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Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools

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Paper I: study design, translation of the NIH-CPSI questionnaire, testing of the questionnaire in population based study, participation in testing of the questionnaire on the patients consulting andrologist, data analysis, writing the paper.

Paper II: clinical evaluation of the patients and controls, cytological analyses of the semen, data analysis, writing the paper.

Paper III: study design, all cytological analyses of the semen, data analysis, writing the paper.

ABBREVIATIONS

AFP	Alpha-fetoprotein
ANOVA	Analysis of Variance
ARE	Androgen Responsive Element
AUA	American Urology Association
BPH	Benign Prostatic Hyperplasia
CAM	Complementary and Alternative Medical Therapies
CBP	Chronic Bacterial Prostatitis
CEA	Carcinoembryonic Antigen
CFU	Colony Forming Unit
CP/CPPS	Chronic Prostatitis/Chronic Pelvic Pain Syndrome
CPCRN	Chronic Prostatitis Collaborative Research Network
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic Acid
ENA-78	Epithelial Neutrophil Activating Factor-78
EPS	Expressed Prostatic Secretion
EU 6th FP	European Union 6th Framework Programme
GPSS	Giessen Prostatitis Symptom Score
HCG	Human Chorionic Gonadotropin
HGF	Hepatocyte Growth Factor
IFN-γ	Interferon-γ
IL	Interleukin
IPSS	International Prostate Symptom Score
kDa	Kilodalton
LUTS	Lower Urinary Tract Symptoms
M	Million(s)
MRS	de Man-Rogosa-Sharpe
MUG	4-Methylumbelliferyl-β-D-Galactopyranoside
NAFA	The Nordic Association for Andrology
NIH	National Institutes of Health
NIH-CPSI	National Institutes of Health Chronic Prostatitis Symptom Index
PAP	Prostatic Acid Phosphatase
PCP	Primary Care Practitioner
PCR	Polymerase Chain Reaction
PPMT	Pre- and Postmassage Test
PSA	Prostate-specific Antigen
ROS	Reactive Oxygen Species
rRNA	Ribosomal Ribonucleic Acid
SD	Standard Deviation
TMP-SMX	Trimethoprim/sulfamethoxazole
TNFα	Tumor Necrosis Factor α
tPSA	Total PSA
VB1	Voided Bladder 1, Initial-stream Urine

VB2	Voided Bladder 2, Midstream Urine
VB3	Voided Bladder 3, Post-prostate-massage Urine
WBC	White Blood Cell
WHO	World Health Organization

INTRODUCTION

Chronic prostatitis is a complex clinical entity that may affect men of all ages, being the most common urological diagnosis in men less than 50 years of age and the third most common diagnosis in men over 50. It has been estimated that up to 50% of men are affected by prostatitis at some point in their lives (McNaughton-Collins *et al*., 1998).

At the same time our present knowledge of this common disease is poor in several ways: the etiology and pathogenesis of chronic prostatitis are still largely unknown, and therefore, no unified diagnostic and treatment criteria have been agreed upon. One of the most common theories of chronic prostatitis etiology has been bacterial infection, yet in most cases the etiology remains unproved, because in up to 80% of these patients, the culture results of pathogenic bacteria in the prostate-specific material are negative (Wolff 1995; Esfandiari *et al*., 2002; Cottell *et al*., 2000). The clinical presentation of chronic prostatitis is not uniform, ranging from asymptomatic inflammation to severe pelvic pain and voiding disturbances, not to mention the overall effect on the patient's quality of life. To further complicate the issue, the symptoms of prostatitis are usually not correlated with the laboratory findings, thus making it difficult to evaluate treatment progress. Lack of knowledge around prostatitis is also reflected by the wide scope of treatment options available that include antibiotics, alpha-blocking agents, anti-inflammatories, herbal and dietary supplements, but also prostate massaging, biofeedback therapy, thermotherapy etc.

Despite of its wide spread and scarce evidence-based data on etiology, pathogenesis and treatment, prostatitis, in comparison with other major prostate diseases – benign prostatic hyperplasia and prostate cancer – has been a largely unresearched area until the last decade when the new prostatitis classification and new diagnostic tools (NIH-CPSI questionnaire, 2-glass test, semen analysis) became available, thus reviving the interest in prostatitis research. Up to now, the aforementioned and most acknowledged questionnaire was not validated for use in Estonian language.

Moreover, a new category of the disease – asymptomatic inflammatory prostatitis – has been included into new prostatitis classification during the last decade. This most neglected and unresearched prostatitis syndrome is defined as the presence of significant amounts of leukocytes and/or bacteria in prostate-specific material (expressed prostatic secretions, semen or prostate tissue sample) and the absence of subjective symptoms. Very few studies are available on the prevalence and etiology of this new form of prostatitis.

Therefore, our primary aims of this research were to 1) determine the prevalence of asymptomatic inflammatory prostatitis in young Estonian men, 2) investigate the etiologic factors of asymptomatic prostatitis by means of quantitative full-microflora analysis of semen, and 3) establish and validate the use of the most acknowledged diagnostic tool available for prostatitis – National

Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) – in Estonian for use in further epidemiological studies and clinical work alike.

In Estonia, chronic prostatitis has been the subject of collaborative research between the Department of Microbiology, University of Tartu and Andrology Centre of Tartu University Hospital since 1999 with the support from Estonian Science Foundation (Kermes *et al*., 2003; Punab *et al*., 2003; Türk *et al*., 2007; Mändar *et al*., 2005).

All studies for the dissertation were carried out in the Department of Microbiology, University of Tartu and in the Andrology Centre of Tartu University Hospital.

REVIEW OF LITERATURE

1. Prostate gland

1.1. Anatomy and histology

The prostate is a firm musculoglandular structure that resembles a chestnut in shape and size, weighing normally about 18–25 grams. It is situated in the pelvic cavity, between the base of the bladder and the deep transverse perineal muscle, 1–1.5 cm behind the pubic symphysis and in front of ampulla of the rectum from which it can be palpated. The prostate surrounds the very beginning of the male urethra, which is called the prostatic urethra. Additionally, two ejaculatory ducts (continued from the vas deferens) traverse through the prostate and enter the prostatic urethra at the level of the verumontanum.

Formerly, the prostate gland had been divided into five lobes: anterior, posterior, median and two lateral lobes. However, this classification has later been revised (McNeal 1970) and a newer classification based on functional and histogenetic aspects has been developed where the glandular elements (making up about 70% of the prostate mass) are divided into the central and the much larger peripheral zone which make up approximately 95 per cent of the whole glandular structure. The remaining 5 per cent forms the transition zone that is located outside the supramontanal muscular segment of the urethra. It is worth mentioning that although the glandular tissue of both transition and peripheral zones is identical, the transition zone is rarely the site of malignancy (at the same time it is assumed to be the origin of all prostatic hyperplasia), whereas the peripheral zone is a common site of prostatic carcinoma (Walsh *et al*., 1992).

The prostate consists of approximately 40 discrete tubuloalveolar glands which form 15–30 secretory ducts that open into the urethra around the seminal colliculus (Leonhardt *et al*., 1993). The ducts and acini of the glandular element are lined by tall columnar epithelium and supported by stromal elements (basal cells, connective tissue fibers, smooth muscle cells).

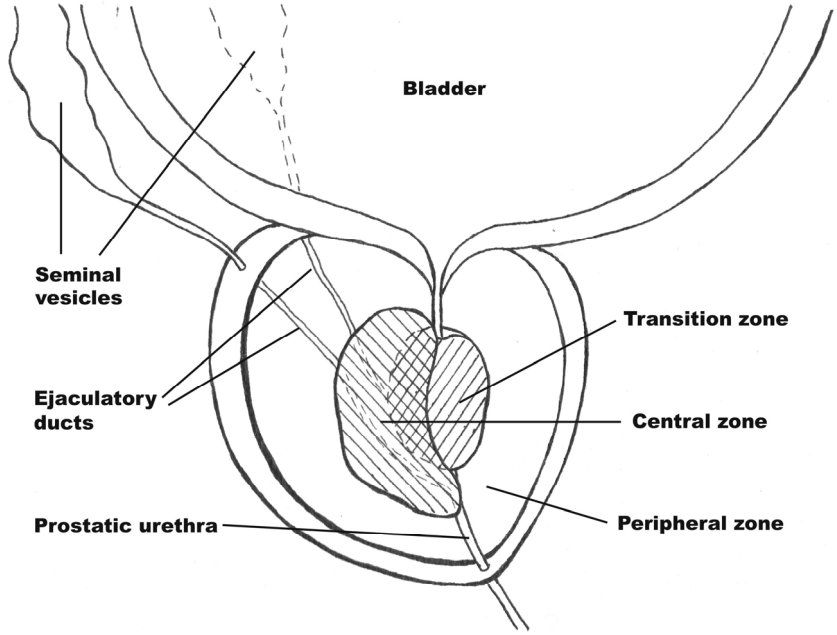

Figure 1. Overview of the prostate anatomy.

1.2. Physiology

The acinar glands (i.e. epithelial cells) respond to androgen (testosterone) stimulation by producing secretions into the acini that drain into the ducts that connect to the urethra. Testosterone that diffuses through the epithelial and stromal cell membranes is metabolized to a more androgenic dihydrotestosterone (DHT) by 5α-reductase and binds specifically to the androgen receptor. The DHT-bound androgen then attaches to a promoter area on DNA sequence called the androgen responsive element (ARE), thus inducing expression of genes responsible for secretory proteins (Robaire *et al*., 1995).

The prostate produces a thin, opaque, weakly acidic secretion (pH 6.6–7.2) that mainly constitutes the first fractions of the human ejaculate. The high zinc content in the seminal plasma originates almost exclusively from the prostate (concentration in seminal plasma 140 μg/ml, concentration in prostatic secretion 488 ± 18 μg/ml). Many possible physiologic roles have been postulated for zinc, including antibacterial activity (Fair *et al*., 1976). The antibacterial mechanism is also supported by studies where it was noted that patients with chronic prostatitis had significantly lower levels of zinc in seminal plasma than the healthy controls (Fair *et al*., 1981; Stamey *et al*., 1968). Zinc levels seen in benign prostatic hyperplasia are elevated, whereas there is a marked decrease in zinc levels in case of prostate malignancies.

Another important constituent of human seminal plasma, an anion citrate, is also mainly secreted by the prostate, with an average content of 376 mg per 100 ml. Citric acid is thought to have a buffer effect in seminal plasma and together with the prostatic acid phosphatase it has been considered a possible chemical indicator of prostate function (Weidner *et al*., 1994).

Prostate-specific antigen (PSA) is secreted by the prostatic epithelial cells and was first identified by Wang *et al*. (1979). It has a molecular weight of 33 kDa and its concentration in seminal plasma is approximately 0.7 mg/ml. PSA can also be detected in serum in much smaller amounts (most frequently used reference range 0–4 μg/l, although lower levels like 2.5 μg/l have been proposed as well (Heidenreich *et al*., 2008) and it has been extensively used as a tumour marker for prostatic carcinoma (Stamey *et al*., 1987; Catalona *et al*., 1991; Schröder *et al*., 2006; Hernandez *et al*., 2004; Han *et al*., 2004). Although its exact function is unknown, it has been proposed that PSA may be important in lysis of the ejaculate clot (Lilja 1985), thus promoting the release and motility of spermatozoa. Elevated serum PSA in case of prostatitis has also been noted in several studies (Nadler *et al*., 2006; Bozeman *et al*., 2002; Jung *et al*., 1998), although it is not yet clear exactly what role PSA plays in the inflammatory process.

Prostatic acid phosphatase (PAP) is a glycoprotein with a molecular weight of 102 kDa, its mean concentration in seminal plasma is 0.3–1.0 mg/ml. The exact function of PAP is unknown, however, it was extensively used as a marker of advanced prostatic carcinomas before more sensitive and specific PSA assay became available.

During ejaculation, sympathetic stimulation from hypogastric plexus causes muscular contraction of the prostate and excretion of the acinar contents into the prostatic urethra and penis to form the ejaculate. The average volume of the human ejaculate is approximately 3 ml, ranging from 2 to 6 ml. The major contribution of the semen volume comes from the seminal vesicles (1.5 to 2 ml); 0.5 ml originates from the prostate and 0.1–0.2 ml from the Cowper's (bulbourethral) glands. During ejaculation, the secretions of these glands are excreted in a sequential manner (Tsai *et al*., 1984). The volume of the spermatozoa component is less than 1 per cent of the total ejaculate.

2. Symptomatic prostatitis

2.1. Concept, clinical features and classification

Prostatitis refers to several clinical syndromes which mainly present a symptom complex of pelvic area pain and a wide range of voiding, psychological and sexual disturbances.

The early classification of prostatitis described four syndromes for which pelvic pain in the male was the common factor (Drach *et al*., 1978) (Table 1). This classification was based on bacteriological localization patterns obtained by the Meares-Stamey 'four glass test' (Meares *et al*., 1968), thus dividing patients into the corresponding categories depending on whether the prostatic secretion contained 1) bacteria and leukocytes (acute/chronic bacterial prostatitis), 2) leukocytes only (chronic nonbacterial prostatitis) or 3) neither of these (prostatodynia).

To improve the diagnosis and treatment of prostatitis, the National Institutes of Health (NIH) established an International Prostatitis Collaborative Network. This group convened 2 consensus conferences (1995 and 1998) to establish a new definition and classification of prostatitis syndromes (Krieger *et al*., 1999) (Table 1).

Acute bacterial prostatitis (NIH category I) and chronic bacterial prostatitis (NIH category II) remained unchanged in the new classification. Acute bacterial prostatitis is a generalized acute infection of the prostate gland with sudden onset. Most usual symptoms include fever, dysuria, malaise, chills, myalgia, abdominal and/or pelvic/perineal pain; physical examination usually reveals a tense and exquisitely tender prostate.

Chronic bacterial prostatitis (NIH group II) typically involves relapsing episodes of urinary tract infections, usually with the same organism seen on urine cultures. Patients with chronic bacterial prostatitis are usually asymptomatic between infections. These two categories comprise only 5–10% of all prostatitis syndromes (McNaughton-Collins *et al*., 2007; Nickel 2000; Naber *et al*., 2000).

Chronic nonbacterial prostatitis was renamed as chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) in order to stress that pelvic pain is the most characteristic symptom and to recognize that the role of the prostate in producing symptoms is in fact still unknown. This category III prostatitis was further divided into 2 subclasses, inflammatory and noninflammatory, depending on the presence or absence of leukocytes in the prostate-specific material (expressed prostatic secretion, post-prostate massage urine, semen). CP/CPPS cannot be rigidly defined and is therefore primarily a diagnosis of exclusion, although it has been proposed that CP/CPPS should be broadly defined as the presence of characteristic symptoms of discomfort of pain in the pelvic region for a period ≥3 months within the past 6 months (Schaeffer *et al*., 2002). In

addition to pain, high proportion of patients with CP/CPPS has some degree of sexual dysfunction (erectile dysfunction, decreased libido, premature ejaculation). A study by Keltikangas-Järvinen *et al*. (1981) showed that 52% of patients with prostatitis experience sexual disturbances (periodic or total erectile dysfunction or decreased libido); Berghuis *et al*. (1996) found that prostatitis reduced the frequency of sexual contacts in 85%, interfered with or ended an ongoing sexual relationship in 67% and prevented the men from establishing new sexual relationships in 43% of men; Mehik *et al*. (2001) reported decreased libido in 24% and erectile dysfunction in 43% of men with symptomatic prostatitis. Although it is clear that sexual dysfunction is higher in prostatitis patients compared to healthy men, the exact mechanisms of these symptoms and their interactions with the infection and/or inflammation are still unknown. Also, variable irritative and/or obstructive urinary symptoms may present with CP/CPPS. All the aforementioned symptoms may be long-lasting, resistant to therapy, thus frustrating to patient (as well as the physician) and as a result can have a significant impact on patient's quality of life.

The new classification also introduced a new category called asymptomatic inflammatory prostatitis, where significant amounts of leukocytes and/or bacteria can be found in prostate-specific samples, but no clinical symptoms are present (see section 3).

Table 1. Classification and defining features of prostatitis syndromes.

New classification (1999)			**Early classification (1978)**
Category	**Type**	**Description**	
I	Acute bacterial prostatitis	Acute febrile infection with pain and dysuria	Acute bacterial prostatitis
II	Chronic bacterial prostatitis	Chronic recurrent infection with pain and dysuria	Chronic bacterial prostatitis
III	Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS)	Chronic genitourinary pain and/or voiding disturbances, no detectable infection	
IIIA	Inflammatory subtype	Significant amount of white blood cells in prostate-specific samples	Chronic nonbacterial prostatitis
IIIB	Non-inflammatory subtype	Insignificant amount of white blood cells in prostate-specific samples	Prostatodynia
IV	Asymptomatic inflammatory prostatitis	Significant amount of white blood cells and/or bacteria in prostate-specific samples, no subjective symptoms	

2.2. Etiology and pathogenesis

Acute bacterial prostatitis (NIH group I) and chronic bacterial prostatitis (NIH group II) are caused by known urinary tract pathogens (mostly Gram-negative bacteria and enterococci). Most likely mechanisms of prostate infection include ascending urethral infection, intraprostatic urinary reflux, direct invasion or lymphogenous spread from the rectum, and direct hematogenous infection. Due to the well-known etiology, these infections can be easily diagnosed using routine bacteriological cultures of mid-stream urine (and/or quantitative segmental bacteriological localization cultures and microscopy of EPS in chronic bacterial prostatitis) (Wagenlehner *et al*., 2003).

At the same time there is continuing debate among researchers as for the possible cause(s) of categories III and IV prostatitis. The category III prostatitis that is the most frequent and problematic among the prostatitis syndromes has gained lot of attention. Although several theories have been proposed regarding its etiology, the nature of this syndrome still remains unknown.

The most usual symptoms of CP/CPPS (NIH group III), pelvic pain and voiding dysfunction, resemble those of bacterial prostatitis, therefore one of the most common theories of CP/CPPS etiology is bacterial infection. However, the diagnostic bacteriological studies for known uropathogens (such as *Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Proteus*, *Serratia*, *Enterococcus* spp.) that are usually found in acute or chronic bacterial prostatitis, are mostly negative in CP/CPPS (Weidner *et al*., 1991), additionally the bacterial counts do not usually correlate with the severity of symptoms (Schaeffer *et al*., 2002). Still, many researchers feel that the majority of patients with prostatitis have a microbial etiology for their disease due to the facts that 1) routine (aerobic) microbiological studies cannot reveal the full complexity of microbiocenosis in the genital tract, and the recent studies (both microbiological and molecular) have suggested that chronic prostatitis may represent an infectious disease in many, if not all cases (Dimitrakov *et al*., 2001; Nickel 2000; Krieger *et al*., 2002; Domingue *et al*., 1998; Tanner *et al*., 1999; Szöke *et al*., 1998); 2) according to both clinical experience and an open-label study by Nickel *et al*. (2001) CP/CPPS patients with negative cultures may still have a positive treatment response with antibiotic therapy. It has been proposed that cryptic, fastidious microorganisms, anaerobes, wall-deficient bacteria, "biofilms" adherent to the prostatic ductal walls or in the obstructed ducts (and therefore inaccessible for culture or treatment by antibiotics) may be important etiologic factors of chronic prostatitis. Other possible causative agents may include viruses and fungi, especially in immunocompromised patients (Domingue *et al*., 1998; Elert *et al*., 2000; Szöke *et al*., 1998; Nickel, 2000). Our own previous study (Kermes *et al*., 2003) has shown that in leukocytospermic CP/CPPS patients the number and total count of different bacterial species was significantly higher than in non-leukocytospermic samples, thus also suggesting that prostatitis might be viewed as an unfavourable shift in the balance of genital microflora.

However, as the causal relationships between infection and prostatitis have not yet been sufficiently researched, several other etiopathogenetic factors have been proposed for prostatitis, such as autoimmune or immunologic (possibly previous bacterial infection), neuromuscular (muscular dysfunction and neuropathic pain), endocrine (low levels of testosterone predisposing the prostate to inflammation), anatomic (dysfunctional high pressure voiding and intraprostatic duct reflux, uncircumcised prepuce) and traumatic (instrumentation, catheterization).

To this date, no single etiopathogenetic mechanism has been proved yet, but an interrelated and multifactorial cascade has been proposed by Nickel (2000) where an initiating event (infection, trauma etc.) may lead to immunologic stimulation, inflammation, neurogenic stimulation, neuropathic damage with afferent nerve upregulation and ultimately, pain.

Although the pathogenesis of (chronic) prostatitis is still largely unknown, the starting point for investigation of CP/CPPS pathogenesis has been inflammation. Traditionally the leukocytes in prostate-specific materials have been studied and considered to be markers for an inflammatory process that contributes to the symptoms. However, as leukocytes can be found also in asymptomatic men and the severity of symptoms in CP/CPPS does not seem to correlate with the leukocyte counts (Schaeffer *et al*., 2002), the use of WBCs as inflammatory markers may be limited.

2.3. Diagnostic procedures

2.3.1. Current diagnostic options

The diagnostic workup of prostatitis stems from the new NIH classification (Table 1) and includes the differentiation between a) acute and chronic prostatitis; b) bacterial and nonbacterial prostatitis; c) inflammatory and non-inflammatory prostatitis and d) symptomatic and asymptomatic prostatitis.

Acute bacterial prostatitis (NIH group I) is clearly associated with bacterial infection and a urine culture that grows uropathogens (McNaughton-Collins *et al*., 2007). Diagnosis of acute bacterial prostatitis is primarily based on clinical findings (fever, pain, dysuria) and a positive urine culture. Prostate massage is not recommended in acute prostatitis due to possible bacteremia.

Chronic bacterial prostatitis (NIH group II) is characterized by culture-documented recurrent urinary tract infection combined with symptoms of acute or chronic pelvic pain without the systemic component of the acute bacterial prostatitis. Although the diagnosis of both conditions (NIH groups I and II) is relatively straightforward, they only account for approximately 10% of all prostatitis cases.

Chronic prostatitis/chronic pelvic pain syndrome (NIH group III, also known as CP/CPPS) accounts for the vast majority of all prostatitis cases (>90%) but in

most cases, the etiologic factor remains unknown. Previous investigators have found that no pathogenic microorganisms can be detected by conventional methods of microbiology in a majority of patients with WBCs in their semen, and no correlation between seminal microbes and raised leukocytes can be found (Wolff 1995; Omu *et al*., 1999; Habermann *et al*., 1999; Esfandiari *et al*., 2002; Cottell *et al*., 2000; Munuce *et al*., 1999; Trum *et al*., 1998). The major presenting symptom and the starting point of clinical evaluation of CP/CPPS is pain/discomfort (pelvic, perineal, penile and/or ejaculatory), but variable irritative and obstructive urinary symptoms and sexual dysfunction (usually ejaculatory disturbances and discomfort) may also be present (Krieger *et al*., 1999; Nickel 2002). It has been suggested that the definition of CP/CPPS should include the presence of characteristic symptoms of discomfort or pain for a period of ≥3 months within the past 6 months (Schaeffer *et al*., 2002). Several symptom questionnaires have therefore been tested and validated for use in CP/CPPS, thus enabling the clinicians and researchers to rate the severity of symptoms numerically, distinguish between symptomatic and asymptomatic forms of the prostatitis syndromes as well as evaluate the treatment effect over time (see section 2.5.2.). Basic examinations of all CP/CPPS patients also include history taking (previous and concurrent illnesses, medications, psycho-social history, sexual history and other possible risk factors) and physical examination. The latter should focus on the lower urinary tract; size, consistency, irregularity and tenderness of the prostate gland should also be evaluated with a careful digital rectal examination (DRE) (Nickel 2003). Urinary flow rate, residual urine determination, transrectal ultrasound of the prostate and serum PSA (prostate-specific antigen) measurement have also been used in evaluation of CP/CPPS, however it should be noted that these procedures are not definitive for prostatitis syndromes and thus should only be used as additional measures – nevertheless, transrectal ultrasound and urinary flow rate are recommended in older subjects in order to rule out possible bladder outlet obstruction and/or prostate cancer component.

CP/CPPS is further divided depending on the presence (inflammatory form IIIA) or absence (noninflammatory form IIIB) of white blood cells (WBC) in semen, expressed prostatic secretion (EPS) or post-prostate-massage urine (VB3).

2.3.2. Prostate-specific specimens

Traditionally, a ‘four-glass test’ (also called Meares-Stamey test) had been used for diagnosis of CP/CPPS (as well as chronic bacterial prostatitis). This quantitative and localizing technique included sequential samples from initial-stream urine (voided bladder 1, VB1), midstream urine (VB2), prostatic secretions obtained by prostate massage (EPS) and post-massage urine (VB3) (Meares *et al*., 1968). However, later surveys (McNaughton-Collins *et al*., 2000) indicated that most urologists never employ this test during lower urinary

tract evaluation for prostatitis, therefore a simpler and cost-effective version of this test (also called pre- and postmassage test (PPMT) or 'two-glass test') was developed by Nickel (1997) that involved the culture and microscopic examination of urine before and after prostatic massage.

The diagnosis of bacterial prostatitis is made when the bacterial colony count in the EPS or VB3 is at least 10-fold greater than in pre-massage urine specimen(s). Leukocytosis (marker of inflammation in EPS or VB3) is defined as 5 to 10 (or more) WBCs per high-power field (Meares *et al.*, 1968; Wright *et al*., 1994), thus enabling to differentiate between categories IIIA and IIIB.

Semen analysis is another frequent tool in the diagnostic workup of prostatitis. Significant number of leukocytes in semen (leukocytospermia) is defined by World Health Organization as >1.0 M WBC/ml of semen (WHO, 1999), however, recent studies (Punab *et al*., 2003; Sharma *et al*., 2001; Lackner *et al*., 2006) have suggested that this limit probably needs to be lowered.

When comparing semen and EPS as diagnostic specimens for prostate inflammation, several important points should be taken into account:

- There are several studies that show loss of sensitivity and/or specificity of cultures in EPS when compared to semen (Budia *et al*., 2006; Pavone-Macaluso 2007).
- Semen sample is easier and more convenient to deliver for the subject than in the case of EPS, being especially relevant for younger men.
- The validity of the cut-off level for WBC in EPS (>5–10 WBC/hpf) has not been uniformly accepted, also EPS cannot be always obtained by prostatic massage, thus the post-massage urine has to be examined as a substitute for EPS (Krieger *et al*., 2000).
- Semen can be regarded as a suitable material for reflecting inflammatory alterations in the prostate, as 30% of ejaculate volume originates from prostate, also, the prostate usually contracts during ejaculation, thus emptying the contents of all acini into the urethra, unlike the case of EPS, where the most heavily represented area is the central part of the prostate. On the other hand, however, semen may be inferior to EPS in case of the ductal obstruction, where EPS is clearly a better option (Budia *et al*., 2006).
- Semen is a better option when evaluating a subfertile patient in order to rule out possible inflammatory causes, whereas EPS cannot be used in these cases. This is also true in healthy individuals who wish to evaluate their fertility status.

Therefore, both semen and EPS have their strengths and weaknesses for diagnostic tests of prostatitis syndromes, however, the combination of these two might yield better results for the diagnostic work-up and clinical management of prostatitis.

2.3.3. Perspectives on the use of new diagnostic tools

In the search for new and more reliable inflammatory markers and mediators, cytokines have been proposed to be more sensitive than WBCs in determining the clinical status of CP/CPPS patients. As for proinflammatory cytokines, several studies have shown that men with CP/CPPS have higher concentrations of tumor necrosis factor α (TNF), interleukin-1β (IL-1β) (Alexander *et al*., 1998; Orhan *et al*., 2001), interleukin-6 (IL-6) (Orhan *et al*., 2001), interleukin-8 (IL-8) (Orhan *et al*., 2001; Hochreiter *et al*., 2000), epithelial neutrophil activating factor-78 (ENA-78) (Hochreiter *et al*., 2000) and interferon-γ (IFN-γ) (Miller *et al*., 2002) in prostate-specific materials compared with controls. However, no clear correlation exists between cytokine concentrations and disease symptoms (mainly pelvic pain) – for example, in one study of NIH category IIIB cases serum and seminal plasma IL-6 increased initially and then decreased, thus correlating with the intensity of clinical symptoms (John *et al*., 2001); in another study, IL-6 correlated inversely with pain (Miller *et al*., 2002). There is also conflicting evidence about the correlation between the concentration of WBCs and cytokines. Nadler and colleagues (Nadler *et al*., 2000) found a clear correlation between IL-1β and TNFα levels, but no correlation between WBC and IL-1β in EPS of men with confirmed chronic prostatitis. On the other hand, several studies have shown strong correlations between IL-8 and WBC (Hochreiter *et al*., 2000; Eggert-Kruse *et al*., 2001), IL-6 and WBC (Eggert-Kruse *et al*., 2001; Ohta *et al*., 2002; Swatowski *et al*., 2002), ENA-78 and WBC (Hochreiter *et al*., 2000). Anti-inflammatory cytokines (IL-1ra, IL-4, IL-10, IL-13) limit the intensity of the inflammatory cascade and suppress the activity of proinflammatory cytokines, therefore, a balance between the effects of proinflammatory and anti-inflammatory cytokines determines the outcome of the inflammatory process (Dinarello 2000). Still, it should be noted that to this date, no validation studies have been performed for cytokine measurement in seminal plasma or EPS.

Another marker of inflammation is the presence of reactive oxygen species (ROS) (in prostate-specific material), which are released by neutrophils in response to antigenic stimulation. The excess amount of ROS and concurrent decrease of antioxidant activity of seminal plasma cause oxidative stress that impairs sperm function. High levels of ROS have been found in men with NIH category III prostatitis (Pasqualotto *et al*., 2000; Shahed *et al*., 2000; Lewis *et al*., 1995; Kullisaar *et al*., 2008). However, it has been proposed that oxidative stress levels are not a marker of leukocytes per se, but rather a marker of tissue injury.

2.4. Treatment options

In acute bacterial prostatitis (NIH category I), parenteral administration of high doses of bactericidal antibiotics, such as a broad-spectrum penicillin derivative, a third-generation cephalosporin with or without aminoglycosides, or a fluoroquinolone, are required until fever and other signs and symptoms of infection subside. Prostatic massage is contraindicated. After initial improvement, a switch to an oral regimen, a fluoroquinolone, is appropriate and should be prescribed for at least 4 weeks. In less severe cases, a fluoroquinolone may be given orally for 2–4 weeks (Naber *et al*., 2001).

In chronic bacterial prostatitis (NIH category II, CBP), selection of an appropriate antimicrobial agent that has optimal pharmacokinetics for prostatic secretion and tissue is important (Stamey *et al*., 1970). Antibiotic groups known for their good penetration in the prostate tissue include fluoroquinolones, trimethoprim/sulfamethoxazole (TMP-SMX), macrolides and tetracyclines, latter two being considered second-line agents (Naber 1999; Nickel *et al*., 2000). The duration of antibiotic treatment is based on experience and expert opinion and is supported by many clinical studies (Naber 1999). In CBP, an oral fluoroquinolone should be given for at least 4–6 weeks after the initial diagnosis. Relatively high doses are needed and oral therapy is preferred (Bjerklund Johansen *et al*., 1998). Chronic bacterial prostatitis can be a relapsing illness and recurrent episodes are best managed by either continuous low-dose suppressive therapy with an effective regimen such as a fluoroquinolone, or intermittent treatment whenever symptoms recur (Wagenlehner *et al*., 2003).

The wide scope of recommended treatments for CP/CPPS (NIH category III) indicates how little is known about what causes the condition and how to diagnose and treat it. Although experts may recommend or support an empirical course of antibiotics for chronic abacterial prostatitis (Nickel 1998; Lipsky 1999; Bjerklund Johansen *et al*., 1998), this practice is not supported by the existing evidence on disease etiology and treatment outcomes (McNaughton-Collins *et al*., 2000). Antibiotics are the most common first-line therapy for all the prostatitis syndromes (Schaeffer *et al*., 2002; McNaughton-Collins *et al*., 2000), despite the consistent finding that only ~10% of these men have definite bacterial infection (Wagenlehner *et al*., 2003). In a recent survey, nearly all urologists who responded reported treating at least 50% of their prostatitis patients with antibiotics (McNaughton-Collins *et al*., 2000). Nickel *et al*. (1998) found that trimethoprim/sulfamethoxazole (TMP-SMX) was the usual first line treatment of prostatitis for 46% of PCPs (primary care practitioners) and 52% of urologists in Canada; fluoroquinolones were the first line treatment choice for 40% of PCPs and 42% of urologists. Other medications that have been prescribed by physicians for treating CP/CPPS are α-blockers and anti-inflammatories (Schaeffer *et al*., 2002), allopurinol (Persson *et al*., 1996), muscle relaxants such as diazepam or baclofen (Osborne *et al*., 1981), and finasteride (Leskinen *et al*., 1999). Dietary supplements such as quercetin have also been

used for treatment of CP/CPPS – in a double-blind placebo trial it was found that 67% of patients receiving quercetin stated significant symptom improvement (Shoskes *et al*., 1999). Repetitive prostate massage has been the traditional and standard therapy for prostatitis for decades and it has become more 'popular' again (Nickel *et al*., 1999) partly because of the failure of traditional medical therapy to improve the symptoms of most patients with prostatitis, but also because the belief that chronic bacterial infection exists in the prostate gland in blocked ducts or microabscesses (Dimitrakov *et al*., 2001). Also, several other complementary and alternative medical (CAM) therapies have been proposed and used in CP/CPPS. These include acupuncture, biofeedback therapy, electrical stimulation, heat/laser therapy, herbal and nutritional agents (e.g. saw palmetto and pollen extract) (Capodice *et al*., 2005). In addition, regular sexual activity is recommended in order to provide symptom relief for men with CP/CPPS (Yavascaoglu *et al*., 1999).

2.5. Epidemiology

Prostatitis syndromes represent a common, but also a complex clinical entity that may affect up to 50% of men at some point in their lives (McNaughton-Collins *et al*., 1998). In the early 1990s, more than 2 million office visits per year in the United States resulted from prostatitis (McNaughton-Collins *et al*., 1998). Furthermore, the devastating effect of prostatitis on the quality of life of the patients could be compared with that of unstable angina, myocardial infarction or Crohn's disease (Wenninger *et al*., 1996). It is the most frequent urological diagnosis in men less than 50 years of age and the third most common urological diagnosis in men over 50 (McNaughton-Collins *et al*., 1998).

2.5.1. Population-based, clinic-based and pathohistological studies

Most commonly used measures of disease occurrence (i.e. frequency) in epidemiology are prevalence and incidence. Prevalence refers to the proportion of people who have the disease at a specific moment in time; incidence, on the other hand, reflects the rate of occurrence of new cases (dos Santos Silva 1999). Because of the varying definitions and study settings used, the literature contains a number of different epidemiological estimates of prostatitis.

In population-based studies where the study subjects received questionnaires (either NIH-CPSI or an unvalidated questionnaire) by mail or were interviewed over the telephone, the overall lifetime prevalence of prostatitis has been estimated to 14% (Mehik *et al*., 2000), the prevalence of a self-reported history of prostatitis, from 5% to 16% (McNaughton-Collins *et al*., 2002; Moon *et al*., 1997; Rothman *et al*., 2004) and the prevalence of chronic prostatitis-like

symptoms, from 2% to 10% (Roberts *et al*., 2002; Tan *et al*., 2002; Ku *et al*., 2001; Cheah *et al*., 2003; Nickel *et al*., 2001).

In clinic-based studies where study data are generally collected from health-care registers and other recorded medical data or the study subjects are limited to referral patients from tertiary care institutions, the prevalence of medically diagnosed prostatitis is estimated to be 9% (Roberts *et al*., 1998) and the incidence of physician-diagnosed CP/CPPS is estimated to be 3.3 per 1,000 person-years (Clemens *et al*., 2005). In a study by McNaughton-Collins *et al*. (1998) it was found that in the United States, prostatitis accounted for almost 2 million annual visits.

Several of the aforementioned studies have certain limitations that have to be noted – for example, in the study by Roberts *et al*. (1998), classification of men was based on a somewhat arbitrary clinical diagnosis of prostatitis, without specific criteria or supporting laboratory findings; in the study by Moon *et al*., 1997, the study population (U.S. National Guard unit) was healthier than average, the study group was relatively small (n=184) and the response rate low (38%) for a population-based epidemiologic study. Also, several prevalence studies of prostatitis-like symptoms that used NIH-CPSI as a study tool have used different case definitions (Nickel *et al*., 2001; Cheah *et al*., 2003; Ku *et al*., 2001). Hence, the true prevalence (and incidence) of symptomatic prostatitis is still largely unknown.

The histologic prevalence of prostatitis is significantly higher than revealed in aforementioned studies ranging from 6% to 44% (autopsy specimens) in a study by Roberts *et al*. (1997) and from 35% to 98% (autopsy and surgical specimens) in a study by Bennett *et al*. (1993). These numbers are even higher than the previously mentioned estimate by McNaughton Collins *et al*. (1998), thus meaning that a considerable portion of all prostatites may be asympto-matic.

2.5.2. Questionnaires used in epidemiological studies

A number of different questionnaires have been developed and used in order to evaluate symptomatic forms of prostatitis. These include the Washington Symptom Score (Krieger *et al*., 1996), a four-item symptom score by Neal and Moon (1994), a ten-item Symptom Severity Index by Nickel and Sorensen (1996), an 18-item Giessen Prostatitis Symptom Score (GPSS, Brähler *et al*., 2001). In a study by Mehik *et al*. (2000), the researchers used a 102-item multiple-choice questionnaire consisting of two parts: personal data (age, marital status, education, profession, job description and outdoor activities) and questions related to urological history, current status of prostatitis symptoms and any procedures carried out to diagnose and treat prostatitis. Also, few studies have used AUA (American Urology Association) symptom index (i.e. International Prostate Symptom Score, IPSS) (Moon *et al*., 1997, Nickel *et al*., 2005), although it is primarily used for evaluation of the obstructive and

irritative urinary symptoms in urological patients and thus not suitable for discriminating among prostatitis and BPH patients.

The original (English) version of the National Institutes of Health chronic prostatitis symptom index (NIH-CPSI) was developed by the NIH Chronic Prostatitis Collaborative Research Network (CPCRN) (Litwin *et al*., 1999) and has been successfully used in both clinical and epidemiological studies (Nickel *et al*., 1999; Nickel, 2003), enabling to differentiate patients with chronic prostatitis (or prostatitis-like symptoms), benign prostatic hyperplasia (BPH) and healthy controls (Litwin *et al*., 1999; McNaughton-Collins 2003). In addition, the translated versions of this questionnaire in Spanish (Collins *et al*., 2001), Japanese (Kunishima *et al*., 2002; Monden *et al*.; 2002), German (Hochreiter *et al*., 2001; Schneider *et al*. 2002), Finnish (Leskinen *et al*., 2003), Malay, Chinese (Cheah *et al*., 2006), Korean (Chong *et al*., 2001), Italian (Giubilei *et al*., 2005), French Canadian (Karakiewicz *et al*., 2005), Turkish (Gonen *et al*., 2005) and Arabic (El-Nashaar *et al*., 2006) have been successfully used. This nine-item questionnaire measures the three most important domains in the chronic prostatitis patients' experience: pain, voiding symptoms and impact of those symptoms on the patient's quality of life. Based on analysis of the index validation study comparing patients with prostatitis to normal controls and those with BPH, the two questions most specific for prostatitis, including perineal and/or ejaculatory pain/discomfort, and a total pain score (0 to 21) 4 or greater have been proposed to identify men with significant prostatitis-like symptoms (Nickel *et al*., 2001).

To this date, the NIH-CPSI has not been validated for use in Estonian language.

3. Asymptomatic inflammatory prostatitis

3.1. Concept and clinical significance

New classification system of prostatitis syndromes (Krieger *et al*., 1999) introduced a new category called asymptomatic inflammatory prostatitis (NIH category IV), where significant amounts of leukocytes and/or bacteria can be found in prostate-specific samples, but no clinical symptoms are present (Table 1). It is often found incidentally during evaluation for other disorders (infertility, suspected prostate cancer) in men without symptoms of prostatitis (Nickel 2003; McNaughton-Collins *et al*., 2007). Though asymptomatic, the increased leukocyte count in semen may not be safe for reproductive function, since it has been associated with a significant decrease in sperm motility, increase in oxidative stress levels, and DNA damage (Saleh *et al*., 2002; Arata de Bellabarba *et al*., 2000; Simbini *et al*., 1998; Fedder 1996), thus suggesting the need for new treatment strategies for leukocytospermia-related infertility. It

has also been hypothesized that a long-lasting inflammation may initiate and/or promote carcinogenesis (Coussens *et al*., 2002), however, no causal relationships have been shown to this date between (asymptomatic) leukocytospermia and infertility, prostate cancer or other pathologic conditions and thus, the clinical relevance of NIH category IV prostatitis is unknown.

3.2. Etiology and pathogenesis

There are only few studies available about asymptomatic inflammatory prostatitis and therefore, the data concerning the possible etiology and/or pathogenesis of NIH category IV prostatitis is scarce. However, it is thought to be associated with subclinical genital tract infection, therefore suggesting a role for bacteria in the etiology of the disease. In those patients where known or potential pathogens have been found, coliforms like *Escherichia coli, Proteus* sp. and *Klebsiella pneumoniae*, β-hemolytic streptococci, *Chlamydia trachomatis*, *Gardnerella vaginalis*, mycoplasmas, *Staphylococcus aureus*, and anaerobes are most frequent. Yet in most cases the etiology remains unproved, because in over 80% of these patients, the culture results of pathogenic bacteria in the prostate-specific material are negative and no correlation can be found between these microorganisms and leukocyte counts (Wolff 1995; Omu *et al*., 1999; Habermann *et al*., 1999; Esfandiari *et al*., 2002; Cottell *et al*., 2000; Munuce *et al*., 1999).

3.3. Diagnostic procedures

Due to its asymptomatic nature, category IV prostatitis can only be detected by laboratory means (significant number of leukocytes in semen, EPS or VB3; inflammation in prostate biopsy). Although the limit of significant leukocytospermia is defined as over one million WBC per ml of semen in WHO guidelines (1999), the diagnostic threshold is not clearly defined for other prostate-specific samples like EPS or VB3 (Nickel *et al*., 2003). Moreover, in several studies (Jedrzejczak *et al*., 1996; Sharma *et al*., 2001; Lackner *et al*., 2006) including our recent study (Punab *et al*., 2003) it has been proposed that this WHO-defined limit of leukocytospermia may be too high to discriminate between patients with or without significant bacteriospermia, and therefore lower limits of leukocytospermia (such as >0.2 M WBC per ml of semen) have been suggested as well.

Previous studies have also indicated that proinflammatory cytokines (e.g. IL-6) are associated with seminal leukocytes (Eggert-Kruse *et al*., 2001; Kopa *et al*., 2005) and may therefore serve as additional inflammatory markers in the diagnostic work-up.

Similarly to other prostatitis categories, asymptomatic inflammatory prostatitis has also been associated with elevated PSA (prostate-specific antigen) levels (Carver *et al*., 2003; Potts 2000), however, the opposite data have been published as well (Nickel *et al*., 1999). Ozden *et al*. (2006) found that PSA levels in men with category IV prostatitis were positively correlated with the aggressiveness of inflammation. Asymptomatic prostatitis has similar effects on total PSA and free PSA levels in serum as prostate cancer (Stancik *et al*., 2004), suggesting that screening for NIH category IV prostatitis (EPS, semen samples) and treating the underlying subclinical inflammation would increase the specificity of PSA screening and thereby reduce the number of potentially unnecessary biopsies in prostatitis patients (Potts 2001). Yet the association between different laboratory markers in case of category IV prostatitis remains to be elucidated.

3.4. Treatment options

For NIH category IV prostatitis, no treatment is currently recommended, except in cases where the underlying condition (infertility or BPH/prostate cancer) requires medical intervention (Gurunadha Rao Tunuguntla *et al*., 2002), thus warranting the use of antibiotics. Antibiotic treatment has also been suggested for men with elevated PSA levels (Potts 2000), however, no consensus exists regarding the treatment of category IV prostatitis due to the lack of studies in this field.

3.5. Epidemiology

Epidemiology of asymptomatic inflammatory prostatitis cannot be studied with symptom questionnaires (e.g. NIH-CPSI), therefore laboratory means must be used. Only few studies have determined the asymptomatic presence of leukocytes in prostate-specific material other than semen: expressed prostatic secretions (Nickel *et al*., 2003; Carver *et al*., 2003), post-prostate massage urine (Nickel *et al*., 2003; Potts 2000) or histological specimens from prostate (Shimomura *et al*., 2003). In these studies, prevalence of asymptomatic inflammation (WBC) ranged from 11 to 42%.

In addition, several studies on chronic symptomatic prostatitis have determined WBC counts in prostate-specific materials (EPS, VB3, semen) of their control subjects (Nickel *et al*., 2003; Christiansen *et al*., 1991) and it may therefore be assumed that the control groups also included men with asymptomatic inflammatory prostatitis. Nickel *et al*. (2003) found that men with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) had statistically higher leukocyte counts in all segmented urine samples and EPS, but not in semen compared to asymptomatic control men (8% prevalence of significant leuko-

cytospermia). However, the control population also had a high prevalence of leukocytes in any prostate-specific material. Christiansen *et al*. (1991) found that 15% of their asymptomatic controls had significant leukocytospermia.

Semen as specimen has also been used in several studies of infertile men with no physical complaints where the authors have found the presence of leukocytospermia to be 10 to 44% (Sharma *et al*., 2001; Omu *et al*., 1999; Wolff 1995; Arata de Bellabarba *et al*., 2000; Alvarez *et al*., 2002; Saleh *et al*., 2002).

No semen-specific population-based studies on the prevalence of asymptomatic inflammatory prostatitis have been published.

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Hence, there are several unsolved problems in understanding and management of chronic prostatitis, especially as for its asymptomatic form. The world-wide acknowledged diagnostic tool for prostatitis syndromes, NIH-CPSI questionnaire, has not been validated for use in Estonian language. The prevalence and etiopathogenesis of the most neglected and under-researched form of prostatitis, asymptomatic inflammatory (NIH category IV) prostatitis, have been largely unknown. In addition, the diagnostic value of inflammatory markers in case of this syndrome needs additional studies to choose the optimal set for routine use.

AIMS OF THE RESEARCH

The general aim of the study was to assess the prevalence, etiological factors and diagnostic markers of asymptomatic inflammatory prostatitis as well as to establish and validate the Estonian version of the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI).

The specific aims of the research were as follows:

1) To establish the Estonian version of the NIH-CPSI questionnaire and to test its validity both in community-based as well as clinic-based study settings;
2) To determine the prevalence of asymptomatic inflammatory (NIH category IV) prostatitis and its possible seasonal variations in young men in Estonia;
3) To identify the possible role of microorganisms in the etiology of asymptomatic inflammatory prostatitis by using quantitative microflora analysis of semen;
4) To determine the possible effect of asymptomatic inflammatory prostatitis on levels of laboratory markers like seminal IL-6 and serum PSA, as well as to determine the possible predictive value of PSA and IL-6 levels in differentiating between subjects with or without significant leukocytospermia;
5) To determine the possible joint effect of asymptomatic inflammatory prostatitis and patient's age on basic semen parameters.

MATERIAL AND METHODS

4. Subjects

Table 2. Summary of study subjects.

Studies	Subjects	Type of investigation
The Estonian version of NIH-CPSI (Paper I)	54 consecutive men with prostatitis-like symptoms	NIH-CPSI questionnaire
	420 community-dwelling men (83 eligible respondents)	NIH-CPSI questionnaire
Seminal microflora in NIH category IV prostatitis (Paper II)	37 men with significant leukocytospermia (>0.2 M WBC/ml), no subjective symptoms	Basic semen analysis, IL-6, cytological analysis, seminal morphology, seminal microbiology
	32 controls (<0.2 M WBC/ml), no subjective symptoms	
Prevalence of NIH category IV prostatitis in young men based on semen analysis (Paper III)	565 community-dwelling young men (558 men met exclusion criteria)	Basic semen analysis, cytological analysis, PSA, IL-6

4.1. Prostatitis patients

4.1.1. Chronic prostatitis/chronic pelvic pain syndrome patients

Consecutive sample of 54 native-speaking Estonian patients (mean age 40.1 ± 10.3 years) with prostatitis-like symptoms attending the Andrology Centre of Tartu University Hospital between April 2004 and November 2005 was used to pre-test the Estonian version of the National Institutes of Health chronic prostatitis symptom index (NIH-CPSI).

4.1.2. Asymptomatic inflammatory prostatitis patients

Thirty-seven men (mean age 26.4 ± 1.1 years) consulting a physician at the Andrology Centre of Tartu University Hospital due to infertility of the couple or for prophylactic purposes and participating in the prospective study of the etiopathogenesis of chronic prostatitis had significant number of leukocytes (>0.2 M per ml) in their semen (Punab *et al*., 2003) while no clinically relevant symptoms of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Therefore they were diagnosed as having NIH IV category prostatitis (asymptomatic inflammatory prostatitis) according to the NIH Classification of the

Prostatitis Syndromes (Krieger *et al*. 1999) and served as a study group in the etiologic study of NIH IV category prostatitis.

4.2. Controls

Thirty-two subjects (mean age 28.7 ± 0.6 years) consulting a physician at the Andrology Centre of Tartu University Hospital due to infertility of the couple or for prophylactic purposes and participating in the prospective study of the etiopathogenesis of chronic prostatitis had insignificant number of leukocytes (<0.2 M WBC per ml) in their semen samples and no clinically relevant symptoms of chronic prostatitis/chronic pelvic pain syndrome. Therefore they served as a control group in the etiologic study of NIH IV category prostatitis.

4.3. Participants of community based studies

4.3.1. Subjects for testing of the Estonian version of the National Institutes of Health chronic prostatitis symptom index (NIH-CPSI)

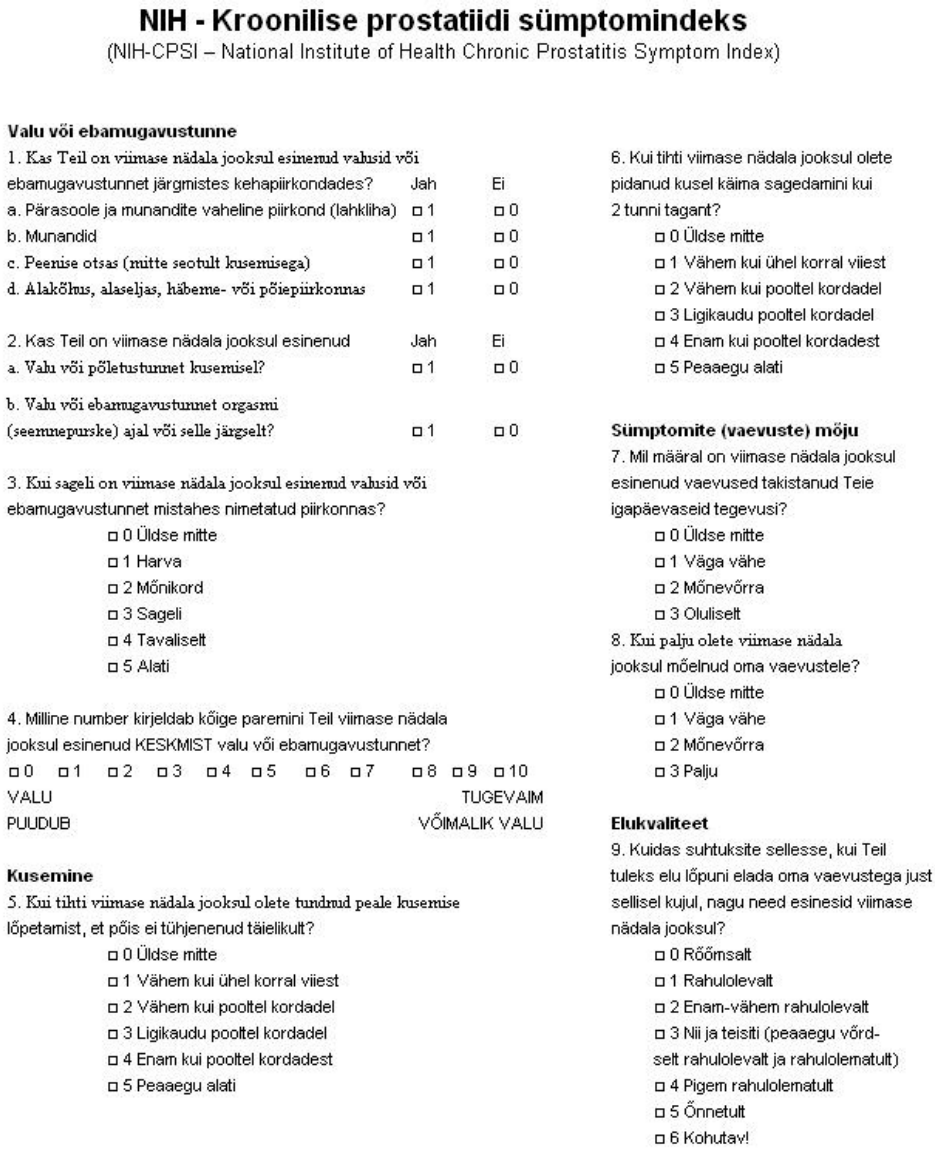
Four hundred and twenty men aged 20–59 years (a stratified sample included equal age groups by 5 years) were invited to participate in a population survey of the prevalence of prostatitis-like symptoms. The survey was conducted on the basis of the patient list of three family practitioners in Tartu.

4.3.2. Young men

Asymptomatic inflammatory prostatitis prevalence study included 565 young men (mean age 18.9 ± 1.8 years) who participated in a prospective study Environment and Reproductive Health (EU 6th FP project QLRT-2001-02911), all men were born and living in Estonia. Initially, 600 young men were planned to be recruited for the study from high schools in different regions of Estonia, but due to the inability of some subjects to produce semen sample and the fact that in part of the stained smear samples it was not possible to perform cytological analysis due to the low quality of the smear, the final number of study subjects was 565. Exclusion criteria were stated according to the suggestions of the NIH workshop on chronic prostatitis in Bethesda, MD, USA, 1995 (Executive Summary, 1995). None of the men had received antimicrobial therapy within 3 months.

4.4. Ethical considerations

Participation in the studies was voluntary. Informed consent was obtained from all study subjects, in accordance with the procedures of the Ethics Review Committee on Human Research of the University of Tartu.



NIH - Kroonilise prostatiidi sümptomindeks

(NIH-CPSI – National Institute of Health Chronic Prostatitis Symptom Index)

Valu või ebamugavustunne

1. Kas Teil on viimase nädala jooksul esinenud valusid või ebamugavustunnet järgmistes kehapiirkondades?

	Jah	Ei
a. Pärasoole ja munandite vaheline piirkond (lahkliha)	□ 1	□ 0
b. Munandid	□ 1	□ 0
c. Peenise otsas (mitte seotult kusemisega)	□ 1	□ 0
d. Alakõhus, alaseljas, häbeme- või põiepiirkonnas	□ 1	□ 0

2. Kas Teil on viimase nädala jooksul esinenud	Jah	Ei
a. Valu või põletustunnet kusemisel?	□ 1	□ 0
b. Valu või ebamugavustunnet orgasmi (seemnepurske) ajal või selle järgselt?	□ 1	□ 0

3. Kui sageli on viimase nädala jooksul esinenud valusid või ebamugavustunnet mistahes nimetatud piirkonnas?

□ 0 Üldse mitte
□ 1 Harva
□ 2 Mõnikord
□ 3 Sageli
□ 4 Tavaliselt
□ 5 Alati

4. Milline number kirjeldab kõige paremini Teil viimase nädala jooksul esinenud KESKMIST valu või ebamugavustunnet?

□ 0 □ 1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ 10

VALU PUUDUB — TUGEVAIM VÕIMALIK VALU

Kusemine

5. Kui tihti viimase nädala jooksul olete tundnud peale kusemise lõpetamist, et põis ei tühjenenud täielikult?

□ 0 Üldse mitte
□ 1 Vähem kui ühel korral viiest
□ 2 Vähem kui pooltel kordadel
□ 3 Ligikaudu pooltel kordadel
□ 4 Enam kui pooltel kordadest
□ 5 Peaaegu alati

6. Kui tihti viimase nädala jooksul olete pidanud kusel käima sagedamini kui 2 tunni tagant?

□ 0 Üldse mitte
□ 1 Vähem kui ühel korral viiest
□ 2 Vähem kui pooltel kordadel
□ 3 Ligikaudu pooltel kordadel
□ 4 Enam kui pooltel kordadest
□ 5 Peaaegu alati

Sümptomite (vaevuste) mõju

7. Mil määral on viimase nädala jooksul esinenud vaevused takistanud Teie igapäevaseid tegevusi?

□ 0 Üldse mitte
□ 1 Väga vähe
□ 2 Mõnevõrra
□ 3 Oluliselt

8. Kui palju olete viimase nädala jooksul mõelnud oma vaevustele?

□ 0 Üldse mitte
□ 1 Väga vähe
□ 2 Mõnevõrra
□ 3 Palju

Elukvaliteet

9. Kuidas suhtuksite sellesse, kui Teil tuleks elu lõpuni elada oma vaevustega just sellisel kujul, nagu need esinesid viimase nädala jooksul?

□ 0 Rõõmsalt
□ 1 Rahulolevalt
□ 2 Enam-vähem rahulolevalt
□ 3 Nii ja teisiti (peaaegu võrdselt rahulolevalt ja rahulolematult)
□ 4 Pigem rahulolematult
□ 5 Õnnetult
□ 6 Kohutav!

Figure 2. The Estonian version of the NIH-CPSI.

5. Establishment of the Estonian version of the NIH-CPSI

5.1. Translation procedure

The original English version of NIH-CPSI was initially translated into Estonian language by two native Estonian andrologists (M.P., P.K.) independently, trying to use very simple language. The translators then compared the two versions with each other, and several phrases and words were revised to enhance the comprehensibility. Next, a professional Estonian-English translator translated the reconciled version back to English. The Estonian andrologists then compared the translated version with the original NIH-CPSI to create the final version. No substantial inconsistencies were found between the versions. During the testing in daily clinical practice no corrections were made. This Estonian version of the NIH-CPSI (Fig. 2) is available online (NIH-CPSI 2006).

5.2. Testing

To examine the comprehensibility, acceptability and psychometric properties of the translated instrument the Estonian NIH-CPSI was pre-tested in a consecutive sample of 54 native-speaking Estonian patients attending the Andrology Centre of Tartu University Hospital with prostatitis-like symptoms. All men were asked to self-complete the NIH-CPSI. They were then interviewed by a study coordinator to determine whether any questions were difficult to comprehend, or irrelevant to their symptoms or health-related quality of life.

Next, a population survey of prostatitis-like symptoms was conducted in 420 men who received the questionnaires with a covering letter explaining the purpose of the study and a stamped, addressed return envelope. Of these 420 men 88 (21%) returned the questionnaire. In addition, 32 men of the same patient list were attempted to contact personally in their homes and 9 (28%) of them agreed to participate in the study. A total of 14 out of 97 respondents were excluded from the study because they only partially filled the questionnaires. In this survey the two questions most specific for prostatitis, including perineal and/or ejaculatory pain/discomfort, and a total pain score (0–21) 4 or greater were used to identify men with significant prostatitis-like symptoms (Nickel *et al*., 2001).

Hence, a total of 137 self-administered questionnaires filled by native-speaking Estonian men were included in the testing of the NIH-CPSI questionnaire.

6. Investigation of etiology and prevalence of asymptomatic inflammatory prostatitis

6.1. Specimens

Semen samples (n=627 in total) were collected by patients following the washing of the glans penis with soap and water and urinating. The samples were obtained by masturbation and ejaculated into a sterile collection tube in a private room near the laboratories. After ejaculation, the semen was incubated at 37°C for 25 to 45 min for liquefaction, followed by detection of basic semen parameters and cytologicial analysis. The subset of samples that were used for microbiological analyses (n=69; 0.2 ml of each) were transported to the microbiology laboratory (located near the Andrology Centre) immediately and processed within 1 hour.

Seminal plasma samples for detection of interleukin-6 were collected into sterile Eppendorf® 0.5 ml plastic tubes, centrifuged and stored at –80°C for later assay.

Blood sampling was performed before the subjects delivered semen samples. Vacuum sterile serum glass tubes with traditional stopper and clot activator (BD Vacutainer™, 10 ml) were used for blood collection. Specimens were clotted at room temperature and centrifuged. Serum samples were then stored at –80°C within 2 hours after venipuncture for later assay.

6.2. Basic semen parameters

The analysis of semen was performed according to WHO guidelines (WHO, 1999). Semen volume was estimated by weighing the collection tube with the semen sample and subsequently subtracting the predetermined weight of the empty tube assuming 1g=1ml. Motility was assessed in order to report the number of motile spermatozoa (WHO motility classes A+B+C). Sperm concentration was assessed using the improved Neubauer haemocytometers. Total sperm count was calculated by multiplying semen volume by sperm concentration.

Etiologic study of asymptomatic inflammatory prostatitis also included morphological evaluation of spermatozoa. Smears for morphology assessment were made and morphology of spermatozoa was evaluated according to strict criteria by Menkveld *et al*. (1990) and NAFA suggestions (Kvist *et al*., 2002), with the lowest limit of normal morphology set at 5% of all spermatozoa. All semen analyses were performed by a qualified laboratory technician.

6.3. Cytological analysis

Semen smears were made for detecting WBC. The smears were air-dried, Bryan-Leishman stained and examined using oil immersion microscopy (magnification = 1000x) by an experienced microscopist. The WBC concentration in semen was calculated using the known sperm concentration (as 10^6/ml) according to the following formula:

$$[\text{WBC}] = \frac{\text{number of WBCs counted}}{\text{number of sperm counted}} \times \text{sperm concentration}$$

One hundred non-sperm round cells were counted and the number of detected WBCs as well as the number of spermatogenetic cells were recorded. The cut-off points for detecting leukocytospermia were 1.0 M WBC/ml according to WHO guidelines (WHO, 1999) and 0.2 M WBC/ml according to our previous study results (Punab *et al*., 2003), where the concentration and the mean number of different microorganisms was compared against WBC concentrations in semen specimens using ROC curve analysis and as a result, it was found that an alternative cut-off level of 0.2 M WBC/ml has the most optimal sensitivity/ specificity ratio to differentiate between men with or without significant bacteriospermia.

The possible deviation of WBC concentrations according to the ejaculate volume was also calculated.

6.4. Detection of interleukin-6 (IL-6)

Interleukin-6 levels of seminal plasma (100 µl of specimen was required for the assay) were measured in serum by chemoluminescent immunoassay IMMULITE 2000 Analyzer (Siemens Medical Solutions Diagnostics, California, US), according to manufacturer's instructions (Kit Catalog Number: L2K6P2). Assays were solid-phase, enzyme-labeled sequential chemiluminescent immunometric tests, which are performed automatically on the IMMULITE 2000 automated analyzer with 2 incubation cycles per 30 minutes, analytic sensitivity of 2 pg/ml for IL-6 and calibration range of up to 1000 pg/ml. Granules coated with antibodies directed towards IL-6 were mixed with the samples. After washing, alkaline phosphatase-labelled antibodies were added. Free antibodies were washed away and chemiluminescent reagent was supplied. The reaction between alkaline phosphatase and the chemiluminescent reagent resulted in light production, which was measured in the Immulite 2000 automated analyzer. The antibody used in assay is highly specific to IL-6 and has no cross-reactivity with IL-1α, IL-1β, IL-2, IL-4, IL-8, TNFα or IFN-γ

(IMMULITE 2000 IL-6, 2006). The use of seminal plasma for detection of IL-6 by chemoluminescent immunoassays has not been yet validated.

6.5. Microbiological analysis

Semen samples were cultured quantitatively to detect anaerobic, microaerophilic, and aerobic bacteria within 1–2 hours from collection. Wilkins-Chalgren agar (Oxoid, Unipath, Basingstoke, UK) supplemented with 5% horse blood, Schaedler medium (Oxoid) supplemented with 5% horse blood, vancomycin and nalidixic acid, Gardnerella vaginalis selective agar (Oxoid), MRS agar for lactobacilli (Oxoid), freshly prepared blood agar, and chocolate agar were used. Wilkins-Chalgren and Schaedler media were incubated in an anaerobic glove box (Sheldon Manufacturing Inc, with a gas mixture: 5% H_2, 5% CO_2, 90% N_2) for 5–6 days. MRS medium, chocolate agar, and Gardnerella vaginalis-selective agar were incubated in a microaerophilic atmosphere (10% CO_2) for 72 hours. Blood agar was incubated aerobically at 37ºC and inspected after 24 and 48 hours.

Colonies with different morphology were Gram stained and examined microscopically. The microorganisms were identified mostly at the genus level. Standard methods were used for identification of enteric and other gram-negative bacteria (Murray *et al*., 1999). A latex test (Oxoid) was employed for differentiation of *Staphylococcus aureus* and coagulase-negative staphylococci. Streptococci and enterococci were identified by the absence of catalase production and differentiated by fermentation of esculin. Group B streptococci were identified with the use of a latex test (Oxoid). *Corynebacterium seminale* was differentiated by testing its beta-glucuronidase activity with the use of MUG medium (Oxoid). *Gardnerella vaginalis* was identified by its ability to grow on selective medium, characteristic morphology, and negative catalase test. The anaerobes were identified by evaluation of their growth on selective media, colony and cellular morphology, Gram stain reaction, and some rapid tests and diagnostic disks. All anaerobic microorganisms were tested for absence of growth under aerobic and microaerophilic conditions. Absence of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* was confirmed by a polymerase chain reaction (PCR) method with the use of Amplicor C. trachomatis/N. gonorrhoeae Test (Roche, Mannheim, Germany) after the DNA extraction, which was performed with the use of the High Pure PCR Template Preparation Kit (Roche).

6.6. Detection of prostate-specific antigen (PSA)

Prostate-specific antigen (PSA) was quantitatively measured in serum by chemoluminescent immunoassay IMMULITE 2000 Analyzer (Siemens Medical Solutions Diagnostics, California, US), according to manufacturer's instructions (Kit Catalog Number: L2KUP6). Assays were solid-phase, sequential chemiluminescent immunometric tests, which are performed automatically on the IMMULITE 2000 automated analyzer with analytic sensitivity of 0.003 ng/ml for tPSA (total PSA) and calibration range of up to 20 ng/ml. The solid phase (bead) is coated with specific murine anti-PSA monoclonal antibody. The patient sample was incubated with the bead during the first cycle, at which time PSA in the sample bound to the monoclonal antibody-coated bead. Unbound serum (0.5 ml of serum required for the assay) was then removed by a centrifugal wash. The reagent containing alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-PSA antibody specific for PSA was introduced in the second cycle (both cycles running for 30 minutes) and bound to PSA on the bead to form an antibody-sandwich complex. Unbound enzyme conjugate was then removed by a centrifugal wash. Finally, chemiluminescent substrate (a phosphate ester of amantyl dioxetane) was added to the bead and signal was generated in proportion to the bound enzyme. The antibody used in assay is highly specific to PSA and has no cross-reactivity with AFP, CEA, ferritin, HCG, PAP or prolactin (IMMULITE 2000 Third Generation PSA, 2006).

7. Statistical analysis

While establishing the Estonian version of the NIH-CPSI questionnaire, for each domain of the questionnaire (pain, voiding symptoms, quality of life), the particular values were summed and the subscores and the total score were calculated. Statistically significant differences among the subject groups were calculated using the Mann-Whitney rank sum test. The correlations between the total NIH-CPSI score and its three subscores were calculated using the Pearson's test. Internal consistency of the total NIH-CPSI score and its three subscores were evaluated using Cronbach's coefficient alpha.

In the prevalence study of asymptomatic inflammatory prostatitis the subjects were divided into 3 study groups by the severity of leukocytospermia as without leukocytospermia (0…0.2 M WBC/ml – Group 1), with moderate (0.2…1.0 M WBC/ml – Group 2) or severe leukocytospermia (>1.0 M WBC/ml – Group 3).

Age, different semen parameters, IL-6 and PSA levels were compared using Kruskal-Wallis test, the group or groups that differed from the others were isolated by using a multiple comparison procedure (Dunn's method). Pearson

product moment correlation was used to find out correlations between PSA levels and leukocytospermia. The calculations that were done for determination of predictive associations of PSA and IL-6 with leukocytospermia were performed using Fisher exact test and predictive value analysis.

In the etiologic study of asymptomatic inflammatory prostatitis, the differences in the prevalence and counts of different microorganisms between the groups were calculated using Fisher exact test and Mann-Whitney rank sum test. The Spearman rank order correlation was used to find the correlation between WBCs and IL-6, and between microbiologic indicators. The clinical parameters (age, period of abstinence, and basic sperm characteristics, including volume, concentration, total sperm count, motility, and morphology) passed a normality test; therefore, a t test was used for comparison of the two groups. Inflammatory markers (WBC count and IL-6) were analyzed by means of the Mann-Whitney rank sum test. In the later analysis the study subjects were regrouped into 3 groups like in prevalence study. The 3 groups were compared using one-way ANOVA or Kruskal-Wallis tests. The group or groups that differed from the others were isolated by using a multiple comparison procedure (Dunn's method).

The differences between the subjects of these two studies were compared using Mann-Whitney rank sum test.

Statistical analyses were performed with the use of the Sigma Stat (Jandel Scientific), Excel (Microsoft Corp, Redmond, OR, USA) and R (R Foundation for Statistical Computing) software programs. Statistical significance was assumed at $p<0.05$ level for all parameters.

RESULTS AND DISCUSSION

8. Establishment of the Estonian version of the NIH-CPSI

We tested the Estonian NIH-CPSI in the patients attending the physician with prostatitis-like symptoms and in the men participating in community-based study to examine its acceptability, discriminative power and psychometric properties. The mean age of the men questioned in the Andrology Centre of Tartu University Hospital was 40.1 years (SD 10.3; range 19–67). The age of the respondents in the mail-based study was 41.1 years (SD 12.1; range 21–59) and that of the men personally contacted in their home was 36.3 years (SD 13.9; range 22–56). Altogether, 137 fully completed questionnaires were available for analysis.

The Estonian translation of the NIH-CPSI could be self-administered to men attending the Andrology Centre (n=54) in a short time (<5 minutes), was well understood by all patients and needed no corrections during the use.

Next, prostatitis-like symptoms were evaluated in the community-based study (in 83 respondents out of 420 study subjects). Frequency and severity of prostatitis-like symptoms as measured by NIH-CPSI in men of the community survey are shown in Table 3. The prevalence of prostatitis-like symptoms in this community-based study was 12% that is similar to results obtained in other prevalence studies of prostatitis (Nickel *et al*., 2001; Mehik *et al*., 2000). However, the sample size in our study was relatively small, therefore these results have to be interpreted cautiously. Men with pain score ≥ 8 (with or without perineal and/or ejaculatory pain or discomfort) were judged to have moderate to severe prostatitis-like symptoms (Nickel *et al*., 2001).

Table 3. Frequency and severity of prostatitis-like symptoms measured by Estonian NIH-CPSI in community survey study subjects (n=83).

Prostatitis-like symptoms and symptom scores	**n (% of total)**
Index pain score < 4	55 (66%)
Index pain score ≥ 4	28 (34%)
Index pain score ≥ 4, perineal and/or ejaculatory pain or discomfort	10 (12%)
Index pain score ≥ 8	14 (17%)
Index pain score ≥ 8, perineal and/or ejaculatory pain or discomfort	6 (7%)

8.1. Validity of the questionnaire

The two most prostatitis-specific domains, pain and quality of life domains, demonstrated high correlation with each other and with the total score (Table 4). The Cronbach's alpha coefficient for the total index of the Estonian NIH-CPSI was 0.82, demonstrating good internal consistency similar to the original English version (Table 5). The alpha coefficients for the domains were slightly lower in comparison with the original version; yet these values are considered sufficient (Sachs, 1999). Most of the questionnaires in our study were obtained from the population survey being filled without the help of medical personnel, and the participants were heterogeneous (with and without complaints). Also the existing published data provide some evidence for the possibility that the cohort itself may have an important influence on the consistency of the findings (Schneider *et al*., 2004).

Table 4. Correlation matrix (Pearson product moment correlation) of the domains and total Estonian National Institutes of Health chronic prostatitis symptom index.

Domain	**Correlations**			
	Total index score	Pain domain	Void domain	Quality of life domain
Total index score	1.00	–	–	–
Pain domain	0.92	1.00	–	–
Void domain	0.62	0.35	1.00	–
Quality of life domain	0.93	0.80	0.47	1.00

Table 5. Internal consistency according to Cronbach's coefficient alpha in different versions of the NIH-CPSI.

NIH-CPSI version	Domain				Reference
	Pain	Void	Quality of life	Total index	
English	0.86	0.79	0.87	0.86	Litwin *et al*., 1999
Estonian	0.67	0.63	0.78	0.82	Paper I
Spanish	0.87	0.81	0.86	0.94	Collins *et al*., 2001
Italian	0.84	0.96	0.86	0.95	Giubilei *et al*., 2005
Japanese	0.83	0.97	0.87	–	Kunishima *et al*., 2002
German	0.60	0.69	0.67	0.74	Schneider *et al*., 2004

Table 6. Comparison between the scores of Estonian version of National Institutes of Health chronic prostatitis symptom index for different study groups.

Domain	Mean scores ± SD [Median scores (quartiles)]			*P* values *	
	A	B	C		
	CP/CPPS patients in Andrology Centre (n=54)	Men of population based study having CP/CPPS (n=10)	Men of population based study without CP/CPPS (n=73)	A *vs* C	B *vs* C
Total index score (0–43)	18.4 ± 7.2 [18 (14–24)]	15.3 ± 5.6 [15.5 (13–19)]	6.0 ± 6.7 [4 (0–8.25)]	<0.001	<0.001
Pain domain (0–21)	8.3 ± 4.1 [8.5 (6–11)]	8.6 ± 2.5 [8.5 (7–10)]	2.2 ± 3.3 [0 (0–3.25)]	<0.001	<0.001
Void domain (0–10)	2.7 ± 2.4 [2.5 (1–4)]	1.2 ± 1.4 [1 (0–2)]	1.3 ± 1.9 [1 (0–2)]	<0.001	0.86
Quality of life domain (0–12)	7.4 ± 2.5 [7.5 (6–9)]	6.1 ± 2.1 [6 (5–8)]	2.5 ± 3.0 [1 (0–4)]	<0.001	0.001

CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome
* Mann-Whitney rank sum test

8.2. Comparison of Estonian NIH-CPSI scores between different study groups

The Estonian NIH-CPSI scores (total score, pain and quality of life domains) differed significantly between the CP/CPPS patients and the men without CP/CPPS, only the void domain scores appeared similar in the two groups of the population-based study (Table 6). When the two prostatitis groups were compared, the differences between scores were not statistically significant, although the void score showed some tendency to be slightly higher in men seeking medical aid (p=0.053). This indicates the good discriminant and content validity of the Estonian version to distinguish the patients with CPPS symptoms from those without.

When the results of the current study were compared with those of the original NIH study (Litwin *et al*., 1999), the scores of the pain domain and quality of life domain were similar for prostatitis patients. Unlike the original and some translated versions (Giubilei *et al*., 2005; Leskinen *et al*., 2003), the Estonian NIH-CPSI showed less distinctive scores for void domain. One possible reason for low void score in CP/CPPS patients seeking for physician might come from the arrangement of medical aid in Estonia: prostatitis patients usually consult andrologists while the patients with voiding disorders attend

urologists. Similarity between the void domain scores of the cases and controls of the community sample likely results from a number of control subjects having lower urinary tract symptoms (LUTS) that were not related to prostatitis. However, as it is accepted that the hallmark of the symptom complex in CP/CPPS is pelvic pain discriminating robustly among chronic prostatitis, benign prostatic hyperplasia and healthy controls (Litwin *et al*., 1999; McNaughton-Collins, 2003), this deviation is negligible as concerns the diagnosis of chronic prostatitis.

9. Prevalence of asymptomatic inflammatory prostatitis in young men

The prevalence of asymptomatic inflammatory (NIH IV category) prostatitis was determined in Estonian young men (n=565). All subjects were examined for possible pathologies in genital region. In 7 men clinical symptoms of genital inflammation were found, and one of them harboured prostatitis-like symptoms. Therefore these 7 men were excluded from further analysis. The final number of eligible study subjects was 558 (mean age 18.9 ± 1.8 years). The prevalence of asymptomatic inflammatory prostatitis according to WHO guidelines (>1.0 M WBC/ml) was 6.0% (n=34). When we used lower threshold (>0.2 M WBC/ml), the prevalence was 19.0% (n=107). Several investigators have proposed that the WHO-defined limit of leukocytospermia (1.0 M WBC/ml) may be too high to discriminate between patients with or without significant bacteriospermia (Jedrzejczak *et al*., 1996; Sharma *et al*., 2001; Lackner *et al*., 2006). This has also been shown in our recent study (Punab *et al*., 2003), where lower limit of leukocytospermia (0.2 M WBC/ml) was significantly better correlated with significant bacteriospermia. Therefore this limit was used in the present study in addition to WHO-defined limit.

We also calculated the possible deviation of WBC concentrations according to the ejaculate volume and no significant differences were seen when compared to the conventional method of expressing WBC concentration per ml of semen.

To this date, WBC counts in genital tract of asymptomatic men have been determined only in few studies, where the prevalence of significant leukocyte counts in prostate-specific specimens ranged from 11 to 42%. Different prostate-specific specimens were used in these studies – expressed prostatic secretions (Nickel *et al*., 2003; Carver *et al*., 2003), post-prostate massage urine (Nickel *et al*., 2003; Potts 2000) or histological specimens from prostate (Shimomura *et al*., 2003), however, no semen-specific population-based studies on the prevalence of asymptomatic inflammatory prostatitis have been published to our best knowledge. Instead, leukocytospermia has been evaluated in two types of studies: 1) several studies of infertile men with no physical

complaints (Lackner *et al*., 2006; Sharma *et al*., 2001; Omu *et al*., 1999; Wolff 1995; Arata de Bellabarba *et al*., 2000; Alvarez *et al*., 2002; Saleh *et al*., 2002), where the significant leukocytospermia (>1.0 M WBC/ml) was found in 10 to 71% of men; 2) several published data on chronic symptomatic prostatitis where WBC counts in semen have been determined in 8 to 15% of asymptomatic controls (Nickel *et al*., 2003; Christiansen *et al*., 1991), thus actually including men with NIH category IV prostatitis, too.

New definition and classification of prostatitis syndromes (Krieger *et al*., 1999) includes description of asymptomatic inflammatory prostatitis as 'significant amount of white blood cells and/or bacteria in prostate-specific samples'. At the same time there is no consensus at present which bacteria should be considered significant in case of prostatitis syndromes, therefore more objective WBC count in semen was used in our study.

It has been shown that environmental factors like cold climate (Mehik *et al*., 2000) and average duration of daily sunlight (Ku *et al*., 2001) may affect prostatitis-like symptoms. Although methodologically different, our study did not reveal any significant seasonal differences in the prevalence of asymptomatic inflammatory prostatitis (Fig. 3), as found formerly in case of symptomatic prostatitis, thus suggesting that seasonal climate changes may influence rather the symptoms (pain or discomfort in pelvic area, dysuria etc.) than the signs (inflammatory reaction in prostate-specific specimens) of inflammation. Possible monthly variations were also analyzed and no significant differences were observed (data not shown).

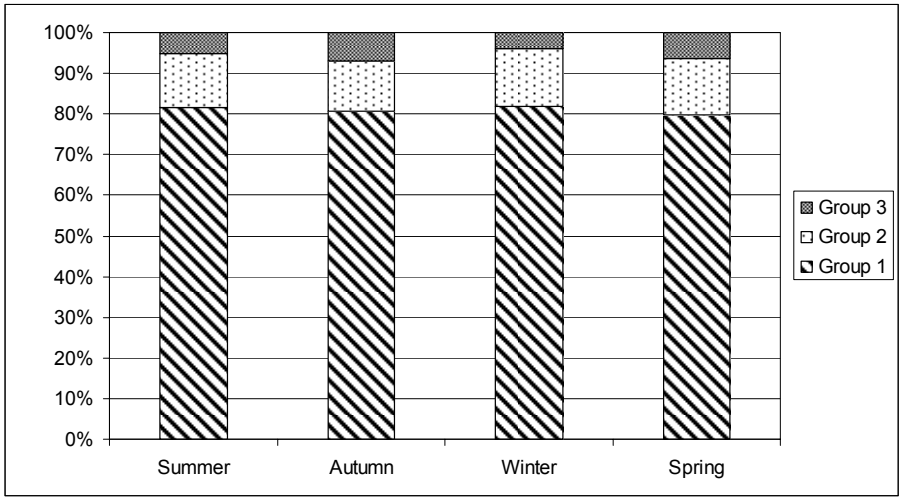


Figure 3. Seasonal variance in the prevalence of asymptomatic leukocytospermia. Group 1 – 0…0.2 million white blood cells (WBC)/ml; Group 2 – 0.2…1 million WBC/ml; Group 3 – >1 million WBC/ml. Every season includes three months, with June being the first month of summer.

10. Changes in laboratory parameters in case of asymptomatic inflammatory prostatitis

10.1. Basic semen parameters

Basic semen parameters were determined in prevalence study (Paper III, n=558) and in etiologic study of asymptomatic inflammatory prostatitis (Paper II, n=69).

In the prevalence study of NIH category IV prostatitis the study subjects were divided into 3 groups – men without leukocytospermia (0…0.2 M WBC/ml, Group 1), men with moderate leukocytospermia (0.2…1.0 M WBC/ml, Group 2) and men with severe leukocytospermia (>1.0 M WBC/ml, Group 3). No significant age differences were observed in 3-group comparison. Sperm concentration and total sperm count were highest in Group 2, differences in sperm concentration being statistically significant (Table 7). Distribution of basic semen parameters vs. leukocytospermia are shown on Figure 4.

In the etiologic study we did not find significant differences in basic semen parameters when all leukocytospermic subjects (>0.2 M WBC/ml) and controls were compared (Table 1 in Paper II). Next, the same study subjects were re-grouped into three groups using the same cut-off points for leukocytospermia as in prevalence study in order to compare the subjects in similar way. We noticed a pattern that was similar to our findings in the prevalence study where sperm concentration and total sperm count were highest in moderate leukocytospermia (Table 7).

Similar tendencies in basic semen parameters were also noted when the data from both studies were combined (data not shown).

Data from available studies show controversial results: some studies clearly indicate the negative impact of leukocytospermia on sperm quality (Ludwig *et al*., 2001; Fedder 1996; Lackner *et al*., 2006; Arata de Bellabarba *et al*., 2000; Simbini *et al*., 1998), whereas the others do not (Tomlinson *et al*., 1993). Some authors (Saleh *et al*., 2002; Zalata *et al*., 2004) have suggested that lack of clinical significance of leukocytospermia may reflect powerful antioxidant properties of the seminal plasma. In a study by Kaleli *et al*. (2000) where also different levels of leukocytospermia were evaluated against basic semen parameters like in our study, the authors found that leukocytospermia may have a favorable effect on some sperm functions at seminal leukocyte concentrations between 1 and 3 million WBC/ml, probably due to dual action of reactive oxygen species (ROS) at different leukocyte concentrations, and perhaps also other mediators. Also, Depuydt *et al*. (1998) have stated that moderate (<2 million peroxidase-positive WBC/ml) numbers of leukocytes may exert beneficial effects on spermatozoa, possibly due to hepatocyte growth factor (HGF – a tissue repairing substance) and the stimulation of immunocompetent cells by cytokines (e.g. IL-6 and IL-8). However, the cut-off points of

leukocytospermia in these studies were different from ours, and the range of 'beneficial' leukocytospermia was much higher than in our study.

Severe leukocytospermia appeared to have a negative effect on the semen quality in our etiologic study but not in prevalence study (Table 7). It may indicate that in young adults the functional reserve of accessory sex glands is much higher than in somewhat older subjects. In addition, regrouping of the study subjects of etiologic study enabled us to compare the subjects from both studies to test whether mean age differences (see also 4.1., 4.2. and 4.3.2.) between the subjects from these studies reflected any changes in basic semen parameters (Table 7). As a result we found that younger study subjects of our prevalence study (mean age 18.9 years, Paper III) tended to have better semen quality (significantly higher sperm concentration, total sperm count, sperm motility) than their older counterparts of the etiologic study (mean age 26.4 years, Paper II), regardless of the leukocytospermia status. Similar tendencies have been described also earlier (Centola *et al*., 1999; Eskenazi *et al*., 2003).

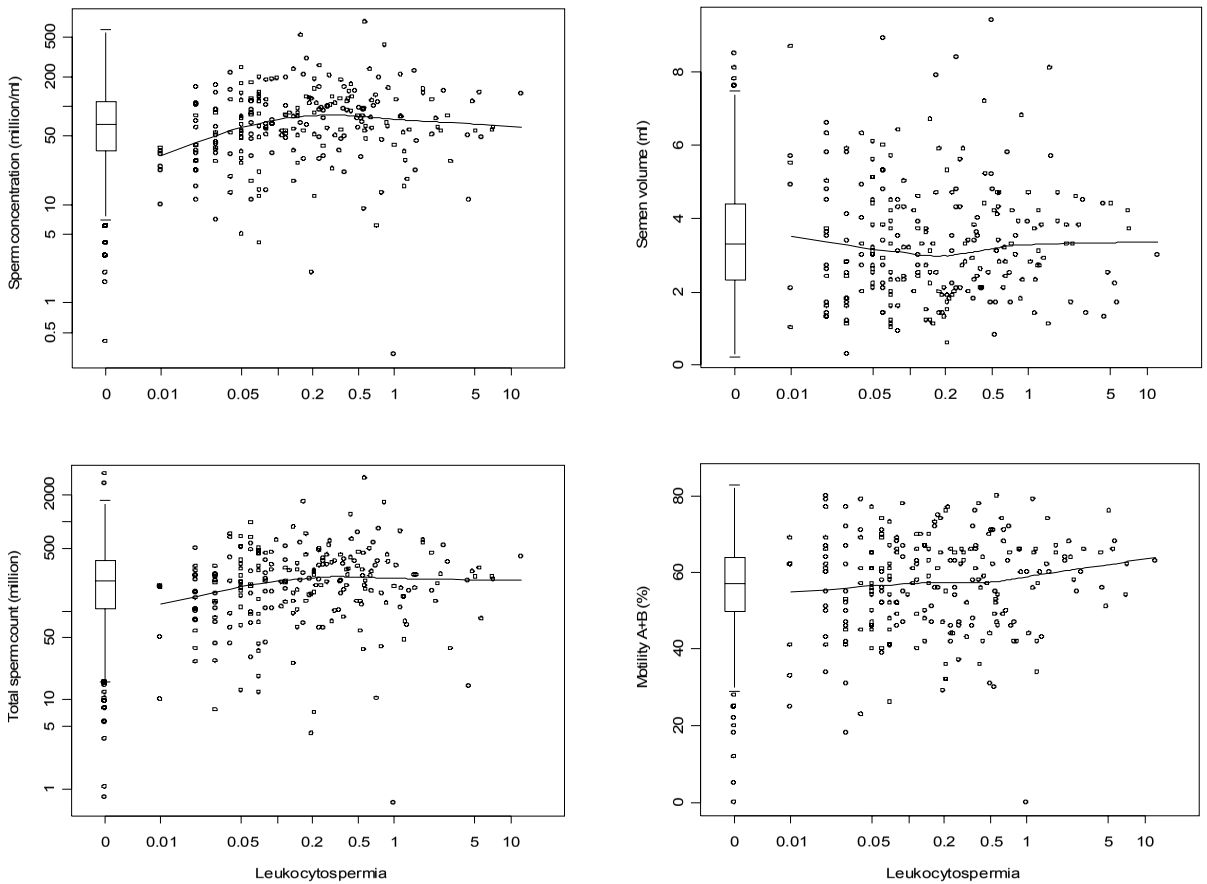


Figure 4. Plot of leukocytospermia (on logarithmic scale, million WBC/ml) versus sperm concentration, semen volume, total sperm count and motility: boxplot of a given variable for 318 individuals with zero level of leukocytospermia, and scatter plot of leukocytospermia vs. the variable with robust lowess regression curve for others.

Table 7. Basic semen parameters, serum PSA and seminal IL-6 levels in case of asymptomatic inflammatory prostatitis – comparison of subjects from two studies

	Etiologic study of asymptomatic inflammatory prostatitis (Paper II)			Prevalence study of asymptomatic inflammatory prostatitis (Paper III)		
	Group 1 (0…0.2 M WBC/ml, n=32)	Group 2 (0.2…1 M WBC/ml, n=20)	Group 3 (>1 M WBC/ml, n=17)	Group 1 (0…0.2 M WBC/ml, n=451)	Group 2 (0.2…1 M WBC/ml, n=73)	Group 3 (>1 M WBC/ml, n=34)
	Median (range)	Median (range)	Median (range)	Median (range)	Median (range)	Median (range)
Semen volume (ml)	3.6 (1.1–8.6)	3.85 (0.9–7.3)	3.9 (1.4–5.7)	3.2 (0.2–8.9)	3.1 (0.6–9.4)	3.5 (1.1–8.1)
Sperm concentration (million/ml)	41.0 (0.0–279.0)[1,9]	77.0 (8.0–259.0)[1,5]	28.0 (1.0–83.0)[1,5,13]	64.0 (0.4–608.0)[2,9]	90.0 (2.0–716.0)[2]	57.0 (0.0–228.0)[2,13]
Total sperm count (million)	150.5 (0.0–921.0)[3,10]	270.1 (7.2–959.0)[3,6]	115.0 (2.5–291.0)[3,6,14]	205.4 (0.8–2675.2)[10]	270.7 (4.2–3078.8)	210.7 (0.0–777.0)[14]
A+B motility (%)	43.5 (9.0–65.0)[11]	45.5 (13.0–65.0)[12]	47.0 (0.0–66.0)[15]	57.0 (0.0–97.0)[11]	56.0 (29.0–80.0)[12]	62.0 (0.0–79.0)[15]
Morphologically normal sperm (%)*	3.0 (0.0–15.0)	4.0 (0.0–11.0)	4.0 (0.0–12.0)	ND	ND	ND
IL-6 (pg/ml)*	7.1 (5.0–72.4)[4,7,8]	28.4 (9.0–708.0)[4,7]	44.5 (14.6–863.0)[4,8]	19.5 (2.6–256.0)[16,18,19]	42.4 (10.1–311.0)[16,18]	61.4 (3.6–1475.0)[16,19]
PSA (µg/l)	ND	ND	ND	0.51 (0.07–3.20)[17,20]	0.60 (0.15–3.73)[17]	0.71 (0.18–4.19)[17,20]

[1] p=0.027; Kruskal-Wallis test
[2] p=0.04; Kruskal-Wallis test
[3] p=0.027; Kruskal-Wallis test
[4] p<0.001; Kruskal-Wallis test
[17] p=0.009; Kruskal-Wallis test
[16] p<0.001; Kruskal-Wallis test
[5-8,18-20] p<0.05; Dunn's method
[9] p<0.001; Mann-Whitney rank sum test
[10] p=0.024; Mann-Whitney rank sum test
[11] p<0.001; Mann-Whitney rank sum test
[12] p<0.001; Mann-Whitney rank sum test
[13] p=0.002; Mann-Whitney rank sum test
[14] p=0.012; Mann-Whitney rank sum test
[15] p<0.001; Mann-Whitney rank sum test

* Indicated parameters were available for 24 men in prostatitis group of etiologic study.
IL-6=interleukin-6; NIH=National Institutes of Health; PSA=prostate-specific antigen; WBC=white blood cells; ND=not determined

10.2. Interleukin-6 in semen

Interleukin-6 was determined in prevalence study (Paper III) and etiologic study of asymptomatic inflammatory prostatitis (Paper II). IL-6 concentration in seminal plasma was significantly higher in NIH IV category prostatitis than in the controls (Table 7) and we found high correlation between leukocytospermia and IL-6 concentration in seminal plasma ($r = 0.74$, $p<0.001$).

Previous studies have also shown that IL-6 reveals significantly higher values in patients with leukocytospermia than in those without (Ohta *et al*., 2002; Swatowski *et al*., 2002). IL-6 is an important mediator of inflammatory processes and a marker of silent genital inflammations in seminal plasma (Eggert-Kruse *et al*., 2001; Kopa *et al*., 2005) where the prostate appears to be its main source (Matalliotakis *et al*., 1998). IL-6 has been shown to be produced mainly by macrophages in response to foreign antigens, pathogens (infection challenge) and also in chronic inflammation (Potashnik *et al*., 2005). It is also known that many types of non-immune cells also produce IL-6, including human spermatozoa (Huleihel *et al*., 2000), thus suggesting that it could be involved in the regulation of physiological function of testis and/or act as a paracrine signal between Sertoli cells and spermatids/spermatozoa in the seminiferous tubules. However, in case of leukocytospermia, the IL-6 concentration increases almost exclusively due to the significant numbers of WBC present and is therefore in good correlation with the latter. Thus detection of IL-6 can be suggested as an additional diagnostic tool for men with fertility problems to rule out possible inflammatory causes, as well as for men with asymptomatic leukocytospermia to gain additional data regarding the activity of inflammation.

10.3. Serum PSA levels

Prostate-specific antigen was detected in prevalence study (Paper III). PSA levels in blood serum were significantly higher in leukocytospermic persons than in those without leukocytospermia (Table 7), and we detected a positive correlation between PSA level in blood serum and WBC count in semen ($r=0.19$, $p<0.001$).

Although prostate-specific antigen has been routinely used as a serum marker for prostate cancer, the effect of (symptomatic) prostatitis on PSA is also well documented (Dalton 1989; Nadler *et al*., 1995; Pansadoro *et al*., 1996; Resim *et al*., 1999). Former studies have associated the NIH category IV prostatitis with elevated PSA levels, too (Carver *et al*., 2003; Potts 2000; Ozden *et al*., 2006), that was confirmed by our study as well. Ozden *et al*. (2006) found that PSA levels in men with category IV prostatitis were positively correlated with the aggressiveness of inflammation. Asymptomatic prostatitis has similar effects on total PSA and free PSA levels in serum as prostate cancer (Stancik *et*

al., 2004), suggesting that screening for NIH category IV prostatitis and treating the underlying subclinical inflammation would increase the specificity of PSA screening and thereby reduce the number of potentially unnecessary biopsies in prostatitis patients. Yet the reports where no correlation has been found between the asymptomatic prostate inflammation and PSA levels can be found as well (Nickel *et al*., 1999).

10.4. Predicting leukocytospermia by serum PSA and seminal IL-6 levels

We evaluated the possible predictive value of blood serum PSA in combination with seminal IL-6 level for prognosticating moderate and severe leukocytospermia (Table 8). We used an arbitrary cut-off of 1 μg/l for PSA and 30 pg/ml for IL-6, the latter being an approximate value that correlates with the lower limit of leukocytospermia (0.2 M WBC/ml).

Table 8. Prevalence of men with or without significant leukocytospermia according to the values of PSA in blood serum and IL-6 in seminal plasma

		No (%) of men with certain combination of parameters		
		Insignificant leukocytospermia	Moderate leukocytospermia	Severe leukocytospermia
		LCS <0.2 M WBC/ml	LCS 0.2–1 M WBC/ml	LCS >1 M WBC/ml
PSA <1 (n=427)*	IL-6 <30 (n=299)	n=281 (94%)	n=13 (4.3%)	n=5 (1.6%)
	IL-6 >30 (n=128)	n=74 (57.8%)	n=36 (28.1%)	n=18 (14.0%)
PSA >1 (n=63)	IL-6 <30 (n=41)	n=34 (83%)	n=7 (17.0%)	n=0 (0%)
	IL-6 >30 (n=22)	n=7 (31.8%)	n=8 (36.3%)	n=7 (31.8%)

LCS = leukocytospermia
PSA = prostate-specific antigen, measured in μg/l
IL-6 = interleukin-6, measured in pg/ml
* The analysis included 490 men of prevalence study (Paper III) in whom both PSA and IL-6 values were available.

As a result, we found that in severe leukocytospermia (>1 M WBC/ml) there was a statistically significant difference between the number of men who had PSA values >1 μg/l and IL-6 whether >30 pg/ml or <30 pg/ml (31.8% vs 0%; p=0.0003). Similarly, we found a significant difference using same cut-offs for

PSA and IL-6 in all men with significant leukocytospermia (>0.2 M WBC/ml) (68.1% vs 17%; p=0.00005). The positive and negative predictive values of the combination PSA+IL-6 (according to the aforementioned cut-off levels) in these cases were 100% and 22% for severe leukocytospermia, respectively, and 68% and 25% for all men with significant leukocytospermia. These results should, however, be interpreted with caution, as the number of subjects used for comparison was relatively small and the alternative cut-offs used for PSA and IL-6 have yet to be tested in further studies.

10.5. Microflora of semen

Microflora of semen was determined in 37 men with leukocytospermia (>0.2 M per ml) and 32 controls. None of the 69 seminal fluids were sterile. More than 20 different microorganisms were isolated in both groups (Table 9). One to eight different microorganisms could be found in any particular semen sample, and the total concentration of microorganisms ranged from 2.0 to 7.5 $\log_{10}$ CFU/ml. Number of different microorganisms and the total concentration of microorganisms were in good correlation ($r = 0.70$, $p<0.001$). Unlike the controls, the NIH IV category prostatitis patients had abundant polymicrobial microbiocenosis in their semen, with both of these parameters being significantly higher in them than in controls (Table 10).

As for individual microorganisms, no statistically significant differences between the groups were observed, except for corynebacteria that are facultatively anaerobic, non-spore-forming Gram positive irregular rods. Some other studies including our recent paper have indicated a possible role for corynebacteria in prostatitis (Tanner *et al*., 1999; Domingue *et al*., 1998; Drach, 1974, Türk *et al*., 2007). As 'coryneform' bacteria form a heterogenous group of morphologically related genera – *Corynebacterium, Arthrobacter, Cellulomonas, Dermabacter, Rhodococcus* and many others (Funke *et al*., 1997; Khamis *et al*., 2005), additional investigations with genus and/or species detection are needed to clarify their importance in prostatitis.

Table 9. Comparison of seminal microorganisms in asymptomatic inflammatory prostatitis patients and the control group

Microorganisms	NIH IV category prostatitis patients (n=37)		Controls (n=32)	
	Median/ range (log)[1]	Occur-rence (%)	Median/ range (log)[1]	Occur-rence (%)
Staphylococcus aureus	<2/<2–6.0	27	<2/<2–4.0	37
Coagulase negative staphylococci	2.0/<2–5.0	68	2.2/<2–4.0	62
ß-haemolytic streptococci	<2/<2–7.0	16	<2/<2–4.0	9
Group B streptococci	<2/<2–4.0	3	<2/<2–3.3	3
Streptococci (α- or γ- haemolytic)	2.0/<2–6.7	51	<2/<2–4.0	37
Enterococci	<2/<2–2.0	3	<2/<2–3.3	9
Coryneform bacteria	3.3/<2–6.7 #	81*	<2/<2–4.0 #	38*
Bacillus sp.	<2	0	<2	0
Gardnerella vaginalis	<2/<2–6.0	14	<2/<2–7.0	16
Coliforms	<2/<2–5.0	16	<2/<2–4.3	3
Haemophilus sp.	<2	0	<2	0
Yeasts	<2	0	<2	0
Actinomyces	<2/<2–3.0	14	<2/<2–3.0	3
Peptostreptococci	<2/<2–7.0	41	<2/<2–6.0	37
Gram-neg. anaerobic rods	1.7/<2–7.0	46	<2/<2–7.0	31
Veillonella	<2/<2–5.7	3	<2/<2–4.0	6
Bifidobacteria	<2/<2–2.0	3	<2/<2–5.0	6
Propionibacteria	<2/<2–3.3	5	<2/<2–6.0	16
Sarcina	<2	0	<2/<2–4.0	6
Eubacteria	<2/<2–4.7	5	<2/<2–3.3	3
Mobiluncus sp.	<2/<2–7.0	11	<2	0
Lactobacilli	<2	0	<2	0

NIH: National Institutes of Health
* $p<0.05$ (Fisher's exact test)
# $p<0.05$ (Mann-Whitney rank sum test)
[1] Counts in $\log_{10}$ CFU/ml (detection level was 2.0)

At the same time it is very important to note the high frequency of anaerobic bacteria in both groups (70% of the samples in NIH IV group and 75% of the samples in the controls). In most of the specimens, the counts of anaerobic bacteria were equal to or outnumbered the aerobic ones (Table 10). Anaerobes occurred frequently concurrently in high counts in leukocytospermic patients. The possible role of anaerobes in chronic prostatitis has been postulated by studies where anaerobic bacteria were identified in prostate biopsy or EPS

(Szöke et al., 1998; Berger *et al*., 1997). These findings are in agreement with those by Tanner *et al*. (1999), too, who demonstrated the presence of a wide spectrum of bacterial species in prostatitis patients using analysis of 16S rRNA sequences, confirming that microorganisms associated with prostatitis generally occur as complex microbial communities. However, it should be noted that all these studies have been performed on symptomatic prostatitis patients.

At the same time numerous other investigators have found that, in most of the patients with leukocytospermia, pathogenic bacteria cannot be cultured from the semen in significant numbers, and no correlation between seminal microbes and raised leukocytes can be found (Wolff *et al*., 1995; Omu *et al*., 1999; Habermann *et al*., 1999; Esfandiari *et al*., 2002; Cottell *et al*., 2000; Munuce *et al*., 1999; Trum *et al*., 1998). However, unlike our investigations, most of these studies have not included suitable methods for a wide spectrum of bacteria and therefore anaerobic bacteria may have been overlooked in them.

Table 10. Comparison of some quantitative parameters of seminal microflora in different study groups

	NIH IV category prostatitis patients (n=37)	Control group (n=32)
Per cent of specimens with:		
aerobes > anaerobes	46% (n=17)	34% (n=11)
aerobes = anaerobes	38% (n=14)	28% (n=9)
aerobes < anaerobes	16% (n=6)	38% (n=12)
Total concentration of microorganisms (log10 CFU/ml) in semen (median [range])	4.8 (2.0–7.5)*	3.9 (2.3–7.3)*
No. of different microorganisms in semen (median [range])	5 (2–8)**	3 (1–7)**

NIH: National Institutes of Health
* $p<0.001$; ** $p = 0.004$ (Mann-Whitney rank sum test)

GENERAL DISCUSSION

Despite of wide spread, chronic prostatitis and especially its new category – asymptomatic inflammatory (NIH category IV) prostatitis – has been a largely unresearched area. Prevalence of (symptomatic) chronic prostatitis is reported to be 10% to 14% (Mehik *et al*., 2000; Nickel *et al*., 2001), but this data does not take into account the NIH category IV that cannot be determined by routine epidemiologic methods (questionnaires). Instead, WBC counts from a prostate-specific material (preferably semen [NIH Executive Summary, Bethesda 1995]) have to be measured in order to determine the prevalence of this subtype of prostatitis.

In our study we aimed to determine the prevalence of asymptomatic inflammatory prostatitis in young Estonian men by measuring WBC counts in semen samples. As the WHO-defined limit of significant leukocytospermia (>1 million WBC/ml) has been a matter of debate lately (Jedrzejczak *et al*., 1996; Sharma *et al*., 2001), we additionally used an alternative cut-off value of 0.2 million WBC/ml suggested by our earlier study results (Punab *et al*., 2003) and found the prevalence of NIH category IV prostatitis to be 6.0% and 19.0%, respectively. Regardless of the diagnostic threshold, men with asymptomatic inflammatory prostatitis still make up a significant part of all men with any form of chronic prostatitis. It is also evident that since asymptomatic inflammatory prostatitis is a subcategory of chronic prostatitis, the prevalence of NIH IV category prostatitis should be taken into account when estimating the total prevalence of chronic prostatitis.

Another methodological problem with comparing the different study results lies in the different methods that are used for detection of white blood cells in prostate-specific specimens. Previous studies have used different approaches for cell counting: WBC count per high-power field, per ejaculate or per 100 sperm, which all yield a different end result and are therefore not comparable. Several methodological problems lie also in the WBC detection itself: 1) several staining methods do not enable to differentiate between spermatogenetic cells and WBC, that may explain the high WBC counts in a study by Maruyama *et al*. (1985); 2) peroxidase technique used for detection of WBC may be inaccurate due to loss of peroxidase activity in some granulocytes during inflammatory process and exocytosis of the cellular content (Villegas *et al*., 2002). These different approaches may largely contribute to existing controversial results, therefore more unified criteria for staining and detection methods, definition of leukocytospermia and specification of the non-sperm cell types that are counted as leukocytes are needed. In our study we used differential counting of white blood cells in Bryan-Leishman stained slides, which enabled us to differentiate neutrophils from spermatogenetic cells as well as avoid possible errors caused by loss of peroxidase activity in WBC, and the results were expressed as the number of WBC per ml of semen.

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Moreover, ejaculate volume shows considerable variation due to physiological and/or pathological conditions, therefore in polyspermic samples (>6 ml) a dilution effect may occur and the WBC concentrations per ml of semen may be relatively lower in these cases. Therefore in our studies, we also calculated the possible deviation of WBC concentrations according to the ejaculate volume and no significant differences were seen when compared to the conventional method of expressing WBC concentration per ml of semen.

As stated earlier (see section 9), no community-based prevalence studies of NIH category IV prostatitis using semen samples have been conducted to this date, therefore it is only possible to make an indirect comparison of our study results with the previous studies where 1) asymptomatic infertile men were evaluated for leukocytospermia and 2) leukocytospermia was detected in control groups of chronic symptomatic prostatitis studies. In the first type of the studies (Sharma *et al*., 2001; Omu *et al*., 1999; Arata de Bellabarba *et al*., 2000) the prevalence of significant leukocytospermia is reported to be considerably higher than our estimate, however it must be noted that infertile men present a highly selected patient group who possibly harbor additional risk factors (e.g. higher rate of damaged spermatozoa which in turn may attract more leukocytes) that may predispose to high WBC counts in prostate-specific materials. This is also reflected in the diagnostic flowcharts of 'WHO manual for the standardized investigation, diagnosis and management of the infertile male' (WHO 2000), which recognizes accessory gland infection as one possible factor leading to male infertility. In the second type of studies (Nickel *et al*., 2003; Christiansen *et al*., 1991), the control groups also reveal somewhat higher prevalence of significant leukocytospermia than our data. At the same time it must be noted that subjects from these two studies were mostly middle-aged, compared to the young adults in our study, thus possibly suggesting higher prevalence of asymptomatic leukocytospermia in older age groups. The very young age of the men studied by us is a limitation mainly because sperm parameters as well as any forms of prostatitis have not been extensively studied in this age group to date and therefore, the value of comparing our data with those from the older subjects may be somewhat limited. On the other hand, this is the strength of our study since it has given valuable information that prostatitis, though in its hidden form, is so frequent in this age group.

In addition to missing data about asymptomatic inflammatory prostatitis in young men, there is lack of data about prevalence of any prostatitis syndromes in that age group. In our study only 7 men out of 565 were excluded due to genital tract inflammation whereas only one of them harboured prostatitis-like symptoms. To our best knowledge, only one study has been published (Ku *et al*., 2001) where the prevalence of prostatitis-like symptoms has been investigated exclusively in young men. In contrast with our data, the prevalence of prostatitis-like symptoms in 20-year-old men was 6% in that study, still it should be noted that the subjects were somewhat older than in our study. Almost negligible prevalence of symptomatic forms of prostatitis in our study

group and on the other hand, relatively high prevalence of asymptomatic prostatitis in the same young men suggests that further studies are needed to clarify the mechanisms of the development of prostatitis symptoms vs. patient's age.

Due to the asymptomatic nature of this prostatitis form, we have no information on the previous duration of the inflammation in our subjects, thus probably including young men at different stages of the disease. Therefore, a follow-up study of our subjects is needed to investigate the dynamics of leukocytospermia vs. time as well as the impact of these dynamics on sperm quality since we found that younger men from prevalence study had better semen quality than their older counterparts from the etiologic study, regardless of their leukocytospermia status. Additional studies are also required to determine the prevalence of NIH IV category prostatitis in other (older) age groups.

A somewhat unexpected byproduct of our study results was that subjects with moderate leukocytospermia had significantly better semen quality than those with lower or higher leukocyte counts. The reason behind the stimulatory effect of moderate leukocytospermia is not clear, but it may be hypothesized that moderate amount of leukocytes may stimulate immunocompetent cells by cytokines (e.g. IL-6 and IL-8) and/or that ROS action may not have deleterious effects in that leukocytospermia range. At the same time moderate leukocytospermia cannot be labeled as not being the 'true' leukocytospermia, because IL-6 concentration was also elevated in those subjects. Furthermore, the accuracy of cytological analysis of Bryan-Leishman stained sperm slides was supported by the determination of IL-6, as the leukocytospermia and the levels of IL-6 were highly correlated and IL-6 analysis was fully automated, thus diminishing the chance of possible errors due to human factor.

Another laboratory marker that was found to be significantly higher in leukocytospermic patients than controls was prostate-specific antigen. This association was evident even despite the fact that in our young men the PSA values remained below any internationally accepted cut-off levels (see 1.2.). PSA is secreted by the prostatic epithelial cells and its physiological function is believed to be participation in lysis of the ejaculate clot. Increased release of PSA is associated with damage of prostatic cells and therefore it has been extensively used as a marker for prostatic carcinoma although the effect of prostatitis on PSA has also been indicated. Therefore it may be suggested that screening for NIH category IV prostatitis and treating the underlying subclinical inflammation would increase the specificity of PSA screening and thereby reduce the number of potentially unnecessary biopsies.

In practice, it is therefore recommended to use the detection of leukocytospermia as a main diagnostic marker for NIH category IV prostatitis, and IL-6 in seminal plasma as an additional diagnostic tool for men with asymptomatic leukocytospermia to gain additional data regarding the activity of

inflammation, as well as for men with fertility problems or high serum PSA values to rule out possible inflammatory causes.

Although asymptomatic inflammatory prostatitis seems to be a frequent condition, its etiology is largely unknown. During routine studies, its infectious genesis is hard to prove due to insufficient laboratory methods. Usually, little attention is paid to anaerobic bacteria as they are sensitive to transportation, and their culture and differentiation is difficult, costly and time-consuming. In our study, which revealed a significant difference between asymptomatic inflammatory prostatitis patients and controls, we used a quantitative microbiologic analysis that included suitable media and environmental conditions for aerobic, microaerophilic, and anaerobic bacteria. Our study showed that asymptomatic leukocytospermia most probably has an important infectious component. Although we found microorganisms in all semen specimens, the total concentration and number of different microorganisms were much higher in asymptomatic inflammatory prostatitis patients than in the controls. Hence, our data support the idea of the polymicrobial nature of prostatitis. In our previous studies, we have seen quite similar microbiologic findings while studying CP/CPPS patients (Kermes *et al*., 2003), thus suggesting that symptomatic (NIH IIIA) and asymptomatic (NIH IV category) inflammatory prostatitis could have similar nature. This knowledge may be important in improving the treatment regimens of leukocytospermic patients.

The question regarding the source of microorganisms arises when semen is used as a specimen. Indigenous microflora exists in the urethra (Spaine *et al*., 2000; Mazuecos *et al*., 1998) as well as on genital skin (Diemer *et al*., 2000), while upper genital tract normally should be sterile, and the presence of normal microflora in the prostate is unlikely (Hochreiter *et al*., 2000). In our study, contamination was minimized by washing the hands and genitals with soap and water before sampling, and urinating. In addition, we have formerly shown significant differences between the first catch urine (that represents microflora of the urethra) and seminal fluid microflora (Kermes *et al*., 2003), indicating that most of the microorganisms in semen do not originate from the urethra, but reflect an infection of the upper genital tract.

To date, a number of different questionnaires have been developed and used in order to evaluate symptoms of prostatitis. The original version of the National Institutes of Health chronic prostatitis symptom index (NIH-CPSI) was developed by the NIH Chronic Prostatitis Collaborative Research Network (CPCRN) and has been successfully used in both clinical and epidemiological studies, enabling to differentiate patients with chronic prostatitis, benign prostatic hyperplasia (BPH) and healthy controls as well as to distinguish between symptomatic and asymptomatic forms of the prostatitis syndromes. This nine-item questionnaire measures the three most important domains in the chronic prostatitis patients' experience: pain, voiding symptoms and impact of those symptoms on the patient's quality of life (Litwin *et al*., 1999). The translated versions of this questionnaire in several other languages have been

successfully used. To this date, the NIH-CPSI has not been validated for use in Estonian language.

We translated the NIH-CPSI into Estonian and tested its validity in men with CP/CPPS and those without in a clinic-based setting as well as in a community-based study. The analysis revealed a high correlation between the two most prostatitis-specific domains (pain and quality of life) as well as between these domains and the total NIH-CPSI score. These scores were similar to those of the original symptom index. Also the internal consistency of the total index was high similarly to that of the original version of the NIH-CPSI. The significant difference of the most important scores of Estonian NIH-CPSI (total, pain and quality of life) between subjects with and without CP/CPPS on one hand and the similarity of those scores between the two (clinic-based and community based) prostatitis groups on the other refers to the good discriminant and content validity of the translated questionnaire when distinguishing patients with and without prostatitis-like symptoms. As for acceptability of the questionnaire, it could be self-administered to men attending the Andrology Centre in a short time and was well understood by all patients.

The Estonian NIH-CPSI is therefore a valid tool for diagnostic evaluation of prostatitis patients as well as for the use in epidemiological studies of chronic prostatitis in Estonia. In addition, the importance of the validated version of the Estonian NIH-CPSI is also reflected by the possibility of proper diagnostic workup of asymptomatic inflammatory prostatitis, by administering the questionnaire to the patients and confirming the lack of any prostatitis-like symptoms that may be overlooked in cases where patient claims to be asymptomatic, but fails to relate some specific symptoms (e.g. perineal discomfort) to prostatitis.

Although asymptomatic inflammatory prostatitis has been considered as true inflammation, its clinical significance is still a matter of debate. However, there is continuously increasing evidence that leukocytospermia may be responsible for many “unexplained” cases of male infertility (Branigan *et al*., 1995) mainly due to increased ROS production (Saleh *et al*., 2002) and ROS-mediated peroxidative damage to spermatozoa that may be observed in a high proportion of men with “unexplained” infertility (Agarwal *et al*., 2002) where the deleterious effects of leukocytospermia cannot be overcome even by assisted reproduction techniques (Saleh *et al*., 2003).

Also, a possible link between prostatic inflammation and carcinogenesis has been suggested (Coussens *et al*., 2002), through ROS-mediated damage of prostatic epithelial cells and the subsequent appearance of mutated cells with anti-apoptotic properties that may lead to the formation of proliferative inflammatory atrophy that has been considered a possible precursor of prostatic carcinoma. Therefore, further studies are needed to come up with better treatment strategies for conditions associated with leukocytospermia, including silent genital tract infections like NIH category IV prostatitis.

CONCLUSIONS

1) The Estonian version of the NIH-CPSI has good discriminant and content validity while distinguishing patients with and without prostatitis-like symptoms both in community-based as well as clinic-based study settings. It is therefore a valid tool for evaluation of both symptomatic and asymptomatic prostatitis patients as well as for the use in epidemiological studies of chronic prostatitis in Estonia;
2) A remarkable proportion of young Estonian men has asymptomatic inflammatory (NIH category IV) prostatitis, whereby symptomatic forms of prostatitis are almost absent in this age group, suggesting that further understanding is needed about the clinical significance of this condition and its possible treatment strategies. Also, the mechanisms of the development of prostatitis symptoms vs. patient's age need to be clarified as well as the possible pathogenetic associations between symptomatic and asymptomatic forms of prostatitis;
3) Asymptomatic inflammatory prostatitis most probably has an important infectious component since these patients harbor abundant polymicrobial microbiocenosis in their semen containing both aerobic and anaerobic bacteria. We have seen similar microbiological findings in CP/CPPS patients, thus suggesting that these two forms of prostatitis could have similar nature;
4) PSA levels in serum and IL-6 concentration in seminal plasma are significantly higher in NIH IV category prostatitis patients than in the controls. Analogous tendencies have been found in symptomatic prostatitis, thus further supporting the similarities between asymptomatic inflammatory prostatitis and symptomatic forms of prostatitis. The combination of these two laboratory markers appears to have a good positive predictive value in differentiating between men with or without significant leukocytospermia;
5) Moderate leukocytospermia appears to increase sperm concentration and total sperm count regardless of age, while severe leukocytospermia tends to have negative effect on the semen quality only in mid-twenties but not in younger men. Moreover, younger study subjects tend to have better semen quality than their older counterparts regardless of their leukocytospermia status. That may indicate age-dependent differences in functional reserve of accessory sex glands.

REFERENCES

Agarwal A, Saleh RA. Role of oxidants in male infertility: rationale, significance, and treatment. Urol Clin North Am 2002;29:817–27.

Alexander RB, Ponniah S, Hasday J, Hebel JR. Elevated levels of proinflammatory cytokines in the semen of patients with chronic prostatitis/chronic pelvic pain syndrome. Urology 1998;52(5):744–9.

Alvarez JG, Sharma RK, Ollero M, Saleh RA, Lopez MC, Thomas AJ Jr, Evenson DP, Agarwal A. Increased DNA damage in sperm from leukocytospermic semen samples as determined by the sperm chromatin structure assay. Fertil Steril 2002;78(2):319–29.

Arata de Bellabarba G, Tortolero I, Villarroel V, Molina CZ, Bellabarba C, Velazquez E. Nonsperm cells in human semen and their relationship with semen parameters. Arch Androl 2000;45(3):131–6.

Bennett B, Richardson P, Gardner WJ. Histopathology and cytology of prostatitis. In: Lepor H, Lawson R, editors. Prostate Diseases. Philadelphia: WB Saunders Co. 1993. pp 399–413.

Berger RE, Krieger JN, Rothman I, Muller CH, Hillier SL. Bacteria in the prostate tissue of men with idiopathic prostatic inflammation, J Urol 1997;157:863–865.

Berghuis JP, Heiman JR, Rothman I, Berger RE. Psychological and physical factors involved in chronic idiopathic prostatitis. J Psychosom Res 1996;41(4):313–25.

Bjerklund Johansen TE, Gruneberg RN, Guibert J, Hofstetter A, Lobel B, Naber KG, et al. The role of antibiotics in the treatment of chronic prostatitis: a consensus statement. Eur Urol 1998;34:457–66.

Bozeman CB, Carver BS, Eastham JA, Venable DD. Treatment of chronic prostatitis lowers serum prostate specific antigen. J Urol 2002;167(4):1723–6.

Brähler E, Kupfer J, Ludwig M, Weidner W. Symptoms of prostatitis in German. The Giessen Prostatitis Symptom Score. Urologe A 2001;40(1):12–5. German.

Branigan EF, Spadoni LR, Muller CH. Identification and treatment of leukocytospermia in couples with unexplained infertility. J Reprod Med 1995;40(9):625–9.

Budía A, Luis Palmero J, Broseta E, Tejadillos S, Benedicto A, Queipo JA, Gobernado M, Fernando Jiménez Cruz J. Value of semen culture in the diagnosis of chronic bacterial prostatitis: a simplified method. Scand J Urol Nephrol 2006;40(4):326–31.

Capodice JL, Bemis DL, Buttyan R, Kaplan SA, Katz AE. Complementary and alternative medicine for chronic prostatitis/chronic pelvic pain syndrome. Evid Based Complement Alternat Med 2005;2(4):495–501. Epub 2005 Oct 10.

Carver, BS, Bozeman, CB, Williams, BJ, Venable, DD. The prevalence of men with National Institutes of Health category IV prostatitis and association with serum prostate specific antigen. J Urol 2003;169(2):589–91.

Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJ, Petros JA, Andriole GL. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med. 1991;324(17):1156–61. Erratum in: N Engl J Med 1991;325(18):1324.

Centola GM, Eberly S. Seasonal variations and age-related changes in human sperm count, motility, motion parameters, morphology, and white blood cell concentration. Fertil Steril. 1999;72(5):803–8.

Cheah PY, Liong ML, Yuen KH, Lee S, Yang JR, Teh CL, Khor T, Yap HW, Krieger JN. Reliability and validity of the National Institutes of Health: Chronic Prostatitis Symptom Index in a Malaysian population. World J Urol 2006;24(1):79–87. Epub 2006 Feb 8.

Cheah PY, Liong ML, Yuen KH, Teh CL, Khor T, Yang JR, Yap HW, Krieger JN. Chronic prostatitis: symptom survey with follow-up clinical evaluation. Urology 2003;61(1):60–4.

Chong CH, Ryu DS, Oh TH. The Korean version of NIH-chronic prostatitis symptom index (NIH-CPSI): validation study and characteristics on chronic prostatitis. Korean J Urol 2001;42:511–20.

Christiansen E, Tollefsrud A, Purvis K. Sperm quality in men with chronic abacterial prostatovesiculitis verified by rectal ultrasonography. Urology 1991;38(6):545–9.

Clemens JQ, Meenan RT, O'Keeffe Rosetti MC, Gao SY, Calhoun EA. Incidence and clinical characteristics of National Institutes of Health type III prostatitis in the community. J Urol 2005;174(6):2319–22.

Collins MM, O'Leary MP, Calhoun EA, Pontari MA, Adler A, Eremenco S, Chang CH, Odom L, Litwin MS; Chronic Prostatitis Collaborative Research Network. The Spanish National Institutes of Health-Chronic Prostatitis Symptom Index: translation and linguistic validation. J Urol 2001;166(5):1800–3.

Cottell E, Harrison RF, McCaffrey M, Walsh T, Mallon E, Barry-Kinsella C. Are seminal fluid microorganisms of significance or merely contaminants? Fertil Steril 2000;74(3):465–70.

Dalton DL. Elevated serum prostate-specific antigen due to acute bacterial prostatitis. Urology 1989;33(6):465.

Depuydt C, Zalata A, Christophe A, Mahmoud A, Comhaire F. Mechanisms of sperm deficiency in male accessory gland infection. Andrologia 1998;30 Suppl 1:29–33.

Diemer T, Ludwig M, Huwe P, Hales DB, Weidner W. Influence of urogenital infection on sperm function, Curr Opin Urol 2000**;**10:39–44.

Dimitrakov J, Diemer T, Ludwig M, Weidner W. Recent developments in diagnosis and therapy of the prostatitis syndromes. Curr Opin Urol 2001;11(1):87–91. Review.

Dinarello CA. Proinflammatory cytokines. Chest 2000;118:503–508.

Domingue GJ Sr, Hellstrom WJ. Prostatitis. Clin Microbiol Rev 1998;11(4):604–13. Review.

dos Santos Silva I. Cancer Epidemiology: Principles and Methods. Lyon: International Agency for Research on Cancer; 1999.

Drach GW, Fair WR, Meares EM, Stamey TA. Classification of benign diseases associated with prostatic pain: Prostatitis or prostatodynia? J Urol 1978;120:266.

Drach GW. Problems in diagnosis of bacterial prostatitis: Gram-negative, Gram-positive and mixed infections. J Urol 1974;111:630–36.

Eggert-Kruse W, Boit R, Rohr G, Aufenanger J, Hund M, Strowitzki T. Relationship of seminal plasma interleukin (IL) -8 and IL-6 with semen quality. Hum Reprod 2001;16(3):517–28.

Elert A, von Knobloch R, Nusser R, et al. Isolated candidal prostatitis. J Urol 2000;163:244.

El-Nashaar A, Fathy A, Zeedan A, Al-Ahwany A, Shamloul R. Validity and reliability of the arabic version of the National Institutes of Health Chronic Prostatitis Symptom Index. Urol Int 2006;77(3):227–31.

Esfandiari N, Saleh RA, Abdoos M, Rouzrokh A, Nazemian Z. Positive bacterial culture of semen from infertile men with asymptomatic leukocytospermia. Int J Fertil Womens Med 2002;47(6):265–70.

Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, Young S, Moore D. The association of age and semen quality in healthy men. Hum Reprod. 2003;18(2):447–54.

Fair WR, Parrish RT. Antibacterial substance in prostatic fluid. In: Murphy GP, Sandberg AA, Karr JP (eds.). Prostatic cell: structure and function, part A. New York: Liss; 1981, pp 247–264.

Fair WR, Wehner N. The prostatic antibacterial factor: identity and significance. Prog Clin Biol Res 1976;6:383–403.

Fedder J. Nonsperm cells in human semen: with special reference to seminal leukocytes and their possible influence on fertility. Arch Androl 1996;36(1):41–65. Review.

Funke G, Graevenitz A, Clarridge JE, Bernard KA. Clinical microbiology of coryneform bacteria. Clinical Microbiology Reviews 1997;10:125–59.

Giubilei G, Mondaini N, Crisci A, Raugei A, Lombardi G, Travaglini F, Del Popolo G, Bartoletti R. The Italian version of the National Institutes of Health Chronic Prostatitis Symptom Index. Eur Urol 2005;47:805–811.

Gonen M, Kalkan M, Cenker A, Ozkardes H. Prevalence of premature ejaculation in Turkish men with chronic pelvic pain syndrome. J Androl 2005;26(5):601–3.

Gurunadha Rao Tunuguntla HS, Evans CP. Management of prostatitis. Prostate Cancer Prostatic Dis 2002;5(3):172–9. Review.

Habermann B, Krause W. Altered sperm function or sperm antibodies are not associated with chlamydial antibodies in infertile men with leucocytospermia. J Eur Acad Dermatol Venereol 1999;12(1):25–9.

Han M, Gann PH, Catalona WJ. Prostate-specific antigen and screening for prostate cancer. Med Clin North Am 2004;88(2):245–65, ix. Review.

Heidenreich A, Aus G, Abbou CC, Bolla M, Joniau S, Matveev V, Schmid HP, Zattoni F. Guidelines on Prostate Cancer. European Association of Urology 2008. http://www.uroweb.org/fileadmin/user_upload/Guidelines/Prostate%20Cancer.pdf

Hernandez J, Thompson IM. Prostate-specific antigen: a review of the validation of the most commonly used cancer biomarker. Cancer 2004;101(5):894–904. Review.

Hochreiter W, Ludwig M, Weidner W, Wagenlehner F, Naber K, Eremenco S, Arnold B. National Institutes of Health (NIH)-Chronic Prostatitis Symptom Index Deutsche Version. Der Urologe 2001;40:16–17.

Hochreiter WW, Nadler RB, Koch AE, Campbell PL, Ludwig M, Weidner W, Schaeffer AJ. Evaluation of the cytokines interleukin 8 and epithelial neutrophil activating peptide 78 as indicators of inflammation in prostatic secretions. Urology 2000;56(6):1025–9.

Hochreiter WW, Duncan JL, Schaeffer AJ. Evaluation of the bacterial flora of the prostate using 16sRNA gene based polymerase chain reaction, J Urol 2000; 163:127–130.

Huleihel M, Lunenfeld E, Horowitz S, Levy A, Potashnik G, Mazor M, Glezerman M. Involvement of serum and lipopolysaccharide in the production of interleukin-1- and interleukin-6-like molecules by human sperm cells. Am J Reprod Immunol. 2000;43(1):41–6.

IMMULITE 2000 IL-6 (PIL2K6P-10, 2006-12-29). Package insert. Siemens Medical Solutions Diagnostics 2006.

16

IMMULITE 2000 Third Generation PSA (PIL2KUP-12, 2006-12-29). Package insert. Siemens Medical Solutions Diagnostics 2006.

Jedrzejczak P, Szumala-Kakol A, Dydowicz P, Szymanowski K, Pisarski T. Usefulness of counting leukocytes and round cells in determination of bacterial infection of semen in infertile men. Ginekol Pol 1996;67(11):569–73. Polish.

John H, Barghorn A, Funke G, Sulser T, Hailemariam S, Hauri D, Joller-Jemelka H. Noninflammatory chronic pelvic pain syndrome: immunological study in blood, ejaculate and prostate tissue. Eur Urol 2001;39(1):72–8.

Jung K, Meyer A, Lein M, Rudolph B, Schnorr D, Loening SA. Ratio of free-to-total prostate specific antigen in serum cannot distinguish patients with prostate cancer from those with chronic inflammation of the prostate. J Urol 1998;159(5):1595–8.

Kaleli S, Ocer F, Irez T, Budak E, Aksu MF. Does leukocytospermia associate with poor semen parameters and sperm functions in male infertility? The role of different seminal leukocyte concentrations. Eur J Obstet Gynecol Reprod Biol 2000; 89(2):185–91.

Karakiewicz PI, Perrotte P, Valiquette L, Benard F, McCormack M, Menard C, McNaughton Collins M, Nickel JC. French-Canadian linguistic validation of the NIH Chronic Prostatitis Symptom Index. Can J Urol 2005;12(5):2816–23.

Keltikangas-Järvinen L, Järvinen H, Lehtonen T. Psychic disturbances in patients with chronic prostatitis. Ann Clin Res 1981;49:809–818.

Kermes K, Punab M, Lõivukene K, Mändar R. Anaerobic seminal fluid micro-flora in chronic prostatitis/chronic pelvic pain syndrome patients. Anaerobe 2003;9(3):117–23.

Khamis A, Raoult D, La Scola B. Comparison between rpoB and 16S rRNA Gene Sequencing for Molecular Identification of 168 Clinical Isolates of Corynebacterium. Journal of Clinical Microbiology 2005;43:1934–36.

Kopa Z, Wenzel J, Papp GK, Haidl G. Role of granulocyte elastase and interleukin-6 in the diagnosis of male genital tract inflammation. Andrologia 2005;37(5):188–94.

Krieger JN, Riley DE. Bacteria in the chronic prostatitis-chronic pelvic pain syndrome: molecular approaches to critical research questions. J Urol 2002;167(6):2574–83. Review.

Krieger JN, Jacobs R, Ross SO. Detecting urethral and prostatic inflammation in patients with chronic prostatitis. Urology 2000;55(2):186–91; discussion 191–2.

Krieger JN, Nyberg L, Nickel JC. NIH consensus definition and classification of prostatitis. JAMA 1999;282:236–7.

Krieger JN, Egan KJ, Ross SO, Jacobs R, Berger RE. Chronic pelvic pains represent the most prominent urogenital symptoms of “chronic prostatitis”. Urology 1996; 48(5):715–21; discussion 721–2. Review.

Ku JH, Kim ME, Lee NK, Park YH. Influence of environmental factors on chronic prostatitis-like symptoms in young men: results of a community-based survey. Urology 2001;58(6):853–8.

Ku JH, Kim ME, Lee NK, Park YH. The prevalence of chronic prostatitis-like symptoms in young men: a community-based survey. Urol Res 2001;29(2):108–12.

Kullisaar T, Türk S, Punab M, Korrovits P, Kisand K, Rehema A, Zilmer K, Zilmer M, Mändar R. Oxidative stress in leucocytospermic prostatitis patients: preliminary results. Andrologia 2008;40(3):161–72.

Kunishima Y, Matsukawa M, Takahashi S, Itoh N, Hirose T, Furuya S, Takatsuka K, Mori M, Tsukamoto T. National Institutes of Health Chronic Prostatitis Symptom Index for Japanese men. Urology 2002;60:74–7.

Kvist U, Björndahl L, eds. Manual on basic semen analysis. ESHRE monographs. Oxford: Oxford University Press; 2002.

Lackner JE, Herwig R, Schmidbauer J, Schatzl G, Kratzik C, Marberger M. Correlation of leukocytospermia with clinical infection and the positive effect of anti-inflammatory treatment on semen quality. Fertil Steril 2006;86(3):601–5. Epub 2006 Jun 16.

Leonhardt H, Kahle W, Platzer W, eds. Color Atlas/Text of Human Anatomy, 4th edition, Vol. 2. Stuttgart: Thieme; 1993.

Leskinen MJ, Mehik A, Sarpola A, Tammela TLJ, Jarvelin MR. The Finnish version of The National Institutes of Health Chronic Prostatitis Symptom Index correlates well with the visual pain scale: translation and results of a modified linguistic validation study. BJU Int 2003;92:251–6.

Leskinen M, Lukkarinen O, Marttila T. Effects of finasteride in patients with inflammatory chronic pelvic pain syndrome: a double-blind, placebo-controlled, pilot study. Urology 1999;53(3):502–5.

Lewis SE, Boyle PM, McKinney KA, et al. Total antioxidant capacity of seminal plasma is different in fertile and infertile men. Fertil Steril 1995;64:868–870.

Lilja H. A kallikrein-like serum protease in prostatic fluid cleaves the predominant seminal vesicle protein. J Clin Invest 1985;76:1899–1903.

Lipsky B. Prostatitis and urinary tract infection in men: what's new; what's true? Am J Med 1999;106:327–34.

Litwin MS, McNaughton-Collins M, Fowler FJ, Nickel JC, Calhoun EA, Pontari MA, Alexander RB, Farrar JT, O'Leary MP. The National Institutes of Health Chronic Prostatitis Symptom Index: development and validation of a new outcome measure. J Urol 1999;162:369–375.

Ludwig M, Dimitrakov J, Diemer T, Huwe P, Weidner W. Prostatitis syndrome. Changes in the ejaculate and effects on fertility. Urologe A 2001;40(1):18–23. Review. German.

Maruyama DK Jr., Hale RW, Rogers BJ. Effects of white blood cells on the in vitro penetration of zona-free hamster eggs by human spermatozoa. J Androl 1985; 6(2):127–35.

Mazuecos J, Aznar J, Rodriguez–Pichardo A *et al.*. Anaerobic bacteria in men with urethritis, J Eur Acad Dermatol Venereol 1998;10:237–242.

Matalliotakis I, Kiriakou D, Fragouli I, Sifakis S, Eliopoulos G, Koumantakis E. Interleukin-6 in seminal plasma of fertile and infertile men. Arch Androl 1998; 41(1):43–50.

McNaughton Collins MM, Joyce GF, Wise M, Pontari MA. Prostatitis. In: Litwin MS, Saigal CS, editors. Urologic Diseases in America. US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases. Washington, DC: US Government Printing Office, 2007; NIH Publication No. 07–5512, pp. 9–42.

McNaughton Collins M. The impact of chronic prostatitis/chronic pelvic pain syndrome on patients. World J Urol 2003;21(2):86–9. Epub 2003 Apr 2. Review.

McNaughton Collins MM, Meigs JB, Barry MJ, Walker Corkery E, Giovannucci E, Kawachi I. Prevalence and correlates of prostatitis in the health professionals follow-up study cohort. J Urol 2002;167(3):1363–6.
McNaughton Collins M, Fowler FJ Jr, Elliott DB, Albertsen PC, Barry MJ. Diagnosing and treating chronic prostatitis: do urologists use the four-glass test? Urology 2000;55(3):403–7.
McNaughton Collins M, MacDonald R, Wilt TJ. Diagnosis and treatment of chronic abacterial prostatitis: a systematic review. Ann Intern Med 2000;133(5):367–81. Review.
McNaughton Collins M, Stafford RS, O'Leary P, et al. How common is prostatitis? A national survey of physician visits. J Urol 1998;159:1224–28.
McNeal, JE. The prostate and prostatic urethra: A morphologic study. J Urol 1970;104:443.
Meares EM, Stamey TA. Bacteriologic localization patterns in bacterial prostatitis and urethritis. Invest. Urol 1968;5:492–518.
Mehik A, Hellstrom P, Sarpola A, Lukkarinen O, Jarvelin MR. Fears, sexual disturbances and personality features in men with prostatitis: a population-based cross-sectional study in Finland. BJU Int 2001;88(1):35–8.
Mehik A, Hellstrom P, Lukkarinen O, Sarpola A, Jarvelin M. Epidemiology of prostatitis in Finnish men: a population-based cross-sectional study. BJU Int 2000; 86(4):443–8.
Menkveld R, Stander FS, Kotze TJ, Kruger TF, van Zyl JA. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Hum Reprod 1990;5:586–92.
Miller LJ, Fischer KA, Goralnick SJ, Litt M, Burleson JA, Albertsen P, Kreutzer DL. Nerve growth factor and chronic prostatitis/chronic pelvic pain syndrome. Urology 2002;59(4):603–8.
Miller LJ, Fischer KA, Goralnick SJ, Litt M, Burleson JA, Albertsen P, Kreutzer DL. Interleukin-10 levels in seminal plasma: implications for chronic prostatitis-chronic pelvic pain syndrome. J Urol 2002;167(2 Pt 1):753–6.
Monden K, Tsugawa M, Ninomiya Y, Ando E, Kumon H. [A Japanese version of the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI, Okayama version) and the clinical evaluation of cernitin pollen extract for chronic non-bacterial prostatitis] Nippon Hinyokika Gakkai Zasshi 2002;93(4):539–47. Japanese.
Moon TD, Hagen L, Heisey DM. Urinary symptomatology in younger men. Urology 1997;50(5):700–3.
Munuce MJ, Bregni C, Carizza C, Mendeluk G. Semen culture, leukocytospermia, and the presence of sperm antibodies in seminal hyperviscosity. Arch Androl 1999; 42(1):21–8.
Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 7th ed. Washington, DC: American Society for Microbiology; 1999.
Mändar R, Raukas E, Turk S, Korrovits P, Punab M. Mycoplasmas in semen of chronic prostatitis patients. Scand J Urol Nephrol 2005;39(6):479–82.
Naber KG, Weidner W. Chronic prostatitis-an infectious disease? J Antimicrob Chemother 2000;46(2):157–61. Review.
Naber KG. Antibiotic treatment of chronic bacterial prostatitis. In: Nickel JC (Ed.). Textbook of Prostatitis. Oxford, Isis Media Ltd. 1999, pp 285–292.

Nadler RB, Collins MM, Propert KJ, Mikolajczyk SD, Knauss JS, Landis JR, Fowler JE Jr, Schaeffer AJ, Alexander RB; Chronic Prostatitis Collaborative Research Network. Prostate-specific antigen test in diagnostic evaluation of chronic prostatitis/chronic pelvic pain syndrome. Urology 2006;67(2):337–42. Epub 2006 Jan 25.

Nadler RB, Koch AE, Calhoun EA, Campbell PL, Pruden DL, Bennett CL, Yarnold PR, Schaeffer AJ. IL-1beta and TNF-alpha in prostatic secretions are indicators in the evaluation of men with chronic prostatitis. J Urol 2000;164(1):214–8.

Nadler RB, Humphrey PA, Smith DS, Catalona WJ, Ratliff TL. Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. J Urol 1995;154(2 Pt 1):407–13.

National Institutes of Health Summary Statement. National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases workshop on chronic prostatitis. Executive Summary, Bethesda, Maryland: National Institutes of Health; 1995.

Neal DE Jr, Moon TD. Use of terazosin in prostatodynia and validation of a symptom score questionnaire. Urology 1994;43(4):460–5.

Nickel JC, Elhilali M, Vallancien G; ALF-ONE Study Group. Benign prostatic hyperplasia (BPH) and prostatitis: prevalence of painful ejaculation in men with clinical BPH. BJU Int 2005;95(4):571–4.

Nickel JC. Recommendations for the evaluation of patients with prostatitis. World J Urol 2003;21(2):75–81. Epub 2003 Apr 9. Review.

Nickel, JC, Alexander RB, Schaeffer, AJ, Landis, JR, Knauss, JS, Propert, KJ; Chronic Prostatitis Collaborative Research Network Study Group. Leukocytes and bacteria in men with chronic prostatitis/chronic pelvic pain syndrome compared to asymptomatic controls. J Urol 2003;170(3):818–22.

Nickel JC. Prostatitis and related conditions. In: Campbell MF, Walsh P, Retik AB (eds) Campbell's urology, 8th edn. WB Saunders, Philadelphia 2002; pp 603–630.

Nickel JC, Downey J, Johnston B, Clark J; Canadian Prostatitis Research Group. Predictors of patient response to antibiotic therapy for the chronic prostatitis/chronic pelvic pain syndrome: a prospective multicenter clinical trial. J Urol 2001; 165(5):1539–44.

Nickel JC, Downey J, Hunter D, Clark J. Prevalence of prostatitis-like symptoms in a population based study using the National Institutes of Health chronic prostatitis symptom index. J Urol 2001;165:842–5.

Nickel JC, Weidner W. Chronic prostatitis: current concepts and antimicrobial therapy. Infect Urol 2000;13:S22–S28.

Nickel JC. Prostatitis syndromes: an update for urologic practice. Can J Urol 2000; 7:1091–1098.

Nickel JC. Prostatitis: the last frontier. World J Surg 2000;24(10):1197–9. Review.

Nickel JC. Chronic prostatitis: an infectious disease? Infect Urol 2000;13(2):31–8.

Nickel JC, Alexander R, Anderson R, Krieger J, Moon T, Neal D, Schaeffer A, Shoskes D. Prostatitis unplugged? Prostatic massage revisited. Tech Urol 1999;5(1):1–7. Review.

Nickel JC, Nyberg LM, Hennenfent M. Research guidelines for chronic prostatitis: Consensus report from the first National Institutes of Health International Prostatitis Collaborative Network. Urology 1999;54:229–233.

Nickel JC, Downey J, Young I, Boag S. Asymptomatic inflammation and/or infection in benign prostatic hyperplasia. BJU Int. 84: 976–81, 1999.

17

Nickel JC, Nigro M, Valiquette L, Anderson P, Patrick A, Mahoney J, Buckley R, Corcos J, Hosking D. Diagnosis and treatment of prostatitis in Canada. Urology 1998;52(5):797–802.
Nickel JC. Effective office management of chronic prostatitis. Urol Clin North Am 1998;25:677–84.
Nickel, JC. The pre and post massage test (PPMT): a simple screen for prostatitis. Tech. Urol 1997;3:38–43.
Nickel JC, Sorensen R. Transurethral microwave thermotherapy for nonbacterial prostatitis: a randomized double-blind sham controlled study using new prostatitis specific assessment questionnaires. J Urol 1996;155(6):1950–4; discussion 1954–5.
NIH-CPSI, Estonian online version 2006. http://meeste.kliinik.ee/index.php?go=nihcpsi June 2nd 2008.
Ohta S, Wada H, Gabazza EC, Nobori T, Fuse H. Evaluation of tissue factor antigen level in human seminal plasma. Urol Res 2002;30(5):317–20. Epub 2002 Sep 10.
Omu AE, Al-Qattan F, Al-Abdul-Hadi FM, Fatinikun MT, Fernandes S. Seminal immune response in infertile men with leukocytospermia: effect on antioxidant activity. Eur J Obstet Gynecol Reprod Biol 1999;86(2):195–202.
Orhan I, Onur R, Ilhan N, Ardicoglu A. Seminal plasma cytokine levels in the diagnosis of chronic pelvic pain syndrome. Int J Urol 2001;8(9):495–9.
Ozden C, Ozdal OL, Guzel O, Han O, Seckin S, Memis A. The correlation between serum prostate specific antigen levels and asymptomatic inflammatory prostatitis. Int Urol Nephrol. 2007;39(3):859–63. Epub 2006 Nov 17.
Pansadoro V, Emiliozzi P, Defidio L, Scarpone P, Sabatini G, Brisciani A, Lauretti S. Prostate-specific antigen and prostatitis in men under fifty. Eur Urol 1996;30(1):24–7.
Pasqualotto FF, Sharma RK, Potts JM, Nelson DR, Thomas AJ, Agarwal A. Seminal oxidative stress in patients with chronic prostatitis. Urology 2000;55(6):881–5.
Pavone-Macaluso M. Chronic prostatitis syndrome: a common, but poorly understood condition. Part II. AU-EBU Update Series 2007;5(1):16–25.
Persson BE, Ronquist G, Ekblom M. Ameliorative effect of allopurinol on nonbacterial prostatitis: a parallel double-blind controlled study. J Urol 1996;155(3):961–4.
Potashnik H, Elhija MA, Lunenfeld E, Potashnik G, Schlatt S, Nieschlag E, Huleihel M. Interleukin-6 expression during normal maturation of the mouse testis. Eur Cytokine Netw. 2005;16(2):161–5.
Potts JM. The four categories of prostatitis: a practical approach to treatment. Cleve Clin J Med 2001;68(5):389–90, 392–3, 397.
Potts JM. Prospective identification of National Institutes of Health category IV prostatitis in men with elevated prostate specific antigen. J Urol 2000;164(5):1550–3.
Punab, M, Lõivukene, K, Kermes, K, Mändar, R. The limit of leukocytospermia from the microbiological viewpoint. Andrologia 2003;35:271–278.
Resim S, Cek M, Gurbuz ZG, Fazlioglu A, Caskurlu T, Uras AR, Sevin G. Serum PSA and age-specific reference ranges in patients with prostatism symptoms. Int Urol Nephrol 1999;31(2):221–8.
Robaire B, Pryor JL, Trasler JM, eds. Handbook of Andrology. Lawrence: Allen Press; 1995
Roberts RO, Jacobson DJ, Girman CJ, et al. Prevalence of prostatitis-like symptoms in a community based cohort of older men. J Urol 2002;168:2467–2471.

Roberts RO, Lieber MM, Rhodes T, Girman CJ, Bostwick DG, Jacobsen SJ. Prevalence of a physician-assigned diagnosis of prostatitis: the Olmsted County Study of Urinary Symptoms and Health Status Among Men. Urol 1998;51:578–84.

Roberts RO, Lieber MM, Bostwick DG, Jacobsen SJ. A review of clinical and pathological prostatitis syndromes. Urol 1997;49:809–21.

Rothman I, Stanford JL, Kuniyuki A, Berger RE. Self-report of prostatitis and its risk factors in a random sample of middle-aged men. Urology 2004;64(5):876–9.

Sachs L. Angewandte Statistik, Anwendung statistischer Methoden. 1999 Springer-Verlag, Berlin.

Saleh RA, Agarwal A, Kandirali E, Sharma RK, Thomas AJ, Nada EA, Evenson DP, Alvarez JG. Leukocytospermia is associated with increased reactive oxygen species production by human spermatozoa. Fertil Steril 2002;78(6):1215–24.

Saleh RA, Agarwal A, Nada EA, El-Tonsy MH, Sharma RK, Meyer A, et al. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. Fertil Steril 2003;79:1597–605.

Schaeffer AJ, Datta NS, Fowler JE Jr, Krieger JN, Litwin MS, Nadler RB, Nickel JC, Pontari MA, Shoskes DA, Zeitlin SI, Hart C; Chronic Prostatitis Collaborative Research Network. Overview summary statement. Diagnosis and management of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Urology 2002;60(6 Suppl):1–4. Review.

Schaeffer AJ, Knauss JS, Landis JR, Propert KJ, et al. Leukocyte and bacterial counts do not correlate with severity of symptoms in men with chronic prostatitis: the national institutes of health chronic prostatitis cohort study. J Urol 2002;168:1048–53.

Schneider H, Brahler E, Ludwig M, Hochreiter W, Collins MF, Eremenco S, Weidner W. Two-year experience with the german-translated version of the NIH-CPSI in patients with CP/CPPS. Urology 2004;63(6):1027–30.

Schneider H, Brähler E, Ludwig M, Hochreiter W, Lenk S, Vahlensieck W, Weidner W. The German version of the NIH-CPSI: evaluation of internal consistency and median scores in 137 patients with CPSS – NIH III a and III b. J Urol 2002;167:110A.

Schröder FH, Roobol MJ, van der Kwast TH, Kranse R, Bangma CH. Does PSA velocity predict prostate cancer in pre-screened populations? Eur Urol. 2006;49(3):460–5; discussion 465. Epub 2006 Jan 17.

Shahed AR, Shoskes DA. Oxidative stress in prostatic fluid of patients with chronic pelvic pain syndrome: correlation with gram positive bacterial growth and treatment response. J Androl 2000;21(5):669–75.

Sharma RK, Pasqualotto AE, Nelson DR, Thomas AJ Jr, Agarwal A. Relationship between seminal white blood cell counts and oxidative stress in men treated at an infertility clinic. J Androl 2001;22(4):575–83.

Shimomura T, Kiyota H, Takahashi H, Madarame J, Kimura T, Onodera S. Prostate histopathology of NIH category IV prostatitis detected by sextant prostate needle biopsy from the patients with high prostatic specific antigen. Kansenshogaku Zasshi 2003;77(8):611–7. Japanese.

Shoskes DA, Zeitlin SI, Shahed A, Rajfer J. Quercetin in men with category III chronic prostatitis: a preliminary prospective, double-blind, placebo-controlled trial. Urology 1999;54(6):960–3.

Simbini T, Umapathy E, Jacobus E, Tendaupenyu G, Mbizvo MT. Study on the origin of seminal leucocytes using split ejaculate technique and the effect of leucocytospermia on sperm characteristics. Urol Int 1998;61(2):95–100.

Spaine MD, Mamizuka EM, Cedenho AP, Srougi M. Microbiologic aerobic studies on normal male urethra, Urology 2000**;**56:207–210.

Szöke I, Török L, Dósa E, Nagy E, Scultéty S. The possible role of anaerobic bacteria in chronic prostatitis. Int J Androl 1998;21:163–168.

Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS, Redwine E. Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med. 1987;317(15):909–16.

Stamey TA, Meares EMJr, Winningham DG. Chronic bacterial prostatitis and the diffusion of drugs into prostatic fluid. J Urol 1970; 103:187–194.

Stamey TA, Fair WR, Timothy MM, Wehner N. Antibacterial nature of prostatic fluid. Nature 1968;218:444.

Stancik I, Luftenegger W, Klimpfinger M, Muller MM, Hoeltl W. Effect of NIH-IV prostatitis on free and free-to-total PSA. Eur Urol 2004;46(6):760–4.

Swatowski D, Jakiel G. The influence of asymptomatic leukocytospermia on interleukin IL-2 and IL-6 levels in male semen plasma. Ginekol Pol 2002;73(10):841–4. Polish.

Tan JK, Png DJ, Liew LC, Li MK, Wong ML. Prevalence of prostatitis-like symptoms in Singapore: a population-based study. Singapore Med J 2002;43(4):189–93.

Tanner MA, Shoskes D, Shahed A, Pace NR. Prevalence of corynebacterial 16S rRNA sequences in patients with bacterial and "nonbacterial" prostatitis. J Clin Microbiol 1999;37(6):1863–70.

Trum JW, Mol BWJ, Pannekoek Y, Spanjaard L. *et al*. Value of detecting leukocytospermia in the diagnosis of genital infection in subfertile men. Fertil Steril 1998;70:315–319.

Tsai YC, Harrison HH, Lee C, Daufeld T, Oliver L, Grayhack JT. Systematic characterization of human prostatic fluid proteins with 2-dimensional electrophoresis. Clin Chem 1984;30:2026–2030.

Türk S, Korrovits P, Punab M, Mändar R. Coryneform bacteria in semen of chronic prostatitis patients. Int J Androl 2007;30(2):123–8. Epub 2006 Nov 29.

Wagenlehner FM, Naber KG. Prostatitis: the role of antibiotic treatment. World J Urol 2003;21(2):105–8. Epub 2003 Apr 10. Review.

Walsh PC, Retik AB, Stamey TA, Vaughan ED, eds. Campbell's Urology, 6th edition, Vol. 1. Philadelphia: W. B. Saunders Company; 1992.

Wang MC, Valenzuela LA, Murphy GP, Chu TM. Purification of a human prostate specific antigen. Invest Urol 1979;17:159–163.

Weidner W, Madsen PO, Schiefer HG (eds.). Prostatitis – etiopathology, diagnosis and therapy. Berlin: Springer-Verlag; 1994.

Weidner W, Schiefer HG, Krauss H, Jantos C, Friedrich HJ, Altmannsberger M. Chronic prostatitis: a thorough search for etiologically involved microorganisms in 1,461 patients. Infection 1991;19 Suppl 3:119–25.

Wenninger K, Heiman JR, Rothman I, et al. Sickness impact of chronic nonbacterial prostatitis and its correlates. J Urol 1996;155:965–968.

Wolff H. The biologic significance of white blood cells in semen. Fertil Steril 1995;63(6):1143–57. Review.

World Health Organization. Laboratory manual for examination of human semen and sperm-cervical mucus interaction, 4th ed. New York: Cambridge University Press; 1999.
World Health Organization manual for the standardized investigation, diagnosis and management of the infertile male, 1st ed. Bath: Cambridge University Press; 2000.
Wright ET, Chmiel JS, Grayhack JT, et al. Prostatic fluid inflammation in prostatitis. J Urol 1994;152(6 Pt 2):2300–3.

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SUMMARY IN ESTONIAN

Asümptomaatiline põletikuline prostatiit: levimus, etioloogilised tegurid, diagnostikavõimalused

Prostatiit e eesnäärmepõletik on kompleksne kliiniline seisund, mis võib mõjutada mehi mistahes eluetapil ning on sagedasim uroloogiline diagnoos alla 50-aastastel ja sageduselt kolmas diagnoos üle 50-aastastel meestel. On pakutud, et ligikaudu 50% meestest esineb prostatiidi sümptomeid vähemalt kord elus (McNaughton-Collins *et al*., 1998).

Hoolimata kõrgest levimusest on meie hetketeadmised kroonilisest prostatiidist ebapiisavad ning haiguse mainimisel viidatakse järjest sagedamini „prostatiidi sündroomile". Kuna etioloogia ja patogenees on suures osas teadmata, puuduvad ka ühtsed diagnostilised ja ravikriteeriumid. Üheks levinumaks kroonilise prostatiidi tekketeooriaks on olnud bakteriaalne infektsioon, samas on aga üle 80%-l kroonilise prostatiidiga patsientidest eesnäärmespetsiifilise materjali külvitulemused negatiivsed (Wolff 1995; Esfandiari *et al*., 2002; Cottell *et al*., 2000). Haiguse kliiniline pilt on mitmekesine, hõlmates nii asümptomaatilist põletikku kui ka tugevate väikevaagnavalude ja urineerimisvaevustega kulgevaid vorme, kusjuures vaevused võivad oluliselt mõjutada patsiendi elukvaliteeti. Lisaks pole prostatiidi kliinilised sümptomid sageli seotud laboratoorse leiuga, mistõttu raviefekti hindamine võib olla komplitseeritud. Teadmiste vähesust prostatiidi kohta peegeldab ka lai valik olemasolevaid ravivõimalusi, mh antibiootikumid, alfa-blokaatorid, mittesteroidsed põletikuvastased preparaadid, taimne ja dieetravi, eesnäärmemassaaž, termoteraapia jne. Samas on ravitulemus sageli puudulik või ajutine. Kõigi nende asjaolude tõttu on prostatiit olnud läbi aegade tõsiseks probleemis nii arstkonnale kui ka patsientidele.

Kõrgele levimusele ja ebapiisavatele teadmistele vaatamata on prostatiiti teiste oluliste eesnäärmehaigustega (eesnäärme healoomuline suurenemine ja eesnäärmevähk) võrreldes vähe uuritud. Uuringute arv on viimase kümnendi jooksul siiski suurenenud, seda paljuski tänu uuele prostatiidi klassifikatsioonile ja uutele diagnostilistele võimalustele (NIH-CPSI küsimustik, 2-klaasi test, sperma analüüs). NIH-CPSI küsimustik kui rahvusvaheliselt enim aktsepteeritud diagnostiline vahend ei olnud siiani eestikeelsena kasutamiseks valideeritud.

Uus prostatiidi klassifikatsioon (ametlikult kasutusel 1998. aastast) määratleb ühe prostatiidivormina ka asümptomaatilist põletikulist prostatiiti (NIH IV kategooria), mille puhul leitakse eesnäärmespetsiifilises materjalis (eesnäärme sekreet, sperma või eesnäärmebiopsia) olulisel määral leukotsüüte, samas kui subjektiivsed kaebused patsiendil puuduvad. Asümptomaatilise põletikulise prostatiidi etiopatogeneesi, levimuse ja kliinilise tähenduse kohta on siiani avaldatud vaid üksikuid teadusuuringuid.

Uurimistöö eesmärgid ja ülesanded

Uurimuse eesmärgiks oli kindlaks teha asümptomaatilise prostatiidi levimus Eestis, etioloogilised tegurid ning mõju diagnostilistele markeritele, samuti välja töötada ning valideerida NIH-CPSI (National Institutes of Health Chronic Prostatitis Symptom Index) eestikeelne versioon.

Uurimistöö täpsemad ülesanded:
1) Välja töötada NIH-CPSI küsimustiku eestikeelne versioon ja hinnata selle valiidsust nii rahvastiku- kui ka kliinikupõhises uuringus;
2) Teha kindlaks asümptomaatilise põletikulise prostatiidi levimus ja selle võimalikud erinevused aastaaegade kaupa Eesti noortel meestel;
3) Määrata kindlaks mikroorganismide võimalik roll asümptomaatilise põletikulise prostatiidi tekkes sperma mikrofloora kvantitatiivse analüüsi abil;
4) Selgitada asümptomaatilise põletikulise prostatiidi mõju PSA tasemele seerumis ja IL-6 kontsentratsioonile spermaplasmas, samuti nende markerite kombinatsiooni kasutatavust olulise leukotsütospermiaga meeste eristamisel leukotsütospermiata meestest;
5) Teha kindlaks asümptomaatilise põletikulise prostatiidi ja vanuse samaaegset mõju sperma põhiparameetritele.

Uuritav materjal ja meetodid

NIH-CPSI valideerimise uuringugrupi moodustasid 54 prostatiidile viitavate sümptomitega SA Tartu Ülikooli Kliinikumi Androloogiakeskusesse androloogi vastuvõtule pöördunud meest ning 420 kolme Tartu perearsti nimistu meest vanuses 20–59 aastat. Kõikides täidetud NIH-CPSI küsimustikes hinnati kaebuste esinemist vastavate kategooriate (valu, urineerimishäired, sümptomite mõju elukvaliteedile) kaupa.

Asümptomaatilise põletikulise prostatiidi etioloogilise uuringu grupi moodustasid: 1) 32 subjektiivsete kaebusteta meest (kontrollgrupp), kes pöördusid SA TÜK Androloogiakeskusesse vastuvõtule paari lastetuse tõttu või profülaktiliste uuringute eesmärgil ning kelle spermaproovis esines alla 0,2 miljoni leukotsüüdi ml kohta; 2) 37 subjektiivsete kaebusteta meest (uuringugrupp), kes pöördusid SA TÜK Androloogiakeskusesse vastuvõtule samuti paari lastetuse tõttu või profülaktiliste uuringute eesmärgil ning kelle spermaproovis esines enam kui 0,2 miljonit leukotsüüti ml kohta.

Asümptomaatilise põletikulise prostatiidi levimust uuriti 565 noorel mehel (keskmine vanus 18,9 ± 1,8 aastat), kes võtsid osa rahvusvahelisest prospektiivsest uuringust Environment and Reproductive Health (EU 6th FP project QLRT-2001-02911), mille eesmärgiks on võrrelda kompleksselt Põhjamaade noormeeste viljakusnäitajaid (munandimaht, sperma põhiparameetrid, vere suguhormoonide sisaldus jm). Kõik uuritavad läbisid androloogilise läbivaatuse

ja küsitluse ning 7 noort meest kõrvaldati uuringugrupist genitaalpiirkonna kaebuste/patoloogia tõttu. Uuringugruppi jäänud 558 meest olid subjektiivsete kaebusteta, eesti päritolu ning vastasid NIH töögrupi nõuetele kroonilise prostatiidi uuringunõuete kohta (Executive Summary 1995; Bethesda, MD, USA). Ükski uuritavatest ei olnud tarvitanud viimase 3 kuu jooksul antibiootikume.

Kõik uuritavad allkirjastasid patsiendi informeeritud nõusolekuvormi, mis oli eelnevalt kooskõlastatud Tartu Ülikooli Inimuuringute Eetikakomiteega.

NIH-CPSI küsimustik tõlgiti eesti keelde kahe sõltumatu versioonina, võrreldi omavahel ning kohandati vastavalt kahe tõlke vahelistele erinevustele ja silmas pidades lihtsamat keelekasutust ning paremat arusaadavust. Lõplikku versiooni testiti 137-l uuritaval, prostatiidile viitavate sümptomite positiivseks kriteeriumiks seati kaks prostatiidile enim spetsiifilisemat sümptomit (perineaalne ja/või ejakulatsioonivalu) ning valuskoor väärtuses 4 punkti või enam (21-st).

Sperma analüüsid teostati vastavalt WHO juhendile (WHO, 1999). Kõik analüüsid viidi läbi SA TÜK Androloogiakeskuses vastavalt rahvusvahelistele standarditele, määrates proovi kaalu grammides, spermatosoidide kontsentratsiooni miljonites milliliitri kohta, koguhulga (kontsentratsiooni ja spermamahu korrutisena) liikuvuse protsentides ning morfoloogilised näitajad vastavalt rahvusvaheliselt aktsepteeritud kriteeriumidele (Menkveld *et al*. 1990; Kvist *et al*., 2002). Asümptomaatilise prostatiidi diagnoosimiseks teostati kõikidest spermaproovidest ka tüstoloogiline uuring Bryan-Leishmani järgi, määrates põletikurakkude (polümorfonukleaaride) arvu 1 ml seemnevedeliku kohta. Lisaks määrati osade uuritavate spermas interleukiin-6 ja PSA sisaldus.

Asümptomaatilise põletikulise prostatiidi etioloogilises uuringus kasutati kvantitatiivseid mikrobioloogilisi meetodeid, et tuvastada anaeroobsete, mikroaerofiilsete ja aeroobsete mikroobide sisaldus spermaproovides.

Andmete statistiliseks töötlemiseks rakendati tarkvaraprogramme Sigma Stat (Jandel Scientific), Excel (Microsoft Corp, Redmond, OR, USA) ja R (R Foundation for Statistical Computing). Kõikide parameetrite statistilist olulisust hinnati tasemel $p<0,05$.

Uurimistöö tulemused ja järeldused

1) Eestikeelne NIH-CPSI versioon eristab prostatiidile omaste sümptomitega patsiente prostatiidikaebusteta patsientidest nii rahvastikupõhises kui ka kliinilises uuringus. Seetõttu on eestikeelne NIH-CPSI sobiv diagnostiline vahend kasutamiseks nii sümptomaatilise ja asümptomaatilise prostatiidiga patsientidel subjektiivse seisundi hindamiseks kui ka kroonilise prostatiidi epidemioloogiliste uuringute läbiviimiseks Eestis;
2) Asümptomaatilise põletikulise prostatiidi levimus Eesti noortel meestel on 6,0% (WHO standardite järgi, s.o >1 milj PMN/ml sperma kohta), samas on madalama läviväärtuse (0,2 milj PMN/l; Punab *et al*., 2003) rakendamisel sama põletiku levimuseks 19,0%. Asümptomaatilise põletikuvormi sage

esinemine ja sümptomaatilise vormi harvaesinemine selles vanuserühmas viitab vajadusele selgitada edasiste uuringute käigus prostatiidikaebuste tekkedünaamikat sõltuvalt patsiendi vanusest ning teha kindlaks võimalikud patogeneetilised seosed prostatiidi sümptomaatiliste ja asümptomaatiliste vormide vahel. Asümptomaatilise põletikulise prostatiidi levimus ei olene (erinevalt sümptomaatiliste prostatiidivormide puhul täheldatust) aasta-aegedest, mis viitab sellele, et klimaatiline keskkond mõjutab pigem prostatiidi sümptomeid kui laboratoorseid näitajaid;

3) Asümptomaatilise põletikulise prostatiidi etioloogias on tõenäoliselt oluline roll infektsioossel komponendil, kuna nende patsientide spermaproovides esineb kõrge kontsentratsiooniga polümikroobne mikrobiotsönoos, mis koosneb nii aeroobsetest kui ka anaeroobsetest bakteritest. Sarnane leid on iseloomulik ka krooonilisele mittebakteriaalsele prostatiidile (CP/CPPS), viidates nende kahe vormi etiopatogeneetilisele sarnasusele;
4) Asümptomaatilise põletikulise prostatiidiga patsientidel on PSA tase seerumis ja IL-6 kontsentratsioon spermaplasmas oluliselt kõrgemad kui kontrolluuritavatel. Analoogseid tendentse on täheldatud varasemates uuringutes sümptomaatilise prostatiidiga patsientidel, mis samuti viitab sarnasustele asümptomaatilise ja sümptomaatilise prostatiidi vahel. Nende kahe markeri kombinatsioonil on potentsiaali kasutamiseks olulise leukotsütospermiaga meeste eristamisel leukotsütospermiata meestest;
5) Mõõdukas leukotsütospermia (0,2–1,0 milj PMN/ml) mõjutab asümptomaatilise põletikulise prostatiidi tingimustes positiivselt sperma kontsentratsiooni ja spermatosoidide koguhulka sõltumata uuritava vanusest (võrreldud keskmised vanused 18,9 ja 26,4 eluaastat), samas kui tugev leukotsütospermia (>1,0 milj PMN/ml) mõjutab sperma kvaliteeti negatiivselt vaid vanemal vanuserühmal. Samuti täheldasime, et noorematel uuritavatel oli sperma kvaliteet parem kui vanematel uuritavatel, seda sõltumata leukotsütospermia tasemest. Eelmainitu põhjal võib oletada, et genitaaltrakti lisasugunäärmete funktsionaalne reserv on noorematel meestel põletiku olemasolul suurem.

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PUBLICATIONS

I

Korrovits P, Punab M, Mehik A, Mändar R. The Estonian version of the National Institutes of Health chronic prostatitis symptom index. Andrologia. 2006 Jun;38(3):106–9.

II

Korrovits P, Punab M, Türk S, Mändar R.
Seminal microflora in asymptomatic inflammatory
(NIH IV category) prostatitis.
Eur Urol. 2006 Dec;50(6):1338–44;
discussion 1344–6. Epub 2006 May 23.

III

Korrovits P, Ausmees K, Mändar R, Punab M.
Prevalence of asymptomatic inflammatory
(National Institutes of Health Category IV)
prostatitis in young men according to semen analysis.
Urology. 2008;71(6):1010–5. Epub 2008 May 2.

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Teadustöö

Peamised uurimisvaldkonnad:

- asümptomaatilise põletikulise prostatiidi etioloogilised tegurid, levimus ja diagnostikavõimalused
- kroonilise prostatiidi epidemioloogilised tegurid
- meeste vananemine

Avaldanud 7 teaduspublikatsiooni (tsiteeritud CC ja Medline rahvusvahelistes andmebaasides) ja 12 konverentsiettekannet.

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