Effect of external environmental factors on PSA concentration

Roman Sosnowski[1], Maria Kowalska[2], Agata Wlazło[1], Janina Kamińska[2], Wojciech Michalski[3], Tomasz Demkow[1]

[1]Uro-oncology Department, The Maria Sklodowska-Curie Memorial Center and Institute of Oncology, Warsaw, Poland
[2]Department of Tumor Markers, The Maria Sklodowska-Curie Memorial Center and Institute of Oncology, Warsaw, Poland
[3]Clinical Trials and Biostatistics Unit, The Maria Sklodowska-Curie Memorial Center and Institute of Oncology, Warsaw, Poland

KEY WORDS

prostate ◗ prostate specific antigen (PSA) ◗ prostate cancer

ABSTRACT

Introduction. Prostate specific antigen (PSA) is an acknowledged marker in various conditions of the prostate including prostatic cancer. The PSA value depends on a given ailment of the prostate gland. It is commonly known that the serum PSA concentration changes under the influence of various drugs, prostate conditions, and urological surgical procedures as well as following ejaculation. Until now it was not clear whether pre-analytical external factors, such as the time and temperature at which the samples are stored from the moment of blood collection, had an influence on the final PSA value.
The purpose of this study was to establish the influence of pre-analytical external factors (temperature and duration of storage before analysis) on the serum level of total PSA (tPSA).
Material and methods. Forty-seven subsequent patients with prostate cancer were included and routine blood samples were collected. The tPSA was determined using the MEIA (Microparticle Enzyme Immunoassay) immunoenzymatic method with Abbott Diagnostics kits. Four measurement series of PSA levels were carried out in each patient dividing them into the following groups: series 1 – following coagulation (about 30 min), measurement after centrifugation – in serum
series 2 – after incubating blood sample for 4 hours at 30°C; series 3 – after incubating blood sample for 4 hours at room temperature; series 4 – after incubating blood sample for 24 hours at 4°C.
Results. The tPSA median and range for series 1 were: 0.656 ng/ml (0.0008 – 152.1 ng/ml). The percentage difference of PSA values between series 2, 3, and 4 and series 1 were analyzed. One-sample T-test was used with H0: assuming zero difference. No statistically significant difference was found between the series. The minimal detectable difference, at 0.9 test power level was 2.8% for all tests.
Conclusions. In the present study, the external factors of blood sample storage had no effect on tPSA concentration.

INTRODUCTION

PSA (prostate-specific antigen) is a monochain glycoprotein with a molecular mass of 34,000 daltons, containing 93% amino acids and 7% hydrocarbons. It is produced by the epithelial cells lining the prostatic tubules and excretory ducts. The PSA-encoding gene is located on the 19th chromosome and belongs to genes from the kallikrein group [1].

PSA is found in serum in a free form (free PSA) in addition to the form bound to α-1-antichymotrypsin (ACT) and α-2-macroglobulin (A2M). Other forms are also distinguished, such as PSA bound to serine inhibitor or PSA bound to α-1-antitrypsin, but their concentrations are extremely low[1]. Free PSA and PSA-ACT demonstrate immunoreactivity in human serum [2]. The determination of total PSA (tPSA) means that the sum is determined by two immunologically active forms, i.e. free PSA and PSA-ACT. PSA is a commonly recognized marker specific to the prostatic gland [3]. The diagnostic value of tPSA in prostatic cancer detection is limited, since the marker's concentration can be elevated also in other, non-malignant prostatic diseases, particularly in benign hyperplasia and in inflammatory conditions of the gland. Free PSA and tPSA concentrations can also be altered by per rectum examination, urinary cytoscopy, biopsy, or surgical operations on the prostatic gland as well as following ejaculation [4]. The method of blood sample storage can also influence tPSA and free PSA concentrations [5].

The aim of the present study was to assess the effect of duration and temperature of blood sample storage on the resulting tPSA concentration.

MATERIAL AND METHODS

The studied group included 47 subsequent patients with mean age of 67 years with prostatic cancer confirmed by histological examination. Blood for clot was collected into four adequately labeled tubes using a vacuum system. The blood samples were centrifuged under typical conditions. The concentration of tPSA was determined by means of the MEIA immunoenzymatic method with Abbott Diagnostics kits. For each patient tPSA concentration determinations were performed according to the study protocol shown in Table 1.

Table 1. Temperature and duration of blood storage before tPSA determination in serum.

Series	External conditions and duration of blood storage
Series 1	Immediately after blood coagulation at room temperature
Series 2	After 4 hours of blood incubation at 30°C
Series 3	After 4 hours of blood incubation at room temperature
Series 4	After 24 hours of blood incubation at 4°C

Table 2. tPSA concentrations in individual series.

No.	Series-1 (ng/ml)	Series-2 (ng/ml)	Series-3 (ng/ml)	Series-4 (ng/ml)
1	0.0008	0.0008	0.0008	0.0008
2	0.166	0.163	0.158	0.15
3	6.336	6.541	6.572	6.565
4	2.618	2.687	2.588	2.476
5	0.003	0.003	0.003	0.004
6	0.105	0.097	0.102	0.1
7	12.858	12.008	12.922	12.445
8	4.065	3.693	3.76	3.723
9	0.162	0.163	0.16	0.15
10	0.0008	0.0008	0.0008	0.0008
11	127.2	134.31	138.48	134.71
12	0.706	0.72	0.741	0.733
13	1.145	1.183	1.172	1.238
14	58.104	53.156	51.771	52.492
15	0.402	0.418	0.398	0.417
16	0.002	0.003	0.003	0.003
17	0.155	0.156	0.158	0.158
18	0.0008	0.005	0.002	0.001
19	0.123	0.133	0.123	0.122
20	0.968	1.067	0.956	1.073
21	27.34	28.538	28.249	29.429
22	50.757	51.315	51.505	52.311
23	89.237	89.527	80.406	86.091
24	8.355	8.629	8.282	8.149
25	23.333	24.891	24.418	23.504
26	0.0008	0.0008	0.0008	0.0008
27	0.78	0.794	0.801	791
28	0.052	0.051	0.049	0.053
29	2.878	2.697	2.805	2.79
30	0.656	0.698	0.679	0.697
31	4.181	4.151	4.264	4.122
32	2.676	2.886	2.924	2.84
33	152.085	155.10	154.67	144.32
34	0.675	0.664	0.642	0.683
35	0.192	0.182	0.181	0.196
36	0.018	0.018	0.021	0.021
37	0.037	0.038	0.036	0.035
38	2.079	2.103	2.208	2.319
39	0.0008	0.0008	0.0008	0.0008
40	0.693	0.634	0.689	0.673
41	0.0008	0.0008	0.0008	0.0008
42	0.127	0.123	0.121	0.116
43	1.159	1.18	1.169	1.213
44	0.403	0.425	0.406	0.418
45	0.0008	0.0008	0.0008	0.0008
46	0.282	0.287	0.291	0.283
47	1.135	1.136	1.177	1.139

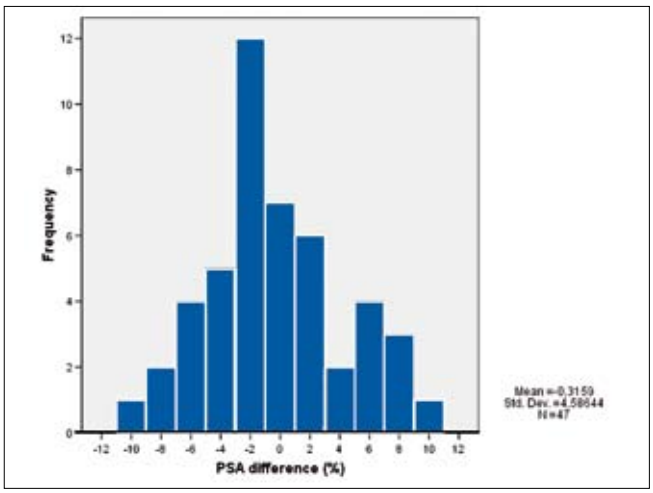

Fig. 1. Distribution of PSA percentage change after 4 hours of storage at 30°C.

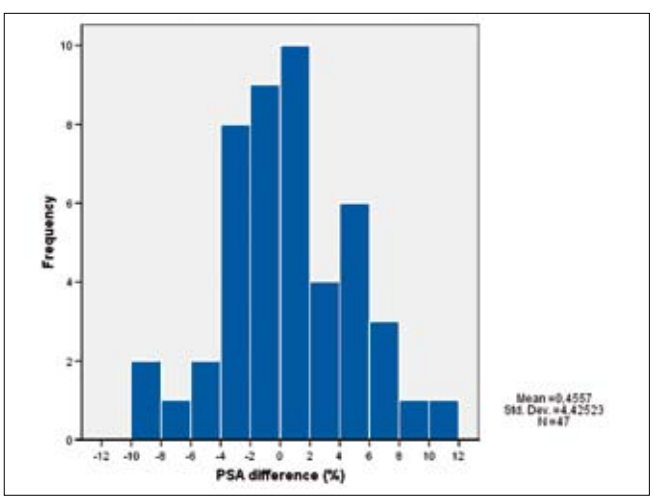

Fig. 2. Distribution of PSA percentage change after 4 hours of storage at 20°C.

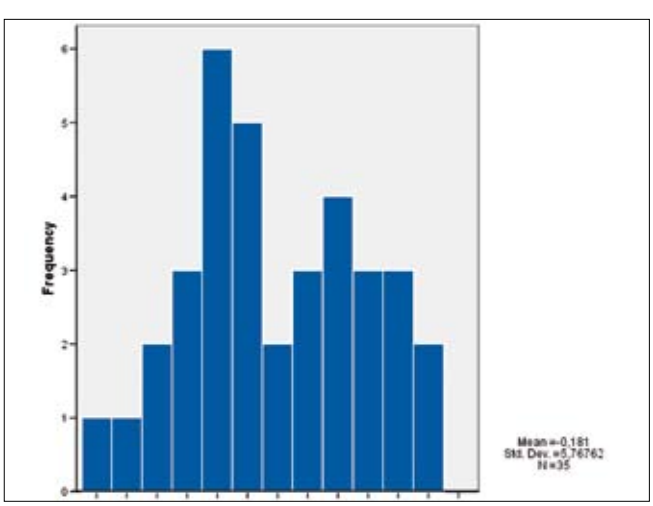

Fig. 3. Distribution of PSA percentage change after 24 hours of storage at 20°C.

The percent differences in PSA measurements between reference level (series 1) and series 2, 3 and 4 was accepted as the influence estimation of temperature and duration of sample storage on the outcomes of PSA measurements. The zero hypothesis assuming zero mean value of those differences was tested using a one-sample T-test.

RESULTS

The tPSA values in series 1 ranged from <0.0008 ng/ml to 152.082 ng/ml, median 0.656. The groups of patients with tPSA value below and above 1,000 ng/ml included 26 and 21 cases, respectively. The values of PSA determinations in all series are presented in Table 2.

No statistically significant difference was found between the measurements in series 2, 3, and 4 and series 1. The respective mean values of the percent differences together with 95% confidence interval were: -0.32 (-1.67; 1.03); 0.46 (-0.84; 1,76); -0.18 (-2.16; 1.80).

One-Sample T-test was used with H0: assuming zero difference. No statistically significant difference was found between the series. The minimal detectable difference, at 0.9 test power level, was less than 2.8% for all tests (Figs. 1, 2, and 3).

DISCUSSION

Our data suggest that tPSA can be reliably measured even when serum was stored for at least 24 hours at room temperature. The character of the prostate pathology, but not the analyzed external factors, has an influence on the obtained changes in the tPSA values measured in the same patient. No papers were found in the available literature on tPSA stability tests under such extreme conditions of blood sample storage. Only Cartledge et al. demonstrated that tPSA is sufficiently stable to permit whole blood samples to remain at room temperature for 24 h before serum is separated [6]. Woodrum et al. characterized the stability of tPSA under various sample collection and storage conditions (serum centrifuged from 1 to 8 hours after blood collection, stored at room temperature or at 4°C) and summarized that tPSA appeared to be stable [7]. Few studies have systematically assessed the stability of tPSA under varying storage conditions of serum, as it was in our case, and not of whole blood. Arcangeli et al. who studied the influence of duration and temperature of serum storage on the results of tPSA concentration determinations, failed to find any statistically significant differences between tPSA concentrations after 24 hours, 2 weeks, or 9 months for storage temperatures of -20°C compared with -70°C [8]. On the other hand, Paus et al. analyzed the effect of storage at 4°C on tPSA in serum and demonstrated that after 7 days of storage at 4°C there was a slight decrease of tPSA in serum. The decrease of tPSA, which was also observed, may be due to a fraction of the free PSA disappearing into α-2-macroglobulin and thus escaping detection by the immunoassays. They cannot ignore the possibility of some PSA being released from α-1-antichymotrypsin during storage [9]. Similarly to other results, we confirmed that tPSA is relatively stable when blood is stored for up to 4 hours at 30°C or 24 hours at 4°C.

CONCLUSIONS

The results of our study indicate a high stability of tPSA. However, such extreme conditions of blood storage are not recommended. Our tests, conducted under such various conditions, aimed at demonstrating the exceptional stability of this marker, which is commonly used in the diagnosis of diseases of the prostate gland.

REFERENCES

1. Qiu SD, Young CY, Bilhartz DL, Prescott JL et al: *In situ hybridization of prostate-specific antigen mRNA in human prostate*. J Urol 1990; 144 (6): 1550-1556.
2. Lundwall A: *Characterization of the gene for prostate-specific antigen, a human glandular kallikrein*. Biochem Biophys Res Commun 1989; 161 (3): 1151-1159.
3. Christensson A, Lilja H: *Complex formation between protein C inhibitor and prostate-specific antigen in vitro and in human semen*. Eur J Biochem 1994; 220 (1): 45-53.
4. Stenman UH, Leinonen J, Alfthan H et al: *A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer*. Cancer Res 1991; 51 (1): 222-226.
5. Stamey TA, Yang N, Hay AR et al: *Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate*. N Engl J Med 1987; 317 (15): 909-916.
6. Cartledge JJ, Thompson D, Verril H et al: *The stability of free and bound prostate-specific antigen*. BJU Int 1999; 84 (7): 810-814.
7. Woodrum D, French C, Shamel LB: *Stability of free prostate-specific antigen in serum samples under a variety of sample collection and sample storage conditions*. Urology 1996, 48 (6): 33-36.
8. Arcangeli CG, Smith DS, Ratliff TL, Catalona WJ: *Stability of serum total and free prostate specific antigen under varying storage intervals and temperatures*. J Urol 1997; 158 (6): 2182-2187.
9. Paus E, Nilsson O, Børmer OP et al: *Stability of free and total prostate specific antigen in serum from patients with prostate carcinoma and benign hyperplasia*. J Urol 1998; 159 (5): 1599-1605.

Correspondence

Roman Sosnowski
1, Cyganeczki Street
02-928 Warsaw, Poland
phone: +48 601 623 018
roman@is.com.pl