European Review for Medical and Pharmacological Sciences 2017; 21: 61-67

Serum BSP, PSADT, and Spondin-2 levels in prostate cancer and the diagnostic significance of their ROC curves in bone metastasis

B.-P. ZHU, Z.-Q. GUO, L. LIN, Q. LIU

Department of Urology, Zhongnan Hospital of Wuhan University, Hubei, China

Abstract. – OBJECTIVE: Bone metastasis is a common complication of prostate cancer. This study investigates serum bone sialoprotein (BSP), prostate specific antigen doubling time (PSADT), and extracellular matrix protein Spondin-2 levels in prostate cancer and the diagnostic significance of their ROC curves in bone metastasis.

PATIENTS AND METHODS: A total of 85 cases of prostate cancer patients, including 43 cases with bone metastases and 42 cases without bone metastases, were enrolled. Serum BSP, Spondin-2, and PSA were tested by ELISA and Electrochemical Luminescence method. PSADT was calculated upon multiplication formula. The diagnostic significances of BSP, Spondin-2, and PSADT on bone metastasis were evaluated by ROC curve.

RESULTS: Serum BSP, Spondin-2, and PSA levels were highest in prostate cancer with bone metastasis, followed by no bone metastasis, hyperplasia, and control ($p < 0.05$). Gleason scores of BSP, Spondin-2, and PSA were highest in low differentiation, followed by moderate differentiation and high differentiation ($p < 0.05$). ROC curve revealed that diagnostic efficiency was in PSADT, Spondin-2, and BSP in the order. Their sensitivity and specificity for the diagnosis of bone metastasis were 79.07, 88.37, 86.05%, and 71.54, 81.30, 83.74%, respectively. Their joint detection elevated the sensitivity to 97.67%, the negative predictive value up to 99.11%, and AUC to 0.973 (95% CI 0.942-0.998). PSADT + BSP exhibited better efficiency among two indicators combination as AUC reached 0.963 (95% CI 0.935-0.992).

CONCLUSIONS: Serum BSP, Spondin-2, and PSADT upregulated in prostate cancer patients with bone metastasis. Their joint detection can improve the diagnostic sensitivity.

Key Words:
Prostate cancer, Bone metastasis, PSADT, BSP, Spondin-2.

Introduction

Bone metastasis is a common complication for prostate cancer patients. It accounts for about 65-70% in the process of prostate cancer development, which mainly involves in the pelvis, spine, and thighbone[1,2]. There are about 80% patients died of prostate cancer suffered from bone metastasis[3,4]. Prostate cancer is often hidden onset without a significant early symptom of bone metastasis. Bone metastasis may lead to pathological fracture, bone pain, and nerve compression. Therefore, bone metastasis symptom is often the most common cause to see a doctor[5,6]. Prostate cancer bone metastasis seriously affects the quality of life and prognosis. Thus, early diagnosis and treatment are of great significance for prostate cancer bone metastases. At present, the whole body bone scan is the main method to diagnose and monitor bone metastasis in prostate cancer. As no signs of bone destruction and low sensitivity in the early stage on bone X-ray, radionuclide bone imaging is the preferred method for the diagnosis of bone metastasis. Compared with traditional X-ray, it exhibits higher sensitivity. However, it subjects to low specificity, high price, and radiation hazard[7]. Following the in-depth investigation of the mechanism of prostate cancer bone metastasis, prostate cancer-related serological markers are applied in disease monitoring because of its repeatability, non-invasion, and cheap. Bone sialoprotein (BSP) has certain diagnostic significance in bone metastasis since it can reflect the bone absorption process and bone cell activity. Serum PSA level can predict the extent of prostate cancer. It significantly elevates in patients with bone metastasis. However, in spite of its high sensitivity, its specificity is limited and cannot distinguish the reason that causes PSA elevation. PSA doubling time

Corresponding Author: Baoping Zhu, MD; e-mail: baopingzhuiop@163.com

(PSADT) is the feature between PSA and time-related curve, which can more accurately reflect tumor metastasis and recurrence compared with simple PSA[8,9]. Spondin-2 is a type of extracellular matrix protein differently expressed in lung cancer cell. It also upregulates in human prostate cancer cell line[10]. This study investigates serum BSP, PASDT, and Spondin-2 levels in prostate cancer and the diagnostic significance of their ROC curves in bone metastasis, aiming to provide the basis for early diagnosis, monitor, and treatment of prostate cancer bone metastasis.

Patients and Methods

General Information

A total of 85 cases of prostate cancer patients between Oct 2013 and Nov 2015 were enrolled in Zhongnan Hospital of Wuhan University. Another 41 patients with benign prostate hyperplasia and 40 healthy controls in the same period were selected. The prostate cancer patients were divided into well, moderate, and low differentiation groups according to Gleason grading at 2-4, 5-6, and 7-10 with 27, 36, and 22 cases in each group. Inclusion criteria: prostate cancer diagnosed by cytological or pathological examination. Exclusion criteria: benign lesions including trauma was excluded from bone metastases, while inflammation, diabetes, or injury excluded from prostate hyperplasia. No patients suffered from significant viscera lesions in heart, liver, and kidney. According to the presence of bone metastases, the patients were divided into bone metastases group with 43 cases and non-bone metastases group with 42 cases. The mean age of bone metastases, non-bone metastases, hyperplasia, and healthy groups was 58.7 ± 4.9, 57.9 ± 4.5, 56.9 ± 4.2, and 57.7 ± 4.6 years old. All the enrolled hyperplasia patients were benign. No statistical significance was observed on general information among four groups ($p > 0.05$). This study was approved by the Ethics Committee in Zhongnan Hospital of Wuhan University, and all the enrolled subjects had signed informed consent.

Detection

No puncture or digital rectal examination was performed on patients at least 3 days before blood sampling. Bone scan was performed within 3 days before blood collection. Peripheral venous blood was extracted and centrifuged at 14000 g for 15 min. The serum was separated and tested within 2 h. Serum BSP, Spondin-2, and PSA were tested by ELISA and Electrochemical Luminescence method. ELISA kits were bought from Aquatic Diagnostics Ltd (Stirling, Scotland, UK). Electrochemical Luminescence kit was purchased from Roche (Basel, Switzerland). UniCel DxI 800 automatic chemiluminescence immune analyzer was got from Beckman Coulter Company (Brea, CA, USA). PSADT was calculated upon multiplication formula. f-PSA and t-PSA were reexamined every 51 days (1-4 months). PSADT = lg(2)/bi, bi = (lg Xi, final-lg Xi, initial)/(ti, final-ti, initial). i = 1, 2,……n, X referred to t-PSA value.

Positive Judgement

The diagnostic significances of BSP, Spondin-2, and PSADT on bone metastasis were evaluated by ROC curve. The positive judgement value was selected from the best point as the largest cut point of Youden index (J = sensitivity + specificity - 1) for the single indicator. BSP > 30.15 μg/L, PSADT > 129, and Spondin-2 > 8 μg/L were treated as positive. For the two indicators joint detection, one item for positive was considered as positive, while two items for negative were treated as negative. For three indicators joint detection, one item for positive was considered as positive, whereas three items for negative were treated as negative[11].

Statistical Analysis

All data analysis were performed on SPSS 19.0 software (Inc. Chicago, IL, USA). The χ^2-test or χ^2 calibration test was used for ratio comparison. Kolmogorov-Smirnov was adopted for normality test, and ± s was selected to depict the measurement data in a normal distribution. ANOVA and LSD test were applied for mean difference comparison between groups. The diagnostic significances of BSP, Spondin-2, and PSADT on bone metastasis were evaluated by ROC curve. Logistic regression model was established according to the indicators combination. Z test was used for the comparison of joint detection. $p < 0.05$ was considered as statistical significance.

Results

BSP, PSA, and Spondin-2 Levels Comparison

Serum BSP, Spondin-2, and PSA levels were highest in prostate cancer with bone metastasis, followed by no bone metastasis, hyperplasia, and

Table I. BSP, PSA, and Spondin-2 levels comparison ($\bar{x} \pm s$).

Group	Cases	BSP (μg/L)	f-PSA (μg/L)	t-PSA (μg/L)	f-PSA/ t-PSA	Spondin-2 (μg/L)
Control	40	7.61 ± 1.26	0.36 ± 0.09	0.94 ± 0.11	0.38 ± 0.09	5.83 ± 1.17
Hyperplasia	41	8.11 ± 1.43*	1.86 ± 0.21*	6.63 ± 0.45*	0.28 ± 0.06*	6.29 ± 1.36*
Non-bone metastasis	42	12.35 ± 3.27*$^{\Delta}$	43.34 ± 8.13*$^{\Delta}$	223.93 ± 81.95*$^{\Delta}$	0.19 ± 0.06*$^{\Delta}$	7.45 ± 1.33*$^{\Delta}$
Bone metastasis	43	37.82 ± 8.54*$^{\Delta\&}$	45.61 ± 7.38*$^{\Delta}$	227.87 ± 82.31*$^{\Delta}$	0.18 ± 0.07*$^{\Delta}$	15.44 ± 3.14*$^{\Delta\&}$
F-value	–	33.63	43.60	45.06	35.03	49.24
p	–	0.00	0.00	0.00	0.00	0.00

*$p < 0.05$, compared with control. $^{\Delta}p < 0.05$, compared with hyperplasia group. $^{\&}p < 0.05$, compared with non-bone metastasis group.

Table II. BSP, PSA, and Spondin-2 levels comparison among prostate cancer patients with different Gleason scores ($\bar{x} \pm s$).

Group	Cases	BSP (μg/L)	f-PSA (μg/L)	t-PSA (μg/L)	f-PSA/ t-PSA	Spondin-2 (μg/L)
Well differentiation	27	11.92 ± 4.39	37.46 ± 7.52	182.32 ± 82.11	0.23 ± 0.05	12.55 ± 3.16
Moderate differentiation	36	22.38 ± 3.93*	43.25 ± 7.27*	222.67 ± 83.14*	0.19 ± 22.36*	17.32 ± 3.45*
Low differentiation	22	40.47 ± 4.74*$^{\Delta}$	48.34 ± 8.21*$^{\Delta}$	245.53 ± 84.95*$^{\Delta}$	0.15 ± 0.04*$^{\Delta}$	19.68 ± 3.41*$^{\Delta}$
F-value	–	4.67	4.15	4.35	4.79	4.41
p	-	0.01	0.02	0.02	0.01	0.02

*$p < 0.05$, compared with control. $^{\Delta}p < 0.05$, compared with hyperplasia group. $^{\&}p < 0.05$, compared with non-bone metastasis group.

control ($p < 0.05$). No statistical difference was found on f-PSA, t-PSA, and f-PSA/t-PSA between bone metastasis and non-bone metastasis groups (Table I).

BSP, PSA, and Spondin-2 Levels Comparison Among Prostate Cancer Patients with Different Gleason Scores

Gleason scores of BSP, Spondin-2, and PSA were highest in low differentiation, followed by moderate differentiation and high differentiation ($p < 0.05$) (Table II).

ROC Curve Analysis of BSP, PSADT, and Spondin-2

Bone metastasis group and control group were selected for ROC curve analysis. ROC curve revealed that diagnostic efficiency was PSADT, Spondin-2, and BSP in the order (Figure 1, Table III).

BSP, PSA, and Spondin-2 Detection Comparison Among Different Groups

According to BSP, PSADT, Spondin-2 levels in healthy and prostate cancer bone metastasis patients, together with ROC curves, the positive

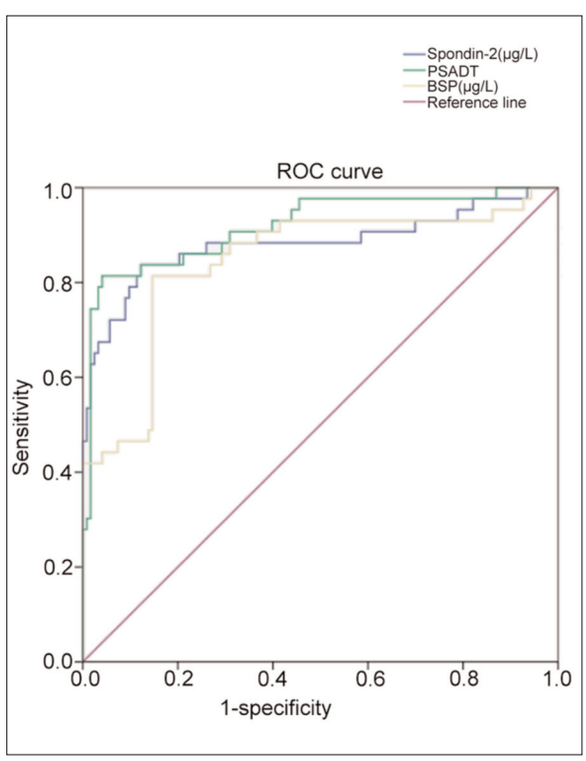

Figure 1. ROC curve analysis of BSP, PSADT, and Spondin-2 in prostate cancer bone metastasis.

Table III. AUC.

Outcome variable	Area	Standard error[a]	Asymptotic Sig.[b]	Asymptotic 95% CI	
				Inferior limit	Upper limit
Spondin-2 (μg/L)	0.882	0.038	0.000	0.807	0.957
PSADT	0.918	0.028	0.000	0.863	0.973
BSP (μg/L)	0.845	0.039	0.000	0.768	0.921

[a]Under nonparametric hypothesis; [b]Null hypothesis: solid area = 0.5.

Table IV. BSP, PSA, and Spondin-2 detection comparison among different groups.

Indicator	Positive threshold value	Sensitivity/%	Specificity/%
BSP	> 30.15 μg/L	79.07 (34/43)	71.54 (88/123)
PSADT	> 129	88.37 (38/43)	81.30 (100/123)
Spondin-2	> 8 μg/L	86.05 (37/43)	83.74 (103/123)

judgement value was selected from the best point as the largest cut point of Youden index (J = sensitivity + specificity - 1) for the single indicator. BSP > 30.15 μg/L, PSADT > 129, and Spondin-2 > 8 μg/L were treated as positive. Their sensitivity and specificity for the diagnosis of bone metastasis were 79.07, 88.37, 86.05%, and 71.54, 81.30, 83.74%, respectively (Table IV).

BSP, PASDT, and Spondin-2 Indicators Joint Detection

Their joint detection elevated the sensitivity to 97.67%, the negative predictive value up to 99.11%, and AUC to 0.973 (95% CI 0.942-0.998). PSADT + BSP exhibited better efficiency among two indicators combination as AUC reached 0.963 (95% CI 0.935-0.992) (Figure 2, Table V-VI).

Discussion

Magnetic resonance imaging and radionuclide bone imaging have relatively high sensitivity for the diagnosis of bone metastases. However, they are limited by radioactivity and high cost. Serological markers of bone can reflect the whole body bone metabolism featured as repeatable, non-invasive, and low expense. Searching for effective and sensitive serological indicators for early diagnosis is of great importance in the treatment of prostate cancer. Bone metabolic markers are mainly produced by bone matrix metabolism or bone cells secretion. Cytokines act on osteoclasts and osteoblasts during malignant tumor cell bone metastasis, leading to normal bone metabolizing material damage and abnormal level of bone metabolic markers. Serum PSA is widely applied in the clinic as a specific bio-

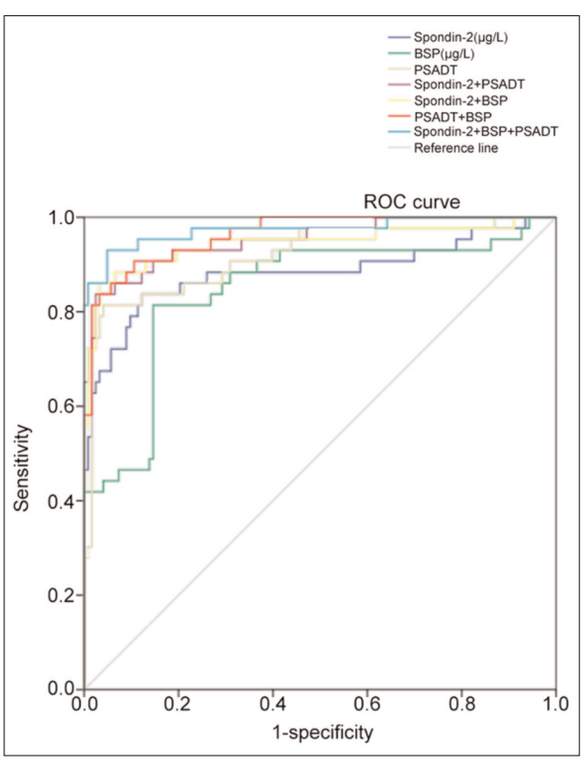

Figure 2. ROC curve of joint detection.

Table V. AUC of joint detection.

Outcome variable	Area	Standard error[a]	Asymptotic Sig.[b]	Asymptotic 95% CI	
				Inferior limit	Upper limit
Spondin-2 (μg/L)	0.882	0.038	0.000	0.807	0.957
BSP (μg/L)	0.845	0.039	0.000	0.768	0.921
PSADT	0.918	0.028	0.000	0.863	0.973
Spondin-2+PSADT	0.952	0.020	0.000	0.912	0.991
Spondin-2+BSP	0.944	0.026	0.000	0.893	0.995
PSADT+BSP	0.963	0.015	0.000	0.935	0.992
Spondin-2+BSP+PSADT	0.973	0.016	0.000	0.942	0.998

[a]Under nonparametric hypothesis; [b]Null hypothesis: solid area = 0.5.

marker since it can predict the tumor lesions of prostate cancer[12]. It is associated with prostate cancer metastasis and prognosis. Bone metastases and bone metabolic state exhibit no direct correlation with PSA, thus joint detection is needed in the diagnosis of bone metastases[13]. Serum PSA reflects the absolute value, whereas its variation trend PSADT has an important significance in the curative effect and prognosis evaluation of prostate cancer. PSADT calculated by early stage PSA is beneficial to disease evaluation in the early stage[14,15]. In this work, no statistical difference was found on f-PSA, t-PSA, and f-PSA/t-PSA between bone metastasis and non-bone metastasis groups. Serum PSA level in bone metastasis group was obviously higher than the non-bone metastasis group, suggesting joint detection was required for the diagnosis of prostate cancer bone metastases.

As a kind of non-collagen protein, BSP can promote bone metastases of breast cancer at a high level and stable expression. Serum BSP has certain significance in the diagnosis of lung cancer bone metastases[16,17]. This study tested the value of BSP for the evaluation of prostate cancer bone metastasis, and the result showed it was markedly higher in bone metastasis group compared with non-bone metastasis group. ROC curve analysis revealed that the sensitivity and specificity of BSP in the diagnosis of prostate cancer bone metastases were 79.07% and 71.54%, respectively. Spondin-2 is a type of secreted extracellular matrix protein upregulated in ovarian cancer. It exhibits higher level in prostate cancer cell line compared with other cancer cell lines[18,19]. Our results demonstrated that serum Spondin-2 level in bone metastasis group was obviously higher than the non-bone metastasis group. It revealed that Spondin-2 may be related to prostate cancer bone metastasis and participate in the pathological process of bone metastases.

Our findings showed that BSP, Spondin-2, and PSA levels in low differentiation group were markedly higher than that in well differentiation group according to Gleason grading. Gleason grading has a critical role in evaluation of postoperative staging. Serum BSP, PSA, and Spondin-2 levels exhibit correlation with malignant tumor differentiation. Bone metastasis is easy to occur in higher malignancy. Prostate epithelial cells hyperplasia and blood barrier lesions lead to local blood transportation abnormal enhancement, resulting in abnormal bone marker. ROC curve revealed that diagnostic effi-

Table VI. Two indicators joint detection.

Indicator	Sensitivity/%	Specificity/%	Positive predictive value/%	Negative predictive value/%
Spondin-2+PSADT	93.02 (40/43)	79.67 (98/123)	61.53	97.02
Spondin-2+BSP	88.37 (38/43)	77.23 (95/123)	57.57	95.00
PSADT+BSP	95.35 (41/43)	88.62 (109/123)	74.54	98.20
Spondin-2+BSP+PSADT	97.67 (42/43)	90.24 (111/123)	77.78	99.11

ciency was PSADT, Spondin-2, and BSP in the order. PSADT and Spondin-2 showed relatively high sensitivity. Their joint detection elevated the sensitivity to 97.67% and the negative predictive value up to 99.11%. PSADT + BSP exhibited better efficiency among two indicators combination, indicating that joint detection can increase the sensitivity and specificity of the diagnosis of prostate cancer bone metastasis. Compared with an imaging examination, serological indicators examination can be repeated and more economical and convenient. Monitoring serum biomarkers of bone metastases contributes to finding early bone metastases and bone metabolic changes in the process of treatment. Joint detection of bone markers is conducive to the early diagnosis of prostate cancer bone metastases[20,21].

Conclusions

Serum BSP, Spondin-2, and PSADT increased in prostate cancer patients with bone metastasis. Their joint detection can improve the diagnostic sensitivity and specificity.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Chang CC, Lee YC, Tsai HW, Yii SC, Yen TH, Chu FY. Diagnostic role of serum free-to-total Prostate Specific Antigen (PSA) ratio in prostate cancer with serum total concentration of PSA below 4 ng/mL. Asian Pac J Cancer Prev 2015; 16: 5261-5264.
2) Koo KC, Park SU, Kim KH, Rha KH, Hong SJ, Yang SC, Chung BH. Predictors of survival in prostate cancer patients with bone metastasis and extremely high prostate-specific antigen levels. Prostate Int 2015; 3: 10-15.
3) Jadaan DY, Jadaan MM, McCabe JP. Cellular plasticity in prostate cancer bone metastasis. Prostate Cancer 2015; 2015: 651580.
4) Takagi T, Katagiri H, Kim Y, Suehara Y, Kubota D, Akaike K, Ishii M, Mukaihara K, Okubo T, Murata H, Takahashi M, Kaneko K, Saito T. Skeletal metastasis of unknown primary origin at the initial visit: a retrospective analysis of 286 cases. PLoS One 2015; 10: e0129428.
5) Mai ZP, Yan WG, Li HZ, Ji ZG, Zhang FQ, Hu K, Xiao Y. Clinical characteristics and outcome of Gleason score 10 prostate cancer on core biopsy treated by external radiotherapy and hormone therapy. Chin Med Sci J 2015; 30: 90-94.
6) Huang Y, Jin C, Hamana T, Liu J, Wang C, An L, McKeehan WL, Wang F. Overexpression of FGF9 in prostate epithelial cells augments reactive stroma formation and promotes prostate cancer progression. Int J Biol Sci 2015; 11: 948-960.
7) Luna A, Vilanova JC, Alcala Mata L. [Total body MRI in early detection of bone metastasis and its indication in comparison to bone scan and other imaging techniques]. Arch Esp Urol 2015; 68: 371-390.
8) Nguyen-Nielsen M, Liede A, Maegbaek ML, Borre M, Harving N, Hernandez RK, Toft Sorensen H, Ehrenstein V. Survival and PSA-markers for mortality and metastasis in nonmetastatic prostate cancer treated with androgen deprivation therapy. Cancer Epidemiol 2015; 39: 623-632.
9) Hvid T, Lindegaard B, Winding K, Iversen P, Brasso K, Solomon TP, Pedersen BK, Hojman P. Effect of a 2-year home-based endurance training intervention on physiological function and PSA doubling time in prostate cancer patients. Cancer Causes Control 2016; 27: 165-174.
10) Parra-Cabrera C, Samitier J, Homs-Corbera A. Multiple biomarkers biosensor with just-in-time functionalization: application to prostate cancer detection. Biosens Bioelectron 2016; 77: 1192-1200.
11) Moslehi M, Cheki M, Salehi-Marzijarani M, Amuchastegui T, Gholamrezanezhad A. Predictors of bone metastasis in pre-treatment staging of asymptomatic treatment-naive patients with prostate cancer. Rev Esp Med Nucl Imagen Mol 2013; 32: 286-289.
12) Pasoglou V, Michoux N, Peeters F, Larbi A, Tombal B, Selleslagh T, Omoumi P, Vande Berg BC, Lecouvet FE. Whole-body 3D T1-weighted MR imaging in patients with prostate cancer: feasibility and evaluation in screening for metastatic disease. Radiology 2015; 275: 155-166.
13) Kitajima K, Murphy RC, Nathan MA, Froemming AT, Hagen CE, Takahashi N, Kawashima A. Detection of recurrent prostate cancer after radical prostatectomy: comparison of 11C-choline PET/CT with pelvic multiparametric MR imaging with endorectal coil. J Nucl Med 2014; 55: 223-232.
14) Verburg FA, Pfister D, Heidenreich A, Vogg A, Drude NI, Voo S, Mottaghy FM, Behrendt FF. Extent of disease in recurrent prostate cancer determined by [(68)Ga]PSMA-HBED-CC PET/CT in relation to PSA levels, PSA doubling time and Gleason score. Eur J Nucl Med Mol Imaging 2016; 43: 397-403.
15) Salman JW, Schoots IG, Carlsson SV, Jenster G, Roobol MJ. Prostate specific antigen as a tumor marker in prostate cancer: biochemical and clinical aspects. Adv Exp Med Biol 2015; 867: 93-114.
16) Wang Y, Zhang XF, Dai J, Zheng YC, Zhang MG, He JJ. Predictive value of serum bone sialoprotein

and prostate-specific antigen doubling time in patients with bone metastasis of prostate cancer. J Huazhong Univ Sci Technolog Med Sci 2013; 33: 559-562.

17) Jokerst JV, Chen Z, Xu L, Nolley R, Chang E, Mitchell B, Brooks JD, Gambhir SS. A magnetic bead-based sensor for the quantification of multiple prostate cancer biomarkers. PLoS One 2015; 10: e0139484.

18) Lucarelli G, Rutigliano M, Bettocchi C, Palazzo S, Vavallo A, Galleggiante V, Trabucco S, Di Clemente D, Selvaggi FP, Battaglia M, Ditonno P. Spondin-2, a secreted extracellular matrix protein, is a novel diagnostic biomarker for prostate cancer. J Urol 2013; 190: 2271-2277.

19) Qian X, Li C, Pang B, Xue M, Wang J, Zhou J. Spondin-2 (SPON2), a more prostate-cancer-specific diagnostic biomarker. PLoS One 2012; 7: e37225.

20) Szot W, Kostkiewicz M, Zajac J, Owoc A, Bojar I. Prostate cancer in patients from rural and suburban areas-PSA value, Gleason score and presence of metastases in bone scan. Ann Agric Environ Med 2014; 21: 888-892.

21) Fizazi K, Massard C, Smith M, Rader M, Brown J, Milecki P, Shore N, Oudard S, Karsh L, Carducci M, Damiao R, Wang H, Ying W, Goessl C. Bone-related parameters are the main prognostic factors for overall survival in men with bone metastases from castration-resistant prostate cancer. Eur Urol 2015; 68: 42-50.