ANGIOSARCOMA - MOLECULAR MARKERS IN HISTOPATHOLOGICAL DIAGNOSIS

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Abstract : Angiosarcoma of the breast represents an uncommon entity. This is a case report of a 36-year-old woman who had a primary angiosarcoma of the right breast. After detecting a mass in her breast, she underwent mastectomy. Routine histopathological techique, followed by immunohistochemistry for factor VIII, CD34, vimentin, desmin, and pan-cytokeratin were performed in order to obtain the positive and differential diagnosis. Six months after the surgery, the patient succumbed to multiple metastases. The clinical course corresponded to general poor prognosis of breast angiosarcoma. Vascular targeting approaches in modern therapies represent promising methods for improvement of angiosarcoma's prognosis.

INTRODUCTION

Primary soft tissue sarcoma of the breast is a rare malignancy that accounts for fewer than 1% of breast malignancies (3, 13, 15), exhibiting a heterogenous array of histologic aspects. Among the most common histologic subtypes are: malignant fibrous histiocytoma, fibrosarcoma, liposarcoma, and angiosarcoma (15). Angiosarcoma of the breast is a rare tumor, with an incidence of approximately 8% of mammary sarcomas (4). It is an extremely aggressive neoplasm of vascular origin, with a five-year survival rate of 8-50% (4). The first case has been described by Schmidt, in 1887 (9).
We report herein the history of a 36-year-old patient who was diagnosed with mammary angiosarcoma, using immunohistochemistry method in order to evidentiate endothelial molecular markers that confirmed the histopathological diagnosis.

MATERIALS AND METHODS

CASE REPORT

Clinical History
The patient was a 36-year-old, married, mother of two childs, with complaint of a painless right breast enlargement, developed in few months. The patient was admitted after the failure of an incisional biopsy, due to marked hemorrhage, in another Hospital. On physical examination, the breast lesion was associated with erythematous suprajacent skin, reflecting hemorrhage and vascularity of the lesion and contralateral breast was clinically normal. Mammography revealed an ill-defined, lobulated tumor, with areas of high and low echogenicity on sonography. After mastectomy, despite the combined therapies, metastatic disease developed within six months, and the disease was lethal.

Pathologic Findings
Gross pathology of the surgical specimen revealed a friable, spongy hemorrhagic mass, with a maximum diameter of about 15 cm, with ill-defined margins, containing large areas of cystic hemorrhagic necrosis and hemorrhagic discoloration in the surrounding tissue, as an indicator of tumoral extension.
For light-microscopy and immunohistochemistry, resected tissues were fixed in 20% buffered formalin solution and embedded in paraffin. Four-µm sections were dewaxed with toluene and rehydrated through a series of graded alcohols. Hematoxylin and eosin staining was performed and alternatively immunohistochemistry technique was applied, as following: endogenous peroxidase activity was blocked by incubation (10 min) in 0.3% H_2O_2 in methanol; sections were gently rinsed with Tris-buffered saline (TBS); incubation for 30 min with antibodies against: factor VIII-related antigen (polyclonal antibody A 0082, Dako), CD34 (mouse IgG1 antihuman, Biogenex), vimentin (clone V9 Biogenex), desmin (Monoclonal, Mouse IgG Biogenex), and cytokeratin cocktail (Monoclonal, Mouse IgG Cocktail Biogenex) was applied; incubation with the secondary antibody (Multilink) for 20 min, using Automatic OptiMax+ (Biogenex) followed; signal detection, using the streptavidin-biotin-peroxidase complex method (LSAB, Strept-Aviden), according to the manufacturer's recommendation (Biogenex), was performed; DAB (3,3 diaminobenzidine hydrochloride) was used as chromogen.

Microscopic pathology in routine staining showed interanastomosing vascular channels intermingled with solid endothelial or spindle cell areas, necrotic foci, marked hemorrhage, cytollogically atypical cells, numerous mitoses, prominent endothelial tufting and solid papillary formations, blood lakes, and infiltrative borders.

RESULTS AND DISSCUSIONS

The atypical cells were immunohistochemically positive for factor VIII related antigen (fig. 1) and for CD34 (fig. 2). These cells were also positive for vimentin and desmin, but negative for keratin. Vascular invasion was common. Final histopathologic diagnosis was that of high grade breast angiosarcoma (type III or grade III or poorly differentiated), exhibiting more than 50% of the tumor high-grade malignant features, associating areas of low-grade and intermediate grade.

Breast malignant tumors are dominantly primary epithelial tumors (carcinomas), mesenchymal tumors being relatively rare (13). Among mesenchymal breast tumors, angiosarcoma represents a highly malignant tumor, composed of neoplastic elements with the morphological properties of endothelial cells (13). Several synonyms were used by different authors, as hemangioendothelioma (12), haemangiosarcoma (11), haemangioblastoma (1), lymphangiosarcoma (7), or metastasing haemangioma (14). Less than 300 cases were described until present (4, 7, 10).

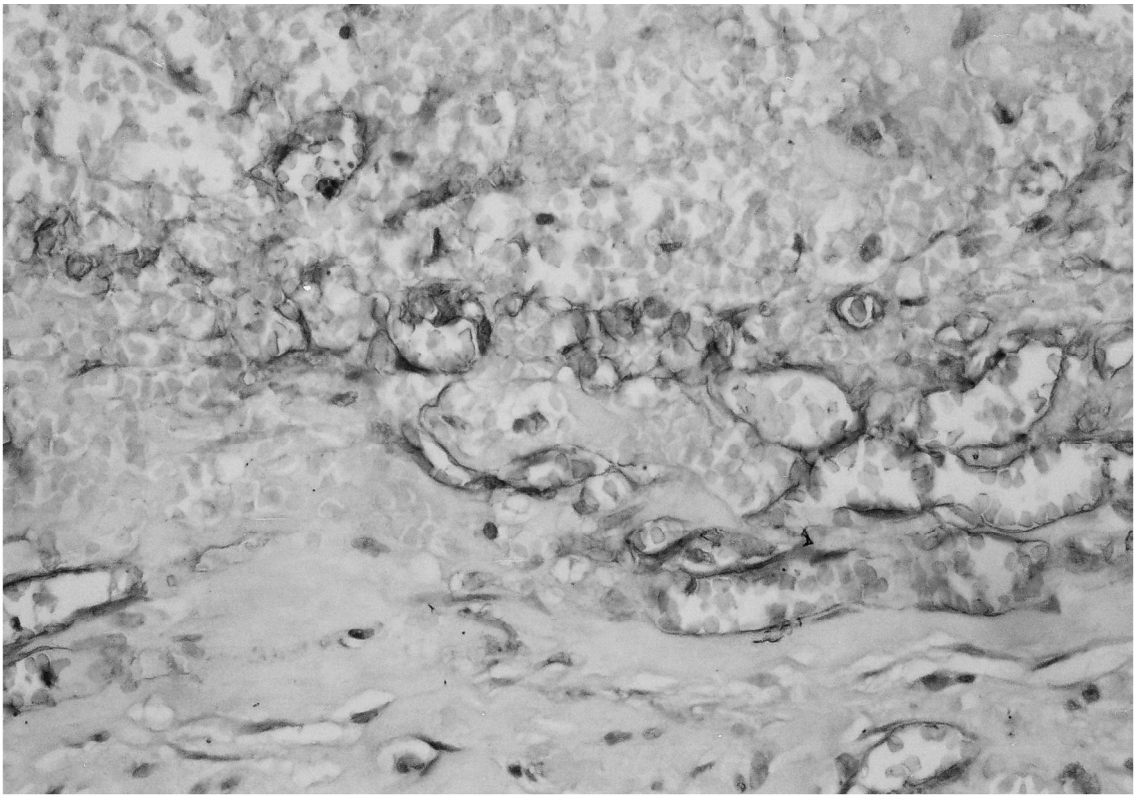

FIG. 1 Immunoreactivity for factor VIII related antigen

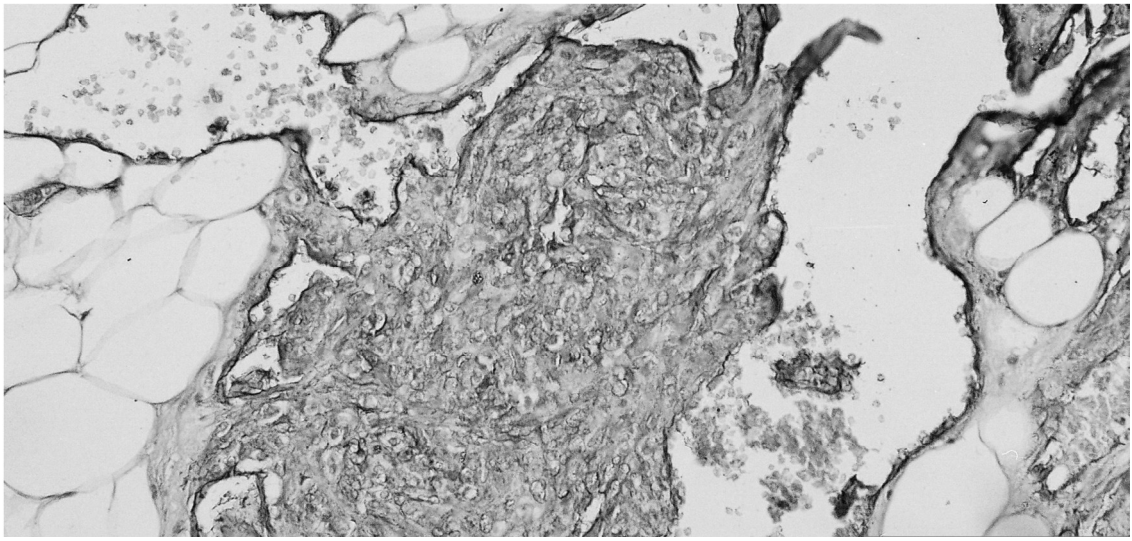

Fig. 2 Immunoreactivity for CD34

Angiosarcoma of the breast occurs during the third and fourth decade of life (4), corresponding to the age of our patient (36-year-old), in contrast with carcinoma that generally arises later (7). This malignant tumor occurs primarily in young women, with 6-12% of the cases diagnosed during pregnancy (7), implying a hormonal determinism. Although in the reported case the malignancy was not intimately associated with pregnancy, a relative correlation with hormonal status was suggested by the onset of the disease after ablactation. The hormonal dependency of angiosarcoma is controversial (4), as numerous tumors do not express estrogen receptors (ERs), but this feature may be explained by the decrease of expression of ERs in high grade tumors.

Several types of angiosarcomas have been described. Primary (*de novo*) forms appear in the breast parenchyma, as was also diagnosed in our patient. Secondary tumors may develop in the arm skin and soft tissues, following ipsilateral radical mastectomy and susequent lymphoedema (Stewart Treves syndrome) (6, 8, 15). Secondary tumors have increasingly been associated with treatment using external beam radiation therapy (15, 16), in the skin and chest wall following radical mastectomy and local radiotherapy, or in the skin or breast parenchyma or both, following conservation treatment and radiotherapy (7, 13).
In reported cases of the literature, the tumor manifested as a painless and rather quickly enlarging, palpable mass without tenderness, with tumor size more than 4 cm in diameter. Only few patients, with tumors less than 4 cm at diagnosis, had a better survival rate (4). The tumor size at discovery had reached 15 cm in diameter, in our reported case. Our patient succumbed to extensive metastases, in 6 months after the surgical treatment. The most common metastases develop after hematogenous spread into the liver, lung, lymph nodes, skeleton, bone marrow, brain, contralateral breast, and less frequently they are detected into ovary, kidney, omentum, adrenal gland, stomach, pancreas, peritoneum, esophagus, skin, spleen, gingiva, placenta and heart (7, 15). Axillary lymph node metastases are occasionally among the previously reported cases (15). Primary ovarian angiosarcoma is an extremely rare tumor, but lacks breast metastases,

so the differential diagnosis with primary breast angiosarcoma with ovarian metastases is facile (5).
Preoperative diagnosis of angiosarcoma of the breast, by aspiration cytology and biopsy, is often difficult, with reported false negative rate of 37% (15). Incisional biopsy failed in the reported case due to an abundant hemorrhage.
Magnetic resonance imaging (MRI) is useful in the diagnosis and control of the opposite breast, as asynchronous contralateral lesions are frequent (7).
The differential diagnosis of the reported angiosarcoma included benign hemangioma, angiomatosis (diffuse angioma), nodular pseudoangiomatous stromal hyperplasia (PASH), fibromatosis, stromal sarcoma, fibrosarcoma, liposarcoma, cystosarcoma phyllodes, metaplastic carcinoma, squamous cell carcinoma with sarcomatoid features, acantholytic squamous cell carcinoma, myoepithelioma, and reactive spindle cell proliferative lesions (7, 13).
Electron microscopy examination can reveal the vascular nature of angiosarcoma and may demonstrate the existence of Weibel-Palade bodies, pinocytotic vesicles, cytoplasmic projections or papillae, discontinuous endothelium and basal lamina, the variable presence of pericytic cells, and the overlapping disorderly fashion of endothelial cells, forming bridges, cord-like structures, and spiderweb pattern along the vessels (4, 7).
The histologic features of angiosarcoma of the breast are classified into three groups (1, 7) (table 1). Hill and Stout (1942) firstly described the three general classes: simple angioma, intermediate type, and clearly and recognizable malignant tumor, and Donnell system (1981) has consequently gained wide impact, as it was proven to be correlated with prognosis (7).
Group I angiosarcoma shows dilated, sinusoid-like vessels surrounding breast ducts; vessels are lined by a single layer of relatively flat endothelial cells, without mitoses (7). Group II exhibits numerous small buds or tufts of endothelial cells projecting into the vascular lumen and papillary growths of endothelial cells (7). Group III shows a focus of growth of spindle and polygonal cells, necrosis and blood lakes. The resected specimen from our patient's breast tumor demonstrated marked hemorrhage and proliferation of atypical polygonal shaped endothelial cells (7). The reported case was considered as high grade breast angiosarcoma (group III), exhibiting more than 50% of the tumor high-grade malignant features, although it exhibited areas of low-grade and intermediate grade.
The clinical course in low-grade and intermediate-grade angiosarcomas shows local or systemic recurrences development. If well differentiating angiosarcoma is excluded, the breast tumor is usually lethal, as was also the clinical course in the reported case. Probabilities of disease-free survival after treatment is generally considered to be correlated with grade, as following: low-grade: 76% survival, intermediate-grade: 70% survival, and high-grade: only 15% survival (7, 15).
The clinical course was compatible with the Group III tumors, in our patient. Mitotic index is considered a useful prognostic factor (2). Younger ages are generally associated with higher grades, as following: grade I: 43 years, grade II: 34 years, and grade III: 29 years (7). Another indicator of poor prognosis is represented by coexistent pregnancy. The development of angiosarcoma after ablactation, in our patient, may be considered as resulting a higher grade (III) than the expected one (II), corresponding to the age of the patient.

Although some patients seemed to benefit from adjuvant chemotherapy, effective treatment is questionable, as was observed in our patient who had approximately 6 months survival after

combined therapy, reflecting the fact that tumor was refractory to chemotherapy and radiotherapy.

CONCLUSIONS AND PERSPECTIVES

Angiosarcoma of the breast is a rare and extremely hostile neoplasm of vascular origin, diagnosis being confirmed by the presence of endothelial molecular markers.
Unfavorable prognosis factors resulted by the investigation of the reported case are: young age, hormonal status, great dimension, and high grade.
Surgical therapy involves total mastectomy. Axillary dissection is not indicated because angiosarcomas spread hematogenously, so may rarely involve lymph nodes.
Distant metastases are frequent and involve most of the organs.
Angiosarcoma is refractory to adjuvant radiation and chemotherapy, but they are justified by few long-term survivors who have been treated with adjuvant chemotherapy.
Modern therapies using immunotoxins, specific antibodies conjugated with efficient cytotoxins, such as ricin, or radioactive isotopes, and monoclonal antibodies with affinity to proliferative endothelium antigens, such as endoglin, represent promising methods in the vascular targeting approach of angiosarcomas (4).

Table 1 Histologic groups of the breast angiosarcomas (Rosen)

Histologic features	Low grade	Intermediate grade	High grade
Lesions involving breast parenchyma	+	+	+
Anastomosing vascular channels	+	+	+
Hyperchromatic endothelial cells	+	+	+
Endothelial tufting	minimal	+	++
Papillary formation	-	Focally +	+
Solid and spindle cell foci	-	-/minimal	+
Mitoses	Rare/-	+ in papillary areas	++ even in low-grade areas
„Blood lakes”	-	-	+
Necrosis	-	-	+

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