Differential Display Code 3 (DD3/PCA3) in Prostate Cancer Diagnosis

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Abstract. *Background: Early diagnosis of prostate cancer (PCa) in an organ-confined stage following radical treatment is the only potential curative approach in PCa. Prostatic-specific antigen (PSA) is very helpful in early diagnosis, but the main disadvantage is that it has a low positive predictive value in the range of the grey zone of 2.5-10 ng/mL , which results in a high number of needless biopsies. For this reason, new tests with better parameters are needed. One promising test is that for differential display code 3 ($DD3^{PCA3}$), which is a prostate-specific non-coding mRNA that is highly overexpressed in prostate tumor cells. The aim of the present study was to evaluate the potential of $DD3^{PCA3}$ for mRNA in PCa diagnosis. Patients and Methods: A total of 186 patients were examined. In a group of patients with suspected PCa, one tissue specimen core was collected for testing $DD3^{PCA3}$ expression. According to the histological verification there were 100 patients with benign prostatic hyperplasia, 12 patients with prostatic intraepithelial neoplasia and 74 patients with PCa. The total RNA was isolated and $DD3^{PCA3}$ and PSA expressions were quantified using quantitative RT real-time PCR method. The $DD3^{PCA3}$/PSA mRNA ratio was determined for all groups. Results: It was found that the levels of the mRNA expression of $DD3^{PCA3}$ were significantly higher ($p<0.045$) in patients with PCa than in patients with benign prostatic hyperplasia. No statistically significant differences in levels of mRNA expression of $DD3^{PCA3}$ between patients with organ-confined and those with advanced or metastatic disease, nor according to Gleason score, were found. Conclusion: $DD3^{PCA3}$ appears to be a promising marker for early detection of PCa and also for differential diagnosis between patients with benign prostate hyperplasia and those with PCa.*

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Prostate cancer (PCa) is the second leading cause of cancer-related deaths in men in Western countries (1). PCa cases continue to rise as the life expectancy of the general population increases. In 2006, worldwide more than 670,000 men were diagnosed with prostate cancer. The highest rates are in the USA, Australia, New Zealand, Western and Northern Europe, whilst the lowest rates are in East and South Central Asia (2, 33). In the Czech Republic in 2005, 5,992 men were newly diagnosed with PCa and 1,839 died from this disease (3). Early detection of prostate cancer is today mainly based on the measurement of total serum prostate-specific antigen (tPSA) and, to a lesser extent, on digital rectal examination (DRE), with an aggressive biopsy policy (34).

A specific test for prostate cancer is not currently available. Serum prostate-specific antigen (PSA) levels have been widely used for diagnostic purposes for more than 25 years, but false-positive and false-negative results are commonplace. When a prostate biopsy is performed, PCa can be found regardless of the PSA result. Thompson *et al*. and Margreiter *et al*. have concluded that no specific PSA level can accurately separate men with PCa from those with only benign prostatic hyperplasia (BPH) (4, 5). In addition, a large population of men with false-positive serum PSA values (*i.e*. the PSA level is elevated for non-PCa reasons such as BPH) has now emerged. These men are at risk of developing clinically significant PCa as they age, but methods such as the PSA velocity and free PSA (fPSA) may not allow for effective treatment of these patients. Consequently, many men with negative biopsy findings undergo repeat biopsies to rule out PCa (6). By

lowering the threshold cut-off where biopsies are performed, the sensitivity of tPSA as a biochemical marker may exceed 90%, but the positive predictive value in the grey zone of 2.5-10 ng/ml drops to <25 % (7). As a result 4 out of 5 biopsies will show no cancer in the biopsy because of tPSA values in this range. The use of the tPSA derivatives, tPSA correlations to prostate volume and changes over time and sophisticated statistical models improve the specifity somewhat, but still do not solve the specificity problem (7).

A better understanding of the molecular mechanisms of PCa oncogenesis and improved analytical techniques such as differential display analysis have identified PCa-specific genes such as *PCGEM*-1 (prostate specific transcript 1 (non-protein coding)), *PDEF* (prostate-derived ets factor), *PSMA* (prostate-specific membrane antigen), prostase *NKX3.1* gene and differential display code *3-DD3^{PCA3}*, a gene closely and specifically associated with PCa, could be a marker of this disease. First described by Bussemakers and colleagues in 1999 (8), *DD3^{PCA3}* encodes a prostate-specific mRNA that is highly overexpressed in PCa tissue compared to benign prostatic tissue (9). The possible use of urinary PCA3 as a PCa marker was first suggested by de Kok *et al*. in 2002 (10). The potential value of urinary PCA3 mRNA testing has been demonstrated in first generation, semiquantitative research assays (9, 11, 12). On the basis of this preliminary evidence, Groskopf and *et al*. developed an investigative PCA3 urinary assay with the potential for generel use in clinical settings (13). This PCA3 assay has been shown to be quantitative, sensitive, and relatively quick and easy to use compared with the earlier versions (13).

DD3^{PCA3} mRNA expression has previously mainly been studied at the tissue level (10) and more recently in urine sediments (9, 12), where it has shown potential as a diagnostic tool leading to the development of a whole urine test with prospects for general clinical use (13). Only a few, and very limited studies of *DD3^{PCA3}* mRNA in circulating cells in the peripheral blood have been reported (14). The results of these studies are partially in conflict with the organ and cancer specificity of *DD3^{PCA3}* based on tissue studies. Clearly, careful design and validation of the methodologies suitable for circulating tumor cells as well as applying them to well-characterized clinical cohorts are needed.

Reverse transcription polymerase chain reaction (RT-PCR) is a very sensitive method for studying gene expression in circulating tumor cells as long as the many potential sources of variation are taken into account. For instance, the yield of RNA extraction varies significantly calling for a convenient procedure to correct for the efficiency of the extraction. Throughout the years, housekeeping genes have been the method of choice to normalize the results. Since it has become evident that the expression of housekeeping genes is not as constant as has been presumed, but is instead quite varied between different experimental conditions, more reliable methods to correct for differences in the RNA extraction yield have been suggested (15).

Table I. *Basic descriptive statistics for DD3^{PCA3} and PSA mRNA expression in PCa and BPH tissue.*

Marker	PCa N=74			BPH N=100			PCa *versus* BPH
	Min.	Median	Max.	Min.	Median	Max.	*p*-Value
Ct_DD3	0	23.163	31.600	0	25.955	32.085	<0.0455
Ct_PSA	0	24.190	33.800	0	28.39	35.085	Ns.
Ct_DD3_PSA	0	0.634	1.583	0	0.022	2.220	Ns.

Ns, Not significant.

Table II. *Basic descriptive statistics for DD3^{PCA3} and PSA mRNA expression in PCa and PIN tissue.*

Marker	PCa N=74			PIN N=12			PCa *versus* PIN
	Min.	Median	Max.	Min.	Median	Max.	*p*-Value
Ct_DD3	0	23.163	31.600	0	28.700	31.380	<0.0234
Ct_PSA	0	24.190	33.800	0	21.713	32.400	Ns.
Ct_DD3_PSA	0	0.634	1.583	0	1.054	1.328	Ns.

Ns, Not significant.

Patients and Methods

A total of 186 patients were examined and included in this prospective study between May 2006 and September 2008 and were referred to two Departments of Urology (the University Teaching Hospital in Plzen and of the First Medical Faculty of Charles University in Prague) for a prostate biopsy. All of them exhibited an elevated serum tPSA level and/or abnormal DRE. The median patient age was 66.5 years (range 48-85 years). The total PSA values ranged from 4.1 to 580 ng/ml. Twenty-four patients in our study group had an abnormal DRE; 145 patients underwent an initial prostate biopsy and 41 patients underwent repeat biopsies.

In the group of 186 patients with suspected PCa, we collected one tissue specimen core for the *DD3^{PCA3}* expression analysis along with the urine. In this work, only the tissue results are evaluated. According to the histological verification, there were 100 patients with BPH, 12 patients with prostatic intraepithelial neoplasia (PIN) and 74 patients with PCa.

Approval was obtained from the Institutional Ethics Committee and written informed consent from each patient. Patients who had had any transurethral manipulation, radiotherapy, were on hormonal therapy,

Table III. *Basic descriptive statistics for $DD3^{PCA3}$ and PSA mRNA expression with the PCa group according to TNM classification (early organ-confined disease versus locally advanced disease with or without metastasis).*

Marker	Organ-confined PCa N=39			Locally advanced or metastatic PCa N=25			Organ-confined PCa *vs.* locally advanced or metastatic PCa
	Min.	Median	Max.	Min.	Median	Max.	*p*-Value
Ct_DD3	0	23.163	31.600	0	22.688	30.270	<0.0234
Ct_PSA	0	25.655	33.800	0	22.270	31.380	Ns.
Ct_DD3_PSA	0	0.732	1.583	0	0	1.198	Ns.

Ns, Not significant.

Table IV. *Basic descriptive statistics for $DD3^{PCA3}$ and PSA mRNA expression in group PCa according to Gleason score (low versus high).*

Marker	Gleason score (0-6) N=35			Gleason score (7-10) N=24			Gleason score (0-6) *vs.* Gleason score (7-10)
	Min.	Median	Max.	Min.	Median	Max.	*p*-Value
Ct_DD3	0	24.075	30.780	0	10.270	31.600	Ns.
Ct_PSA	0	24.335	33.800	0	22.368	32.560	Ns.
Ct_DD3_PSA	0	0.366	1.283	0	0.669	1.583	Ns.

Ns, Not significant.

Table V. *Basic descriptive statistics for $DD3^{PCA3}$ and PSA mRNA expression in BPH and PIN tissue.*

Marker	BPH N=100			PIN N=12			Organ-confined PCa *vs.* locally advanced or metastatic PCa
	Min.	Median	Max.	Min.	Median	Max.	*p*-Value
Ct_DD3	0	25.955	32.085	0	28.700	31.380	<0.0141
Ct_PSA	0	28.390	35.085	0	21.713	32.400	Ns.
Ct_DD3_PSA	0	0.022	2.220	0	1.054	1.328	Ns.

Ns, Not significant.

had an indwelling catheter or acute urinary infection before the biopsy were excluded from the study. The study group with suspected PCa represents the typical patient population having a prostate biopsy today. A previous biopsy was not a cause for exclusion, providing it had been performed at least three months prior to the study.

The tumor tissue samples were obtained from a bioptic sample and were frozen at –70˚C until used.

Quantitative estimation of mRNA using RT real-time PCR. Total RNA was isolated from approximately 10 mg of tissue using fastRNA Pro Green Kit (Q BIOgene, USA). A volume of 10 µl of isolated total RNA were used for reverse transcription (RT), which was performed with Superscript III Reverse Transcriptase (Life Technologies, USA) and oligo d(T)21 used as a primer. The primers for *PSA* and $DD3^{PCA3}$ were used as described by Bussemakers and colleagues (8). The sequence of primers for $DD3^{PCA3}$ were modified as follows: forward primer: 5'-TGGTGGGAAGGACCTGATGATACAG-3' and reverse primer: 5'-TCTCCCAGGGATCTCTGTGCTTCC-3'. A real-time PCR was performed on an iCycler apparatus (BioRad, USA); amplification for the $DD3^{PCA3}$ gene was monitored with 0.5× Sybr-Green I (Molecular Probes, USA). Amplification for PSA was performed using Supermi mix (BioRad, USA). The specificity of the PCR reaction was verified with the melting curve and agarose gel electrophoresis.

The results are presented as values crossing threshold (Ct, lower Ct=higher number of copies) for $DD3^{PCA3}$ and PSA and also as $DD3^{PCA3}$/*PSA* mRNA ratio (29).

Statistical analysis. Statistical analysis was performed using SAS 8.02 software (SAS Institute Inc., Cary, NC, USA) to estimate the curve parameters for each patient. The number of $DD3^{PCA3}$ mRNA

copies and $DD3^{PCA3}$/*PSA* mRNA ratio distribution was determined for all groups. We further assessed the correlation of *PSA* and $DD3^{PCA3}$ expression in tissue specimens with histological findings, tumor grade and tumor extension.

Results

It was found that mRNA expression of $DD3^{PCA3}$ (Ct) was significantly higher ($p<0.045$) in patients with PCa than in those with BPH (Table I). Messenger RNA of $DD3^{PCA3}$ (Ct) was significantly higher in patients with PIN ($DD3^{PCA3}$, $p<0.023$) (Table II) than in those with PCa. When values of $DD3^{PCA3}$ and *PSA* mRNA expression in the group PCa were assessed according to TNM classification (organ confined *vs.* local advanced and metastatic PCa), there was no statistical difference among any of the groups (Table III). Furthermore, we evaluated the values of the $DD3^{PCA3}$ and *PSA* mRNA expressions in the PCa group according to the Gleason score (low risk *vs.* high risk). There were no statistical differences according to the Gleason score (Table IV).

Finally, we assesed the $DD3^{PCA3}$ and *PSA* mRNA expression in the BPH and PIN tissue. There was a significantly ($p<0.0141$) higher level of mRNA expression of $DD3^{PCA3}$ (Ct) in the group with BPH (Table V).

Discussion

PCa may present as two main clinical forms, organ-confined and locally advanced, with or without metastases, but only if the disease is still confined to the prostate can it be cured by radical surgery or radiation therapy. To reduce the mortality rate, there have been numerous efforts to detect this malignancy at an early stage. Since its discovery twenty years ago, PSA has been considered to be the most valuable tool in the early detection, staging and monitoring of PCa during follow-up (16). However, PSA has turned out not to be PCa-specific since elevated serum PSA levels have been detected in patients with BPH and prostatitis. One of the limitations of PSA as a tumor marker is that there is a substantial overlap in serum PSA values for men with BPH with those for men with PCa. The great majority of these men underwent prostate biopsy because of an elevated serum PSA level. The positive predictive value of a serum PSA level of 4.0 ng/ml or more, the level currently recommended by the American Urological Association and also by the European Association of Urology (17, 29-32) for biopsy, is approximately 24% for men in their seventh decade (18). Thus, about one million men in the U.S. underwent a prostate biopsy in 2006 to detect PCa in one-fourth of them. Furthermore, men with negative biopsy findings but elevated PSA levels may still have PCa, because 25% of PCa cases remain undiagnosed after a single set of biopsy cores (19).

Novel approaches in molecular technology seem to overcome hurdles in detecting PCa cells in urinary samples and therefore, PCa diagnosis from urine is moving into the realm of clinical practice (20, 21). Analysis of urinary sediments after prostate massage in order to detect PCa was performed for *GSTP1* (the gene encoding the pi-class glutathione *S*-transferase hypermethylation) and showed a specificity of 98% and a sensitivity of 73% in 92 patients (22, 23). Telomerase activity is known to be expressed in at least 90% of PCa. Bensalah *et al.* gave a plethora of candidate PCa biomarkers as candidate blood-based biomarkers, including human glandular kallikrein, early prostate cancer antigens, insulin-like growth factor-I (IGF-I) and its binding proteins (IGFBP-2 and IGFBP-3), urokinase plasminogen activation system, transforming growth factor-beta 1, interleukin-6, chromogranin A, prostate secretory protein, prostate-specific membrane antigen, PCa-specific autoantibodies and alpha-methylacyl-CoA racemase (26, 27). The method of $DD3^{PCA3}$ determination, as described in our and several other studies is a substantial change from earlier versions and provides improved analytical sensitivity. Because BPH (and normal) prostate cells express low levels of $DD3^{PCA3}$ mRNA, the assay results are reported as copies of $DD3^{PCA3}$ per copies of mRNA of PSA, with the latter as a measure of prostate RNA present in the specimen.

A time-resolved fluorescence-based quantitative RT-PCR assay was developed based on the principle of quantitative RT-PCR for *PSA* mRNA recently described by Ylikoski and colleagues (24). The results of the accurate quantification of $DD3^{PCA3}$ transcripts in normal tissue specimens showed that $DD3^{PCA3}$ was exclusively expressed in the prostate. This was in concordance with earlier published data (8, 10). The accurate quantification power of this assay allowed the determination of the median up-regulation of $DD3^{PCA3}$ in prostate tumors. In the radical prostatectomy specimens of 7 patients, the $DD3^{PCA3}$ expression in tumor areas was compared to the $DD3^{PCA3}$ expression in the adjacent non-neoplastic prostate tissue. A 6-to 1,500-fold up-regulation of $DD3^{PCA3}$ was found in the prostate tumors compared to the adjacent non-neoplastic prostate tissue. In the non-matched group of tissue specimens a median 66-fold up-regulation of $DD3^{PCA3}$ in PCa was found. These data are in agreement with the 10-100-fold overexpression of $DD3^{PCA3}$ in tumor areas compared to adjacent non- neoplastic prostate tissue, based on Northern blot analysis (8, 24).

Tao *et al.* present results of mRNA $DD3^{PCA3}$ tissue expression of 27 non-PCa patients (patients with other malignant tumors), 21 with PCa, 39 with BPH and 15 with normal prostate. Messenger RNA expression was not detected in the non-PCa tissues. The median expression of $DD3^{PCA3}$ mRNA in PCa, BPH and normal prostate tissues

were 7.2×10^6, 2.5×10^4 and 1.5×10^4 copies/mg tissue, respectively. The $DD3^{PCA3}$ mRNA expression levels were significantly lower in nonmalignant than in malignant tissues ($p<0.01$). No significant differences in $DD3^{PCA3}$ mRNA expression were detected between the NP and BPH tissues and no significant correlation was found between the $DD3^{PCA3}$ mRNA expression and clinical pathological parameters (28).

The specificity of the $DD3^{PCA3}$ assay for prostate cancer would seem to be useful for the early detection of PCa and also for differential diagnosis between patients with BPH and patients with prostate cancer. As a next step, we will evaluate the possible usage of $DD3^{PCA3}$ for the early detection of the development of prostate cancer in patients with BPH.

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References

1 Landis SH, Murray T, Bolden S and Wingo PA: Cancer statistics, 1999. CA Cancer J Clin *49(1)*: 8-31, 1999.
2 Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C and Thun MJ: Cancer statistics, Cancer statistics, 2006. CA Cancer J Clin *56(2)*: 106-130, 2006.
3 SVOD (System for Visualization of Oncological Dates version 6); http://www.svod.cz
4 Thompson IM, Ankerst DP, Chi C, Lucia MS, Goodman PJ, Crowley JJ, Parnes HL and Coltman CA Jr: Operating characteristics of prostate-specific antigen in men with an initial PSA level of 3.0 ng/ml or lower. JAMA *294*: 66-70, 2005.
5 Stamey TA, Caldwell M, McNeal JE, Nolley R, Hemenez M and Downs J: The prostate specific antigen era in the United States is over for prostate cancer: what happened in the last 20 years? J Urol *172*: 1297-1301, 2004.
6 Karazanashivili G and Abrahamsson PA: Prostate-specific antigen and human glandular kallikrein 2 in early detection of prostate cancer. J Urol *169*: 445-457, 2003.
7 Punglia RS, D'amico AV, Catalona WJ, Roehl KA and Kuntz KM: Effect of verification bias on screening for prostate cancer by measurement of prostate-specific antigen. N Engl J Med *46*: 307-310, 2003.
8 Bussemakers MJ, van Bokhoven A, Verhaegh GW, Smit FP, Karthaus HF, Schalken JA, Debruyne FM, Ru N and Isaacs WB: *DD3*: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res *59*: 5975-5979, 1999.
9 Hessels D, Klein Gunnewiek JM, van Oort I, Karthaus HF, van Leenders GJ, van Balken B, Kiemeney LA, Witjes JA and Schalken JA: DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol *44(1)*: 8-15; discussion 15-16, 2003.
10 De Kok JB, Verhaegh GW, Roelofs RW, Hessels D, Kiemeney LA, Aalders TW, Swinkels DW and Schalken JA: DD3(PCA3), a very sensitive and specific marker to detect prostate tumors. Cancer Res *62*: 2695-2698, 2002.
11 Fradet Y, Saad F, Aprikian A, Dessureault J, Elhilali M, Trudel C, Mâsse B, Piché L, Chypre C and Fradet Y: uPM3, a new molecular urine test for the detection of prostate cancer. Urology *64*: 311-316, 2004.
12 Tinzl M, Marberger M and Horvath S: *DD3/PCA3* RNA analysis in urine a new perspective for detecting prostate cancer. Eur Urol *46*: 182-187, 2004.
13 Groskopf J, Auhin SMJ and Deras IL: APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. Clin Chem *52*: 1089-1095, 2006.
14 Marangoni K, Neves AF, Cardoso AM, Santos WK, Faria PC and Goulart LR: The endothelial nitric oxide synthase Glu-298-Asp polymorphism and its mRNA expression in the peripheral blood of patients with prostate cancer and benign prostatic hyperplasia. Cancer Detect Prev *30:* 7-13, 2006.
15 Huggett J, Dheda K, Bustin S and Zumla A: Real-time RT-PCR normalisation; strategics and considerations. Genes Immun *6*: 279-284, 2005.
16 Polascik TJ, Oesterling JE and Partin AW: Prostate-specific antigen: a decade of discovery what we have learned and where we are going. J Urol *162(2)*: 293-306, 1999.
17 Thompson IM, Carroll P and Coley C: Prostate-specific antigen (PSA) best practice policy. Oncology *14*: 267-286, 2000.
18 Vollmer RT: Predictive probability of serum prostate-specific antigen for prostate cancer: an approach using Bayes rule. Am J Clin Pathol *125*: 336-342, 2006.
19 Roehl KA, Antenor JA and Catalona WJ: Serial biopsy results in prostate cancer screening study. J Urol *167*: 2435-2439, 2002.
20 Clements JA, Rhode P, Allen V, Hyland VJ, Samaratunga ML and Tillev WD: Molecular detection of prostate cells in ejaculate and urethral washings of men with suspected prostate cancer. J Urol *161*: 1337-1342, 1999.
21 Muller M, Goessl C, Krause H and Miller K: Molecular diagnostics in urologic oncology. Detection of nucleic acids in urine samples. Urologe A *42*: 660-668, 2003.
22 Goessl C, Muller M, Heicappell R, Krause H, Straub B and Schrader M: DNA-based detection of prostate cancer in urine after prostatic massage. Urology *58*: 335-338, 2001.
23 Cairns P, Esteller M, Hermann JG, Schoenberg M, Jeronimo C and Sanchez-Cespedes M: Molecular detection of prostate cancer in urine by GSTP1 hypermethylation. Clin Cancer Res *7*: 2727-2730, 2001.
24 Ylikoski A, Karp M, Lilja H and Lovgren T: Dual-label detection of amplified products in quantitative RT-PCR assay using lanthanide-labeled probes. Biotechniques *30(4)*: 832-836, 2001.
25 Margreiter M, Stangelberger A, Valimberti E, Herwig R and Djavan B: Biomarkers for early prostate cancer detection. Minerva Urol Nefrol *60(1)*: 51-60, 2008.
26 Bensalah K, Lotan Y, Karam JA and Shariat SF: New circulating biomarkers for prostate cancer. Prostate Cancer Prostatic Dis *11(2)*: 112-120, 2008.
27 Sardana G, Dowell B and Diamandis EP: Emerging biomarkers for the diagnosis and prognosis of prostate cancer. Clin Chem *54(12)*: 1951-1960, 2008.
28 Tao ZH, Mao XL, Wang CH, Chen XD, Yu KY, Weng ZL, Hu YP, Zhang XH, Xie H, Wang OC, Song QT, Li CD and Chen ZG: Quantitative detection of *DD3* mRNA in prostate cancer tissues by real-time fluorescent quantitative reverse transcription polymerase chain reaction. Zhonghua Nan Ke Xue *13(2)*: 130-133, 2007.

29 Bustin SA: Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J Mol Endocrinol *25(2)*: 169-193, 2000.

30 Nickel JC, Herschorn S, Corcos J, Donnelly B, Drover D, Elhilali M, Goldenberg L, Grantmyre J, Laroche B, Norman R, Piercy B, Psooy K, Steinhoff G, Trachtenberg J, Saad F and Tanguay S: Canadian Prostate Health Council; Canadian Urological Association Guidelines Committee. Canadian guidelines for the management of benign prostatic hyperplasia. Can J Urol *12(3)*: 2677-2683, 2005.

31 Kuvibidila S and Rayford W: Correlation between serum prostate-specific antigen and alpha-1-antitrypsin in men without and with prostate cancer. J Lab Clin Med *147(4)*: 174-181, 2006.

32 Aus G, Abbou CC, Bolla M, Heidenreich A, Schmid HP, van Poppel H, Wolff J and Zattoni F: European Association of Urology: EAU guidelines on prostate cancer. Eur Urol *48(4)*: 546-551, 2005.

33 Jemal A, Siegel R, Ward E, Hao Y, Xu J and Thun MJ: Cancer statistics, 2009. CA Cancer J Clin *59(4)*: 225-249, 2009.

34 Suzuki H, Flanigan RC and Okuyama A; American Urological Association: Prostate-specific antigen (PSA)-based screening. Int J Urol *15(9)*: 757-762, 2008.

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