Prostate specific antigen (PSA) blood test represents the standard procedure in prostate cancer (CaP) diagnosis and follow-up. However, determination of PSA in the urine, where PSA is present in much higher concentrations than in the blood, still remains in the field of research.

Objectives: To determine urinary concentrations of PSA (uPSA) in different groups of patients (pts.), and to estimate is it possible to differentiate benign and malignant prostate diseases and to follow-up the results of treatment.

Methods: Between January 2001 and November 2003, urinary concentrations of PSA were determined at 142 pts. divided in seven groups: 1. young and healthy volunteers, 2. "BPH-24": pts. with benign prostatic hyperplasia (BPH) who collected the sample of 24-hour voided urine, 3. "BPH-I": pts. with BPH who collected the first portion of first urinary voiding, 4. "TRUS-CaP": pts. with CaP which gave the first portion of urine just prior to transrectal ultrasound-guided prostate biopsy (TRUS-biopsy), 5. "TRUS-non-CaP": pts. who gave first portion of urine prior to TRUS-biopsy, but biopsy did not prove the presence of CaP, 6. "RRP": pts. who underwent radical retropubic prostatectomy (RRP), 7. "AAT": pts. who underwent androgen deprivation therapy.

Results: Average uPSA value in the group of young and healthy volunteers, was 13.8±19.6 ng/ml, in "BPH-24": 38.0±44.4 ng/ml, in "BPH-I": 140.8±140.9 ng/ml, in "TRUS-CaP": 234.8±277.7 ng/ml, in TRUS-non-CaP: 113.1±148.5 ng/ml, and in the group "RRP": 4.4±4.7 ng/ml. There was no statistically significant difference of average uPSA values between "BPH-I" and "TRUS-CaP" groups. The significant difference was found between the group of young volunteers and "BPH-I". In "TRUS-CaP" group, there was strong correlation between tumour size and aggressiveness and uPSA concentration. Finally, PSA and uPSA decline during androgen deprivation therapy, strongly correlated (up to r=0.95).

Conclusions: Determination of uPSA cannot differentiate BPH and CaP. However, in the group of pts. with proven localized CaP, uPSA can provide additional information concerning T-staging. Moreover, simultaneous monitoring of PSA and uPSA response on hormonal therapy, can provide an early recognition of androgen-in indifferent CaP (AIPCA) and hormone-resistant CaP (HRPCA).

Key words: urinary prostate specific antigen, tumor volume antiandrogen therapy.

INTRODUCTION

Prostate specific antigen (PSA) is the proteolitical enzyme which physiological function is liquefaction of seminal coagulum, the process that allows the progressive motility of spermatozoa. The PSA concentration in seminal plasma is 0.3-3.0 mg/ml, which makes PSA one of the most abundant proteins synthesised in the prostate. The determination of PSA concentrations in blood is one of the most important tools in prostate diseases detection and follow-up. On the other hand, PSA measurement in urine took place in the sporadic clinical researches.

1.1. PSA in the prostate

The concentration of PSA in the prostate is similar in normal, hyperplastic and malignant tissue and counts 0.01-0.08 mg/ml tissue. That means that prostate of 20g contains 0.2-1.6mg PSA.

Major form of PSA in the prostatic tissue is free PSA, that makes more than 98% of total PSA. There is no apparent physiological function of PSA in the prostatic tissue: after the synthesis in the cytosol, PSA molecules are preserved in secretory granules (SG). Prostatic secretory epithelial cells empty the contents of SG into the acinar and ductal lumina. The amount of the synthesis depend on androgens and sexual stimulation, which is under parasympathetical control. From prostatic ducts, PSA molecules arrive in the prostatic urethra, from where are been washed during the micturition. Different to this "basal
PSA leakage, massive acinar and ductal emptying take place during the ejaculation. Central and transition zone ducts enter the prostatic urethra in the sharp angle, lateral to the verumontanum. Peripheral zone ducts enter the urethra perpendicularly, along the distance between verumontanum and distal urethral sphincter. These differences play an important role in the drainage of seminal fluid into the urethra.

1.2. PSA in the periurethral glands and "minor prostatic glands"

The use of new fixative in the pathology, glutaraldehyde, enabled the identification of prostatic secretory granules (SG) in epithelial cells out of the prostate. Prostatic SG were identified in all periurethral glands in penile urethra, and occasionally, in the urinary bladder, in more than 50% cases of cystitis cystica and glandularis. These "minor prostatic glands" are made of prostatic cells, or mixed prostatic and mucinous epithelium. Minor prostatic glands are responsible for urethral PSA production after radical prostatectomy and cystoprostatectomy.

1.3. PSA in seminal plasma

The concentration of PSA in seminal plasma is 0.2-3 mg/ml; PSA concentration in expressate, after prostatic massage, is 1-2mg/ml. The ratio between free and total PSA (f/T) is 0.76-0.81, and is similar in healthy men, and the patients with CaP. High concentration of active PSA forms is the result of low concentrations of extracellular protease inhibitors in seminal plasma, like protein-C-inhibitor (PCI), which has only 5-10% molar concentration of PSA.

1.4. Urinary PSA determination in clinical use

In one of the first reports about uPSA, in 1992, DeVere White found average uPSA concentration in the group of BPH patients, of 216 ng/ml. Moreover, he found elevated concentrations of uPSA in 77% patients after RRP, and concluded that the majority of patients after RRP has residual prostatic tissue. However, next year, from Stanford University came the evidence that PSA in the urine originates from periurethral glands, but not from residual prostatic tissue. In this issue, the authors found average uPSA in the group with localized CaP, of 915.1ng/ml in the first urinary stream, and 245.9 ng/ml in the middle stream. In the group with RRP, average uPSA was 21.4 ng/mL in the first stream, and 1.8 ng/mL in the middle stream. Similar uPSA levels were found in patients without CaP, after cystoprostatectomy with bladder substitution: 15.5 ng/ml and 1.2 ng/ml. Similar results were presented by Taka-yama. Breul found higher uPSA concentrations in BPH, than in CaP group, using Tandem-E test: 748.78±246.3 ng/ml, versus 214.57±121.2 ng/ml. The serum/urinary PSA ratio was 177±28.17, versus 15.76±5.47.

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Irani collected the 24 hour urinary samples and expressed uPSA concentration in micrograms / mMol of urinary creatinine; he also found higher uPSA concentration in BPH, than in CaP group: 30.7±4.7 microg. / mMol creat., versus 4.6±1.6 microg. / mMol creat. In the group of patients with serum PSA in "grey zone" (4-10 ng/ml) he found average uPSA of 18.5 microg. / mMol creat. In BPH patients, and 5.2 microg. / mMol creat. In CaP patients. These results differed significantly. In the recent issue, the same author found significantly different
PSA / PSA ratio in BPH and CaP group, in 12-hour collected urine: 4.2 versus 1.2. All patients had "grey zone" PSA.

Hillenbrand found similar results and concluded that uPSA increased total specificity of PSA in the screening of CaP. However, authors from Johns Hopkins Institute reported different results, with no statistical differences in total, free, and ACT- bound uPSA between BPH and CaP group.

The concentration of PSA could be measured in the prostatic expressate, as well. Kim found different PSA concentration in expressate from BPH and CaP patients: 1.42 mg/ml and 2.25 mg/ml, respectively. Some authors found elevated PSA in expressate in the group of patients with residual disease, after RRP.

Very interesting fact is that female-to-male transsexuals could maintain PSA synthesis after testosterone substitution. The glands of Skene, or "female prostate" are responsible for urinary PSA concentrations, which are comparable with that of young and healthy males.

METHODS

1. Between January 2001, and November 2003., urinary concentrations of PSA were determined at 142 patients divided in seven groups: 1. young and healthy volunteers, 2. "BPH-24" group: patients with benign prostatic hyperplasia (BPH) who collected the sample of 24-hour voided urine, 3. "BPH-I" group: patients with BPH who collected the first portion of first urinary voiding, 4. "TRUS-CaP" group: patients with CaP which gave the first portion of urine just prior to transrectal ultrasound- guided prostate biopsy (TRUS-biopsy), 5. "TRUS-non-CaP" group: patients who gave first portion of urine prior to TRUS-biopsy, but biopsy did not prove the presence of CaP, 6. "RRP" group: patients who underwent radical retropubic prostatectomy (RRP), 7. "AAT" group: patients who underwent anti-androgen, or androgen deprivation therapy. (Table 1).

All patients were diagnosed and controlled in the Institute for Urology and Nephrology, Clinical Centre of Serbia, in Belgrade. Control group consisted of young and healthy volunteers. Patients with BPH had minimal voiding problems, or received medical therapy for BPH; all patients had PSA 4.0 ng/ml and no suspect lesions on TRUS and digitorectal examination (DRE). In "TRUS-CaP" and TRUS-non-CaP groups, TRUS- guided biopsy was performed due to PSA 4.0 ng/ml, and/or suspect findings on TRUS/DRE. These patients gave first 70-80 ml of fresh urine just prior to biopsy. Urine was kept on – 4 C. Ultrasound examinations and TRUS- biopsies were performed on Toshiba- Tosbee machine, with transrectal probe IVE-506S. Prostate volume was calculated on usual manner: a x b x c x pi/6. Biopsy was performed with needles of 160mm/16G, using Gallini biopsy gun. Pathohistological preparations were performed in Institute for Pathology, Clinical Centre of Serbia, Belgrade. All PSA concentrations could be measured in the prostatic expressate, as well. Kim found different PSA concentration in expressate from BPH and CaP patients: 1.42 mg/ml and 2.25 mg/ml, respectively. Some authors found elevated PSA in expressate in the group of patients with residual disease, after RRP.

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### TABLE 2

<table>
<thead>
<tr>
<th>No</th>
<th>Group</th>
<th>uPSA (ng/ml)</th>
<th>PSA (ng/ml)</th>
<th>Prostate volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Young/healthy</td>
<td>13.8±19.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>BHP-24</td>
<td>38.0±44.4</td>
<td>1.9±1.1</td>
<td>37.8±19.3</td>
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<td>3</td>
<td>BHP-I</td>
<td>140.8±140.9</td>
<td>1.7±0.8</td>
<td>32.8±21.5</td>
</tr>
<tr>
<td>4</td>
<td>TRUS-CaP</td>
<td>234.8±277.7</td>
<td>40.3±118.6</td>
<td>35.5±11.4</td>
</tr>
<tr>
<td>5</td>
<td>TRUS-non-CaP</td>
<td>113.1±148.5</td>
<td>8.9±4.1</td>
<td>42.7±15.6</td>
</tr>
<tr>
<td>6</td>
<td>RRP</td>
<td>4.4±4.7</td>
<td>0.14±0.2</td>
<td>30.3±18.6</td>
</tr>
</tbody>
</table>

### TABLE 3

<table>
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<tr>
<th>Group I</th>
<th>Group II</th>
<th>Statistical difference</th>
</tr>
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<tbody>
<tr>
<td>BPH-I</td>
<td>young</td>
<td>+</td>
</tr>
<tr>
<td>BPH-I</td>
<td>BPH-24</td>
<td>+</td>
</tr>
<tr>
<td>Young</td>
<td>RRP</td>
<td>+</td>
</tr>
<tr>
<td>TRUS-CaP</td>
<td>TRUS-non-CaP</td>
<td>+</td>
</tr>
<tr>
<td>TRUS-CaP</td>
<td>BPH-I</td>
<td>-</td>
</tr>
<tr>
<td>BPH-I</td>
<td>TRUS-non-CaP</td>
<td>-</td>
</tr>
</tbody>
</table>
concentrations in serum and urine were determined using IRMA-PSA test, produced in Institute for Nuclear Energy Application (INEP), Zemun, Belgrade.

RESULTS

3.1. Average uPSA concentrations

Average uPSA in control group was 13.8 - 19.6 ng/ml, in "BPH-24", 38.0 - 44.4 ng/ml, in "BPH-I", 140.8 - 140.9 ng/ml, in "TRUS-CaP", 234.8 - 277.7 ng/ml, in "TRUS-non-CaP", 113.1 - 148.5 ng/ml, and in "RRP", 4.4 - 4.7 ng/ml. (Table 2,3)(Fig 1,2)

3.2. Average uPSA Density (uPSAD)

Table 4. Average uPSA Density (uPSAD).

Average uPSAD differed statistically between groups "TRUS-non-CaP" and "TRUS-CaP" (p > 0.05).

3.3. Correlations

3.3.1. Correlation between uPSA and prostate volume

There was a correlation between uPSA and prostate volume in the group TRUS-CaP, but not in other groups. (Table 6).

3.3.2. Correlation between uPSA level and the tumour size and aggressiveness

There was a correlation in group "TRUS-CaP", between uPSA level and tumour size and aggressiveness. Tumour size was estimated according to number of cancer-positive cores and the percentage of infiltration of the material. Tumour aggressiveness was estimated according to tumour grade and Gleason score.(Table 7, Fig 3).

3.3.3. The correlation between PSA and uPSA during antiandrogen therapy

There is a graduate decline in PSA concentration during antiandrogen therapy, followed by uPSA decline. In some cases the correlation is very strong, up to 0.95 (p 0.01). In other cases, PSA decline does not follow uPSA decline, pointing out different response on therapy. (Fig. 4,5).

DISCUSSION

Prostate specific antigen is today one of the most frequently used tumour markers in medicine. Together with its use in early cancer detection, i.e. screening of male population older than 50 years, PSA has a major role in preoperative staging, the choice of the therapy, and the monitoring of the therapy.

PSA molecules enter the systemic circulation through the rim capillaries around the acini. However, the physiological pathway of PSA is through the prostatic ducts and urethra, from where the PSA molecules are expelled within seminal fluid in the process of ejaculation. Between the ejaculations, prostatic fluid rich in PSA molecules, leaks through the ducts into prostatic urethra, in which is collected until first micturition.

So, the concentration of PSA in the urine is much higher than in serum, but more liable to different influences. Generally, urinary PSA concentration depend on synthesis in prostatic epithelial cells and the drainage through the ductal system. The strength of the synthesis depend on an-
due to PSA instability on room temperature due to bacterial action. Groups with BPH, CaP, and group "TRUS-non-CaP", in which the chronic prostatitis was common pathological finding, had similar uPSA levels which did not differ statistically. This is not completely clear, but, most probably, other factors influenced the results by compromising ductal drainage. In the "TRUS-CaP" group we found a correlation between tumour size and aggressiveness. The correlation was negative, i.e. with the increase of tumour size and dedifferentiation, uPSA levels were lower. This phenomenon is, most probably, the consequence of tumorous ductal obstruction.

In the group "AAT", there was extremely high correlation between PSA and uPSA levels during androgen deprivation, in various stages of the disease. As it can be postulated that uPSA reflects the function of the normal prostatic cells, that drain its content in the system of normal ducts, and serum PSA shows the activity of malignant cells, simultaneous monitoring of uPSA and PSA shows the degree of the malignant cells androgen dependance. So, strong correlation between uPSA and PSA respond means that malignant cell clones are still androgen-sensitive, bad correlation depicts the beginning of androgen indifference i.e. the moment for the modulation of therapy.

**CONCLUSION**

It is not possible to differentiate BPH and CaP only with urinary PSA, due to numerous factors that take place in benign and malignant prostate diseases. However, in the group of patients with CaP, uPSA levels have correlation with size and aggressiveness of the tumour and can provide additional information for T- staging, i.e. determination of local stage of the disease: very low uPSA means great tumour volume and local spread.

Finally, simultaneous monitoring of uPSA and PSA during androgen deprivation, enables early recognising of androgen-indifferent, and further on, hormone-resistant prostate cancer, and prompt change of therapy.

**SUMMARY**

Iako se daleko veće količine PSA mogu naći u urinu nego u serumu ispitanika, određivanje PSA u urinu (uPSA) do danas nije našlo kliničku primenu. Cilj ovog istraživanja bio je da se odredi nivo uPSA kod različitih grupa ispitanika i da se vidi da li je na osnovu njegove moguće razlikovati različite bolesti prostate i pratiti rezultate lečenja. Nivo PSA u urinu određen je kod 142 ispitanika, medju kojima su se nalazili zdravi ispitanici, bolesnici sa benignom hiperplazijom prostate (BPH) kao i bolesnici sa karcinomom prostate (CaP) u različitim kliničkim stadijumima. Prosečna vrednost uPSA kod mladih ljudi bila je 13,8 ng/ml, kod bolesnika sa BPH 140.8 ng/ml a kod bolesnika sa CaP 234,8 ng/ml. U grupi CaP je na osnovu kliničkog materijala nadalje korelacija izmedju veličine i agresivnosti tumora i nivoa uPSA. Najzad, kontinuirano praćenje nivoa uPSA u toku antiandrogene terapije predstavlja kontrolu za kretanje PSA u serumu i pruža korisne informacije u vezi androgen-senzitivnosti tumora.
REFERENCES


