Bone Metabolism of the Patient with a Malignant Melanoma during the Entry Examination and the Check-up of Whole-body Bone Scintigraphy

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Abstract: Malignant melanoma is a malignancy located predominantly in the skin and the incidence of melanoma increases. We compared the markers of bone metabolism – osteocalcin (OC), beta-carboxyterminal cross-linked telopeptide of type I collagen (β-CrossLaps, β-CTx) and tumour marker – human epididymis protein 4 (HE4) in the serum with finding during the entry examination and the check-up of whole-body bone scintigraphy of the patient with a malignant melanoma. Serum concentrations of OC, β-CTx, HE4 were determined in 1 patient (female, age 64 years) with malignant melanoma and correlated with the presence of equivocal bone metastases detected by whole-body bone scintigraphy (the entry examination and check-up after 6 months). Concentrations of bone metabolism markers decreased during six months and we observed progress in bone metastases. The change of the markers levels during the entry examination and the check-up of the whole-body bone scintigraphy with equivocal finding of bone metastases could be a sign of a possible initiating progression of malignant melanoma despite a clinically negative finding that does not prove the progression of the disease.

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Introduction

Malignant melanoma, a neoplasm of melanocytes or a neoplasm of the cells that develop from melanocytes is the most dangerous type of skin cancer (Dummer et al., 2012). The etiology of malignant melanoma is combination of sun exposure (particularly UVB radiation), genetic and environmental influences (Koh et al., 1993). Metastases develop in regional lymph nodes, as satellite or in-transit lesions or in distant organs (Zbytek et al., 2008). The lung is most commonly involved followed by brain, liver, bone marrow, and intestine (Deepali et al., 2007). Bone marrow metastases of melanoma are in 5–7% of patients with disseminated disease but in up 45% when an autopsy-staging procedure is performing (Serrier and Lesesve, 2013). Typical bone metastases are osteolytic with medullary origin. Lesion growth caused erosion and destruction, pathologic fractures and soft-tissue involvement. Atypical skeletal metastases exhibit as a mixed osteolytic-osteoblastic pattern or hardly ever as a completely osteoblastic (Potepan et al., 1994; Brountzos et al., 2001). Whole-body bone scintigraphy is commonly used for detection of bone metastases in cancer patients and it is more sensitive than X-rays. It is nuclear imaging procedure, offering of total body examination, low cost, and high sensitivity (Brenner et al., 2012). The recording is performed 2 to 4 hours after intravenous application of radioactive tracer (Technecium-99m methylene disphosphonate, Tc-99m MDP) with a double head gamma camera as a whole-body scan in anterior and posterior projection (Kane et al., 2013; Ghosh, 2014). Osteocalcin (OC) is the most important noncollagen protein in bone matrix, accounts for approximately 1% of the total protein in human bone and OC is produced by osteoblasts and is widely accepted as a marker of bone osteoblastic activity. Elevated levels of osteocalcin indicate metabolic bone disease including osteoporosis, osteomalatia, hyperparathyroidism, renal osteodystrophy, thyrotoxicosis, fractures and bone metastasis (Kardamakis et al., 2009). Osteoclasts secrete during bone resorption a mixture of acid and neutral proteases that degrade the collagen fibrils into molecular fragments including carboxyterminal cross-linked telopeptide of type I collagen (CTX). As bone ages, the alpha form of aspartic acid present in CTX converts to the beta form. Beta form is released into the bloodstream and serves as a specific marker for the degradation of nature type I collagen. Beta-carboxyterminal cross-linked telopeptide of type I collagen (β-CTx) levels are increased by bone resorption and are associated with osteoporosis, osteopenia, Paget's disease, hyperparathyroidism and hyperthyroidism (Biver, 2012). Human epididymis protein 4 (HE4), also known as WFDC2 (WAP four – disulphide core domain 2), is a secretory protein detectable in human serum and was originally identified in human epididymis (Li et al., 2013; O'Neal et al., 2013). This protein is a useful tumour marker for ovarian cancer and endometrial cancer. Recent studies have suggested that HE4 is overexpressed also in other malignant tissues and in a number of tumour cell lines including melanoma cell line (Ross et al., 2000; Garber et al., 2001; Ryu et al., 2002; Escudero et al., 2011; Iwahori et al., 2012; Karlsen et

al., 2014). Human epididymis protein 4 may be a novel, useful tumour marker and the increased concentration of HE4 may be a marker for metastasis and a negative prognostic marker for patients after chemotherapy.

Case report

A 64-year-old patient (woman, Caucasian) was examined at the Department of Dermatology with a suspected malignant melanoma of skin (the diameter of 6 mm) on the lateral side of the right arm and a malignant melanoma of skin (the size of 12×6 mm) on the medial side of the right arm. Lymph node on the neck and in the axillae were not palpable. The lymph nodes were not affected during the sonographic examination of the axillae and the neck; struma nodosa bilateral present, gallstones present during the sonography of stomach, steatosis of liver, hepatopathy. Myopathic, reciprocally dilated heart and multiplied bronchovascular marking on an X-ray picture of the chest. She fought off common childhood disease, embolisation into lungs. She undergoes a long-term treatment of arterial hypertension and she is being observed for the struma within a replacement therapy. The value of protein S-100 was 0.093 (standard: <0.105). The total extirpation of malignant melanoma with a 10mm-protective edge recommended by the Melanoma Committee. The intervention was realized in local anaesthesia. The histological examination of dermal lesion situated on the lateral side of the right arm discovered a regressively changed malignant melanoma and a melanocyte lesion (nevus) with epithelioid features and atypical junction proliferation from the dermal lesion on the medial side of the right arm. The proliferation activity of the cells was examined in the immune-chemical way Ki-67 and a higher share of cells expressing Ki-67 was detected in the middle and upper layers of the lesion, less in the base. The wounds were healed with no complications after a surgical intervention. During the examination by computed tomography (CT) – without deposit changes of the metastases character on brain and thoracic and abdominal organs. Older hypodense deposits of the post-ischemic lesions nature and moderate cerebral atrophy were present on the brain in the front part and in the area of basal ganglia on the left. There was also a finding of hypodense deposit in the right lobe of the thyroid gland and a calcified wall of the gallbladder. The entry examination of the whole-body bone scintigraphy was realized 1 month after the operation. The recording was acquired 2 hours after intravenous application of 600 MBq of radioactive tracer Tc-99m – MDP as a whole-body scan of the skeleton in anterior and posterior projection with scanning speed of 10 centimetres per minute. There was the finding of equivocal bone metastases in the bones of the proximal part of the right forearm. Before the examination itself we realized the blood collection, we acquired the serum from a peripheral vein that was consequently stored in sterile plastic test tubes at –50 °C. A check-up was realized 6 months after the entry examination of the whole-body bone scintigraphy with a finding of new equivocal bone metastases in the area of Th7 vertebra and a

moderate regression of the finding in the bones of the proximal part of the right forearm was detected. The blood was again collected and serum stored. Then the patient was observed at the Department of Oncology and Dermatology. The scars after the surgical intervention are unchanged with no palpable resistance. The serum acquired from entry examination and the check-up was used at the workplace of Clinical Biochemistry for the determination of osteocalcin, beta-carboxyterminal cross-linked telopeptide of type I collagen and human epididymis protein 4 in the serum by electrochemiluminescent immunoanalysis – ECLIA method (Elecsys, Roche Diagnostics, at the Department of Clinical Biochemistry, University Hospital in Martin, Comenius University in Bratislava, Slovakia) (Hasanbegovic et al., 2015). Reference values: OC women: 15–46 ng/ml, β-CTx women: 0.33–0.78 ng/ml, HE4: ≤140 pmol/l.

Results

The concentrations of bone metabolism markers in the serum of the patient with a malignant melanoma were: OC: 25.22 ng/ml, β-CTx: 0.513 ng/ml and the concentration of HE4 was 55.33 pmol/l, after the first entry examination of the whole-body bone scintigraphy, and the concentrations were after the second check-up: OC: 17.99 ng/ml, β-CTx: 0.321 ng/ml, HE4: 67.76 pmol/l (Table 1).

We observed the reduction of the concentration of the markers of bone metabolism of OC and β-CTx and the moderate increase of the HE4 concentration comparing the concentrations of OC, β-CTx and HE4 in the serum during the entry examination and the check-up of the whole-body bone scintigraphy with equivocal finding of bone metastases.

Table 1 – The concentrations of osteocalcin (OC), beta-carboxyterminal cross-linked telopeptide of type I collagen (β-CrossLaps, β-CTx) and human epididymis protein 4 (HE4) in the serum during the entry examination and the check-up scintigraphic examination of the patient with the malignant melanoma

Examination	Bone scintigraphy	OC (ng/ml)	β-CTx (ng/ml)	HE4 (pmol/l)
Entry examination	Equivocal metastases in the bones of the forearm	25.22	0.513	55.33
Check-up after 6 months	Equivocal metastases in the area of the Th7 vertebra, moderate regression in the bones of the forearm	17.99	0.321	67.76

Th – thoracic vertebra; referential values: OC women: 15–46 ng/ml, β-CTx women: 0.33–0.78 ng/ml, HE4: ≤140 pmol/l

Conclusion

We observed increased bone metastasis by whole-body bone scintigraphy in patient with melanoma after one month after total extirpation of lesion and bone metastasis progressed after next six months. The changes of the bone metabolism markers levels were observed during six months as well and could be a sign of a possible initiating progression of malignant melanoma, either in a local or distant form of bone metastases and the metastases in the structures of soft tissues and organs despite a clinically negative finding that does not prove the progression of the disease. It would be appropriate to examine the set containing a higher number of patients with a malignant melanoma in order to evaluate the finding more precisely as well as to observe them during a longer period and correlate the given parameters in the serum with the finding during the whole-body bone scintigraphy. There is no available information about the determination and observation of these parameters in the serum of the patients with a malignant melanoma in the literature. Des Grottes et al. (2001) observed the increased concentration of osteocalcin in patients with melanoma and Escudero et al. (2011) observed the increased concentration of HE4 in the case of the group of 9 patients with a malignant melanoma.

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