Original Research Article: full paper

(2017), «EUREKA: Health Sciences»
Number 5

DYNAMICS OF BONE TRAP-5B LEVEL IN PATIENTS WITH BONE METASTASES OF RENAL CELL CANCER AT COMBINED TREATMENT

Serhiy Boichuk
Department of Oncological Orthopedics, Skin Tumors and Soft Tissue Tumors
National Cancer Institute
33/43 Lomonosova str., Kyiv, Ukraine, 03022
sergeyboichuk@yahoo.com

Anatoliy Diedkov
Department of Oncological Orthopedics, Skin Tumors and Soft Tissue Tumors
National Cancer Institute
33/43 Lomonosova str., Kyiv, Ukraine, 03022
an.dedkov@gmail.com

Viktor Kostiuk
Department of Oncological Orthopedics, Skin Tumors and Soft Tissue Tumors
National Cancer Institute
33/43 Lomonosova str., Kyiv, Ukraine, 03022
doc.kostiuk@gmail.com

Vasyliy Ostafiychuk
Department of Oncological Orthopedics, Skin Tumors and Soft Tissue Tumors
National Cancer Institute
33/43 Lomonosova str., Kyiv, Ukraine, 03022
lugnik2007@gmail.com

Abstract

The objective of this study was to determine the sensitivity of tartrate-resistant acid phosphatase (Bone TRAP-5b) for early detection of bone metastases (BM) and to investigate the efficacy of bisphosphonates (BF) (zolendronic acid-ZA) in prevention of bone metastases in patients with Renal Cell Carcinoma (RCC). The 60 patients with RCC with proven BM were investigated to assess the sensitivity and specificity of Bone TRAP-5b. 95 patients with RCC with high level of tartrate-resistant acid phosphatase (Bone TRAP-5b) (8,5±0,2 IU/L) after radical surgical treatment were divided into two groups: 1-st group: (n=44) received zolendronic acid (ZA) (BF+), and 2-nd group (n=51) patients didn't receive ZA (BF–). Patients of both subgroups were similar by age, sex, stage of disease. The levels of Bone TRAP-5b, Ca++, alkaline phosphatase, LDG were accessed every 3 months, and MRI imaging, bone scan with 99mTc every 6 month in both groups. We determined the high correlation between bone TRAP-5b and the presence of bone metastases (r=0,9; p <0,05), but its level wasn't dependent with the number of BM. The results showed the high sensitivity and specificity of Bone TRAP-5b at the critical value of 5.2 IU/L (98,3 % and 90,0 %), (χ^2=64,6; p<0.01). Using BF for the prevention of bone metastases in high risk group patients with RCC provides a significant difference in the incidence of bone metastases in patients.

Keywords: tartrate-resistant acid phosphatase-5b, renal cell cancer (RCC), bone metastases, bisphosphonates, zolendronic acid, serum level.

DOI: 10.21303/2504-5679.2017.00407

1. Introduction

The metastatic tumors of musculoskeletal system are more prevalent than the primary, and take up to 96 % of all tumors of skeletal system [1]. Bone metastases are usually diagnosed in 30–50 % of cases of RCC and take the third place in frequency after lung and liver metastases. In 48 % of patients RCC is diagnosed because of BM presence [2]. Usually BM are localized in the vertebrae in 40–50 % cases, long bones in 30–40 % cases and pelvic bones in 20–30 % of all cases, beside femoral bone destruction occurs in 45–60 % of cases [3].

The BM significantly complicates the course of the disease, impairs the quality of life of patients due to the presence of severe pain, pathological fractures of the bones, significant limb dysfunction, the risk of hypercalcemia and spinal cord compression [4].

Diagnosis of BM is based both on the results of clinical and radiological methods of investigations. Both computer tomography (CT) and MRI can reveal the nature of bone structure changes, the prevalence of tumor components, their location, but despite the high informative properties of these modern methods of examination it can detect the tumor lesions when bone demineralization reaches 30–40 %, rather late stages of the disease [5].

The importance of detecting, identifying bone resorption markers is essential for prognosis of treatment effectiveness, the duration of disease-free period and overall survival of the patients [6]. The intensive search for possible biological markers of the bone resorption as predictors of bone metastases in patients with RCC has been carried out in recent years.

The main biochemical markers, usually used as indicators of bone resorption are products of degradation of collagen I and II types, N- and C-telopeptides, tartrate-resistant acid phosphatase (bone TRAP 5B), pyridinolin and deoxypyridinoline [7].

Many reports has been presented in recent years about detecting of products of degradation types 1 and 2 collagen, N- and C-telopeptides in patients with bone metastases at prostate and breast cancer, thyroid cancer as indicators of BM, especially during treatment with bisphosphonates [8].

Acid phosphatases are widely distributed enzymes from the class of hydrolases and manifested their properties to phosphorus monoetheres of orthophosphate in acidic area. It differs from other lysoforms with the resistance to the tartrate's inhibitory action, released from osteoclasts during resorption, participates in the destruction of extracellular matrix and excreted into the blood serum in right proportion regarding to the intensity of resorption processes [9]. The high activity of serum TRAP-5b reflects an increasing of intensity of bone resorption [9]. The scientific literature describes changes in the serum concentration of bone-TRAP-5b with metastatic bone lesions in patients with prostate, breast cancer, multiple myeloma, lung cancer with a sensitivity and specificity level from 85 to 95 % [10].

The high sensitivity and specificity of TRAP-5b as a predictor of bone lesions in patients during treatment with bisphosphonates was reported ranging from 71 to 90 % [11].

2. Aim of research

To determine the sensitivity of tartrate-resistant acid phosphatase (bone TRAP-5b) for early detection of bone metastases and to investigate the efficacy of bisphosphonates (ZA) in prevention of BM in patients with RCC and high risk of bone metastases.

3. Materials and methods

The bone-TRAP 5b activity was determined in patients with RCC which were divided into two groups: 1-st 60 patients with RCC with clinical and radiologically proven BM and 2-nd – 95 patients with RCC after surgical treatment with elevated level of tartrate-resistant acid phosphatase (Bone TRAP-5b) (8,5±0,2 IU/L) (**Table 1**), as the high risk of bone metastases.

Osteolytic metastases were detected in 39 (65 %) patients, osteoblastic – in 9 (15 %) patients, mixed lesions – in 12 (20 %) cases. In this group of patients, at least 1 skeletal event was registered in 18 (30,0 %) patients, two in 31 (51,7 %) cases and in 11 (18,3 %) more than 3 skeletal events were registered. Combined treatment including operative was performed in 42 (70.0 %) of patients with BM, conservative – 18 (30.0 %) of cases.

Patients from 2-nd group were divided into two groups: 1-st group: (n=44) (46.32 %) were treated with ZA (BF +), and 2-nd group (n=51) patients (53.68 %) did not receive ZA (BF–). Patients of both subgroups were similar by age, sex, stage of disease (**Table 2**).

Patients of 1-st subgroup of the main group (44 subjects) received adjuvant immunotherapy (IFN) and bisphosphonates and patients of the 2nd subgroup from the main group (51 individuals), received only adjuvant immunotherapy. The control group consists of 25 volunteers with RCC with normal ranges of TRAP-5b, treated with adjuvant immunotherapy.

Table 1

Patient's characteristic with bone metastases of Renal Cell Carcinoma

Data	Patients with BM n=60 (%)	Patients without BM n=95 (%)
Sex		
Men	45 (75,0)	62 (51,7)
Women	15 (25,0)	58 (48,3)
Mean age, y.o	56,3±1,3	55,1±1,1
Tumor stage		
T1b	2 (3,3)	6 (5,0)
T1a	14 (23,3)	32 (26.7)
T2a	12 (20,0)	23 (19,2)
T٢b	10 (16,7)	20 (16,7)
T3a	12 (20,0)	19 (15,8)
T٣b	7 (11,0)	10 (8,3)
T4	3 (5,0)	11 (9,2)
Histologic gradation		
G2	6 (10,0)	59 (49,2)
G3	39 (65,0)	51 (42,5)
G4	15 (25,0)	10 (8,3)
Lymphatic nodes		
N0	38 (64,1)	80 (67,0)
N1	19 (32,0)	29 (25,0)
N2	3 (5,1)	11 (8,0)
Lung metastases	23 (38,3)	0
Other organs	10 (16,7)	0

Table 2

Patient's characteristic without bone metastases of Renal Cell Carcinoma

Data	1-st group BF (+) (n=44)	2-nd group BF (–) (n=51)
Sex		
Men	23 (52,2)	24 (47,0)
Women	21 (47,7)	27 (52,9)
Mean age, y.o	53,1±1,9	56,1±1,6
Tumor stage		
I	13 (29,5)	13 (25,5)
II	18 (40,9)	17 (33,3)
III	12 (27,2)	20 (39,2)
IV	1 (2,3)	1(1,9)
Morphology, Fuhrman's criteria, n (%)		
II	23 (52,3)	25 (49,0)
III–IV	21 (47,7)	26 (50,9)
Bone TRAP 5b IU/l	7,3±0,3	6,5±0,2
Alkaline Phosphatase, U/l	1564,74±66,6	1827,9±217,3

Observational period, months	18,5±0,7	14,6±0,6

Zolendronic acid was administered at dose 4 mg intravenously monthly (every 28 days) under the control of tartrate-resistant acid phosphatase every three months, within 6 months after the normalization of bone TRAP 5b BF was discontinued.

The levels of alkaline phosphatase (U/L), serum calcium (mmol/l), lactate dehydrogenase (U/l) were accessed using modern, generally accepted methods in the biochemical and immunological laboratories of the National cancer institute;

The quantitative determination of TRAP-5b active isoform in serum samples was performed by immunoassay (ELISA) method with monoclonal antibodies specific for TRAP-5b osteoclasts according to the Bone TRAP® Assay kit, SB-TR 201A series, production USA. The measurement wavelength was 405 nm, measuring range – 0,5–10 units/l, sensitivity – 0,5 units/l.

All biochemical values were accessed every 3 months. The CT and MRI imaging, bone scan with 99mTc were performed every 6 months.

4. Results

As shown in **Table 3** the levels of alkaline phosphatase (APh), serum calcium (Ca++) and lactate dehydrogenase (LDH) concentrations didn't differ significantly in different groups ($p > 0.05$).

The TRAP-5b serum level varied from 5.3 to 16.2 units/l in the first group and from 3.5 to 10.2 IU/l in the second group. It was noticed that the bone TRAP-5b concentration in patients of 1-st group (9.8±0.3) IU/l on average was significantly higher than in patients of the second group – (6.1±0.1) units/l and with practically healthy subjects of the control group – (2.9±0.3) IU/l on average $p < 0.05$ (**Table 3**).

Table 3

Biochemical Markers of Bone Turnover

Marker	Patients		Control group (n=20)
	I-st group with BM (n=60)	2 nd group without BM (n=95)	
BoneTRAP-5b IU/l	9,8±0,2*	6,1±0,1*	2,9±0,3
Alcaline Phosphatase U/l	1890,6±100,3	1651,01±97,6	1458,6±88,3
Ca^{++} mmol/l	2,3±0,01	2,3±0,01	2,3±0,03
LDG U/l	608,7±29,1	430,1±18,8	423,5±37,7
Observation, months	18,5±0,7	14,6±0,6	18,4±1,2

*Note: * – p<0,05 with CG*

Despite the absence of skeletal metastatic lesion in patients of the second group, the bone TRAP-5b concentration was also significantly higher comparing with control group ($p \leq 0.05$).

The maximum sensitivity and specificity of Bone TRAP-5b we determined at value of 5.2 IU/L (98,3 % and 90,0 %), ($\chi^2=64,6$; $p<0.01$). When the concentration increased up to 9.8 IU/l, the sensitivity decreased to 26,7 % ($\chi^2=6,6$; $p=0.01$). We determined high correlation between levels of boneTRAP-5b and presence of bone metastases ($r=0,9$; $p < 0,05$), but its level didn't dependent with the number of BM. There was no correlation of alkaline phosphatase, LDH with the presence of bone lesions ($r=0,19$; $p<0,05$) (**Table 4**).

The mean level of TRAP-5b was elevated to 8,5±0,2 IU/l in all patients. The average period of follow-up was 18.5 months. During the treatment period in patients of 1st subgroup (BF+) we received the marked tendency to normalization of bone TRAP-5b from (7,3±0,7) IU/L to (5,7±0,6) IU/l after 18 months.

In patients of 2nd subgroup (BF–) we observed the increase of bone TRAP-5b from (6,40±0,4) IU/L, (8,3±0,4) IU/l, (8,1±0,3) IU/l, on average by 6, 9 and 18 months follow-up respectively (**Fig. 1**).

Table 4

The Sensitivity and Specificity of Bone TRAP-5b

Critical value	The Sensitivity, % (95 % CI)	The Specificity, % (95 % CI)	χ^2	P
5,2	98,3 (92,9–99,9)	90,0 (73,8–94,7)	64,6	<0,01
6,1	93,3 (87,3–96,0)	90,1 (72,0–98,0)	52,2	<0,01
7,1	76,7 (70,0–79,4)	90,0 (70,1–98,2)	27,7	<0,01
9,8	26,7 (21,0–26,7)	99,8 (83,0–100,0)	6,6	=0,01

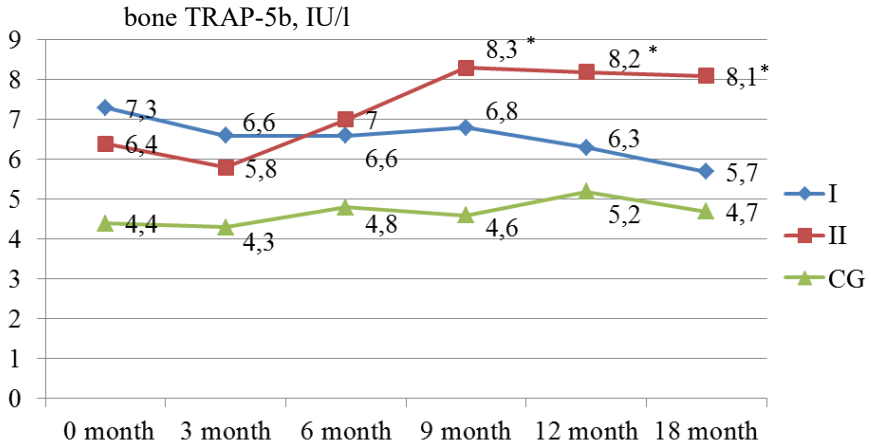

Fig. 1. The BoneTRAP-5b dynamics during the treatment with ZA every 4 week regimen (IU/l)

The significant difference was observed in the incidence of BM in patients of (BF+) and (BF–) groups (20.5 and 54.9 %) respectively, χ^2=11,78; p <0.05; but time of appearance of BM in patients did not differ between the two subgroups (7,8±0,9) and (7,8±0,4) months (**Fig. 2**).

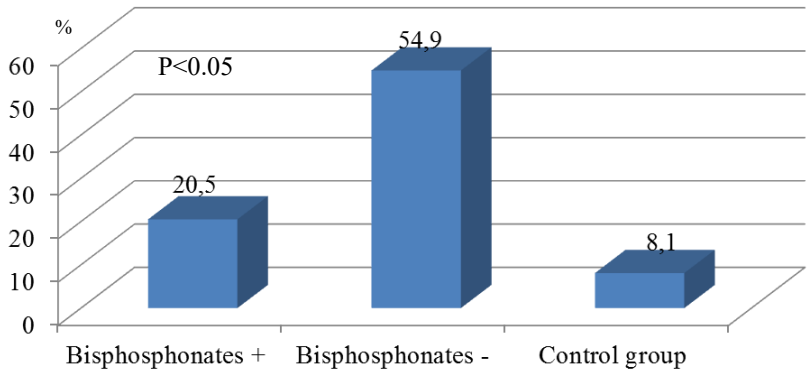

Fig. 2. Frequency of appearance of bone metastases in patients during the treatment with BF

5. Discussion

The metastatic lesions of a skeleton become a serious problem of the modern oncology. Nearly in one-third part of patients the RCC is usually diagnosed with synchronous metastatic process and in 20 % progression of the disease occurs one month after nephrectomy, according to our results, kidney cancer was diagnosed simultaneously with bone metastases in 23.3 % of patients [12].

The main goal of the treatment strategy of these patients is increasing of life expectancy, because the average survival rate for patients with RCC with multiple BM is 12 months, where 50 % of patients dying within 10 months and only 10 % of patients have five years survival rate [12].

The surgical treatment is mainly performed with symptomatic aim to decrease and to prevent complications, associated with the destruction of bone tissue. In patients with BM we have diagnosed multiple bone lesions three times more often than in the case of a solitary cancer – 73.0 % and 27.0 % respectively, moreover, in most of them, there was a pathological fracture (44.0 %), and it's threat was diagnosed in 27.0 % of patients.

The scientific interest to bone resorption markers in patients with RCC has been increased in recent years [9]. Numerous studies were devoted to the influence of metastatic cells on the bone tissue and searching for biochemical markers of its destruction. Ozu C. et al. [13] argue that the alkaline phosphatase and bone TRAP-5b have a high ability to detect early bone lesions of patients with breast cancer – in almost 70 % of cases.

Smith M. et al. [14] in the study with 1,468 patients with prostate cancer demonstrated the reliable effectiveness of an increased concentration of bone TRAP-5b in the early diagnosis of BM and its effectiveness on bisphosphonates treatment.

Our results obtain a close correlation between elevated levels of TRAP-5b and the presence of metastatic lesions ($r=0.9$; $p<0.05$), without dependence of its level on the number of bone lesions.

We investigated the sensitivity and specificity of the marker at its various critical values and the optimum value of Bone TRAP-5b level was determined at 5.2 units/l. It has been shown that the sensitivity of the marker has decreased from 98.3 % (95 % CI (92.9–99.9) to 26.7 % (95 % CI (21.0–26.7), with its lowest value established at the bone TRAP concentration at 9.8 units / l, the specificity of the marker remained at 90 (95 % CI (73.8–94.7) – up to 99 % (95 % CI (85–100 %).

However, in his observations, R. Preussner (2014) found out that the sensitivity and specificity of TRAP-5b in patients with RCC was ambiguous: the sensitivity of marker was reduced to 47 % with a critical marker level rising from 5 units/l to 50 units/l, but the specificity elevated from 36.7 to 96.7 % [9].

Korpela J. et al. (2006) provided a study that illustrates the significant growth of TRAP-5b with the presence of bone metastases in patients with breast carcinoma, with the results confirming a sensitivity of 87 % and a specificity of 62 % at a bone TRAP-5b serum level of 3.65 units/l [15]. According to Yao N. S., the diagnostic value of TRAP-5b with a sensitivity of 63.9 % and a specificity of 76.8 % is important in patients with small cell lung cancer at a concentration of 2.55 units/l [16].

Thus our results coincide with the literature data, as we have shown a significant increase of TRAP-5b serum level in patients with bone metastases, comparing to the other group.

Thus taking into account the presence of pathological resorption of bone tissue bisphosphonates (BF) have a key role in the treatment and prevention of possible skeletal events [17].

Zaghloul M.S., Boutrus R. (2010) demonstrated 12-month survival increase in patients with BM of bladder cancer within the treatment with zolendronic acid [18].

In our study, the main group of patients (with a serum level of boneTRAP-5b more than 5.2 units/l) 3 months later the introduction of zolendronic acid the bone TRAP -5b decrease was to 9 % comparing with the baseline, and at the end of the study it was (5.7±0.6) IU/l. In the second subgroup of patients, received adjuvant therapy only, bone TRAP-5b was (7.0±0.3) IU/L and (8.1±0.4) at 6 and 18 months respectively ($p=0.03$).

It should be noted that patients of main group who had experienced BM in the future the average level of TRAP-5b was increased to 29 % comparing with the control group nearly 6 months before their radiological confirmation (8.7±0,7) IU/l and (6.8±0.3) IU/l.

Consequently, the obtained data show the high diagnostic sensitivity and specificity of TRAP-5b as a marker of metastatic skeletal defeat in patients with RCC.

The determination of the level of this marker in patients with RCC provides the opportunity to determine the risk group of patients with the possible development of skeletal lesions, which in general determines the patient's survival [19].

The high sensitivity of the marker can increase the accuracy of the early preclinical diagnosis of skeletal metastasis, assessing the degree of metastatic process, as well as monitoring the effectiveness with BF treatment [20].

The study shows promising feasibility in finding and developing adequate methods for predicting the onset of BM in patients with RCC, as well as in treatment monitoring.

6. Conclusions

1. The diagnostic sensitivity and specificity of bone TRAP-5b as a marker of metastatic lesions was proven in patients with skeletal lesions in RCC at its various meanings: the highest sensitivity and specificity levels obtained by bone TRAR-5b level (5,1–5,3) IU /l (98,3 and 90.0 % respectively). When boneTRAP-5b level was 7,1 IU/l sensitivity was 76.7 %; (95 % CI (70,0–79,4)) and specificity was 90.0 %; (95 % CI (70,1–98,2)); χ^2=27,7; p <0.01.

2. The mean critical value of bone TRAP-5b was (5,2 IU/l) with a high probability of bone lesions appearance in patients with RCC after the radical surgery of the primary tumor.

3. There was a significant difference in the incidence of bone metastases in patients with (1st subgroups (BF+) and 2nd subgroup (BF–) – 20.5 and 54.9 % respectively; p <0.05).

4. The administration of 4 mg of zolendronic acid in q4 week regimen in patients with elevated levels of bone TRAP-5b leads to normalization of its serum level within 18 months.

5. The ZA administration in patients with RCC with the high risk of bone lesions provides a significant difference in the incidence of bone metastases in patients with (1st subgroups (BF+) and 2nd subgroup (BF–) – 20.5 and 54.9 % respectively; p <0.05).

6. BF can prevent and reduce the risk of skeletal lesions and improve patient's outcomes.

References

[1] Volkov, N. (2011). The Mechanism of bone metastases development. Practical oncology, 12 (3), 97–102.

[2] Alekseev, B., Kaplinskiy, A. (2009). The new options for target therapy of the metastatic kidney cancer. Onkourology, 3, 8–12.

[3] Semkov, A., Mahson, A., Peterson, S. et. al. (2010). The surgical treatment of bone metastases of kidney cancer. Oncourology, 4, 10–15.

[4] Bolshakova, S. (2011). The skeletal metastases of breast cancer: a mechanism of development, complications, a modern view of the combination therapy with bisphosphonates and radiotherapy. The Bulletin of the Russian Scientific Center of Roentgenology, 3 (11). Available at: http://vestnik.rncrr.ru/vestnik/v11/papers/bolsh_v11.htm

[5] Reinus, W. R., Khurana, J. S. et. al. (2010). Diagnostic Imaging of Musculoskeletal Diseases. A Systematic Approach. Philadelphia: Springer science+Business media, LLC, 302–308.

[6] Snegovoy, A., Mandzyuk, L. (2011). The importance of biomarkers for determination of the treatment options and prognosis of malignant tumors. Practical Oncology, 2 (4), 166–170.

[7] Lyubimova, N. V., Kushlinskiy, N. E. (2015). Biochemical markers of bone metastasis. Advances in Molecular Oncology, 2 (1), 61–75. doi: 10.17650/2313-805x.2015.2.1.61-75

[8] Lyubimova, N. V., Kozharskaya, G. V., Portnoi, S. M., Kushlinskii, N. E. (2014). Biochemical Markers of Bone Metabolism in Breast Cancer. Bulletin of Experimental Biology and Medicine, 157 (6), 769–772. doi: 10.1007/s10517-014-2663-1

[9] Preussner, R., Sauer-Eppel, H., Oremek, G. (2014). Tartrate-resistant acid phosphatase 5b as a diagnostic marker of bone metastases in patients with renal cell carcinoma. Integrative Cancer Science and Therapeutics, 1 (3), 35–38.

[10] Chung, Y.-C., Ku, C.-H., Chao, T.-Y., Yu, J.-C., Chen, M. M., Lee, S.-H. (2006). Tartrate-Resistant Acid Phosphatase 5b Activity Is a Useful Bone Marker for Monitoring Bone Metastases in Breast Cancer Patients after Treatment. Cancer Epidemiology Biomarkers & Prevention, 15 (3), 424–428. doi: 10.1158/1055-9965.epi-04-0842

[11] Wu, Y.-Y., Janckila, A. J., Ku, C.-H., Yu, C.-P., Yu, J.-C., Lee, S.-H. et. al. (2010). Serum tartrate-resistant acid phosphatase 5b activity as a prognostic marker of survival in breast cancer with bone metastasis. BMC Cancer, 10 (1), 158. doi: 10.1186/1471-2407-10-158

[12] Santoni, M., Conti, A., Procopio, G., Porta, C., Ibrahim, T., Barni, S. et. al. (2015). Bone metastases in patients with metastatic renal cell carcinoma: are they always associated with poor prognosis? Journal of Experimental & Clinical Cancer Research, 34 (1), 10. doi: 10.1186/s13046-015-0122-0

[13] Ozu, C., Nakashima, J., Horiguchi, Y., Oya, M., Ohigashi, T., Murai, M. (2008). Prediction of bone metastases by combination of tartrate-resistant acid phosphatase, alkaline phosphatase and prostate specific antigen in patients with prostate cancer. International Journal of Urology, 15 (5), 419–422. doi: 10.1111/j.1442-2042.2008.02029.x

[14] Smith, M. R., Cook, R. J., Coleman, R., Brown, J., Lipton, A., Major, P. et. al. (2007). Predictors of Skeletal Complications in Men with Hormone-Refractory Metastatic Prostate Cancer. Urology, 70 (2), 315–319. doi: 10.1016/j.urology.2007.03.071

[15] Korpela, J., Tiitinen, S. L., Hiekkanen, H. et. al. (2006). Serum TRACP 5b and ICTP as a markers of bone metastases in breast cancer. Anticancer Research, 26, 3127–3132.

[16] Yao, N.-S., Wu, Y.-Y., Janckila, A. J., Ku, C.-H., Hsieh, A.-T., Ho, C.-L. et. al. (2011). Serum tartrate-resistant acid phosphatase 5b (TRACP5b) activity as a biomarker for bone metastasis in non-small cell lung cancer patients. Clinica Chimica Acta, 412 (1-2), 181–185. doi: 10.1016/j.cca.2010.09.038

[17] Volosnev, L. V. (2013). The mechanismes of metastatic skeletal lesions and role of treatment with N-bosphosphonates. Oncology. P. A. Hertzen' Journal, 1, 73–77.

[18] Zaghloul, M. S., Boutrus, R., El-Hossieny, H., Kader, Y. A., El-Attar, I., Nazmy, M. (2010). A prospective, randomized, placebo-controlled trial of zoledronic acid in bony metastatic bladder cancer. International Journal of Clinical Oncology, 15 (4), 382–389. doi: 10.1007/s10147-010-0074-5

[19] Sahi, C., Knox, J. J., Clemons, M., Joshua, A. M., Broom, R. (2010). Renal cell carcinoma bone metastases: clinical advances. Therapeutic Advances in Medical Oncology, 2 (2), 75–83. doi: 10.1177/1758834009358417

[20] Nenonen, A., Cheng, S., Ivaska, K. K., Alatalo, S. L., Lehtimaki, T., Schmidt-Gayk, H. et. al. (2005). Serum TRACP 5b Is a Useful Marker for Monitoring Alendronate Treatment: Comparison With Other Markers of Bone Turnover. Journal of Bone and Mineral Research, 20 (10), 1804–1812. doi: 10.1359/jbmr.050403