Prostatic Diseases and Male Voiding Dysfunction

Seminal Interleukin-6 and Serum Prostate-specific Antigen as Possible Predictive Biomarkers in Asymptomatic Inflammatory Prostatitis

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OBJECTIVES
To determine the possible predictive values of seminal interleukin-6 (IL-6) and serum prostate-specific antigen (PSA), as well as their combined values, in differentiating between subjects with or without asymptomatic inflammatory prostatitis.

METHODS
The study group consisted of 490 men (mean age 18.9 ± 1.8 years, range 16-25). Cytologic examination of all ejaculates (using Bryan-Leishman-stained slides) and clinical examination for possible pathologic findings in the genital region were performed. The subjects with any clinical symptoms of inflammation were excluded. The levels of PSA in the blood serum and IL-6 in the seminal plasma were also determined. The IL-6 and PSA levels for different leukocytospermia status were statistically compared, and receiver operating characteristic curves were designed to determine the sensitivity versus specificity and the positive and negative predictive values of IL-6 and PSA levels against different thresholds of leukocytospermia (0.2, 0.5, and >1.0 × 10⁶ leukocytes/mL).

RESULTS
The levels of both IL-6 in the seminal plasma and PSA in the blood serum were significantly greater in National Institutes of Health prostatitis IV than in the controls. The receiver operating characteristic curves for seminal IL-6 and serum PSA showed high negative prognostic values for all 3 leukocytospermic subgroups, and positive prognostic values were seen only with IL-6 in the lower leukocytospermic range.

CONCLUSIONS
Both seminal IL-6 and serum PSA are excellent negative predictive markers for asymptomatic inflammatory prostatitis in young men, although positive predictive values of these biomarkers remain less indicative in this age group. UROLOGY 78: 442–446, 2011. © 2011 Elsevier Inc.

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symptomatic inflammatory (National Institutes of Health [NIH] group IV) prostatitis (AIP) is defined as the presence of significant amount of leukocytes in prostate-specific samples (expressed prostatic secretion, semen, and postprostatic massage urine), possible bacterial component, and the lack of subjective symptoms.¹ Because the current mainstay of diagnostic options does not provide enough insight into the etiology and pathogenesis of the prostatitis syndromes, there is a constant search for new diagnostic options.

Recently, increasing evidence has shown a role of proinflammatory cytokines in prostatitis.² These mediators (especially interleukin [IL]-6 and IL-8) are associated with seminal leukocytes³,⁴ and might therefore serve as additional inflammatory markers in the diagnostic workup of inflammatory prostatitis.

Although elevated prostate-specific antigen (PSA) levels in the serum is considered a marker of prostate cancer, several recent studies have indicated its association with various forms of chronic prostatitis, including AIP.⁵,⁶ Asymptomatic prostatitis has been found to have effects on total and free PSA levels in serum similar to those in prostate cancer,⁷ and it has been noted that the PSA levels in men with AIP correlate positively with the aggressiveness of the inflammation.⁸
The aim of our study was to determine the possible predictive values of seminal IL-6 and serum PSA (as well as their combined values) in differentiating between subjects with or without AIP.

**MATERIAL AND METHODS**

**Study Group**
The study was performed at the Andrology Centre of Tartu University Hospital from May 2003 to June 2004 and included 565 young men (mean age 18.9 ± 1.8 years, range 16-25) who participated in a prospective study Environment and Reproductive Health (European Union Sixth Framework Programme project QLRT-2001-02911). The principles of the study group formation have been described previously. All subjects were examined for possible pathologic findings in the genital region, and 7 men were excluded from additional analysis because of clinical symptoms of genital inflammation. Additionally, in several cases, ≥1 of the study parameters could not be measured for technical reasons; therefore, the final number of eligible study subjects was 490; all men were born and living in Estonia. None of these men had any complaints of chronic pelvic pain or discomfort. The exclusion criteria were stated according to the suggestions of the National Institutes of Health Workshop on Chronic Prostatitis (Bethesda, Maryland) in 1995. None of the men had received antimicrobial therapy within the previous 3 months.

**Samples**
The semen samples were collected by the patients after washing 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 25-45 minutes for liquefaction. Blood samples were obtained by venipuncture, and the serum was obtained by centrifugation at 3000g for 5 minutes and was analyzed within 2 hours.

**Cytologic Analysis**
Semen smears were made to detect the white blood cells (WBCs). The smears were air-dried, Bryan-Leishman stained, and examined using oil immersion microscopy (magnification ×1000) by an experienced microscopist. The WBC concentration in the semen was calculated using the known sperm concentration (as 106/mL) according to the following formula: (number of WBCs counted/number of sperm counted) × semen sperm concentration. A total of 100 nonsperm round cells was counted, and the number of detected WBCs and the number of spermatogenic cells was recorded. The cutoff points for detecting leukocytospermia were selected according to the results of our previous study as 0.2 × 106 WBCs/mL and according to the World Health Organization guidelines as 1.0 × 106 WBCs/mL. For receiver operating characteristic (ROC) curve analysis, an additional cutoff point of 0.5 × 106 WBC/mL was applied.

**Detection of PSA**
The PSA levels in the blood serum (0.5 mL of serum required for the assay) were quantitatively measured using chemiluminescence (DPC IMMULITE 2000, Siemens Medical Solutions, CA), according to the manufacturer’s instructions.

**Detection of IL-6**
The IL-6 levels of seminal plasma (100 μL of specimen required for the assay) were measured using the Immulite automated chemiluminescence immunoassay analyzer (DPC IMMULITE 2000), according to the manufacturer’s instructions.

**Statistical Analysis**
Statistical analyses were performed using SigmaStat (Jandel Scientific, Corte Madera, CA), Excel (Microsoft, Redmond, WA), and R (R Foundation for Statistical Computing) software programs. The IL-6 and PSA levels for different leukocytospermia status were compared using the Kruskal-Wallis test. The group or groups that differed from the others were isolated using a multiple comparison procedure (Dunn’s method). The ROC curves were designed using R software to determine the sensitivity versus specificity and the positive predictive value (PPV) and negative predictive value (NPV) of IL-6 and PSA levels against different thresholds of leukocytospermia (0.2, 0.5, and >1.0 × 106 WBCs/mL).

Statistical significance was assumed at P < .05 for all parameters.

**Ethical Considerations**
Participation in the present study was voluntary. All study subjects provided informed consent. The Ethics Review Committee on Human Research of the University of Tartu approved the present study.

**RESULTS**
The levels of both IL-6 in the seminal plasma and PSA in the blood serum were significantly greater in NIH IV category prostatitis than in the controls (Table 1). The ROC curves for seminal IL-6 (Fig. 1) showed high area under curve (AUC) values (0.81-0.84), excellent

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**Table 1.** IL-6 and PSA levels stratified by white blood cell count in semen

<table>
<thead>
<tr>
<th>Variable</th>
<th>No LCS (0-0.2 × 10⁶ WBCs/mL, n = 396)</th>
<th>Moderate LCS (0.2-1 × 10⁶ WBCs/mL, n = 64)</th>
<th>Severe LCS (&gt;1 × 10⁶ WBCs/mL, n = 30)</th>
<th>Total (n = 490)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA (μg/L) in blood serum</td>
<td>0.50 (0.07-3.20)††</td>
<td>0.61 (0.15-3.73)*</td>
<td>0.73 (0.18-4.19)††</td>
<td>0.52 (0.07-4.19)</td>
</tr>
<tr>
<td>IL-6 (ng/L) in seminal plasma</td>
<td>19.8 (2.0-144.0)††</td>
<td>42.2 (10.1-311.0)††</td>
<td>69.3 (10.2-1475.0)††</td>
<td>21.4 (2.0-1475.0)</td>
</tr>
</tbody>
</table>

IL-6, interleukin-6; PSA, prostate-specific antigen; LCS = leukocytospermia; WBC, white blood cells.

* P = .004; Kruskal-Wallis test.
† P < .05; Dunn’s method.
‡ P < .001; Kruskal-Wallis test.

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NPVs and good sensitivity and specificity for all 3 leukocytospermic subgroups, as well as an increasing PPV of the test in the case of the lower cutoff values for leukocytospermia. The optimal cutoff values for IL-6 in different subgroups ranged from 31 to 43 ng/L.

The ROC curves for serum PSA (Fig. 2) showed the greatest AUC value in the subjects with severe leukocytospermia (AUC 0.65), with the most optimal PSA cutoff point at 0.52 μg/L and high NPVs for all 3 leukocytospermic subgroups (85.7%-97.2%). However, the PPVs of PSA in the different subgroups were low (9.5%-29.0%).

Subsequently, combined ROC curves of seminal IL-6 and serum PSA versus leukocytospermic status were constructed using a logistic regression model (Fig. 3), with AUC values (0.82-0.84), sensitivity, specificity, and positive and NPVs similar to those for IL-6 alone.

**COMMENT**

In our study, we found that both seminal IL-6 and serum PSA had a high NPV in all 3 study groups, with greatest prediction observed in severe leukocytospermia. The PPVs of both markers were greatest in lower leukocytospermic range, although the extent of the positive prediction was not comparable to that of the negative (29.1%-50.4% vs 92.5%-97.9% for IL-6, respectively).

The combined predictive model of IL-6 and PSA did not yield significant added value to the PPV and NPV of IL-6 alone, although the PPV of the combined model was slightly better for the lower range of leukocytospermia. The possible reason for the former might have been the low PPV of PSA in our study group, which, in turn, reflected the relatively low variability of PSA values in this age group (mean age 18.9 ± 1.8 years in our study) and might be the most important limitation of our study. Whether the PPV of PSA (and the combined predictive value of IL-6 and PSA) would be greater in older age groups in which the PSA values are more diverse, is a matter for future studies.

Very few studies have been done on interleukins and PSA as predictive biomarkers in prostate inflammation. Penna et al.14 in 2007, studied both IL-6 and IL-8 in patients with the NIH prostatitis subtypes IIIA and IIIB and concluded that IL-8 has the capacity to discriminate between these 2 types. Additionally, they found a significant correlation between seminal IL-8
and the serum PSA level. However, IL-6 was also found to be significantly greater in those patients, with lower discriminative capacity than that of IL-8. Data on the NPVs of these biomarkers was not presented in their study.

Similar results for seminal IL-6 and IL-8 in chronic prostatitis/chronic pelvic pain syndrome have also been reported in some earlier studies. To the best of our knowledge, only 1 study evaluating the role of cytokines in prostate inflammation included patients with asymptomatic inflammatory prostatitis. They found that IL-8 was significantly greater in patients with NIH IIIA and NIH IV (asymptomatic) prostatitis compared with controls. However, that study used expressed prostatic secretions and not seminal plasma as the study specimen.

Stancik et al in 2004 investigated the serum PSA concentrations in patients with NIH IV prostatitis and found that asymptomatic prostatitis had effects on serum PSA similar to those with prostate cancer. A study by Nadler et al in 2006 found only a slight increase in the serum PSA levels in patients with chronic prostatitis/chronic pelvic pain syndrome compared with controls. However, as the investigators themselves stated, their study population was a highly screened group of patients with chronic prostatitis/chronic pelvic pain syndrome, not reflecting the situation in the general population. Although very few studies have been done on the relationship of serum PSA and prostate inflammation and even those results have been contradictory, it has been admitted that considerable evidence has shown that prostatitis can be a significant confounder to the intelligent use of the PSA level for prostate cancer screening.

In the present study, we investigated seminal IL-6 and serum PSA as possible predictive biomarkers for AIP, mainly for 2 reasons. First, AIP cannot be diagnosed clinically, and, because its only laboratory diagnostic criteria is the prevalence of significant numbers of WBCs in the semen and/or expressed prostatic secretions, additional diagnostic biomarkers would be helpful for diagnosing and better understanding the AIP pathogenesis.

Second, cytologic examination of WBCs in ejaculate/prostatic secretions is a subjective method that could be prone to inter- and intraobserver variabilities, as opposed to automated detection of both seminal IL-6 and serum PSA.

CONCLUSIONS
Both seminal IL-6 and serum PSA are excellent negative predictive markers for asymptomatic inflammatory prostatitis in young men, but the PPVs of PSA and IL-6 remain less indicative. We emphasize that these results are applicable only to this age group and any such correlations for older age cohorts should be studied separately.

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References


