatments with analgesics, corticosteroids, anti-allergics, amitriptyline, PPS, antibiotics, prostaglandins, L-arginine, immunosuppressants, anticholinergics and gabapentin, intravesical heparin, intravesical hyaluronic acid, intravesical DMSO, intravesical BCG, intravesical clorpactin, and intravesical vanilloids (Fall et al. 2007). Surgical treatments, such as bladder distension, transurethral resection of the bladder, or reconstructive surgery are also included in the EAU urological guidelines.

278 randomized clinical trials comparing pharmacological treatments were found in a systematic review of the literature since 1966. Of those 278 studies only 21 (7.6%) met the demands (controlled/placebo controlled trial, randomization, prospective study, adequate report of treatment response, disease defined, more than 10 patients included in treatment arms) of critical analysis (**Table 4**) (Dimitrakov et al. 2007).

**Table 4**. *Randomized controlled trials in the treatment for PBS/IC.*

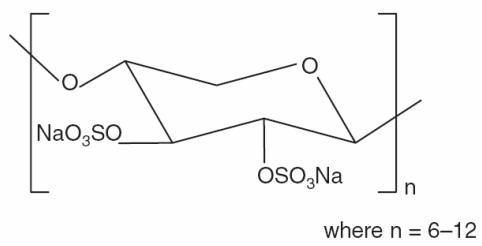
| Treatment | Number of patients | reference |
|---|---|---|
| Amitriptyline | 50 | (van Ophoven et al. 2004) |
| Antibiotics | 50 | (Warren et al. 2000) |
| BCG, intravesical | 319 | (Mayer et al. 2005, Peeker et al. 2000c, Peters et al. 1997) |
| Cimetidine | 36 | (Thilagarajah et al. 2001) |
| DMSO, intravesical | 54 | (Peeker et al. 2000c, Perez-Marrero et al. 1988) |
| Hydroxycine | 121 | (Sant et al. 2003) |
| L-Arginine | 69 | (Cartledge et al. 2000, Korting et al. 1999) |
| Oxybutynine, intravesical | 36 | (Barbalias et al. 2000) |
| Oxygen, hyperbaric | 21 | (van Ophoven et al. 2006) |
| PPS | 569 | (Holm-Bentzen et al. 1987b, Mulholland et al. 1990, Parsons and Mulholland 1987, Parsons et al. 1993, Sant et al. 2003) |
| PPS, intravesical | 20 | (Bade et al. 1997) |
| Resinaferotoxin, intravesical | 203 | (Chen et al. 2005, Lazzeri et al. 2000, Payne et al. 2005) |

## Pentosan polysulfate sodium

Pentosan polysulfate sodium is a semi-synthetically produced heparin-like macromolecular carbohydrate derivative which, chemically and structurally, resembles glycosaminoglycans (**Figure 1**). It has a mean molecular weight of 4,7 kDa. Pentosan polysulfate is obtained from an extract of European beech wood (*Fagus sylvatica*). The hemicellulose is isolated from the wood and is then subjected to sulphate esterification using chlorosulfonic acid (Ghosh 1999). PPS was previously used as an anticoagulant to prevent thromboembolic complications (Bergqvist and Ljungner 1981), but it is no longer used regularly for this indication as more effective drugs are available. However, PPS has been investigated, mainly in animal models, in prion diseases, neuronal ischemia, prevention of urinary tract calcium stones and in the treatment of malignant tumours (Barthlen et al. 2003, Jones and Monga 2003, Kocisko et al. 2006, Parry et al. 2007, Sakurai-Yamashita et al. 2006). These studies have not led to clinical applications.

PPS has no effect on pharmacokinetics of warfarin (Modi et al. 2005). Interactions with other drugs are not studied. The bioavailability of PPS is low 0.5–1% (Simon et al. 2005). Only 3% of PPS is eliminated unchanged in the urine, while more than half of the drug is delivered unchanged to faeces (Simon et al. 2005). PPS is desulfated in liver and in spleen and caution is recommended in the use of PPS when a liver or spleen disorder is known (Anderson and Perry 2006).

***Figure 1***. *Chemical structure of PPS*




## Mechanism of action of PPS in PBS/IC

The proposed mechanism of how PPS might be benefit patients with PBS/IC involves its capability to bind to the bladder wall mucus. PPS is a sulphated polysaccharide, structurally resembling the GAG-rich bladder surface mucus. Intravesical PPS was shown to replace normal bladder GAG layer as bacterial adherence barrier in an animal model, in which normal mucin layer of rabbit bladder was destroyed with acid (Parsons et al. 1980). In an animal model, PPS was resisted bladder washings (Odlind et al. 1987). PPS reduced the urea uptake in mucin-deficient bladders in rabbits by restoring the epithelial permeability-barrier function (Nickel et al. 1998).
Another postulated mechanism of PPS action is the ability to inhibit mast cell secretion, which was shown in the rat peritoneal mast cell culture (Chiang et al. 2000). PPS may inhibit unspecified stimulants of bladder inflammation and, in this way, indirectly reduce urothelial responses to inflammatory stimuli (Sadhukhan et al. 2002).

## Pentosan polysulfate sodium (PPS) in the treatment of PBS/IC

The replacement therapy of GAG layer with oral PPS was introduced in 1983 (Parsons et al. 1983). In this pilot study, 24 patients with PBS/IC were treated for 6 to 24 months with a dose of 50 mg 4 times a day or 150 mg twice a day. An excellent response was reported in 71% of the patients (80% decrease in pain, nocturia, and urinary urgency).
Since then, five placebo controlled trials have evaluated the efficacy of PPS. The first one, published as an abstract (Squadrito et al. 1985), reported that only 1 patient out of 12 responded to PPS treatment. Neither a statistically or clinically significant effect of PPS (200 mg twice a day for 4 months) was found in comparison with placebo in 115 patients with painful bladder disease (Holm-Bentzen et al. 1987b). Parsons and Mulholland randomized 62 patients to 3 months PPS treatment with 100 mg three times a day, 200 mg twice a day, or placebo. The patient selection also included patients who had no pain related to the bladder. PPS had no effect on urinary diaries and was not significantly superior to placebo for the parameters urgency, frequency, or nocturia. PPS had significantly more impact on pain, as 44% of patients in PPS arm and 15% in the placebo

arm reported at least 50% decrease in pain (Parsons and Mulholland 1987). Non-responders had a tendency to have more severe symptoms than responders.

Mulholland and associates randomized 110 patients to either 100 mg PPS three times a day or placebo for three months (Mulholland et al. 1990). A global response assessment (GRA) was obtained at three months and patients could choose from no change, slight improvement, moderate improvement, great improvement, or to be completely cured. Patients choosing one of the three latter categories were considered to be responders. At three months, 28% of PPS treated patients and 13% of placebo treated patients responded according to GRA ($p = 0.04$). No change in urinary diaries was seen in either treatment. The effect on pain was statistically equal with PPS or placebo.

In a multicenter study, 148 patients were randomized to either PPS (100 mg three times a day) or placebo. At three months, 32% of patients in PPS group responded to treatment according to GRA compared with 16% of patients in placebo group ($p=0.01$) (Parsons et al. 1993). PPS showed benefit in patient's self evaluation for the parameters pain and urgency but failed to affect the urinary diaries statistically.

The NIDDK criteria were not used in any of the above mentioned five studies for patient selection. Only in the study by Mulholland were the patients obviously comparable with those fulfilling the NIDDK criteria.

The long term effects of PPS therapy outside randomized trials are somewhat less favourable than those reported from the trials. It is reported that in the long run, between 6.2% and 18.7% of patients with PBS/IC benefit from PPS (Jepsen et al. 1998).

PPS is well tolerated. The side effects are usually related to the alimentary system. In a 32-week dose-finding study with 380 patients the side effects were dose dependent. Of those who received a daily dose of 300 mg, 14.9% had diarrhoea, and in those who received a daily dose of 900 mg the percentage was 40.4% (Nickel et al. 2005). Other common side effect included abdominal pain, dyspepsia, nausea, and alopecia. Increasing the PPS dose from 300 mg/day to 900 mg/day did not improve the clinical effect. It was concluded that the duration of the treatment is more important than the dose (Nickel et al. 2005). It is generally suggested that treatment with PPS should be carried out for six months before conclusions are drawn on its efficacy. In addition, the placebo effect apparent at the beginning of a trial may be less evident at six months.

Recently, an idea on simultaneous therapy with both oral and intravesical PPS was introduced (Davis et al. 2008).

In September 1996, PPS received approval from the FDA for use in the treatment of PBS/IC (Manhattan 2001).

## Cyclosporine A

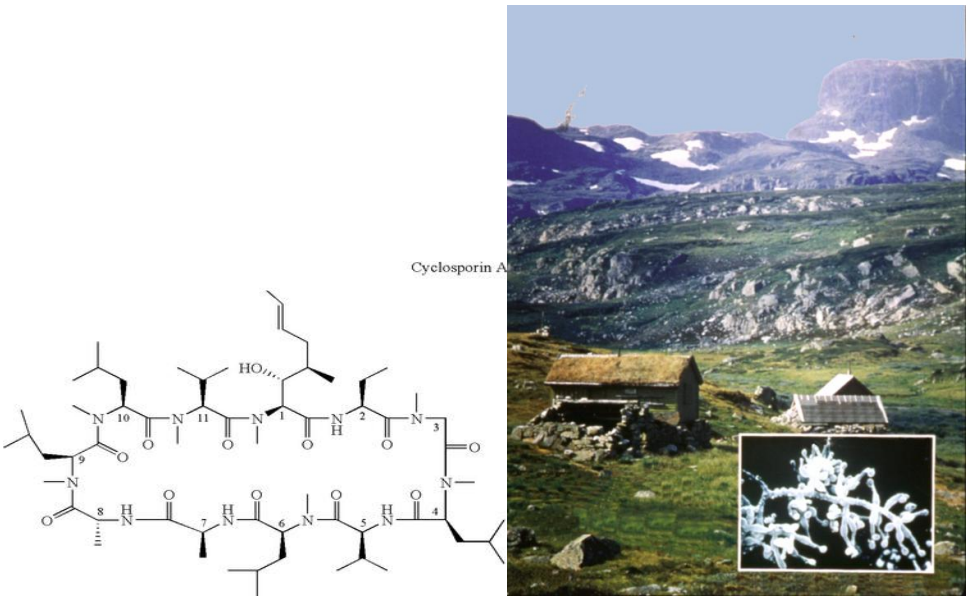
For decades the pharmaceutical companies have collected soil samples from all over the world to screen soil for interesting new drugs (Borel and Kis 1991). In September 1969, an employee of a Swiss company collected soil samples from Norway including samples from a mountain plateau Hardangervidda, 1200 m above sea level (Figure 2.). He returned to Switzerland carrying the soil in a plastic bag. A fungus named Tricoderma polysporum

was discovered in the soil sample. Later, the fungus was found to be Tolypocladium inflatum, which had been first described in Denmark in 1916. Tolypocladium inflatum is a widespread fungus, and was later also isolated from soil samples in the USA, Sweden, Canada, and Nepal.

Cyclosporine A (CyA) was originally isolated from a fungus collected from Wisconsin, US, but the Norwegian fungus was used in industrial development of the drug (Svarstad et al. 2000).

CyA is a cyclic polypeptide consisting of 11 amino acids (MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-(D)Ala-MeLeu-MeLeu-MeVal). It has a molecular weight of 1202.6 Daltons and its molecular formula is $C_{62}H_{111}N_{11}O_{12}$ (**Figure 2**).

**Figure 2**. *Chemical structure of CyA and the area where Tolypocladium inflatum was found.*




Hargangervidda, Norway. 1200 m above the sea level

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The immunosuppressive properties of CyA were first demonstrated by a delay in skin graft rejection in mice and graft-versus-host disease in mice and rats (Borel et al. 1976). Clinical use of cyclosporine began in 1978 in the context of renal cadaveric transplantation in patients with renal insufficiency (Calne et al. 1978) and in graft-versus-host disease after bone-marrow transplantation from sibling donors (Powles et al. 1978). CyA may still be used to prevent graft-versus-host disease, but newer, safer drug regimens have replaced it to a large extent.

CyA is a calcineurin inhibitor that interferes with T-lymphocyte action. Antigen presentation to T cell results in an increased intracellular calcium level. Calcineurin, which is calcium and calmodulin-dependent phosphatase, is activated. The nuclear factor of activated T cells becomes dephosphorylated by calcineurin. It then translocates into the nucleus and stimulates production of cytokines (Ruhlmann and Nordheim 1997, Wiederrecht et al. 1993). CyA binds to cyclophilin protein and this complex inhibits the

dephosphorylation of nuclear factor by binding to calcineurin. Secretion of cytokines such as interleukin-2 (IL-2), IL-3, IL-4, tumor necrosis factor-α and interferon-γ from T lymphocytes is inhibited.

The therapeutic window for CyA is narrow. Below the therapeutic range the immune system may not be sufficiently suppressed and excessive levels of CyA may lead to adverse events due to the intra- and inter-individual variations in the bioavailability of CyA (Kahan 2004).

CyA is widely distributed in the whole body, concentrations in the liver, kidney, endocrine glands, and skin are exceeding those in the blood or plasma (Maurer et al. 1984). The drug is metabolised to at least 30 metabolites. Elimination is mainly biliary. 6% of each dose of CyA is excreted to urine, and only 0.1% is in urine as unchanged drug (Dunn et al. 2001). Reports of the exact concentration of CyA in the bladder do not exist, even though the distribution of CyA in various other organs has been reported (Boland et al. 1984).

CyA has been shown to augment the relaxation response of the bladder to isoproterenol, and the contractile response of the urethra to phenylephrine and clonidine (Kitani et al. 1996). This animal model was used to show whether CyA is responsible for increased urethral resistance, thus causing urinary disturbance. In another animal model, urinary outlet obstruction was created to 16 rabbits leading to secondary detrusor decompensation. Rabbits received CyA after the procedure and as a result the hypertrophic bladder mass decreased and expression of atriac natriuretic factor-mRNA decreased together with normalization of myosin heavy chain A to B ratios (Clement et al. 2006). CyA was further shown to inhibit stress induced activation of protein kinases (c-Jun $Nh_2$-terminal kinase) in an animal model - acting as a blocker of Ca2+ ions – showing direct action to bladder smooth muscle cells (Kushida et al. 2001).

## Cyclosporine A in autoimmune diseases

All chronic autoimmune diseases are characterized by the presentation of an autoantigen by the antigen presenting cells to the autoreactive T cells (Sykulev et al. 1996). After this, the cascade of activation of co-stimulatory receptors leads to gene activation and synthesis of pro-inflammatory cytokine. The antigen presenting cells may belong to different cell lineages (macrophages, B cells or dendritic cells) in different tissues (Shlomchik et al. 2001). In autoimmune diseases, the autoimmune response can extend to intermolecular as well as to intramolecular epitopes, as T cells lose reactivity to the initial epitope (Ferraccioli et al. 2005). IL-2 and interferon-γ synthesis is crucial in all autoimmune diseases, whether T or B cell dependent. CyA may help to keep chronic autoimmune diseases under autoimmune threshold through inhibition of IL-2 and interferon-γ and by downregulating CD40L on T-cells (von Herrath and Harrison 2003). This mechanism of action makes CyA an attractive drug for the treatment of autoimmune diseases. CyA has been used with variable success in almost all autoimmune diseases (Ponticelli 2005). Psoriasis, atopic dermatitis, rheumatoid arthritis, uveitis, systemic lupus erythematous,

sympathetic ophtalmia, Sjögren's syndrome, and nephrotic syndrome are the most frequent indications for CyA use.

The initial dose of CyA used in the treatment of autoimmune diseases should not exceed 5 mg/kg/day. The dose should then be reduced to a maintenance dose of 2-3 mg/kg/day, or to the lowest dose at which the symptoms are still under control. By keeping the dose low, the safety of the drug is increased.

Cyclosporine is a substrate of the cytochrome enzyme P450 CYP3A4 and interacts with many other drugs (Campana et al. 1996). Of special concern are those drugs that increase the nephrotoxicity of CyA. These include diltiazem, verapamil, nicardipine, ketoconazole, erythromycin, clarythromycin and high dose methyl prednisolone (Ponticelli 2005). CyA may also increase the concentration of other drugs. Such important interactions of CyA include the increase in digoxin concentrations (Dorian et al. 1988) and the exacerbation of complications with lipid lowering medication (Neuvonen et al. 2006). In the treatment of autoimmune diseases, the CyA blood levels may be decreased without compromising its therapeutic effect; a phenomenon which allows dose reduction of CyA when drug interactions are suspected.

## Adverse effects of CyA therapy

Nephropathy is an important potential adverse effect of cyclosporine therapy. Nephrotoxicity is dose dependent and patients should be checked regularly. The higher the serum creatinine, the higher the risk of severe renal toxicity. The risk of nephrotoxicity is minimal if the increase of creatinine is less than 20–30%. Nephrotoxicity increases as serum creatinine increases and lowering of the dose or even withdrawal of CyA is advised in cases where the increase over the baseline serum creatinine level is greater than 30% (Feutren and Mihatsch 1992). With careful monitoring of the patient's creatinine values, nephrotoxicity can be limited to reversible changes such as tubulopathy. Irreversible changes follow longer exposure to CyA in patients with impaired renal function, leading to histological changes in the kidneys (Mihatsch et al. 1995).

The most common adverse effects of CyA are listed in **Table 5**.

**Table 5**.

| Side effects reported in more than 10% of patients | Side effects reported in more than 1% but less than 10% of patients |
|---|---|
| Renal function impairment | Paresthesia |
| Hypertension | Nausea, abdominal pain, diarrhoea |
| Tremor | Gingival hyperplasia |
| Headache | Hepatotoxicity |
| Hyperlipidemia | Hyperurikemia, hyperkalemia, hypomagnesemia |
| | Hirsutism |
| | Fatigue |
| | Myalgia |

Cyclosporine has not been shown to be mutagenic, but a higher rate of lymphoproliferative disorders and skin malignancies are observed in organ-transplant recipients treated with cyclosporine A than in the general population (Cockburn and Krupp 1989).

## Dimethyl sulfoxide

Dimethyl sulfoxide (DMSO) is a by-product of wood pulping. It is a compound with the chemical formula $(CH_3)_2SO$. DMSO is an efficient solvent for water insoluble compounds and is a hydrogen bond disrupter (Santos et al. 2003). DMSO has many different applications in the chemical industry and also has various pharmacological applications. Furthermore, DMSO is used in cryopreservation of hematopoietic stem cells and as a fixative in electron microscopy (Egorin et al. 1998, Fassel et al. 1997).
The clinically interesting pharmacological applications of DMSO include treatment of systemic and local amyloidosis (Iwasaki et al. 1994, McCammon et al. 1998) and topical treatment of cutaneous herpes zoster and other dermatological disorders (Bertelli et al. 1995, Swanson 1985). New indications are currently under investigation.

### DMSO in the treatment of PBS/IC

The use of DMSO in PBS/IC was first introduced in 1967 (Stewart et al. 1967) and later a success rate of 65% was reported after DMSO instillations (Stewart et al. 1972).
The FDA approved DMSO in the treatment of PBS/IC in 1978 on the basis of the data obtained in open-label, prospective trials (Parkin et al. 1997).
The only placebo controlled trial with DMSO showed marked improvement in PBS/IC symptoms in 53% of 33 patients treated with DMSO, whereas 18% of patients benefited from placebo (Perez-Marrero et al. 1988). In a randomized, double-blind study with DMSO and BCG, DMSO had a significant effect on pain, and in a subset of patients with ulcerous disease, on urinary frequency (Peeker et al. 2000c). This study did not report the overall success rate of either treatment and did not include a validated symptom questionnaire.

The mechanisms of action by which DMSO is beneficial in PBS/IC are partly unknown. The effect is not related to histamine release by mast cells (Stout et al. 1995). DMSO has the ability to dissolve compounds like amyloid and collagen, which may play a role in the treatment of PBS/IC (Melchior et al. 2003). DMSO has an anti-inflammatory effect by inhibiting intracellular hydroxyl radicals and interleukin-8 in whole blood (DeForge et al. 1992). This anti-inflammatory action may be useful in PBS/IC. DMSO can alter the ATP release of urothelial cells in PBS/IC by blocking in vitro the stretch induced expression of this purinergic nocio-neurotransmitter (Sun and Chai 2002). DMSO is further shown to modulate the nerve conduction of C-fibers that are responsible for the pain sensation. DMSO concentration of 9% was the minimum dose that interfered C-fiber sensitivity when DMSO was applied directly to exposed peripheral nerves (Evans et al. 1993).

Higher concentrations of DMSO may result in harmful, irreversible changes in bladder smooth cells, thus causing fibrosis, and it is advised that the usual DMSO concentration of 50% be reduced to 25% for the treatment of PBS/IC (Melchior et al. 2003).

## Bacillus Calmette-Guerin

In the period 1908-1921, Calmette and Guerin achieved passage of a strain of Mycobacterium bovis, the pathogen of bovine tuberculosis. They researchers developed a live vaccine against human tuberculosis. It consisted of several substrains of the original strain, which are now known as BCG (Brandau and Suttmann 2007). Observations of occasional remissions of lymphosarcomas following systemic bacterial infection and the low prevalence of malignant neoplasms in patients with tuberculosis, inspired efforts to treat carcinomas with BCG (Mathe et al. 1969). In 1976, BCG was introduced as intravesical treatment of non-muscle invasive bladder carcinoma (Morales et al. 1976). Today the following indications for BCG therapy in superficial bladder carcinoma are widely accepted: Adjuvant induction cycle for intermediate risk tumors, adjuvant induction cycle plus maintenance therapy for high-risk tumors, induction cycle plus maintenance therapy for primary treatment of carcinoma in situ (Oosterlinck et al. 2004).

Intravesical BCG is an immunotherapy in which the normal cells are activated to attack cancer cells. In response to BCG stimulation, urothelial cells secrete pro-inflammatory cytokines (de Reijke et al. 1993). A complex inflammatory cascade is followed after instillation of BCG leading to induction of neutrophils, monocytes, and lymphocytes. Attracted immunocompetent cells form granuloma-like structures in the bladder wall (Bohle et al. 1990). With repeated instillations, these granuloma-like infiltrates progress for several weeks. In time, cytotoxic CD4 and CD8 cells as well as Natural Killer (NK) cells are activated, and these are essential for eliminating the bladder tumor (Brandau and Suttmann 2007).

### BCG in the treatment of PBS/IC

Use of intravesical BCG in the treatment of PBS/IC was first reported in 1994 (Zeidman et al. 1994). In this report, five patients had benefited from BCG added to standard therapy. Later, two placebo controlled, randomized evaluated the effect of BCG on PBS/IC. In the first study involving 15 patients, the response rate for BCG was 60%, which was not significantly different from that of placebo (Peters et al. 1997). In the second study with 131 patients in BCG arm, the response rate for BCG was 21%, which was equal to placebo (Mayer et al. 2005).

The mechanism of induction of immunological inflammation after BCG instillation is the same in PBS/IC as in bladder carcinoma. The reason why this is beneficial in PBS/IC is unknown.

# AIMS OF THE STUDY

There is no known medical therapy with a major impact on the symptoms of painful bladder syndrome/interstitial cystitis when the disease is refractory. Therefore, ideas for new treatment options are needed. Treatment with a potent anti-inflammatory agent, immunosuppressant cyclosporine, has been previously tested in a non-randomized, open, pilot trial with good clinical effect.

The promising results of CyA therapy encouraged us to continue use of this agent, first in selected patients and then in the context of a prospective, randomized, comparative trial.

The specific aims of this study were

i. to evaluate the long-term results of CyA therapy on PBS/IC related symptoms in patients who had received CyA therapy for at least one year.

ii. to compare the clinical effect and safety of CyA therapy with an approved treatment, pentosan polysulfate sodium (PPS), in a randomized, unblinded trial.

iii. to evaluate the treatment effect of CyA and PPS on the potassium sensitivity test (PST) in order to obtain further evidence of the drug related alleviation of symptoms and to test whether conducting repeated PST is of any value.

iv. to measure the concentrations of urinary biomarkers, epidermal growth factor (EGF), and interleukin-6 (IL-6) in PBS/IC and to compare the changes with their pre- and post-treatment levels after CyA or PPS therapy.

v. to measure the impact of CyA, PPS, DMSO and BCG therapy on the health-related quality of life questionnaire and to evaluate the usefulness of that questionnaire in PBS/IC.

# METHODS

## PATIENTS AND METHODS (I)

The patients included in the various parts of this thesis study were patients with painful bladder syndrome/interstitial cystitis (PBS/IC) who visited a urologist in several community hospitals. For publication I, no systematic recruitment for a prospective study was performed. From 1993 to 2001, patients attending the urological departments of Helsinki University Hospital, Kymenlaakso Central Hospital (Kotka), or Päijät-Häme Central Hospital (Lahti) were offered cyclosporine (CyA) therapy for PBS/IC after failure of other conventional conservative treatments. Of the original 26 patients, three refused to start cyclosporine therapy. Publication I is a retrospective analysis on 23 patients (20 females and 3 men) all of whom received CyA therapy for at least one year (**Table 6**). All patients fulfilled the NIDDK criteria of the PBS/IC.

**Table 6**. *Demographics of the 23 patients in publication I. Values are mean±(SD).*

| | |
|---|---|
| Age of the patients ± SD | 65±7.6 years |
| Months on CyA therapy | 60.8±36 months |
| PBS/IC symptoms lasted before starting CyA | 64±56 months |
| Bladder capacity in anaesthesia before CyA | 449±187 ml |
| Mean voided volume before CyA | 101±43 ml |
| Maximal single voided volume before CyA | 160±76 ml |

Usual failed treatments the patients had before were: Hydrodistensions, DMSO, pentosan polysulfate sodium, oxychloroquine, intravesical heparin, intravesical cortisone, buprenorphine, alpha-blockers, urethral Hegar dilatations, transcutaneous neutrostimulation, anticholinergics and intravesical BCG-instillations.

All patients had normal renal function based on determination of serum creatinine and creatinine clearance. They also had normal liver function and blood pressure. None had a history of malignant tumor. Any suspicion of a contraindication for cyclosporine treatment ruled out participation of the patient.

Patients were informed about the potential side effects before starting CyA therapy. Control visits were carried out by the same urologists at intervals of 4 weeks for the first three months. If treatment was without complications, the control intervals were gradually lengthened up to one year. At every control visit, blood pressure, serum creatinine, and cyclosporine concentration were measured. If the aminotransferase values were normal after one year, they were checked thereafter only occasionally. Voiding diaries of the previous two days, subjective analysis of pain, concomitant medication, and side effects were recorded at every control visit.

Maximum bladder capacity was defined as the largest single voided volume according to the voiding diaries. Severity of pain was estimated on the basis of the patient's verbal comment on this parameter. Patients were allowed to use additional pain medication, but no other treatment for PBS/IC was permitted during follow-up.

The initial cyclosporine dose was 3 mg/kg divided into two daily dosages. When symptoms were alleviated, the dose was lowered gradually to as low as 1 mg/kg, given as a single daily dose. If patients became asymptomatic, there was no pain, and the bladder capacity improved, they were offered the option of stopping the medication. If the symptoms reappeared the medication was restarted.

## PATIENTS AND METHODS (II-III)

Patients reported in publications II and III are patients who were recruited to a prospective, randomized, multicenter study comparing the clinical effect of CyA and pentosan polysulfate sodium (PPS). The study protocol was approved by the Ethics Committee of Helsinki University Hospital. For publication II, 64 patients meeting the NIDDK criteria for PBS/IC were randomized in a 1:1 ratio to either CyA (Sandimmun Neoral ®, Novartis Ringaskiddy Ltd., Cork, Ireland) or to PPS (bene-Arzneimittel GmbH, Munich, Germany) treatment for 6 months. The CyA dose was 3 mg/kg divided into two daily doses, while that of PPS was 100 mg t.i.d. Randomization was centralized. Closed envelopes were divided into two identical blocks, and the envelopes containing the name of the drug were opened by a nurse not otherwise involved in the study.

The first patient was randomized in October 2002, after which the enrolment of all 64 patients took 17 months. Seven Finnish urological units participated in this study. Exclusion criteria for the study were a history of cancer in the last ten years, untreated hypertension, renal insufficiency, liver dysfunction, or severe hypercholesterolemia. Serum creatinine and hepatic transaminases were followed throughout the study for CyA safety. All patients underwent urodynamic studies to rule out detrusor overactivity prior to randomization. All patients gave their written informed consent. No other specific treatments for PBS/IC were accepted during the study. If the patients were using NSAID or other painkillers or medication for insomnia at the time of entrance into the study, they were allowed to continue the co-medication.

Baseline voiding symptoms were recorded in two-day voiding diaries, visual analogue scale (VAS) for worst pain, and O`Leary-Sant symptom and problem indices for PBS/IC (appendix). Control visits were at one month, three months and six months. At these visits, patients returned two-day voiding diaries, marked the VAS score, filled out symptom and problem questionnaires, and gave the global response assessment (GRA). GRA was defined as: 1 = worse, 2 = no change, 3 = slightly better, 4 = moderately better, 5 = much better, 6 = completely cured. Participants who reported categories 4-6 were evaluated as treatment responders.

Due to possible nephrotoxicity and development of hypertension in the CyA group, serum creatinine and blood pressure were controlled monthly. If blood pressure or serum creatinine were elevated, the CyA dose was reduced by half. In case of subjective

intolerable side effects, it was also possible to reduce the CyA dose. To rule out urinary bacterial infection, urine analysis was conducted at all visits.

The primary end point of the study was micturition frequency in 24 hours. Secondary endpoints were maximum bladder capacity, mean voided volume, number of nocturia episodes, O`Leary-Sant symptom and problem scores, VAS score, and GRA.

### Potassium sensitivity test (III)

Publication III is based on the same patient population as publication II. In addition, a potassium sensitivity test was conducted before and after the treatment in all volunteer patients.

The patients were not aware of the sequence of the two intravesically instilled solutions. A single use small catheter was inserted into the bladder, which was emptied prior to instillation. 40 ml of 0.9% saline was administered slowly into the bladder and was left in situ for 3 minutes. The patient was asked to grade the sensations of urgency and pain on a scale of 0 to 5 (0 = no sensation, 1 = slight, 2 = moderate, 3 = clear, 4 = intense and 5 = maximal). After 3 minutes the bladder was emptied. Then 40 ml of 0.4 M KCL was instilled into the bladder. Instillation and the grading of sensations were performed in the same way as with the saline. Only if the patient experienced immediate, intense pain was the instillation stopped and the bladder emptied. PST was considered positive if urgency or pain score was 2 points or more and it was also more provocative than with saline.

## PATIENTS AND METHODS (IV)

Study IV is based on 37 patients who were recruited in the departments of Urology at Helsinki and Tampere University Hospitals for the comparative trial with CyA and PPS. The patients gave a voided urine sample before randomization to CyA or PPS therapy; none of them were on specific PBS/IC treatment. In the last week of the six months' treatment, a second urine sample was obtained from 34 patients.

A urinary dipstick test was performed on all samples to rule out bacterial urinary tract infection, after which the samples were centrifuged and stored at -80°C until analysis of IL-6, EGF and creatinine.

Clinical response was determined by subjective global response assessment (GRA) at the end of the study. Participants reporting GRA 5 or 6 were evaluated as treatment responders.

### Determination of IL-6 and EGF (IV)

Urinary creatinine was determined by routine enzymatic assay. The concentration of IL-6 in urine was determined using a solid phase, two-site immunometric assay (Immulite IL-6, DPC) and that of EGF by modification of a previously described time-resolved immunofluorometric assay (IFMA) (Vuorela et al. 2002). A monoclonal antibody (200 µL, 5 mg/L) against human EGF (Mab #636, R&D Systems) was absorbed to the walls of microtitre wells (Combiplate 12, Labsystems, Helsinki, Finland) by incubation at room temperature (RT) overnight in Tris-buffered saline (TBS; 0.05 mol/L Tris-HCl, 0.15 mol/L NaCl, pH 7.7). The solution was collected and the wells were saturated with 1% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO, USA) with 6% sorbitol in TBS for 2 hours at RT. In the assay, 100 µL of calibrators (recombinant human EGF R&D Systems) and samples were pipetted into the wells in duplicate followed by addition of 100 µL of assay buffer (equal parts of Delfia buffer (PerkinElmer-Wallac, Turku, Finland) and 0.05 mol/L Tris-HCl, pH 7.7, 0.9% NaCl, 0.05% NaN3, 5 g/L bovine serum albumin, 0.05 g/L bovine globulin, 0.01 ml/L Tween 40) and incubated for 1 hour at RT on a shaking plate. The wells were washed twice with Wash Buffer (PerkinElmer-Wallac), and 50 ng of goat anti-EGF (AF 236, R&D Systems) labeled with europium (PerkinElmer-Wallac) was then pipetted into the wells in 200 µL of assay buffer and incubated for 1 hour at RT on a shaking plate. The wells were then washed four times, 200 µL of Enhancement Solution (PerkinElmer-Wallac) was added, and time-resolved fluorescence measured on a Victor II Fluorometer (PerkinElmer-Wallac)). The detection limit of both assays was 5 ng/L. The intra- and inter-assay coefficients of variation of the EGF assay were 3.2% and 4.5% at the relevant concentrations, respectively, and those of the IL-6 assay were 9.6% and 7.7%, respectively. To account for varying urine concentration, the concentrations of IL-6 and EGF were normalized against the creatinine concentration.

## PATIENTS AND METHODS (V)

Publication V comprises 151 patients who participated in two separate prospective, randomized, open-label studies conducted by FinnIC study group. The first prospective study included 87 patients who were randomized to intravesical BCG or DMSO therapy. The recruitment of patients took place from September 1999 to June 2002. Six Finnish urological units participated in this study. Patients were randomized in a 1:1 ratio to six weekly instillations with 50 ml 50% DMSO or 50 ml Tice strain BCG. All patients filled the health-related quality of life (HRQOL) questionnaire developed by Cleary and co-workers (Cleary et al. 1995) before treatment and at the three-month follow-up. At the follow-up they also marked their subjective global assessment of treatment response

(GRA). At three months, the patients were allowed to change to the other treatment group if subjective treatment response had not been achieved. In this case, a new baseline QoL questionnaire was obtained and a further outcome questionnaire after three months.
The study population of publication II is included in study V. The HRQOL questionnaire was filled by all the 64 patients in publication II at baseline and the final evaluation of the same questionnaire was performed after six months' treatment with CyA or PPS.
The health-related quality of life questionnaire is shown in the appendix.

## STATISTICAL METHODS (I – V)

Baseline factors were compared with t- and Mann-Whitney rank sum tests and the differences between treatment outcome were calculated with the Mann-Whitney rank sum test. The proportions of responders were calculated with the Fisher Exact Test. Wilcoxon's signed rank test was used in calculating variations in blood pressure during CyA treatment. The proportions of patients who had changed PST after treatment were calculated with the Fisher Exact Test which was also used in calculating the probability of response to treatment among PST positive and negative patients. The Mann-Whitney Rank Sum Test was used in comparison with the distribution of positive and negative PST in different symptom groups after treatment.
The differences in urinary concentrations of EGF and IL-6 before and after treatment were compared using the Wilcoxon's signed rank test and the paired t-test. The connection between subjective treatment response, type of treatment (CyA or PPS), age of the patient, and urinary levels of the markers was assessed by the Mann Whitney U test, Fischer's exact test or Kendall's tau-b test for correlation. All tests were two-sided and *p*-values below 0.05 were considered statistically significant.

In the comparison of QoL points between different GRA categories, the Wilcoxon Rank Sum test was applied. Differences in the response rates between the treatments were calculated by Fisher's exact test. The T-test was used to compare the treatment efficacy in the different domains of QoL.

Statistical analysis was performed with SigmaStat and SPSS (version 13.0) statistical software (Jandel corp., SPSS Inc., Chicago, Illinois).

# RESULTS

## Long-term CyA therapy had an impact on voiding diaries (I)

Twenty-three patients received CyA treatment at least for one year. The mean follow-up for all patients was 60.8 (range: 14-123) months. Patients' maximum bladder capacity and mean voided volume increased, while the 24-hour voiding frequency decreased ($p < 0.001$, for all three parameters) (**Table 7**, **Figure 3**, and **Figure 4**).

**Table 7**. *Changes in micturition patterns after one year of treatment.*

| | Before CyA therapy | On Cya therapy | Statistical significance |
|---|---|---|---|
| Mean voided volume (ml) | 101.4 (SD=42.7) | 246.4 (SD=97.9) | $p<0.001$ |
| Maximal bladder capacity awake (ml) | 161.8 (SD=74.6) | 360.7 (SD=99.3) | $p<0.001$ |
| Voiding frequency in 24 hours | 20.8 (SD=6.3) | 10.2 (SD=3.8) | $p<0.001$ |

**Figure 3**. *Mean maximum bladder capacity awake (ml) during the follow-up.*

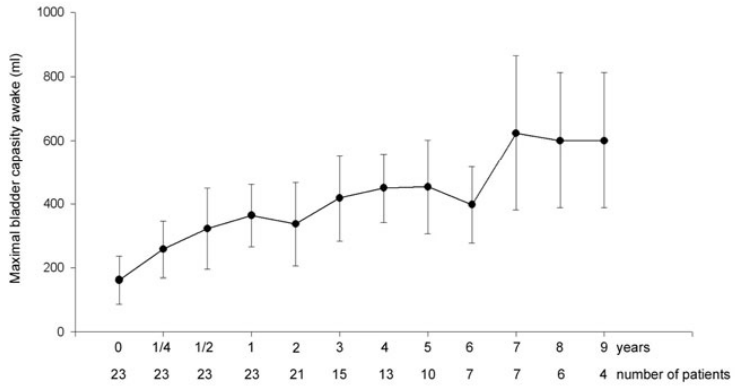
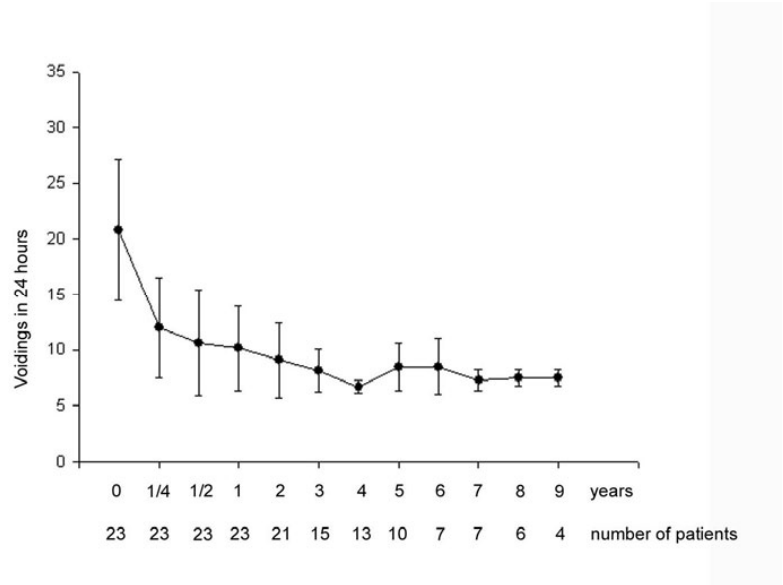



**Figure 4**. *Mean number of voidings in 24 hours during the follow-up.*




In 22 out of 23 (96%) patients the maximum voided volume increased by at least 50% of the baseline volume. Twenty patients out of 23 (87%) had the same percentage increase in mean voided volume.

The maximum bladder capacity awake exceeded pre-treatment anesthesia bladder capacity in 10 patients out of 23 (43%). In one patient the mean voided volume (405 ml) exceeded her pre-treatment bladder capacity in anesthesia (350 ml).

Treatment effect on pain was recorded only in the form of the patient's subjective statement. Twenty patients reported that they were totally free of pain on CyA therapy. In three patients some bladder pain persisted, but only one used painkillers regularly.

Cessation of treatment was encouraged in patients who had been asymptomatic for at least one year. Eleven patients stopped CyA therapy, but in 9 patients the symptoms recurred within 3 months, leading to restart of the CyA medication. The patients who started CyA again after a pause had equal benefit from it, as after initial treatment.

CyA therapy was well tolerated. No clinical nephrotoxicity was noticed. Typical side effects reported were gingival hyperplasia and hirsutism, which were regarded as mild. In three patients antihypertensive drug therapy was started during CyA therapy because of an increase in blood pressure.

One patient underwent surgery for facial basalioma after 5 years of CyA therapy. In one patient local breast cancer was diagnosed after more than 7 years of CyA therapy.

## CyA had superior efficacy in PBS/IC-related symptoms compared with PPS (II)

Sixty-four patients were randomized to this prospective, six-months treatment trial. The baseline characteristics and treatment results are presented in **Table 8**.

**Table 8**. *Patient characteristics and treatment results. Parameters are presented as mean±standard deviation (SD).*

| | Cyclosporine A | | Pentosan polysulfate sodium | |
|---|---|---|---|---|
| No of patient (women, men) | 32 ( 27 , 5 ) | | 32 ( 26 , 6 ) | |
| Age of patients±SD (yrs.) | 56.2±14.7 | | 59.7±13.0 | |
| Duration of symptoms±SD (yrs.) | 7.8±7.0 | | 8.9±6.7 | |
| Cystometric capacity±SD (ml) | 232±99 | | 201±99 | |
| | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment |
| Frequency in 24 hrs.±SD | 16.7±4.4 | 10.7±4.1 | 19.1±8.4 | 17.4±6.8 |
| Nocturia episodes±SD | 3.9±2.2 | 1.9±1.5 | 4.2±3.3 | 4.2±3.2 |
| Maximum bladder capacity awake±SD (ml) | 230±81 | 309±107 | 189±78 | 191±80 |
| Mean voided volume±SD (ml) | 122±39 | 178±69 | 106±46 | 106±39 |
| O`Leary-Sant symptom score±SD (0-36) | 29.4±4.8 | 15.2±8.8 | 30.2±3.7 | 27.5±4.7 |
| O`Leary-Sant symptom index±SD (0-20) | 15.7±2.8 | 7.8±4.2 | 16.6±2.0 | 14.9±2.9 |
| O`Leary-Sant problem index±SD (0-16) | 14.0 ±1.7 | 7.1±4.4 | 14.0±1.8 | 12.7±2.2 |
| Visual analogue scale VAS±SD (0–10 cm.) | 6.8±2.0 | 2.4±2.4 | 7.0±2.1 | 5.5±2.9 |

Cya had more effect than PPS on all measured parameters. $p<0.001$, except for maximal bladder capacity awake $p=0.003$.

Twenty-nine patients out of 32 (91%) completed the study in both study arms. Three patients dropped out of CyA arm because of intolerable side effects within one month of start of treatment. The three dropouts in PPS arm were due to lack of efficacy in two cases and sudden hematuria in one case.

The predicted efficacy in 24-hour micturition frequency, which was our primary end point, was not achieved. We had calculated that in 70% of patients treated with CyA the reduction in micturition frequency would be at least 50%. The corresponding assumption for PPS treated patients was that in 35% of patients the reduction in frequency would be at least 50%. A 50% reduction in frequency was observed in 11 patients (34%) in the CyA group and in none in the PPS group ($p < 0.001$). The O`Leary-Sant score was reduced by at least 50% from baseline in 17 patients out of a total 29 (59%) completing the six-month CyA arm. The same effect was not achieved in any of the patients in the PPS arm.

According to GRA (category 4 to 6), 24 patients (75%) responded to CyA therapy while a response was seen in 6 patients (19%) in the PPS arm ($p< 0.001$).

Adverse effects were more frequent in the CyA arm (**Table 9**).

**Table 9**. *Participants with at least 1 adverse event (worst reported).*

| | None (%) | Mild (%) | Moderate (%) | Significant (%) | Total No. (%) |
|---|---|---|---|---|---|
| CyA | 2 (6.3) | 23 (72) | 4 (13) | 3 (9.4) | 30 (94) |
| PPS | 14 (44) | 14 (44) | 3 (9.4) | 1 (3) | 18 (56) |

At 6 months, the patients were asked whether they wanted to proceed with their current treatment at their own cost. Nineteen patients continued CyA therapy and four patients continued PPS therapy.

## Six months' treatment on PBS/IC with CyA may change positive PST to negative (III)

The challenge test was performed prior to starting the study drug and at six months when administration of the medication was completed. The PST test was performed in 63 on inclusion and six patients refused the test at six months.

In 56 patients (89%) the PST test was positive at the time of entering the study, while it was negative in 7 patients (11%).

Of the 24 responders to either CyA or PPS therapy, the PST test turned negative in 15 (63%), while the percentage in the 26 non-responders to treatment was 12% ($p<0.001$).

The probability of having a negative PST at six months was higher among patients with less symptoms (**Table 10**).

**Table 10**. *PST and symptoms at 6 months. Differences in positive PST were significant for the O`Leary-Sant questionnaire (p< 0.001), for the voiding frequency (p=0.006) and for VAS (p=0.014).*

| | O`Leary-Sant symptom points 8 or less n=21 | O`Leary-Sant symptom points >8 n=36 | Voiding frequency 10 or less n=18 | Voiding frequency >10 n=40 | VAS < 5 cm n=33 | VAS 5 cm or more n=25 |
|---|---|---|---|---|---|---|
| Positive PST at 6 months | 3 (14%) | 24 (65%) | 3 (17%) | 24 (59%) | 11 (33%) | 16 (64%) |
| Negative PST at 6 months | 17 (81%) | 8 (22%) | 13 (72%) | 12 (29%) | 20 (61%) | 5 (20%) |
| Refused repeated PST | 1 (5%) | 5 (13%) | 2 (11%) | 4 (12%) | 2 (6%) | 4 (16%) |

PST changed more often to negative after CyA therapy than after PPS therapy (**Table 11**).

**Table 11**. *Patients with positive or negative PST before and after 6 months treatment. At 6 months more patients changed PST negative in CyA group (p = 0.002).*

| | CyA group | | PPS group | |
|---|---|---|---|---|
| | PST positive | PST negative | PST positive | PST negative |
| at entry | 28 | 4 | 28 | 3 |
| at 6 month | 8 | 18 | 19 | 7 |

## PBS/IC treatment with CyA but not with PPS reduces urinary EGF levels (IV)

Our previous studies showed a reduction of clinical symptoms of PBS/IC after treatment with CyA (I-II). We wanted to test whether the effect of treatment could be measured objectively in the reduction of urinary EGF or IL-6.

Pre-treatment levels of EGF and IL-6 were comparable in both CyA (18 patients) and PPS (19 patients) arms. The reduction of baseline level of urinary EGF was significant after 6 months of treatment with CyA. No such effect was seen in the PPS arm (**Table 12**).

**Table 12**. *Concentrations of EGF and IL-6 in CyA and PPS treated patients.*

| Treatment | CyA | | | PPS | | |
|---|---|---|---|---|---|---|
| | Before | After | p* | Before | After | p* |
| n | 18 | 18 | | 19 | 16 | |
| Urine marker, mean (median; range) | | | | | | |
| EGF (ng/mg creatinine) | 35 (30;10-70) | 28 (20; 10-60) | 0.034 | 33 (30;10-70) | 32 (30;10-50) | 0.81 |
| IL-6 (pg/mg creatinine) | 7.1 (4.2; 0.5-25) | 3.6 (2.7; 0.5-18) | 0.18 | 11 (5.8; 0.6-63) | 11 (5; 0.9-50) | 0.3 |

* Wilcoxon signed ranks test

To evaluate the possible effect of patient age on pre-treatment EGF and IL-6, we compared these levels in two age groups. There were 11 patients below 53 years and their mean age was 39 years (range: 22–52 years). The mean age of the 26 patients at least 53 years was 63 years (range: 53–75 years). In the older patient group the mean IL-6 concentration 12.3 (SD 13.8) pg/mg creatinine was significantly higher than the mean IL-6 concentration of 2.3 (SD 1.9) pg/mg creatinine seen in the younger patient group (p= 0.001).

In this older patient group the IL-6 concentration decreased after CyA therapy from 10.3 (SD 8.1) pg/mg creatinine to 4.1 (SD 5.0) pg/mg creatinine (p = 0.044). The IL-6 concentration was 2.1 (1.7) pg/mg creatinine before treatment in the younger group and 2.9 (1.7) pg/mg creatinine after CyA therapy (p=0.477).

Patient's age had no effect on the pre-treatment EGF concentrations.

## The HRQOL questionnaire was sensitive to treatment response after CyA, PPS, BCG or DMSO therapy (V)

Forty-four patients were randomized to DMSO therapy and 43 to BCG therapy. Six patients in the BCG group changed to DMSO group at three months, and 7 patients in the DMSO group changed to the BCG group. The total number of treatments with either instillation was 100 (50 for each group). Twenty-nine patients out of 32 in both CyA and PPS groups completed the study resulting in 58 patients for analysis. One hundred and fifty-eight (158) HRQOL questionnaires were obtained before treatment and 138 questionnaires at follow-up. The missing 20 coupons were due to 19 drop-outs during the treatment and one form that was lost.

There was a higher response rate after DMSO therapy than after BCG therapy ($p<0.01$), and CyA therapy had significantly higher response rate than PPS therapy ($p<0.001$) (**Table 13**).

**Table 13**. *Demographics of the treatment groups and the response rates in them.*

| | DMSO (n=50) | BCG (n=50) | CyA (n=32) | PPS (n=32) |
|---|---|---|---|---|
| Mean age of patients (SD) | 60.7 (12) | 59.2 (13) | 56.2 (15) | 59.7 (13) |
| Sex distribution (females/males) | 48/2 | 46/4 | 27/5 | 26/6 |
| Number of patients completing the trial (%) | 44 (88%) | 43 (86%) | 29 (91%) | 29 (91%) |
| Number of responders to treatment (GRA 5 or 6) | 16 (32%) | 4 (8%) | 19 (59%) | 4 (13%) |

The questionnaire was filled out conscientiously, but the questions concerning sexuality remained unanswered by half of the patients. In our cohort, the sexual activity was low. Seventy patients out of 158 (44%) reported having had sexual activity within the past month. There were no differences in sexual activity between the four treatment groups, and in treatment responders it remained at the same level.

Post-treatment GRA correlated with the change in the HRQOL questionnaire (Ta**ble 14**). If the patient responded to the treatment (GRA 5 or 6), the HRQOL questionnaire improved significantly in the aspects of general health perceptions, pain, general well-being, vitality, social functioning, and physical capacity. Disability days and activity limitations days were also significantly changed in those patients. The parameter pain was also improved in patients in the category GRA 4. Furthermore, patients with impaired symptoms after treatment, experienced worse HRQOL in social functioning and had more disability days.

DMSO, BCG, CyA and PPS treatments had different impact on the HRQOL questionnaire. The results are listed in **Table 15**.

**Table 14**. *Results in the HRQOL questionnaire after treatment with any of the four study medications according to the patient expressed GRA.*

| Measure | Baseline (n=158) | GRA 1 (n=11) | GRA 2 (n=27) | GRA 3 (n=27) | GRA 4 (n=23) | GRA 5 (n=34) | GRA 6 (n=16) |
|---|---|---|---|---|---|---|---|
| General Health perceptions (0-10) | 5.2 (1.9) | 4.2 (1.9) | 5.0 (2.6) | 5.3 (1.9) | 5.7 (2.0) | 6.7 (2.1) * | 7.3 (2.2) * |
| Pain (1-10) | 5.4 (1.8) | 6.1 (2.2) | 5.9 (2.2) | 4.0 (2.2) | 3.7 (1.4) * | 2.5 (1.4) * | 1.8 (1.5) * |
| Emotional well-being (0-100) | 62.0 (18.0) | 50.4 (14.4) | 59.7 (17.1) | 62.5 (15.1) | 72.7 (14.7) | 79.8 (16.2) * | 81.5 (13.5) * |
| Vitality (0-100) | 55.0 (16.7) | 48.5 (12.8) | 55.3 (18.0) | 55.5 (16.0) | 65.1 (17.7) | 68.1 (14.9) * | 75.1 (14.1) * |
| Social functioning (1-6) | 3.2 (1.3) | 2.1(1.2) # | 3.3 (1.5) | 3.7 (1.4) | 4.0 (1.5) | 5.0 (1.1) * | 5.4 (1.0)* |
| Physical capacity (1-5) | 2.1 (0.7) | 2.7 (1.0) | 2.0 (0.9) | 1.9 (0.9) | 1.7 (0.7) | 1.4 (0.5) * | 1.6 (1.0) |
| Sexual interest (1-5) | 1.7 (0.8) | 1.5 (0.7) | 1.7 (0.8) | 1.9 (0.8) | 1.7 (0.9) | 1.8 (0.8) | 2.1 (1.1) |
| Sexual functioning (1-5) | 2.5 (1.3) | 3.3 (1.6) | 3.1 (1.6) | 2.0 (0.7) | 2.7 (1.3) | 2.7 (1.1) | 1.6 (0.7) |
| Disability days (0-7) | 1.1 (1.7) | 2.1(1.9) # | 1.2 (1.4) | 0.4 (0.9) | 0.5 (1.0) | 0.2 (0.6) * | 0.6 (1.8) |
| Activity limitation days (0-7) | 3.3 (2.2) | 4.3 (2.0) | 3.3 (2.1) | 2.5 (2.5) | 2.2 (1.9) | 1.0 (1.4) * | 0.3 (0.7) * |

# = significant difference compared with baseline towards impairment.
* = significant difference compared with baseline towards improvement.
Wilcoxon Rank Sum test used in all measurements.

**Table 15**. *Results in the HRQOL questionnaire after the four treatments.*

| | DMSO | | BCG | | CYA | | PPS | |
|---|---|---|---|---|---|---|---|---|
| | Pre-treatment (n=50) | Post-treatment (n=44) | Pre-treatment (n=50) | Post-treatment (n=43) | Pre-treatment (n=32) | Post-treatment (n=29) | Pre-treatment (n=32) | Post-treatment (n=32) |
| General health perceptions 0-10 | 5.2 (1.9) | 5.9 (2.2) | 5.2 (2.0) | 5.4 (2.7) | 5.2 (1.7) | 6.4 (2.2) | 5.4 (1.9) | 5.9 (2.0) |
| Pain 1-10 | 5.2 (1.9) | 3.1 (2.0) | 5.3 (1.9) | 4.2 (2.5) | 5.5 (1.7) | 2.9 (2.1) | 5.8 (1.6) | 4.9 (2.3) |
| Emotional well being 0-100 | 63.7 (19) | 70.6 (18) | 65.5 (20) | 65.4 (21) | 58.5 (13) | 75.9 (15) | 58.8 (18) | 65.4 (15) |
| Vitality 0-100 | 57.0 (17) | 65.2 (16) | 56.7 (19) | 58.0 (21) | 50.3 (12) | 63.9 (16) | 53.8 (16) | 60.0 (16) |
| Social functioning 1-6 | 3.2 (1.5) | 4.2 (1.6) | 3.2 (1.3) | 3.8 (1.6) | 3.3 (1.1) | 5.0 (1.3) | 3.1 (1.3) | 3.4 (1.4) |
| Physical capacity 1-5 (1 best, 5 worst) | 2.1 (0.8) | 1.8 (0.8) | 2.1 (0.8) | 2.0 (1.1) | 2.2 (0.5) | 1.5 (0.7) | 1.9 (0.7) | 1.7 (0.7) |
| Disability days 0-7 | 1.3 (1.9) | 0.7 (1.5) | 1.1 (1.7) | 0.9 (1.5) | 1.2 (1.3) | 0.5 (1.1) | 0.8 (1.5) | 0.5 (0.8) |
| Activity limitation days 0-7 | 3.2 (2.3) | 2.0 (2.1) | 3.2 (2.2) | 2.7 (2.3) | 4.0 (2.0) | 1.5 (2.0) | 3.3 (2.1) | 2.6 (2.3) |
| Sexual interest 1-5 (1 best, 5 worst) | 1.6 (0.8) | 1.6 (0.8) | 1.7 (0.9) | 1.7 (0.9) | 2.0 (1.0) | 2.2 (1.0) | 1.6 (0.6) | 1.8 (0.8) |
| Sexual functioning 1-5 (1 best, 5 worst) | 2.0 (1.0) | 2.7 (1.4) | 2.4 (1.2) | 2.5 (1.5) | 2.7 (1.0) | 2.3 (1.2) | 2.9 (1.3) | 2.7 (1.4) |
| Number of patients (%) having sex (masturbation or intercourse) | 18 (36%) | 19 (43%) | 22 (44%) | 18 (42%) | 11 (34%) | 15 (52%) | 16 (50%) | 16 (55%) |

The effect of DMSO and BCG therapy on the HRQOL questionnaire was comparable.

CyA had statistically more impact on emotional well being, social functioning, activity limitation days, pain and physical capacity than PPS treatment ($p<0.05$; t-test).

# DISCUSSION

## Therapeutic effect of CyA on PBS/IC (I-II)

In our two separate studies (I-II) the therapeutic effect of CyA was excellent. The promising results which the preliminary, open, non-randomized, short-term study provided (Forsell et al. 1996) were shown to be consistent in our work. In study I, the 24-hour voiding frequency decreased from baseline 20.6±6.3 (mean±SD) to 10.2±3.8 ($p<0.001$). In 19 patients out of 23 (83%), the micturition frequency in 24 hours was reduced by more than 50% and in 20 patients out of 23 (87%) the mean voided volumes according to urinary diaries increased by at least by 50%. In 19 patients the maximum voided volume increased by at least by 100% (unpublished data). No such long-term results have been reported with any other drug therapy before, when all patients fulfilled the NIDDK criteria of PBS/IC.

The placebo effect of this kind of open treatment, which includes regular follow-up visits with enthusiastic urologists, is most likely substantial. However, all our patients had failed therapies in their medical history and during CyA treatment only the clinical effect improved. No flare-ups of the disease were seen if the medication was being administered. However, symptoms recurred in 9 patients out of 11 in whom CyA therapy was ceased (I). In a longitudinal follow-up study in 637 patients with PBS/IC, it was observed that although all PBS/IC related symptoms fluctuate, there was no evidence of significant long-term change in overall disease severity (Propert et al. 2000). Patients included in that database had different kinds of treatments; most common were hydrodistension, amitriptyline, phenatzopyridine, special diet, intravesical heparin, hyoscyamine, oxybutinine, and oral PPS (Rovner et al. 2000).

In study I, only changes in micturition charts are available. In addition, the reduction of pain is also apparent, as the frequent use of additional pain medication was reduced that only one of the 23 patients required pain medication while on CyA therapy (I). The reason why validated O`Leary-Sant questionnaire was not used in our study I is that the introduction and validation of it came out just after the time of initiation of CyA to patients.

In study II, the number of responders increased during the treatment. That is in accordance with the results of study I. The positive effect of CyA increases over time in those who respond to it. This is probably due to the slow process of re-modulation of active inflammation in those patients with severe symptoms. Based on our results, a six-month trial with CyA is sufficiently long to reach a conclusion as to whether the patient should continue with the drug.

No other peer reviewed articles on CyA therapy, beside our own, have been published to date. Two abstracts on CyA therapy in PBS/IC were presented at the AUA 2007 conference. In the first abstract, 34 patients in Sao Paulo, Brazil had taken CyA for one year with results comparable to ours (Chade et al. 2007). In the second abstract, six patients with classic type PBS/IC were treated for 16 weeks with CyA in Stockholm. After treatment, a decrease in the symptom questionnaire scores and reduced levels of

intravesical nitric oxide were seen in all patients (Ehren et al. 2007). Based on our experience some patients, like in Århus, have empirically taken CyA for their refractory PBS/IC (personal communication). Because it is difficult for a single center or a low-population country to recruit enough patients for clinical trials, efforts should be combined internationally to conduct large-scale studies in the future.

The mean age of the patients at the start of CyA medication in study I was 60.8 years (range: 49 to 76 years) and the mean age of CyA treated patients in study II was 56.2 years (range: 27 to 79 years). Thus the age of our patients was somewhat higher than usually reported for patients with PBS/IC. In the NIDDK sponsored Interstitial Cystitis Database (ICDB) study the 424 patients included had a mean age of 44.3 years (Simon et al. 1997). Inclusion criteria in this study did not follow the strict NIDDK criteria used in our studies. Our age distribution matches a Finnish study reporting population based prevalence of clinically confirmed PBS/IC (Leppilahti et al. 2005). The higher mean age of patients in Finnish studies may reflect the unawareness of PBS/IC among general practioners, which results in delays in consultation with specialists.

Erickson and associates found that patients of more advanced age had a tendency to more severe inflammation in bladder biopsies and reduced bladder capacity (Erickson et al. 1997). Age, symptom severity, and resistance of symptoms to previous therapies, might reflect some special etiological features in our patients with PBS/IC, such as ongoing autoimmune process. In study II, the best therapeutic effect (GRA 5 or 6) was achieved among patients with a higher mean age (59.6 ± 14.6 years) compared with those with those demonstrating less effect (mean age 47.2 ± 12.1 years). CyA therapy is of particular help in older patients. The potential side effects of CyA restrict its use of in risk groups (examples: hypertensive patients, multipharmacy, recent malignancy, impaired renal function).

## Therapeutic effect of PPS on PBS/IC (II)

PPS was selected as a comparator drug to CyA as it is FDA approved in the indication PBS/IC. As PPS is not available in Finland without special permission from the National Agency for Medicines, it is seldom used in Finland and the patients were naive to PPS.
In the present study II the patients had severe symptoms, fulfilled the NIDDK criteria, and the majority of them had a history of multiple treatment failures. In patients of this category, good therapeutic effects of PPS could be considered optimistic.

Our results show that PPS was inferior to CyA in all the symptom parameters measured. Response rate to PPS was not affected by time. At three months, 25% of patients responded to PPS and at six months 19%. Our results do not support the hypothesis that a prolonged duration of PPS medication would have an effect on the outcome (Nickel et al. 2005). A three-month trial with PPS would most likely have been sufficient to demonstrate the efficacy in our population. The VAS score was the only clinical symptom marker that showed a benefit of treatment after PPS therapy. It was reduced by a mean value of 1.6±3.3 cm at six months. However, study II was designed to report only differences in the two treatment groups. Four patients wanted to continue with

PPS after the trial, but one of them underwent reconstructive surgery for PBS/IC just a few weeks after the end of the trial (personal communication). We do not regard PPS as an effective treatment, at least not for patients fulfilling the NIDDK criteria of PBS/IC.

## Safety of CyA and PPS (I-II)

In study II, 29 patients out of 32 completed the trial in both drug arms. However, drop-out causes were different between the groups. In the CyA group all three drop-outs were due to adverse effects of the drug. These included emesis, abdominal pain, gingival hyperplasia, headache, and paresthesia in the hands. After stopping CyA the side effects vanished. In the PPS arm, two drop-outs were due to lack of efficacy in the relief of symptoms and the third drop-out (male patient) was caused by massive hematuria. This was resolved after stopping PPS. The mechanism responsible for the hematuria was not necessarily dependent on PPS, but it resulted in hospitalization and discontinuation of PPS. No cases of hematuria were reported in a study in which the dosages of PPS were three times higher than ours (Nickel et al. 2005). Use of PPS in study II resulted in an expected number of adverse events, but these were mainly mild.

In study II, 94% of patients taking CyA reported some adverse events. The high proportion of adverse events is related to the drug itself, but may also be due to the careful follow-up of the patients who were asked about, and reported all symptoms. No irreversible adverse events were seen. In two patients blood pressure increased resulting in lowering of the initial CyA dose by half. In one patient, increase of serum creatinine persisted still at 6 months, despite CyA dose was lowered by half at three months. In a previous study with CyA in rheumatoid arthritis (RA) with a larger dose (5 mg/kg/d), increased serum creatinine was observed in 78% of patients (n=60), and six patients dropped out because of toxic events (Gerards et al. 2003). Although these patients had normal serum creatinine at baseline, it is known that severe RA is associated with renal dysfunction (de Groot 2007). In a prospective study with pustulosis palmoplantaris patients, CyA (dose 1–4 mg/kg/d) resulted in an increase of serum creatinine in 7% of patients and blood pressure changes in 15% (Erkko et al. 1998). It has been proposed that a proportion of the population is more sensitive to CyA and are more prone to develop increased blood pressure (Feutren et al. 1990). CyA therapy was shown to cause progressive renal structural injury and reduced glomerular filtration rate when psoriasis patients used the drug for up to three years at an average dose of 3.9 mg/kg/day (Young et al. 1994).

We monitored the CyA concentration in patients of study I, as is done after organ transplantation, but did not find any correlation between the concentration of the drug and the clinical response. Therefore the results for concentration monitoring in study I were not reported and omitted in study II. The safety measurements, including regular check-ups of serum creatinine and blood pressure, are sufficient. Likewise, drug concentration measurement is not recommended if the indication for drug use is an autoimmune disease (Tsunoda and Aweeka 1996).

Long-term safety of CyA was good in study I. In addition, the individual doses of CyA were lowered from baseline to the lowest level at which symptoms were still controlled. In this long-term study we did not see any increases in serum creatinine, but in three patients, antihypertensive treatment was started during the course of Cya therapy. One skin basalioma and one breast cancer occurred. These are common disorders in the elderly and, most likely, the low dose CyA did not play a role in these cases. Despite the good tolerability in study I, we recommend regular follow-ups in patients on chronic CyA. The patients must be warned of the possible adverse effects which show great interindividual variance.

## Necessity of conducting PST in patients with PBS/IC (III)

Study III showed that the majority of the patients with PBS/IC that fulfil the NIDDK criteria had a positive PST. However, it was negative in 11% of patients and we believe that the test cannot diagnose whether a patient has PBS/IC or not. PST simply reflects the sensitivity to potassium ions, possibly due to increased permeability of the urothelium.
Our study plan to repeat PST in a randomized prospective trial was new. A previous non-randomized study showed that patients who had a positive PST were more likely to respond to intravesical hyaluronate therapy than those with a negative test (Gupta et al. 2005). However, in this study, patients were regarded as responders even if there was a mild improvement in symptoms. In a retrospective study with 25 PST positive and 17 PST negative patients, treatment response was more likely with combined heparinoid and tricyclic antidepressant medication when PST was positive (Teichman and Nielsen-Omeis 1999). The number of PST negative patients in this study was high and the retrospectively collected data may have led to some bias. Previously, it was shown that 50% (n= 40) of selected patients who had positive PST and good clinical response to intravesical heparin therapy, changed to PST negative (Kuo 2001).

Our results showed that baseline positive PST turned into negative at 6 months in 63% (n=18) of treatment responders, 14 of these were in the CyA arm. In 8 patients the PST remained positive at 6 months. Six of these (75%) showed good clinical response to treatment. Thus the sensitivity of the test in the follow-up of CyA patients is poor. PST is a subjective test with certain bias in the repeated test. If the test was painful, the memory of it may affect the experience and the result of a repeated test.

We showed that symptom severity predicts the PST result. O`Leary-Sant symptom index points of less than 8, voiding frequency of less than 10, and VAS score for pain of less than 5 cm after six months therapy were associated with negative PST.

The concept of using PST in the follow-up of patients with PBS/IC is not supported by our results. 15% of patients refused a repeat PST due to pain during the initial test. Of the non-responders (PST positive at entry) tested, 73% remained PST positive. A repeated PST will not give further evidence of the disease; it causes unnecessary pain to patients and can be omitted in the follow-up.

Positive PST is considered to be a sign of increased epithelial permeability. There could be an effect on the results of PST, also with CyA therapy, which is not a direct effect on the glycosaminoglycan lining of the bladder. On the contrary, PPS therapy, which is glycosaminolayer replenish therapy, had less effect on the PST test. Overall, we suggest that the positive PST in PBS/IC is not related to the glycosaminoglycan layer in the bladder, but rather to hypersensitized nerve endings following chronic inflammation.

## Changes in urinary markers reflect the treatment effect of CyA on PBS/IC (IV)

Concentrations of urinary EGF are higher in patients with PBS/IC than in asymptomatic controls (Keay et al. 1997, Keay et al. 2007, Keay et al. 2001, Zhang et al. 2005a). In our study IV, concentrations of urinary EGF were at the same level as in earlier studies and were significantly reduced after CyA therapy but not after PPS therapy. Prospective evaluation of urinary EGF levels has been performed earlier with intravesical BCG, but no significant change in EGF levels or in the other tested urine markers was seen (Keay et al. 2007). One reason possibly leading to negative results was the low response rate of PBS/IC to BCG.

The reasons for our finding of reduced EGF levels after CyA therapy are speculative. Most probably, EGF is over-expressed in PBS/IC as it is needed for re-epithelialisation in response to inflammatory damage in the epithelia (Keay et al. 1997). Most of the urinary EGF is produced in the distal tubuli of the kidneys (Harris 1991), but in cultured PBS/IC bladder urothelial cells EGF is also over-expressed (Keay et al. 2000). Application of exogenous EGF to normal bladder urothelial cells caused acute inhibition of the inward potassium current in a dose dependent fashion (Sun et al. 2007). Whether this change in the cell potassium current is responsible for the hypersensitivity in PBS/IC to the potassium sensitivity test is unknown. Our results showed reduction in EGF concentrations and reduced sensitivity to potassium after CyA therapy with anti-inflammatory effects in the same patients.

CyA has been shown to have an effect on EGF in different models. In rat gingiva CyA leads to higher mRNA and protein expressions of EGF (Chin et al. 2006). It has been suggested that an increased level of EGF plays a role in gingival hyperplasia, a common side effect related to CyA. An anti-EGF effect was seen, as CyA was able to inhibit proliferation of keratinocytes in vitro when their proliferation was driven by EGF (Karashima et al. 1996, Sharpe et al. 1989).

It is possible that the reduction of EGF seen in our study is caused by a direct action of CyA in the kidneys. In CyA induced nephrotoxicity, EGF levels decline, and after withdrawal of CyA they increase again. After kidney transplantation, administration of CyA leads to reduced EGF levels in urine (Di Paolo et al. 1997, Kvist and Nexo 1989). In our study IV, patients not responding to CyA did not show any reduction in post-treatment EGF levels. We therefore believe that CyA is not directly responsible for changes in the EGF levels, but it is due to anti-inflammatory changes in CyA responders. We believe that

the reduction in EGF levels reflects alleviation of symptoms, and at least part of the EGF reduction is due to decrease of EGF induction at the bladder level.

In CyA induced nephropathy, urinary IL-6 levels are increased and EGF levels are reduced (Di Paolo et al. 1997). We did not notice any significant increase in the IL-6/EGF ratio.

Knowledge of urinary IL-6 in autoimmune diseases or after CyA therapy is lacking in indications other than the prevention of graft-versus-host disease.

We could not demonstrate significant reduction in IL-6 levels after CyA or PPS therapy. Interestingly, we noticed that a patients' age reflects the urinary IL-6 concentration. This is in accordance with previous reports where patients with severe inflammation were older than those with milder inflammation and were more likely to have increased IL-6 levels (Erickson et al. 1997). In our study, the severity of symptoms in older patients was equal to that in younger patients, but their disease history was longer and, most likely, the inflammatory changes might have been different. Using a cut-off point age of 53 years, higher IL-6 concentrations were seen, and these levels also decreased after CyA therapy.

There is major interindividual variability in the IL-6 levels and the same concentrations of this cytokine may reflect different inflammatory responses. Therefore, results of urinary IL-6 measurement have to be interpreted with caution and no direct conclusions can be drawn from them. We did not include any asymptomatic control group in the EGF or IL-6 comparison. The patients were acting as their own controls when we compared a clinically effective (CyA) and an ineffective (PPS) treatment.

PBS/IC is a pain syndrome and inflammatory processes are suggested to be responsible for every chronic pain syndrome (Omoigui 2007). In our study, we were able to show that inflammatory markers were recognized in urine and that they could be affected by the anti-inflammatory medication.

The reduction in EGF levels and in the high pre-treatment levels of IL-6 in patients responding well to treatment can provide objective support to the subjective symptom evaluation.

## The need for assessment of health-related quality of life in the follow-up of patients with PBS/IC (V)

According to our study V, the treatment responders (defined as GRA 5 and 6 categories) have improved QoL assessed by a generic health-related questionnaire. This is expected, as it has been previously shown that patients with severe PBS/IC symptoms have poorer scores in various aspects of life than patients with milder symptoms (Rothrock et al. 2002). Our prospective study also showed that poor outcome (GRA 1) was associated with impaired QoL even compared with baseline results. Additionally, we found that DMSO therapy was more effective than BCG as defined by the response rate. Similar results were previously reported by Peeker and co-workers (2000c), as they were unable to show any benefit from BCG therapy. In study V, CyA was superior to PPS adjusted by treatment

response, and this effect was also seen in the HRQOL questionnaire. Despite better results in the GRA, DMSO was equal to BCG in the change of the pre-treatment HRQOL questionnaire. This may be due to the low response rates to DMSO (32%) and BCG (8%) treatment, while the high response rate to CyA (59%) differed even more significantly to that of PPS (13%).

The results in the QoL between CyA or PPS and DMSO or BCG therapies are not directly comparable. We presented in study V the combined data of two separate trials. The DMSO-BCG study was initiated by physician Mikael Leppilahti, MD, and was named the FinnIC I-study. It was the first national multicentre study conducted on PBS/IC in Finland. It stopped recruiting patients four months before the CyA-PPS study (so-called FinnIC II-study) started. The route of administration varies, as DMSO and BCG are intravesical drugs requiring repeat visits to hospital for instillations. CyA and PPS are oral drugs, which route provides more convenience to patients. CyA had more side effects than PPS in our publication II, but the adverse events did not reflect the QoL. The inclusion and exclusion criteria for the FinnIC-I and FinnIC-II studies were the same as the NIDDK criteria were followed. In these two similar study populations, CyA had most impact on the pre-treatment HRQOL questionnaire. Other data of the FinnIC-I study than that reported here are not yet available, so the effect of DMSO and BCG on clinical signs other than GRA cannot be reported or compared with CyA or PPS.

The HRQOL questionnaire was well adapted to the patients. An exception was the questions concerning sexuality, which remained unanswered by most patients. These questions should undergo revision. But as only 40% of our cohort reported having sexual activity during the last month, the revised questions about sexual function and interest may be of little benefit. In a population younger than ours (mean age of 59.2 years) this would naturally be an aspect of particular interest.

Including a generic QoL questionnaire in interventional studies provides additional information on symptoms and symptom relief compared to the usual parameters noted in urinary diaries or specific symptom scores. If the questionnaire is designed to be symptom specific, it will have a certain overlap with urinary diaries, symptom scores, or VAS score. It is recommended that a multidimensional measure of quality of life be incorporated in future trials (Michael et al. 2000), either solely or in the form of a disease specific, gender targeted questionnaire.

## Future research in the field of PBS/IC

Our results obtained with CyA concerning clinical symptoms, the PST test, urinary markers, and the HRQOL questionnaire in PBS/IC warrant future therapeutic studies also with other better tolerated immunosuppressants. These future trials should be placebo controlled studies. One placebo controlled study with Mycofenolate mofetil is underway in the USA. A placebo controlled study with CyA has not yet been possible, but would also be necessary. In future trials with any immunosuppressive agent, evaluation of a urinary marker panel should be included. Antiproliferative factor (APF) is among the most

interesting parameters. Treatment effect on bladder histology and immunomorphometric studies on cytokines in the bladder wall, as well as the effect on non-specific serum autoantibodies offer interesting future prospects. A quality of life questionnaire which also covers gender dependent aspects of life is required and it should undergo validation for patients with PBS/IC. The data obtained from our studies is of great benefit in attempts to define the elusive pathology behind this obscure disease.

# SUMMARY AND CONCLUSIONS

Painful bladder syndrome/interstitial cystitis (PBS/IC) is a chronic urinary bladder disorder characterized by symptoms of bladder pain and urinary frequency. In diagnosing PBS/IC, any confusable disease which might lead to similar symptoms must be excluded. PBS/IC has a typical appearance in cystoscopic examination. Biopsies taken from the bladder may show various inflammatory changes, but are not diagnostic.
PBS/IC is a chronic disease in which drug therapy has not led to significant success over the course of time. If the symptoms of PBS/IC are refractory to standard treatments, a possible cure might demand surgical intervention involving cystectomy.
The eventual autoimmune etiology in mind, and to avoid both acute and late complications related to major surgery, immunosuppressive drug therapy with cyclosporine A (CyA) was started to patients with refractory PBS/IC.

CyA is a potent anti-inflammatory drug, a calcineurin inhibitor which inhibits T lymphocyte IL-2 production. T cells are present in abundance in inflammation of the bladder in PBS/IC.

On the basis of a pilot, short-term study with CyA on PBS/IC, use of CyA was continued empirically over the long term. We conducted a prospective, randomized, six-month study in 64 patients comparing the effect of CyA with the FDA approved treatment, pentosan polysulfate sodium (PPS). We measured the drug effect on patient's symptoms, the potassium sensitivity test, and on urinary biomarkers. We further tested the impact of CyA, PPS, DMSO and BCG therapy on a health-related quality of life questionnaire and evaluated the response rate to treatment with these therapies.

According to our results we conclude that

1. Long-term use of CyA was safe and effective in PBS/IC patients in whom it was started empirically. The good clinical effect matured individually during the years in which CyA was continued, resulting in less frequent voiding and larger amounts of urine at single voidings. Cessation of medication led to the reappearance of symptoms, and restarting CyA to renewed alleviation, so that CyA was administered as continuous medication. The dosage of CyA could be lowered in order to enhance tolerability.

2. CyA was associated with greater alleviation of symptoms than PPS. The response rate to CyA increased during the study period, comprising 75% of CyA patients at six months. 19% of patients responded to PPS therapy. Withdrawals were equally common for both therapies. Adverse effects were more common in the CyA group, underlining the importance of monitoring the drug safety and appropriate titration of the dose. CyA therapy can be recommended in patients with severe PBS/IC.

3. The potassium sensitivity test (PST) is positive in the majority of PBS/IC patients fulfilling the NIDDK criteria of the disease. Successful therapy of PBS/IC can alter nerve sensitivity to external potassium. This effect was seen more often after CyA therapy.

Patients with refractory symptoms after therapy should not undergo a repeat PST in the follow-up of PBS/IC.

4. Successful treatment of PBS/IC with CyA resulted to decreasing urinary levels of EGF, reflecting the good clinical response achieved. IL-6 levels in urine were higher among older patient with a longer history of PBS/IC. In these patients, reduced levels of urinary IL-6 were measured after CyA therapy.

5. Patients who experience the best treatment response have improved quality of life (QoL) according to the post-treatment health-related quality of life (HRQOL) questionnaire. CyA had more impact on the majority of the aspects of QoL than PPS. Despite DMSO therapy being more successful than BCG in the count of responders, DMSO and BCG had equal effects on the HRQOL questionnaire.

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# APPENDIX

## The O`Leary-Sant symptom and problem indices

**Symptom Index**

Q1. During the past month, how often have you felt the strong need to urinate with little or no warning

0. not at all
1. less than 1 time in 5
2. less than half the time
3. about half the time
4. more than half the time
5. almost always

Q2. During the past month, have you had to urinate less than 2 hours after you finished urinating?

0. not at all
1. less than 1 time in 5
2. less than half the time
3. about half the time
4. more than half the time
5. almost always

Q3. During the past month, how often did you most typically get up at night to urinate?

0. none
1. once
2. 2 times
3. 3 times
4. 4 times
5. 5 or more times

Q4. During the past month, have you experienced pain or burning in your bladder?

0. not at all
2. a few times
3. almost always
4. fairly often
5. usually

Add the numerical values of the checked entries; total score:_____

**Problem Index**

During the past month, how much has each of the following been a problem for you?

Q1. Frequent urination during the day

0. no problem
1. very small problem
2. small problem
3. medium problem
4. big problem

Q2. Getting up at night to urinate?

0. no problem
1. very small problem
2. small problem
3. medium problem
4. big problem

Q3. Need to urinate with little warning

0. no problem
1. very small problem
2. small problem
3. medium problem
4. big problem

Q4. Burning, pain, discomfort, or pressure in your bladder?

0. no problem
1. very small problem
2. small problem
3. medium problem
4. big problem

Add the numerical values of the checked entries; total score:_____

# Finnish version of the O`Leary-Sant questionnaire

**Oirepisteet**

1. Kuinka usein viimeksi kuluneen kuukauden aikana Te olette tuntenut äkillisesti voimakkaan virtsaamisen tarpeen.

0 En laisinkaan.
1 Harvemmin, kuin joka viides kerta.
2 Harvemmin, kuin puolella kerroista.
3 Noin puolella kerroista.
4 Useammin, kuin puolella kerroista.
5 Melkein aina.

2. Kuinka usein viimeksi kuluneen kuukauden aikana Te olette joutunut virtsaamaan uudelleen alle kahden tunnin kuluttua edellisen virtsaamisen jälkeen?

0 En laisinkaan.
1 Harvemmin, kuin joka viides kerta.
2 Harvemmin, kuin puolella kerroista.
3 Noin puolella kerroista.
4 Useammin, kuin puolella kerroista.
5 Melkein aina.

3. Kuinka usein viimeksi kuluneen kuukauden aikana Te olette tavallisesti noussut virtsaamaan yöllä?

0 En kertaakaan.
1 Muutaman kerran.
2 2 kertaa.
3 3 kertaa.
4 4 kertaa.
5 5 kertaa.

Onko Teillä viimeksi kuluneen kuukauden aikana tuntunut kipu tai poltetta rakossa?

0 Ei laisinkaan.
2 Muutaman kerran.
3 Melko usein.
4 Tavallisesti
5 Melkein aina

**Haittapisteet**

1. Kuinka paljon viimeisen kuukauden aikana kuikin seuraavista oireista on vaivannut Teitä?
Tiheä virtsaaminen päiväsaikaan.
0 Ei ole laisinkaan vaivannut
1 Vaivannut hyvin vähän
2 Vaivannut vähän
3 Vaivannut kohtalaisesti
4 Vaivannut kovasti

2. Nouseminen yöllä virtsaamaan.

0 Ei ole laisinkaan vaivannut
1 Vaivannut hyvin vähän
2 Vaivannut vähän
3 Vaivannut kohtalaisesti
4 Vaivannut kovasti

3. Äkillisesti ilmaantuva virtsaamisen tarve.

0 Ei ole laisinkaan vaivannut
1 Vaivannut hyvin vähän
2 Vaivannut vähän
3 Vaivannut kohtalaisesti
4 Vaivannut kovasti

4. Rakossa tuntuva polte, kipu, vaiva tai paine

0 Ei ole laisinkaan vaivannut
1 Vaivannut hyvin vähän
2 Vaivannut vähän
3 Vaivannut kohtalaisesti
4 Vaivannut kovasti

Pisteiden kokonaissumma _____

## Health-related quality of life questionnaire

1. What are your initials? / *Mitkä ovat nimikirjaimenne?*

2. In what hospital are you being seen? / *Missä sairaalassa Teitä on nyt tutkittu?*

3. What is today's date (DD/MM/YY) / *Kyselyynvastaamispäivämäärä (pv/kk/v)*

4. Please circle the number that best describes how much pain you have had on average since yesterday. (The number 10 would indicate pain so severe as to prohibit all activity – the worst pain you can imagine.) / *Ympyröikää se numero, joka parhaiten kuvaa miten paljon KIPUA Teillä on keskimäärin ollut EILISEN JÄLKEEN. (Numero 10 kuvaa niin kovaa kipua kuin voi kestää-pahinta kipua, jonka voitte kuvitella)*

no pain / *ei kipua* pain as bad / *pahin kipu jota*
as you can imagine / *voitte kuvitella*

1 2 3 4 5 6 7 8 9 10

5. Please circle the number that best describes your worst pain during the past 7 days. (The number 10 would indicate pain so severe as to prohibit all activity – the worst pain you can imagine.)
/ *Ympyröikää se numero, joka parhaiten kuvaa SUURINTA KIPUA VIIMEISEN 7 PÄIVÄN aikana. (Numero 10 kuvaa niin kovaa kipua kuin voi kestää-pahinta kipua, jonka voitte kuvitella)*

no pain / *ei kipua* pain as bad / *pahin kipu jota*
as you can imagine / *voitte kuvitella*

1 2 3 4 5 6 7 8 9 10

6. Please circle the number that best describes your least pain during the past 7 days. (The number 10 would indicate pain so severe as to prohibit all activity – the worst pain you can imagine.) /

*Ympyröikää se numero, joka parhaiten kuvaa SUURINTA KIPUA VIIMEISEN 7 PÄIVÄN aikana. (Numero 10 kuvaa niin kovaa kipua kuin voi kestää-pahinta kipua, jonka voitte kuvitella)*

| no pain / *ei kipua* | | | | | | | | | pain as bad / *pahin kipu jota* as you can imagine / *voitte kuvitella* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

7. Please circle the number that best describes how much your pain interfered with your activities during the past 7 days. / *Ympyröikää se numero, joka parhaiten kuvaa MITEN PALJON KIPUNNE HÄIRITSI TOIMINTAANNE VIIMEISEN 7 PÄIVÄN aikana.*

| not at all / *ei häirinnyt* | | | | | | | | | extremely / *häiritsi voimakkaasti* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

8. How much of the time, during the past month, has your health limited your ability to visit with close friends or relatives? / *Kuinka paljon VIIMEISEN KUUKAUDEN AIKANA on sairautenne rajoittanut vierailujanne läheisten ystävien tai sukulaisten luona?*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

9. How much of the time, during the past month, has your health limited your ability to participate in other social activities? / *Kuinka paljon VIIMEISEN KUUKAUDEN AIKANA on sairautenne rajoittanut osallistumistanne muuhun sosiaaliseen kanssakäymiseen?*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

10. How much of the time, during the past month, have you been a very nervous person? / Kuinka usein *VIIMEISEN KUUKAUDEN AIKANA olette ollut hyvin hermostunut*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

11. During the past month, how much of the time have you felt calm and peaceful? / *Kuinka usein olette tuntenut itsenne tyyneksi ja rauhalliseksi VIIMEISEN KUUKAUDEN AIKANA?*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

12. How much of the time, during the past month, have you felt downhearted and blue? / *Kuinka usein olette tuntenut itsenne masentuneeksi ja alakuloiseksi VIIMEISEN KUUKAUDEN AIKANA?*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

13. During the past month, how much of the time have you been a happy person? / *Kuinka usein olette ollut onnellinen VIIMEISEN KUUKAUDEN AIKANA?*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

14. How often, during the past month, have you felt so down in the dumps that nothing could cheer you up? / *Kuinka usein VIIMEISEN KUUKAUDEN AIKANA olette tuntenut itsenne niin masentuneeksi, ettei mikään piristäisi?*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

15. How often, during the past month, did you feel dull or sluggish? / *Kuinka usein VIIMEISEN KUUKAUDEN AIKANA olette tuntenut itsenne laiskaksi ja saamattomaksi?*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

16. During the past month, did you have or feel energy, pep, or vitality? / *Kuinka usein olette tuntenut itsenne energiseksi. aikaansaavaksi tai elinvoimaiseksi VIIMEISEN KUUKAUDEN AIKANA ?*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

17. How often, during the past month, have you felt tired, worn out, used up, or exhausted? / *Kuinka usein VIIMEISEN KUUKAUDEN AIKANA olette tuntenut väsymystä, liikarasittuneisuutta, uupumusta tai loppuun kulumista?*

| all of the time/kaiken aikaa | most of the time /suurimman osan aikaa | a good bit of the time/ paljon | some of the time/ jonkin verran | a little bit of the time vähän | none of the time/ ei lainkaan |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |

18. Please circle the number of days during the past 7 days that you cut down on the things that you usually do because of your health. / *Ympyröikää NIIDEN PÄIVIEN LUKUMÄÄRÄ VIIMEISTEN 7 PÄIVÄN aikana jolloin Teidän täytyi sairautenne vuoksi vähentää niiden asioiden tekemistä, joita tavallisesti teette.*

1 2 3 4 5 6 7

19. Please circle the number of days during the past 7 days that you stayed in bed for all or most of the day because of your health. / *Ympyröikää NIIDEN PÄIVIEN LUKUMÄÄRÄ VIIMEISTEN 7 PÄIVÄN aikana jolloin olitte vuoteen omana koko tai suurimman osan päivästä sairautenne vuoksi.*

1 2 3 4 5 6 7

20. Please circle the number that best describes your overall health during the past month. / *Ympyröikää se numero, joka parhaiten kuvaa TERVEYTTÄNNE YLEENSÄ VIIMEISEN KUUKAUDEN AIKANA.*

0 1 2 3 4 5 6 7 8 9 10

21. Please circle the number that best describes how much difficulty you had because of your health during the past month doing vigorous activities, like lifting heavy objects, running, or participating in sports. / *Ympyröikää se numero, joka parhaiten kuvaa sitä, KUINKA VAIKEAA TEIDÄN ON VIIMEISEN KUUKAUDEN AIKANA sairautenne vuoksi ollut tehdä voimaa vaativia tehtäviä, kuten nostaa painavia esineitä, juosta tai urheilla.*

| no difficulty / *ei vaikeuksia* | a little difficulty/*kohtalaisen vaikeaa* | moderate difficulty/ *vaikeaa* | a great deal of difficulty/ *hyvin vaikeaa* | unable to do/ *mahdotonta* |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

22. Please circle the number that best describes how much difficulty you had because of your health during the past month doing moderate activities, like moving a table, carrying shopping, or bowling. / *Ympyröikää se numero, joka parhaiten kuvaa MITEN VAIKEAA Teillä on ollut sairautenne vuoksi VIIMEISEN KUUKAUDEN AIKANA liikkua ja toimia kohtuullisesti, kuten siirtää pöytää, tai kantaa ostoksia.*

| no difficulty / *ei vaikeuksia* | a little difficulty/*kohtalaisen vaikeaa* | moderate difficulty/ *vaikeaa* | a great deal of difficulty/ *hyvin vaikeaa* | unable to do/ *mahdotonta* |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

23. Please circle the number that best describes how much difficulty you had because of your health during the past month walking uphill or climbing a few flights of stairs. / *Ympyröikää se numero, joka parhaiten kuvaa MITEN VAIKEAA Teillä on ollut sairautenne vuoksi VIIMEISEN KUUKAUDEN AIKANA kävellä ylämäkeä tai nousta muutama kerros portaita.*

| no difficulty / *ei vaikeuksia* | a little difficulty/*kohtalaisen vaikeaa* | moderate difficulty/ *vaikeaa* | a great deal of difficulty/ *hyvin vaikeaa* | unable to do/ *mahdotonta* |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

24. Please circle the number that best describes how much difficulty you had because of your health during the past month bending, lifting, or stooping. / *Ympyröikää se numero, joka parhaiten kuvaa MITEN VAIKEAA Teidän on ollut sairautenne vuoksi VIIMEISEN KUUKAUDEN AIKANA nostaa tai kumartua.*

| no difficulty / *ei vaikeuksia* | a little difficulty/*kohtalaisen vaikeaa* | moderate difficulty/ *vaikeaa* | a great deal of difficulty/ *hyvin vaikeaa* | unable to do/ *mahdotonta* |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

25. Please circle the number that best describes how much difficulty you had because of your health during the past month going for a short walk outdoors. / *Ympyröikää se numero, joka parhaiten kuvaa MITEN VAIKEAA Teillä on ollut sairautenne vuoksi VIIMEISEN KUUKAUDEN AIKANA tehdä pieni kävelylenkki ulkona.*

| no difficulty / *ei vaikeuksia* | a little difficulty/*kohtalaisen vaikeaa* | moderate difficulty/ *vaikeaa* | a great deal of difficulty/ *hyvin vaikeaa* | unable to do/ *mahdotonta* |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

26. Please circle the number that best describes how much difficulty you had because of your health during the past month shaving, dressing, bathing, or showering. / *Ympyröikää se numero, joka parhaiten kuvaa MITEN VAIKEAA Teidän on ollut sairautenne vuoksi VIIMEISEN KUUKAUDEN AIKANA ajaa partaa, pukeutua, kylpeä tai käydä suihkussa.*

| no difficulty / *ei vaikeuksia* | a little difficulty/*kohtalaisen vaikeaa* | moderate difficulty/ *vaikeaa* | a great deal of difficulty/ *hyvin vaikeaa* | unable to do/ *mahdotonta* |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

27. Please circle the number that best describes how much the following statement applied to you during the past month: "I was interested in having sex". / *Ympyröikää se numero, joka parhaiten kuvaa kuinka hyvin seuraava lause sopii Teihin VIIMEISEN KUUKAUDEN AIKANA:" Olen ollut kiinnostunut seksistä".*

| not at all/ ei ollenkaan | a little/ hieman | some/ jonkin verran | quite a bit/ melko paljon | a great deal/ paljon |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

28. Please circle the number that best describes how much the following statement applied to you during the past month: " I thought others found me sexually attractive". / *Ympyröikää se numero, joka parhaiten kuvaa kuinka hyvin seuraava lause sopii Teihin VIIMEISEN KUUKAUDEN AIKANA:" Luulen, että toiset pitävät minua seksuaalisesti puoleensavetävänä".*

| not at all/ ei ollenkaan | a little/ hieman | some/ jonkin verran | quite a bit/ hyvin | a great deal/ erittäin hyvin |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |

29. Please circle the number that best describes how much the following statement applied to you during the past month: “I felt sexually attractive”. / *Ympyröikää se numero, joka parhaiten kuvaa kuinka hyvin seuraava lause sopii Teihin VIIMEISEN KUUKAUDEN AIKANA:” Olen tuntenut itseni seksuaalisesti puoleensavetäväksi”.*

| not at all/ | a little/ | some/ | quite a bit/ | a great deal/ |
|---|---|---|---|---|
| ei ollenkaan | hieman | jonkin verran | hyvin | erittäin hyvin |
| 1 | 2 | 3 | 4 | 5 |

30. Have you tried engage in any type of sexual activity, including masturbation or intercourse, during the past month? / *Oletteko yrittäneet harjoittaa seksuaalista toimintaa, mukaanlukien itsetyydytys ja sukupuoliyhdyntä, VIIMEISEN KUUKAUDEN AIKANA*

| yes / *kyllä* | no / *ei* |
|---|---|
| 1 | 2 |

31. Please circle the number that best describes how much the following statement applied to you during the past month: “I had difficulty becoming sexually aroused”. / *Ympyröikää se numero, joka parhaiten kuvaa kuinka hyvin seuraava lause sopii Teihin VIIMEISEN KUUKAUDEN AIKANA:” Minun on ollut vaikea kiihottua seksuaalisesti”.*

| not at all/ | a little/ | some/ | quite a bit/ | a great deal/ |
|---|---|---|---|---|
| ei ollenkaan | hieman | jonkin verran | hyvin | erittäin hyvin |
| 1 | 2 | 3 | 4 | 5 |

33. Please circle the number that best describes how much the following statement applied to you during the past month: “I had difficulty reaching orgasm”. / *Ympyröikää se numero, joka parhaiten kuvaa kuinka hyvin seuraava lause sopii Teihin VIIMEISEN KUUKAUDEN AIKANA:” Minun oli vaikeaa saada orgasmi”.*

| not at all/ | a little/ | some/ | quite a bit/ | a great deal/ |
|---|---|---|---|---|
| ei ollenkaan | hieman | jonkin verran | hyvin | erittäin hyvin |
| 1 | 2 | 3 | 4 | 5 |