Immunohistochemistry in diagnosis and surgical treatment of femoral bone metastasis

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Abstract

Background: When the primary tumor is unknown, the biopsy and the histopathological examination (associated with immunohistochemistry and molecular profiling) will identify the type and subtype of the tumor and, if possible, the site of origin. The classification in type and subtype will allow to assess the prognosis and to indicate the most appropriate therapeutic approach. Aim: Identification of the unknown primary tumor by biopsy and immunohistochemistry from the femoral bone metastasis, the clinical results and the survival rate after osteosynthesis ± cement stabilization of the lesion. Patients and Methods: 52 patients with femoral metastasis were included in a prospective study. The gender ratio was 30 women/22 men (average age – 64 years and six months; range: 33–82 years). Thirty-three patients had pathologic bone fracture, while 19 had osteolytic lesions without fracture. The mean follow-up for survivors was 34 months (range: 17–56 months). Surgical treatment consisted in hemiarthroplasty, osteosynthesis with DHS (Dynamic Hip Screw), proximal femoral nail, locked centromedullary nail or DCS (Dynamic Condylar Screw) ± cement. In 19 (36.54%) cases, the primary tumor was not known. In these cases, biopsy and histological examination with immunohistochemistry were performed. Twenty-three (52.27%) patients underwent chemotherapy. Clinical and radiological check-ups were performed every three months in the first year and every six months after that. Results: Pain was ameliorated in all cases. Deambulation was achieved in 45 out of 52 (86.54%) patients. Survival rate was 76.92% (40/52) at six months and 59.61% (31/52) at 12 months. At the end of the follow-up period, 18 (34.61%) patients were alive, 24 (46.15%) were deceased and 10 (19.23%) were lost to follow-up. The survival rate was 33.33% (9/27) in the pathologic fracture group and 60% (9/15) in the osteolytic lesion without fracture group. Conclusions: Most of the patients (86%) could be mobilized immediately after surgery. The expected survival rate one year after surgery is around 60%. The goals of osteosynthesis are the same, regardless the location of the lesion and the implant used: pain amelioration, appropriate stability for immediate full weight bearing, durability for patient’s life expectancy. All extended osteolytic lesions must be reinforced at the time of the surgical procedure. The presence of a pathologic fracture is a negative prognosis factor for the medium term survival rate.

Keywords: bone metastasis, femur, biopsy, immunohistochemistry.

Introduction

Skeletal metastasis is quite common, especially in some types of malignant tumors. Breast, lung, thyroid, renal cancer and prostate carcinoma are known to provide the highest rate of bone metastasis [1–4]. Unfortunately, in some cases, the skeletal metastatic lesion (with or without fracture) may be the first sign of a cancer, with unknown primary tumor [5, 6]. When such a condition occurs, the patient undergoes to an advanced medical investigation protocol, in order to detect the primary tumor. Even so, in a number of cases this tumor cannot be detected. Biopsy from the bone metastatic lesion and histological examination of this tissue may be of great help for the diagnosis of these tumors.

The aim of this study consists in identification of the unknown primary tumor by biopsy and immunohistochemistry from the femoral bone metastasis and analysis of the clinical results and the survival rate after osteosynthesis ± cement stabilization of the lesion.

Patients and Methods

Fifty-two patients with femoral metastasis were included in a prospective study. The gender ratio was 30 women/22 men (average age – 64 years and six months; range 33–82 years). Thirty-three patients had pathologic bone fracture, while 19 had osteolytic lesions without fracture (Figure 1).

Figure 1 – Multiple metastatic osteolytic lesions of the femur: preoperative and postoperative antero-posterior X-ray views.

The mean follow-up for survivors was 34 months (range: 17–56 months). Surgical treatment consisted in hemiarthroplasty, osteosynthesis with DHS (Dynamic Hip Screw), proximal femoral nail, locked centromedullary nail or DCS (Dynamic Condylar Screw) ± cement.
In 19 (36.54%) cases, the primary tumor was not known. In these cases, biopsy and histological examination with immunochemistry were performed. If a closed centromedullary osteosynthesis was indicated for fixation, the biopsy was performed through a small incision on the lateral aspect of the thigh.

All patients underwent pulmonary X-ray and abdominal ultrasound examination. When the primary tumor was unknown and the pulmonary X-ray views and the abdominal ultrasound exam did not identify any tumoral lesion, thoracic and abdominal CT-scans were indicated.

Bone scintigraphy was performed in 11 cases. Scintigraphy identified other osseous metastatic determinations and helped the surgeon to assess the extent of the femoral metastasis. The latter imagistic method showed other occult femoral lesions in four cases. In these cases, the osteosynthesis was extended in order to bypass and fix both lesions (the radiological visible osseous lesion and the radiological occult one). When the primary tumor could not be identified by imagistic methods, its diagnosis was based solely on histological examination with immunochemistry.

Twenty-three (52.27%) patients underwent chemotherapy. Clinical and radiological check-ups were performed every three months in the first year and every six months after that.

**Results**

Pain was ameliorated in all cases. Deambulation was achieved in 45 out of 52 (86.54%) patients. Survival rate was 76.92% (40/52) at six months and 59.61% (31/52) at 12 months. At the end of the follow-up period, 18 (34.61%) patients were alive, 24 (46.15%) were deceased and 10 (19.23%) were lost to follow-up. After eliminating the patients lost to follow-up, the survival rate was 33.33% (9/27) in the pathologic fracture group and 60% (9/15) in the osteolytic lesion without fracture group. Of the 19 cases in which the primary tumor was unknown, imagistic examinations successfully diagnosed it in seven (36.84%) cases: four pulmonary tumors, one kidney tumor, one liver tumor, one colonic tumor. Histopathology examination from the metastatic tissue clearly identified the primary tumor in two (10.53%) cases (one thyroid tumor, one melanoma), in which imaging examinations were unsuccessful and also confirmed the primary tumor identified by imaging in five (26.31%) cases. Immunohistochemistry diagnosed the primary tumor in seven (36.84%) cases (clear cell renal carcinoma – one case, prostate adenocarcinoma – one case, breast ductal carcinoma – one case), and in three (15.79%) cases the primary tumor remained unidentified. In our series, the immunohistochemistry-limited number of kits interfered with its ability to identify the primitive tumor (Figures 2–7).
Discussion

Almost all malignant tumors have the ability to provide bone metastases and the site may be any bone of our skeleton. Approximately 50% of malignant tumors show bone metastases at necropsy. Bone metastases are the most common condition of destructive bone lesions in adults. After the lungs and the liver, bone is the third most common location of metastases. Carcinomas that most commonly cause bone metastases are breast, lung, thyroid, renal and prostate cancers. They occur more frequently after the fourth decade of life.

The most common sites are the axial skeleton (vertebrae, ribs, pelvis) and proximal segments of the limbs (femur and humerus). Bone metastases rarely occur in the hands and feet, but lung carcinomas and melanomas can cause such lesions [7].

Depending on the type of the primary tumor, the most common skeleton localizations are:

- Prostate: spine, femur, pelvis, skull, ribs, sternum;
- Renal: humerus, spine, femur, pelvis, ribs, foot;
- Thyroid: skull, ribs, sternum, spine, humerus;
- Breast: spine, pelvis, proximal femur, skull, ribs, humerus;
- Lung: thoracic spine, ribs [8].

The population growth in absolute numbers, the increase in life expectancy, the increase of older segments of the population and the continuous exposure to various oncogenic factors, all lead to an increased incidence of malignant tumors. Therefore, as these facts become more and more common, the orthopedic surgeon must treat pathological fractures secondary to bone metastases or metastatic bone lesions, which threaten the structural integrity of bone and induces an imminent risk of fracture.

In front of a metastatic bone lesion (+ pathologic fracture), the orthopedic surgeon’s objectives are:

- diagnosis of the primary lesion (if not already known) and referring the patient to the oncologist in order to treat it;
- assessment of the patient general health status and exact determination of the lesion (type, location, size, associated injuries);
- treatment of the bone lesion (if the general status and associated pathology of the patient allows it) in order to achieve sufficient stability of the fixation site, allow immediate loading of the limb and enough durability for the remaining life expectancy of the patient.

Surgical treatment goals are: stopping or slowing bone tumor progression, pain control, mobilization of the segment (walking with support for the lower limb) and maintaining patient’s quality of life as long as possible.

In women, the main malignant tumors that metastasize in the skeleton, are cancers of the breast and lungs (they are responsible of about 80% of bone metastases), while in men, the most common sources of bone metastases are lung and prostate cancers (approximately 80% of bone metastases). The remaining percentages are represented by metastases from renal, gastrointestinal, thyroid and other tumors.

Bone tumor imaging examination consisted in antero-posterior and lateral view radiographs of the femur (performed in all cases), to which, in some cases, CAT scan, MRI and angiography of the respective segment were added. At X-ray examination, bone metastases may appear as osteolytic or osteocondensant lesions, or a combination of the two. Namely, renal and thyroid tumors produce osteolytic metastases, lung and breast tumors may lead to mixed osteolytic/osteocondensant bone lesions and metastatic prostate tumors appear osteocondensant.

If the primitive tumor is already known and under treatment at the time when bone metastasis is diagnosed, the oncologist must be immediately informed about the new condition in order to adjust his therapy. If bone metastasis is the first manifestation of a malignant tumor, besides bone lesion treatment (osteosynthesis), efforts are made to identify the primitive tumor. In our series, the steps followed were: complete medical history and medical examination (breast, rectal examination – prostate/rectum, thyroid, etc.), biochemical panel, chest X-ray, abdominal ultrasound. In some cases, we performed thorax, abdomen and pelvis CAT scans, whole skeleton scintigraphy, mammography, prostate antigen determination (PSA and free PSA), thyroid scintigraphy and ultrasound, etc. We did not perform any PET-CT. Rougraff et al. [9] proposes a simple imaging protocol consisting of the aforementioned, which would allow the identification of the primitive tumor in 85% of cases.

In all cases where the primitive tumor was unknown,
tissue fragments from the bone metastasis were collected for pathology and immunohistochemistry. Pathology examination goals were: to determine whether we are in front of a benign/malignant lesion, tumor type differentiation (carcinoma, sarcoma, melanoma, lymphoma, myeloma, etc.) and to identify the origin site of malignancy.

If pathology reveals an adenocarcinoma structure and imaging tests do not detect any tumor, then most likely a lung cancer is the primitive tumor.

The immunohistochemical examination helps to identify the primary tumor by using a large panel of antibodies [10]. They can be located on the cell membrane, in the cytoplasm and in the nucleus. The method uses both positivity antibodies and negativity antibodies.

Depending on the type of the primitive tumor, the immunohistochemical markers initially make a differential diagnosis between tumors with different embryological origins. Hence, carcinoma which derives from ectoderm is positive for cytokeratin (CK), carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA), etc., melanoma is positive for S100 protein (normally found in cells derived from the neural crest), human melanoma black which identifies immature melanosomes (HMB-45), Melan A, etc. Lymphoma stains positive for cutaneous lymphocyte-associated antigen (CLA), leukocyte common antigen (LCA/CD45), which differentiates between malignant lymphoma and poorly differentiated non-hematopoietic tumors (CD45-). B-cell markers (CD20 and CD79a), T-cell markers (CD3 and CD5), etc. and sarcoma, which originates in mesoderm is positive for vimentin (intermediate filaments found in conjunctive tissues), smooth muscle actin (SMA), actin, S100 protein, etc. [11–13].

Depending on the site of the primary tumor and its histology, we present the algorithms used for detecting the most frequent types of tumors.

Squamous cell pulmonary carcinoma is immunohistochemistry positive for cytokeratin 5/6, p63, CEA, thrombomodulin and negative for vimentin, thyroid transcription factor-1 (TTF1), napsin A [14].

Pulmonary adenocarcinoma is positive for TTF1 (Figure 2), EMA, CEA, CK7 and negative for CK5, CK20. Bronchioalveolar type pulmonary adenocarcinoma is positive for CK20 but negative for TTF1.

Small cell pulmonary carcinoma is positive for CD56/ N-CAM (neural cell-adhesion molecule), chromogranin, synaptophysin and negative for leukocyte common antigen (LCA); in this case, the differential diagnosis is made between lymphoma and metastasis of small cell carcinoma.

Clear cell renal carcinoma is positive for common acute lymphoblastic leukemia antigen (CD10/CALLA), renal cell carcinoma marker (RCC-MA), PAX2 (homeogene expressed during kidney development), CK8, CK18, AE1 (which reacts with the high molecular weight cytokeratins 10, 14, 15, and 16, and the low molecular weight cytokeratin 19), vimentin (Figure 3) and negative for CK7, CK20, CEA, TTF1 [15].

Prostate adenocarcinoma is positive for prostate specific antigen (PSA) (Figure 4), prostatic acid phosphatase (PAP), prostate specific membrane antigen (PSMA), prostein (P501S), AMACR (alpha methyl CoA racemase), AR (androgen receptor) and negative for CK7, CK20, thrombomodulin, TTF1 [16].

Breast ductal carcinoma is positive for gross cystic disease fluid protein 15 (GDFP15), mammaglobin [17], estrogen receptor (ER), E-cadherin, CK8, CK18, CK19, EMA and is negative for p63, CK20 (Figures 5 and 6). However, lobular breast carcinoma is negative for E-cadherin.

Colon adenocarcinoma is positive for CK20 (Figure 7), mucin 1 (MUC1), mucin 3 (MUC3), CEA, CDX2 and is negative for CK7, DPC4 (termed from deleted in pancreatic cancer, locus 4), hepatocyte paraffin-1 (HepPar1), cancer antigen 125 (CA125).

Thyroid papillary carcinoma is positive for thyroglobulin, PAX8 (relative specific marker for Müllerian tumors), CK19, mesothelioma marker (HBME), galectin and negative for CK20, estrogen receptor (ER), progestosterone receptor (PR) [18].

Serous ovarian carcinoma is positive for CA125, Wilms’ tumor protein 1 (WT1), ER, CK5/6, inhibin and negative for CEA [19, 20].

Although pancreatic carcinoma rarely metastasis in skeletal system, if does, it stains positive for antytripsin (specific pancreatic zymogen), CEA and CA19A [21].

Conclusions

Most of the patients (86%) could be mobilized immediately after surgery. The expected survival rate one year after surgery is around 60%. The goals of osteosynthesis are the same, regardless the location of the lesion and the implant used: pain amelioration, appropriate stability for immediate full weight bearing, durability for patient’s life expectancy. All extended osteolytic lesions must be reinforced at the time of the surgical procedure. The presence of a pathologic fracture is a negative prognosis factor for the medium term survival rate. Classical pathology examination certainly identifies the primary tumor in 10% of cases, while immunohistochemistry achieves the same objective in approximately 37% of cases.

References


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