Histol Histopathol (2007) 22: 373-377

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Primary mammary osteogenic sarcoma

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Summary. A 78 year-old female patient underwent a total mastectomy with axillary lymph node dissection for a primary breast osteosarcoma. Microscopically the tumor was identical to grade II skeletal osteosarcoma. Immunohistochemically no reactivity was detected, either for the epithelial markers EMA, AE1/AE3, CK8, 18, 19, or for HER-2/neu, estrogen and progesterone receptors, as well as fluorescent IN SITU hybridization for HER-2/neu. The diagnosis of this tumor fulfills certain clinicopathological criteria. Mammary osteosarcoma is usually developed in phyllodes tumors or carcinosarcomas of the breast as a result of metaplasia of the epithelial component. This rare tumor of the breast is occasionally associated with prior radiation therapy or well documented trauma. Mammary osteosarcoma is a biologically aggressive neoplasm with a 38% five-year survival rate. Surgical resection is the most effective therapy to date. Adjuvant treatment -chemotherapy or radiotherapy- has shown no clear benefit. An extensive review of the literature is also presented.

Key words: Extraskeletal osteosarcoma, Mammary, Histology, Diagnosis, Treatment

Introduction

Extraskeletal osteosarcoma (EsOs) is by definition a non-skeletal malignant mesenchymal neoplasm located in the soft tissue producing osteoid, bone or chondroid. This rare entity, which accounts for 1-2% of all soft tissue sarcomas, is found in such diverse locations as the skin, the urinary bladder, the prostate and the breast (Weiss and Goldblum, 2001). Some cases originate in tissue associated with a prior history of radiation therapy or well-documented trauma. Mammary osteosarcoma usually arises in phyllodes tumors or carcinosarcomas. Primary osteosarcoma of the breast without an epithelial component present or prior irradiation is extremely rare. Mammary osteosarcomas account for 12% of all mammary sarcomas (Tavassoli and Devilee, 2003). Most reports in the literature are limited to presentation of single cases but Silver and Tavassoli have reported a series of 50 cases of primary osteogenic sarcoma of the breast, focusing mainly on the clinicopathological findings of this rare entity (Silver and Tavassoli, 1998). Surgical excision of the tumor is the mainstay of treatment. The ipsilateral axillary nodes are occasionally involved by direct tumor extension to the axilla rather than by metastatic deposits through lymphatic vessels. Soft tissue sarcomas without a curative surgical option can be submitted to palliative chemotherapy, which represents the best currently available treatment (Weiss and Goldblum, 2001). As to the systemic management of this rare tumor, because of the limited number of publications relating to treatment, there is no reproducible data about the effectiveness of post-operative chemotherapy.

In the current report we present a case of primary mammary extraskeletal osteosarcoma, as well as an extensive review of the relevant literature.

Materials and methods

A single case of a 78 year-old female was admitted to the hospital with a large, painless mass of the left breast that had grown large over the years. The lesion was located in the outer lower quadrant of the breast. The past medical history of the patient was insignificant and no history of trauma or malignancy was present. Physical examination revealed a normal looking breast with no nipple discharge, skin tethering, or nipple retraction. A large movable palpable hard mass was felt in the left breast presenting no continuity with the bony elements of the chest wall. The mass was entirely located within the mammary gland with no evidence of axillary or (supraclavicular) lymphadenopathy. Mammography revealed a benign looking, well circumscribed lesion with a surrounding rim of calcification. Detailed clinical and laboratory examination revealed no bone malignancy. The patient underwent an open biopsy procedure on which the

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frozen section examination of the tumor showed neoplastic osteoid and chondroid matrix as well as areas of undifferentiated mesenchymal cells. Total mastectomy with a level I axillary lymph node dissection followed.

The lesion was fixed in 10% buffer formalin for 24 hours, dehydrated in ethanol and embedded in paraffin. A decalcification procedure was not performed on the specimen because the trabeculi were thin and 5mm slices were easily achieved. Microscopical 5µm sections of the lesion were obtained for haematoxylin/eosin staining, immunohistochemistry and fluorescent in situ hybridization (FISH).

Immunohistochemistry staining (Table 1) was performed using automated staining systems. Over-night incubation at 37°C and xylene for 10 min at room temperature was used for deparaffinization, the dehydration completed with degradated alcohols and phosphate buffer saline, and pretreatment achieved with citrate buffer for 10min in a microwave. Completion of the immunostaining procedure was accomplished at room temperature with DAKO Autostainer for (EMA, AE1/AE3, HER-2/neu, Estrogen receptor and Progesterone receptor) antibodies and I-6000 BioGenex Automated Staining System (Menarini, Chevilly Larue, France) for CK 8,18,19 antibody. Envision/HRP kit and Super Sensitive Detection kit with blocking serum were used respectively for secondary antibody and Streptavidin complex, concentrations and duration of incubation were followed according to manufacturer's instructions. Color was developed by incubating in a dark chamber in diaminobenzidine (DAB) for 10min, and counterstained with Harris hematoxylin for 45sec. Finally, the section was dehydrated with ascending alcohols and coverslipped. For staining quality control the appropriate tissues were used for positive and buffer saline for negative controls.

Also fluorescent in situ hybridization (FISH) for HER-2/neu was examined by using the automated Ventana BenchMark platform (Ventana Medical System, Tucson, AZ, USA). Slides (case and control) were bar-coded and placed onto Ventana platform. Using a bar-coded detection program, sections were heated to 37°C, deparaffinized with EZ Prep buffer, treated with protease (Protease III, Ventana Medical Systems) and washed with SSC. The sample/probe was denaturated at 75°C, and the HER-2/neu probe hybridization was performed at 37°C. Post hybridization stringency washes were made with 2X, 2X and 1X SSC at 42, 42 and 50°C respectively. The detection of the probe was done with mouse FITC anti-Biotin antibody and FITC-anti mouse antibody. The slides were removed from the instrument and washed briefly in detergent, equilibrated in reaction buffer (Tris-based buffer pH 7.6-7.8), drained, counterstained with 4,6-diamidine, 2-phenylindole dihydrochloride solution (DAPI) and coverslipped. The samples were visualized using a Zeiss Axioskop microscope equipped with x10, x20, x40 dry and x100 oil objectives, a 100 watt mercury lamp, a DAPI filter and a triple band pass DAPI/FITC/Rhodamine. The whole staining procedure was completed approximately in 13 hours.

Table 1. Immunohistochemical stains.

ANTIBODIES	SOURCE	DILUTIONS
EMA	Dako	1:200
AE1/AE3	Dako	1:400
CK 8, 18, 19	BioGenex	1:100
HER-2/neu (CB11)	Dako	Herceptest kit
Estrogen receptors	Dako	1:40
Progesterone receptors	Dako	1:40

Results

The gross appearance of the cut-surface of the tumor was well-circumscribed, measuring 7.5 cm in maximum diameter; it was a whitish-gray color with sporadic necrosis, variably mineralized with central trabeculation (Fig. 1). The remainder of the breast was normal. From

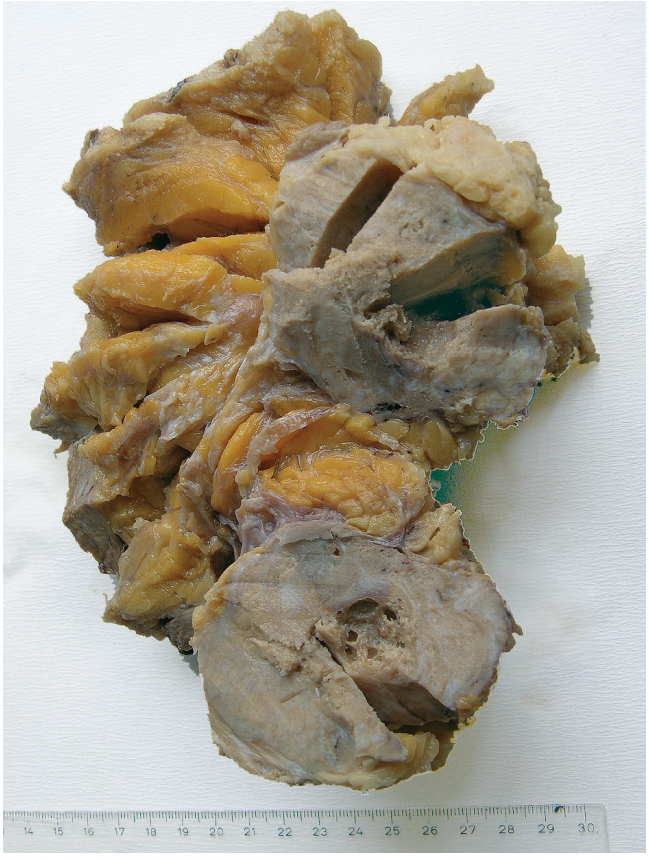

Fig. 1. Macroscopical image of the tumor, a well circumscribed lesion with central trabeculation and whitish-gray areas of chondroid matrix to the periphery. Sporadic necrotic speckles were also noticed.

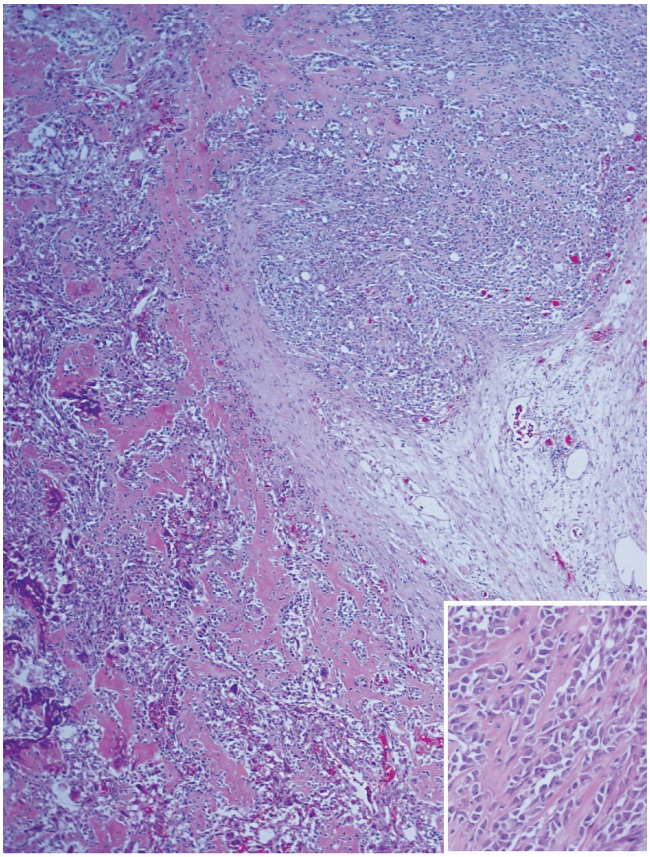

Fig. 2. Primary mammary osteosarcoma. Photograph showing neoplastic osteoid, areas of undifferentiated mesenchymal cells and mammary adipose tissue. H/E x 8. Inset: image showing lace-like osteoid deposition bordered by neoplastic osteoblasts. H/E x 40

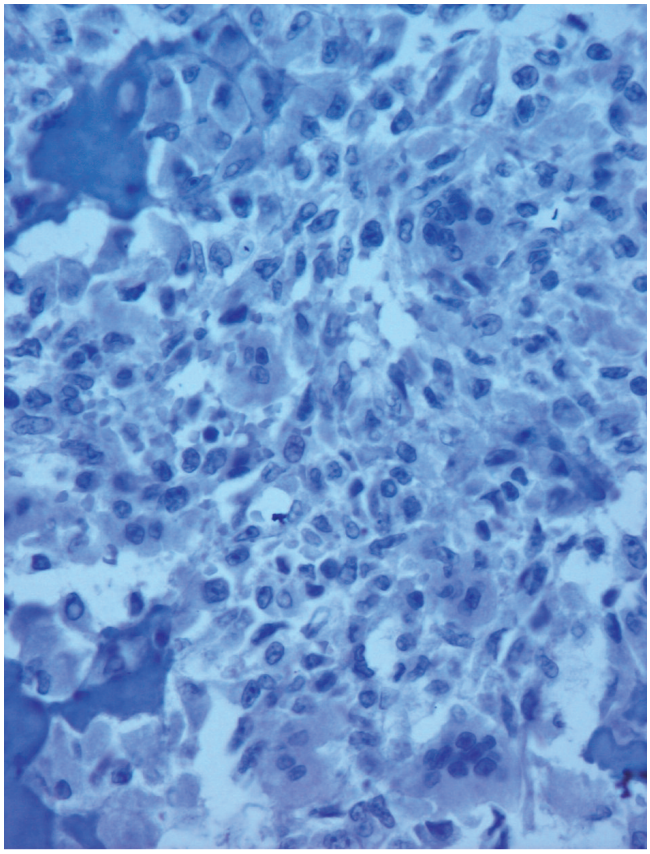

Fig. 3. Primary mammary osteosarcoma. Immunohistochemical staining which is negative for HER 2/neu. x 80

the axillary fibroadipose tissue 12 lymph nodes were dissected. *Histologically*, several samples of the lesion were carefully reviewed. The tumor consisted of osteoid in lace-like forming reticular trabeculi, focally calcified, bordered by atypical osteoblasts, with foci of chondroid matrix and marked atypia (Fig. 2). The fibroblasts and the osteoclastic giant cells were organized in bands or compact fascicles accompanied by areas of undifferentiated mesenchymal cells. Necrosis, muscle infiltration, neuronal and vascular invasion and frequent mitoses were present. The examined lymph nodes were free from any tumor deposits. No other malignancy, such as carcinoma, phyllodes tumor or even a mere epithelial component in the tumor was noticed. The *immunohistochemical staining* was negative for EMA, AE1/AE3, CK 8, 18, 19, HER-2/neu (CB11) (Fig. 3) and estrogen and progesterone receptors. No reactivity was shown with fluorescent in situ hybridization (FISH) for HER-2/neu. Based on medical history, microscopic findings and immunohistochemical profile, a final diagnosis of primary osteogenic sarcoma was secured.
The patient refused any further adjuvant therapy and she is alive and well with no recurrent disease 24 months post-operatively.

Discussion

For the diagnosis of the extraskeletal mammary osteosarcoma the criteria proposed by Allan and Soule should be fulfilled: 1) exclusion of bone origin of the tumor 2) presence of bone or neoplastic osteoid and 3) absence of an epithelial component (Allan and Soule, 1971). Our case fulfills the above requirements. In primary osteosarcoma of the breast the history of trauma as an antecedent risk factor is implicated in 6%-13% and radiation therapy in 2%-10% (Silver and Tavassoli, 1998). Microscopically, the morphology of our lesion corresponds completely to grade II skeletal osteosarcoma. The deposition of lace-like osteoid distributed in a sarcomatous stroma, the mineralization of neoplastic osteoid and the presence of osteoblastic, chondroblastic and fibroblastic differentiation with marked atypia and numerous mitotic figures constitute the essential microscopic elements necessary for diagnosis.

Benign and malignant breast lesions (fibroadenoma,

carcinoma or carcinosarcomas) occasionally contain bone, osteoid or giant cells resembling osteoclasts (Silver and Tavassoli, 1999). Therefore, extensive sampling is required in order to exclude benign or carcinomatous epithelial components, and additional immunohistochemistry is necessary to distinguish these conditions from primary mammary osteosarcoma. Although extra-skeletal osteosarcoma in general reacts with epithelial markers (8% with cytokeratin and 52% with EMA) (Fletcher et al., 2002), in our case no reactivity was noticed to AE1/AE3, CK 8, 18, 19 and EMA.

The prognostic significance of HER-2/neu in breast cancer is well-documented; and the overexpression of the oncogene correlates positively with poor prognosis and decreased survival. The oncogene overexpression is observed in patients with lung metastasis and less tumor necrosis after pre-operative chemotherapy (Morris et al., 2001). Detection of this oncogene in osteosarcoma is associated with poor histological response to chemotherapy and with decreased survival rate (Gorlick et al., 1999). Based on HER-2/neu expression, therapeutic protocols have been proposed and used for systemic tumor treatment (Gorlick et al., 1999; Morris et al., 2001). In phase II trials, which are still going on, administration of rhuMAB HER2 weekly to patients with refractory or relapsed osteogenic osteosarcoma has been proposed (Gorlick et al., 1999; Morris et al., 2001). Pilot trials were also designed using Trastuzumab therapy in recurrent osteosarcomas that overexpress HER-2/neu (Kilpatric et al., 2001).

The estimation of immunohistochemical staining on breast tissue for HER-2/neu is based on complete membranous expression of the oncogene. Reports on HER-2/neu immunostaining of organs other than the breast are controversial (Kilpatric et al., 2001). Those studies support the significance of cytoplasmic staining to indicate its prognostic value (Fellenberg et al., 2004). As negative immunostaining does not necessarily mean absence of the HER-2/neu oncogene, the importance of establishing anew more sensitive methods of detection, such as the fluorescent *in situ* hybridization (FISH) and the real-time reverse transcription-polymerase chain reaction (RT-PCR) becomes obvious (Gorlick et al., 1999; Kilpatric et al., 2001; Fellenberg et al., 2004). However, Anninga et al. (2004) have used all available methods and they have concluded that the detection of HER-2/neu expression in osteosarcomas is not reliable and of no clinical significance, so that a trial with Trastuzumab therapy is unlikely to be of therapeutic benefit. In our case no Real-time quantitative (RT-PCR) was performed since the results of immuno-histochemistry and FISH were regarded as sufficient on a specimen that had not undergone decalcification.

In contrast to osteosarcoma of the bone occurring mainly in children and adolescents, extra-skeletal osteosarcoma tends to appear in elderly women with a median age 64.5 years (Tavassoli and Devilee, 2003). Mammary osteosarcoma is an extremely aggressive tumor with an 38% overall 5-year survival rate (Tavassoli and Devilee, 2003). The overall recurrence rate for extraskeletal osteosarcoma is about 43% at a median of 1 year. The role of adjuvant chemotherapy is unclear (Silver and Tavassoli, 1998). In contrast, the histological response to pre-operative chemotherapy remains the strongest prognostic factor of osteosarcoma (Gorlick and Meyers, 2003). Despite the fact that the intensification of pre-operative chemotherapy on patients suffering from osteosarcoma increases the histological response of the tumor, no improvement in event-free survival was achieved (Meyers et al., 1998; Gorlick and Meyers, 2003). Chi et al. (2004) concluded that alteration in osteosarcoma treatment did not change the relapse pattern or the survival rate. For extraskeletal osteosarcoma combined therapy with radical surgery, radiotherapy, and sequential preoperative or postoperative chemotherapy should be carried out in the hope of improving survival. The detection of chemosensitivity and administration of the appropriate chemotherapeutic agents is of great clinical importance (Gorlick and Meyers, 2003; Hawkins and Arndt, 2003).

In summary, future clinical research efforts should be directed toward developing reliable prognostic markers or techniques for osteosarcomas. Targeted specific therapies, perhaps biologically based, are probably required for those patients who are destined for failure with the current approaches.

References

Allan C.J. and Soule E.H. (1971). Osteogenic sarcoma of the somatic soft tissues. Clincopathologic study of 26 cases and review of the literature. Cancer 27, 1121-1133.

Anninga J.K., van de Vijver M.J., Cleton-Jansen A.M., Kristel P.M., Tamimniau A.H., Nooij M., Egeler R.M. and Hogendoorn P.C. (2004). Overexpression of HER-2 oncogene dose not play a role in high-grade osteosarcomas. Eur. J. Cancer 40, 963-970.

Chi S.N., Conklin L.S., Qin J., Meyers P.A., Huvos A.G., Healy J.H. and Gorlick R. (2004). The patterns of relapse in osteosarcoma: the Memorial Sloan-Kettering experience. Pediatr. Blood Cancer 42, 46-51.

Fellenberg J., Krauthoff P., Delling G. and Parsch D. (2004). Evaluation of the predictive value of HER-2/neu gene expression on osteosarcoma therapy in laser-microdissected paraffin-embedded tissue. Lab. Invest. 84, 113-121.

Fletcher D.M., Unni K.K. and Mertens F. (2002). World Health Organization Classification of Tumours. Pathology & Genetics. Tumours of Soft Tissue and Bone. IARC. Lyon. pp 182.

Gorlick R. and Meyers P.A. (2003). Osteosarcoma necrosis following chemotherapy: innate biology versus treatment-specific. J. Pediatr. Hematol. Oncol. 25, 840-841.

Gorlick R., Huvos A.G., Heller G., Aledo A., Beardsley G.P., Healey J.H. and Meyers P.A. (1999). Expression of HER2/erbB-2 correlates with survival in osteosarcoma. J. Clin. Oncol. 17, 2781-2788.

Hawkins D.S. and Arndt C.A.S. (2003). Pattern of diseases recurrence and prognostic factors in patients with osteosarcoma treated with contemporary chemotherapy. Cancer 98, 2447-2456.

Kilpatric S.E., Geisinger K.R., King T.S., Sciarrotta J., Ward W.G., Gold

S.H. and Bos G.D. (2001). Clinicopathologic analysis of HER-2/neu immunoexpression among various histologic subtypes and grades of osteosarcoma. Mod. Pathol. 14, 1277-1283.

Meyers P.A., Gorlick R., Heller G., Casper E., Lane J., Huvos A.G. and Healey J.H. (1998). Intensification of preoperative chemotherapy for osteogenic sarcoma: results of the Memorial Sloan-Kettering (T12) protocol. J. Clin. Oncol. 16, 2452-2458.

Morris C.D., Gorlick R., Huvos A.G., Heller G., Mayers P.A. and Healey J.H. (2001). Human epidermal growth factor receptor 2 as a prognostic indicator in osteogenic sarcoma. Clin. Orthop. 382, 59-65.

Silver S. and Tavassoli F. (1998). Primary osteogenic sarcoma of the breast: a clinicopathologic analysis of 50 cases. Am. J. Surg. Pathol. 22, 925-933.

Silver S.A. and Tavassoli F.A. (1999). Osteosarcomatous differentiation in phyllodes tumors. Am. J. Surg. Pathol. 23, 815-821.

Tavassoli F.A. and Devilee P. (2003). World health organization classification of tumours. Pathology and genetics. Tumours of the breast and female genital organs. IARC. Lyon. pp 97-98.

Weiss S.W. and Goldblum J.R. (2001). Enzinger and Weiss's Soft Tissue Tumors. 4th ed. Mosby. St. Louis. pp1405, 41.

Accepted October 10, 2006