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Case Report

Primitive neuroectodermal tumor of the breast: a case report and review of the literature

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Abstract: Background: Primitive neuroectodermal tumor (PNET) is a rare aggressive type of sarcoma characterized by translocation involving the EWS gene. Peripheral PNET is rarely observed in female breast. So far, only 11 cases of PNETs of breast have been reported in literatures, and no Chinese case has ever been reported. We presented, here, a case of primary PNET of breast occurred in a 32-year-old Chinese woman. At her first visit to the hospital, this woman complained of a palpable mass in her right breast noticed by herself for 6 months. No lesion of metastasis was found by imaging. The diagnosis was established by surgery, immunohistochemical staining and fluorescence *in situ* hybridization (FISH). The patient received 6 cycles of chemotherapy after surgery using a regimen of cyclophosphamide (CTX) 500 mg/m^2 + doxorubicin (ADM) 50 mg/m^2 + vincristine (VCR) 2 mg. Since then, she had been on regular follow-up and remained disease free for 51 months after surgery. Conclusion: PNET of breast mainly affects young females. In spite of its extream rarity, PNET of breast should be taken into consideration upon differential diagnosis of breast tumors and positively treated by multidisciplinary planning advisory teams.

Keywords: PNET, breast, multidisciplinary treatment

Case report

A 32-year-old unmarried woman come to the Fifth People's Hospital of Leqing Country, Wenzhou City for a consultation about a mass in her breast that had already persistented for 6 months. The mass appreciably increased in size in the first month after notice. No other symptoms had been found beside the breast lump. She denied any history of antecedent trauma, inflammation and ibroadenoma.

On examination, the lump was found in the upper medial quadrant of the right breast, approximately 2.0 cm in size. The tumor was firm in consistency and did not showed any adhesion to the surrounding chest wall and the skin. The remaining areas of the right breast, left breast, and both axillae were normal. The patient was subjected to sonomammography of breasts which revealed the lump to be a well-defined, round, soft tissue density lesion of size 2.0*2.0 cm. Provisional diagnosis of breast cancer was made and simple mastectomy of the right breast was carried out on April 29th, 2011. The histopathologic analysis of hematoxylin and eosin-stained sections revealed that the tumor was composed of monotonous, small, blue cells in the shape of round to oval, with hyperchromatic nuclei and scanty cytoplasm. Furthermore, vague rosette-like patterns of tumor cells were observed in the form of perivascular pseudorosettes, beside occasionaly true rosettes (**Figure 1**). Immunohistochemistry analysis of the tumor cells found that they expressed vimentin, cluster of differentiation 99 (CD99), and neural cell adhesion molecule (NCAM, also known as CD56). Stains for pan cytokeratin (AE1/AE3), cytokeratin (CAM5.2), myogenin, cytokeratin, chromogranin, synaptophysin (Syn), s 100 protein (S100), desmin, and myogenic differentiation *1* (myoD1) were all negative (**Figure 2**). Based on

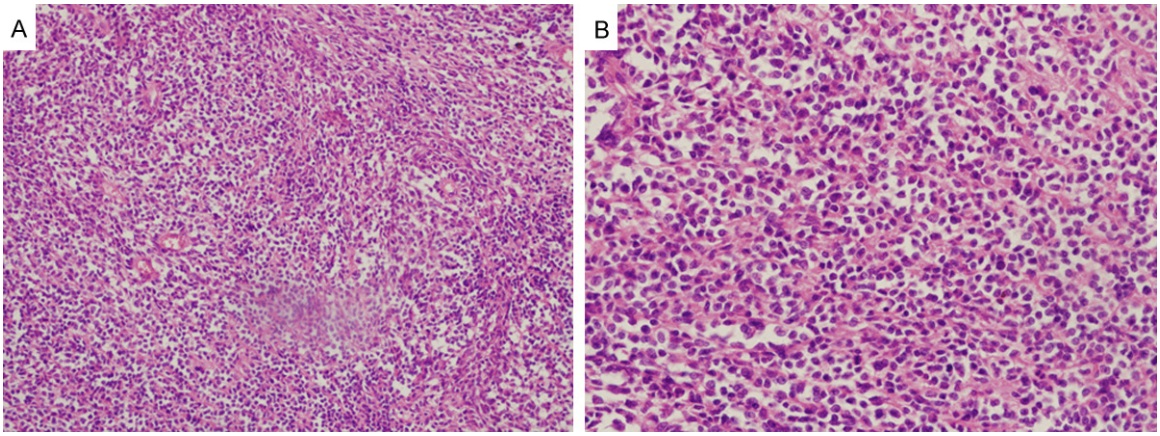

Figure 1. PNET in breast. A. Photomicrograph showing monomorphic population of small blue cells (hematoxylin and eosin) (original magnification ×200). B. Small, round tumor cells with scanty cytoplasm and round nuclei (original magnification, ×400).

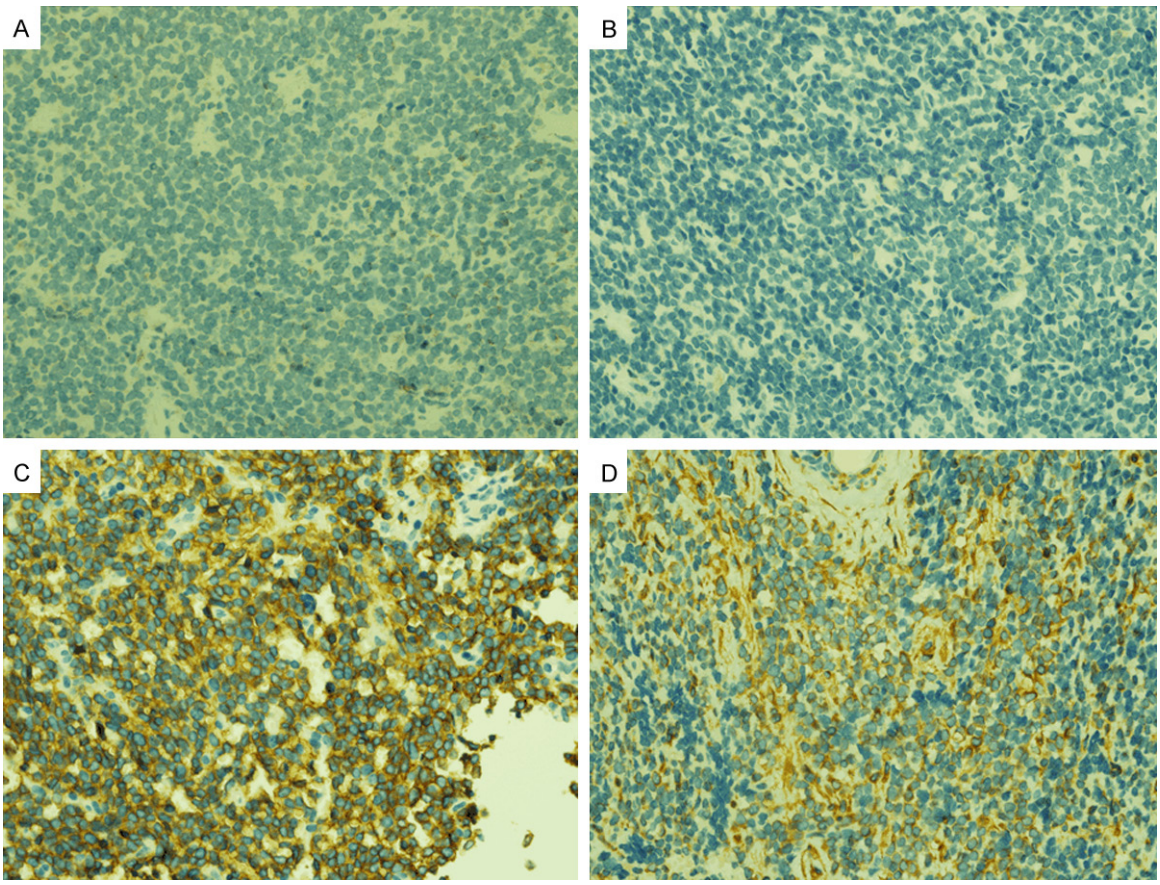

Figure 2. Immunohistochemical staining. The tumor cells were negative for synaptophysin (A), and negative for AE1AE3 immunostain (B), positive for cluster of differentiation 99, immunostaining for CD99 was positive with a membranous pattern (C) and vimiten immunostain with nuclear location (D) (original magnification, ×400).

the findings of histopathology and immunochemistry, a diagnosis of PNET of the breast was made. Fluorescence *in situ* hybridization (FISH) revealed the characteristic EWSR1 translocation, confirming the diagnosis of PNET of the breast (**Figure 3**). A staging work-up with

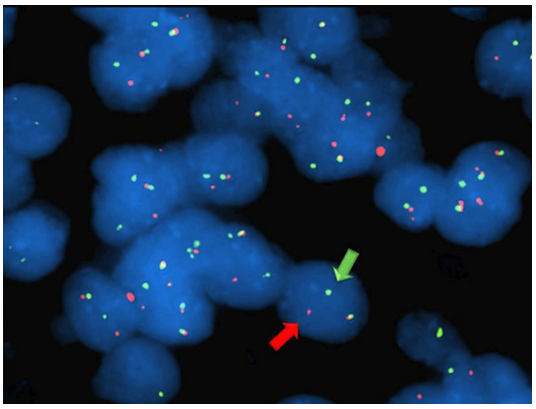

Figure 3. Fluorescence in situ hybridization (FISH) analysis of ES/pPNET. The probes on the centromeric and telomeric sides of the chromosome 22 *EWS* gene. One pair of green-red probe signals (arrows) is split apart due to rearrangement of the *EWS* region.

PET-CT scan after surgery was done to exlude metastasis lesions. The patient was given adjuvant chemotherapy and received six cycles of cyclophosphamide (CTX) 500 mg/m^2, doxorubicin (ADM) 50 mg/m^2, and vincristine (VCR) 2 mg postoperatively. Thereafter, the patient was treated with traditional Chinese medicine. She has been followed up with scheduled computerized tomography (CT) and ultrasonography (USG) scans, as well as periodic tumor marker monitoring every 6 months. During 51 months follow-up since surgery and chemical therapies, no evidence of recurrence or metastasis has been detected.

Study selection and collection of individual patient data

A comprehensive search for literatures about PENT of breast tumor was performed. For literatures pulished in English, the Pubmed National Library of Medicine searcher was employed, whereas for those in Chinese, the Chinese Biomedical Literature Database and the Chinese Biology and Medicine Database were employed using "primitive neuroectodermal tumor and breast" as key words. The search covered the period from 1989 to 2015.

All references used in these reports were retrieved, reviewed and evaluated. Clinical variables were collected including age, clinical presentation, tumor size, metastasis, surgery, chemotherapy regimen, radiotherapy, recurrence or progression, overall survival and the vital status. A total of 12 cases including the one reported here by the authors were analyzed.

Statistical analysis

Findings were analyzed using SPSS for Windows, Version 16. The relationship between clinico-pathological parameters and biomarkers was tested with the Chi-square and Fisher exact tests. Survival outcome was estimated using the Kaplan-Meier method, and comparison between groups was made by using log-rank test. *P*-value <0.05 was considered to indicate statistical significance.

Discussion

Peripheral primitive neuroectodermal tumor/Ewing's sarcoma (pPNET/EWS) occurs mainly in children and young adults and shows predilection for bones and soft tissues in the trunk, para spinal region and lower extremities. Peripheral PNETs, first described by Askin et al in 1979, are tumors that originate in the soft tissue of the chest wall, occasionally in bone, and rarely in lung, kidney, ureter, bladder, testis and seminal vesicles, and in many other visceral organs, i.e., the ovary, pancreas, uterus, parotid gland, and lung [1]. Moreover, PNET that occur in breast is extremely rare, and so far only isolated cases have been reported in literatures. By a comprehensive survey, we found only 11 cases [2-12] that had been published since the first report in 2006 [2]. PNET metastasis to breast following a previously PENT in the back and bone had been reported in 2002 [13]. To the best of our knowledge, the present case is the first one in Chinese women. The clinical pathological features of the total 12 cases of primary PENT of breast cases including the one we report here are summarized in **Table 1**.

PNET of breast-clinical and pathological features

PNET of breast is a relatively aggressive malignancy occurring predominantly in young women. In the present study (**Table 2**), we found the median age of patients with PNET of breast was 35.00±7.22 years in the range of 24-47 years. The courses of the disease from establishment of diagnosis to death ranged from 1 to 24 months, with a mean of 7.33 months. The primary tumors arose from the left breast with

Table 1. Summary of primary breast primitive neuroectodermal tumors reported in literature

Reference	Age	Presentation	Size (cm)	Lymph node	Metastasis	Treatment	Outcome
Maxwell et al 2006	35	Breast lump	1.8	/	None	Lumpectomy+chemo	DFS 30 M
Tamura et al 2007	47	Breast lump	2.1*1.8	Negative	NA	Mastectomy	NA
Da Silva et al 2008	35	Breast lump	12*7.5	Negative	None	Chemo+Radio	Local+lung R; Die 24 M
Ko et al 2009	33	Breast lump	2.5*2	Negative	None	Lumpectomy	DFS 6 M
Vindal et al 2010	26	Breast lump	3*2	Negative	None	Wide local excision+chemo	DFS 36 M
Chuthapisith et al 2012	46	Breast lump	12*12	3/24	Lung	Chemo+radio+modified radical mastectomy with local skin flap coverage	Local+lung R; Die less than 24 M
Sahoo et al 2013	36	After lumpectomy	8*5	Negative	Local	Incision and drainage+Chemo+radio	DFS1 2 M DFS2 18 M
Majid et al 2013	30	Breast lump	7 4	Positive	Lung+pleural effusion	Chemo	Die 2 M
SM Ikhwan et al 2013	33	Left breast lump	20	Positive	Lung+pleural effusion	Chemo	Die 3 M
Meddeb et al 2014	43	Breast lump	9.7	0/13	None	Modified radical mastectomy+chemo	DFS2 M OS 20 M
TAŞLI et al 2014	24	Breast lump	13*11*9	Positive	Right axillary region	Wide excisional biopsy chemotherapy Radiotherapy modified radical mastectomy and axillary lymph node dissection	DFS2 M OS 8 M

Table 2. Baseline characteristics of all the 12 patients

Characteristics	Group	Number of patients (n=12)
Source	Our hospital	1 (8.33%)
	Consultation	11 (91.67%)
Year of diagnosis	2006	1 (8.33%)
	2007	1 (8.33%)
	2008	1 (8.33%)
	2009	1 (8.33%)
	2010	1 (8.33%)
	2012	1 (8.33%)
	2013	3 (25.00%)
	2014	2 (16.67%)
Age	35.00±7.22	(24-47)
Course	7.80±7.06	1-24 mo
Age group	≤30 years	3 (25.00%)
	>30 years	9 (75.00%)
Site	Left	6 (50.00%)
	Right	5 (41.70%)
	Left+right	1 (8.30%)
Site	Outer upper quadrant	4 (33.30%)
	Inner upper quadrant	2 (16.70%)
	Central	3 (25.00%)
	Missing	3 (25.00%)
Surgery	Without surgery	4 (33.30%)
	With radical surgery	8 (66.70%)
Surgery	1	7 (87.50%)
	≥2	1 (12.50%)
Tumor size	7.87±5.84	1.8-20 cm
Tumor size group	≤5 cm	5 (41.70%)
	>5 cm	7 (58.30%)
Stage	I-III	8 (66.70%)
	IV	4 (33.30%)
LN metastasis	Negative	8 (66.70%)
	Positive	4 (33.30%)
Far away metastasis	Negative	8 (66.70%)
	Positive	4 (33.30%)
Chemotherapy	Salvage	4 (33.33%)
	Adjuvant	6 (50.00%)
	Without adjuvant	2 (16.67%)
Radiotherapy	Without	8 (66.67%)
	Salvage	2 (16.67%)
	Adjuvant	2 (16.67%)
Combined chemoradio	Negative	8 (66.67%)
	Positive	4 (33.33%)
Combined surgerychemo	Negative	6 (50.00%)
	Positive	6 (50.00%)
Combined S-C-R	Negative	10 (83.33%)
	Positive	2 (16.67%)

6 patients (50.00%), the right breast with 5 patients (41.70%), and one case (8.30%) in both breasts. All the tumors presented as palpable abnormalities, and with an average of 7.87±5.84 cm in size at presentation, varying from 1.8 to a maximum of 20.0 cm. The disease occured absolutely in young women with a high metastatic potential. Four patients had evidence of systemic metastasis at their presentation, the metastasis were restricted involving the local axillary region, lung, and pleural effusion. 2 patients suffered recurrence or progression during the follow-up period. The most common site of distant metastasis for early stage PNET of breast was the lung (2/2, 100.00%) (**Table 1**).

Histopathologically, they are characterized by a rather uniform population of small, dark cells, with or without Homer Wright rosettes. Furthermore, PNET of breast must still be differentiated from other small round-cell tumors, such as malignant lymphoma, small cell carcinoma, poorly differentiated sarcoma and desmoplastic small round-cell tumors. Immunohistochemically, the tumor cells expressed one or more of the characteristic neural markers, including CD99, which is a cell surface glycoprotein involved in cell adhesion [14]. Friend leukemia integration 1 transcription factor (FLI1) positivity also intensifies the diagnosis of PNET of breast [15]. Strong membranous positivity of CD-99 and nuclear staining of FLI1 are two characteristic features of PNET, as they expressed in 100% of cases in this series of patients (**Table 3**). Besides, other relevant specific markers, such as vimentin (88.89%), syn (62.50%), neuron specific enolase (NSE) (100.00%) are also recommended (**Table 3**). In contrast, negative immunohistochemical stains for destin, epithelial membrane *antigen* (EMA) and leucoyte common *antigen* (LCA) can assist in distinguishing PNET from the tumors above mentioned.

Table 3. Analysis of the immunohistochemical and cytogenetic markers in PNET of breast

No.	Antibody marker	Positive	Negative	Missing	Percentage of positive	
1	Fli-1	4	/	8	4/4	100.00%
2	CK	2	7	3	2/9	22.22%
3	AE1/AE3	1	3	8	1/4	25.00%
4	EMA	0	3	9	0/00	0.00%
5	Vimentin	8	1	3	8/9	88.89%
6	Desmin	0	7	5	0/7	0.00%
7	NSE	4	0	8	4/4	100.00%
8	Syn	5	3	4	5/8	62.50%
9	Cg A	1	7	4	1/8	12.50%
10	S-100	2	4	6	2/6	33.33%
11	CD56	2	5	5	2/7	28.57%
12	LCA	0	6	6	0/6	0.00%
13	CD99	11	0	1	11/11	100.0%
14	FISH	3	/	9	3/3	100.0%

PNET is characterized by the t(11;22)(q24;q12) translocation resulting in the production of the EWS/FLI1 fusion gene. The EWS gene on chromosome 22 encodes an RNA-binding protein that is disrupted by the t(11;22)(q24;q12) translocation. The diagnosis of 3 cases among 12 cases were verified by FISH (**Table 3**).

In this study, a case of breast PNET in a 32-year-old Chinese female is presented. To the best of our knowledge, it is the first reported PNET of breast in China. Histological analysis of hematoxylin and eosin-stained sections revealed that the tumor was composed of monotonous, small, blue round cells, two to three times the size of lymphocytes, with hyperchromatic nuclei and scanty cytoplasm (**Figure 1**). An immunohistochemical panel demonstrated strong, diffuse membranous positivity for CD99 (**Figure 2**) and FISH which revealed the characteristic EWSR1 translocation (**Figure 3**), further confirming the diagnosis of PNET of breast. Although rare, the possibility of PNET should be kept in mind while evaluating a palpable abnormality in young females, especially if pathology shows presence of cells of non-breast origin. In all cases of suspected PNET of breast, imaging should be carried out to define the primary location of the tumor in order to differentiate primary from metastatic disease. The computed tomography scan of the chest, abdomen, and pelvis are recommended to find out a possible primary site such as bone, soft tissue, or other organs. Our case refers to a young adult patient with a primary PNET of her right breast and no evidence of neoplasia in any other part of her body was found. Therefore, it is considered a PNET primarily arising from the breast.

PNET of breast-treatment and prognosis

PNET is an aggressive disease with high metastatic potential, however, the prognosis of most of the patients is relatively good after surgery. Among 11 cases that had so far been reported in literatures, 5 patients were eventually succumbed because of huge tumors at presentation (4 cases) or a relapse 2 months after the surgery (1 patient) (**Table 1**).

The choice of treatment of PNET depends on the size of tumor, faraway metastasis, age and general conditions of the patients. Effective therapies that combines surgery, chemotherapy and radiotherapy have been reported. Surgery is recommended for cases without extensive metastasis. **Table 1** summarizes the general features of the 11 patients reported in the literature including age of, status of the tumor, therapy received and outcome. In this series of patients (**Table 1**), 8 patients were operabale (66.70%) and all had received radical surgery. The percentage of patients treated with adjuvant chemotherapy or adjuvant radiotherapy was 50.00% and 16.67%, respectively. The other 4 cases received first-line chemotherapy. As far as multidisciplinary treatment was concerned, 4 cases received combined chemoradiotherapy, 6 cases received combined surgery and chemotherapy, and 2 cases had been treated with surgery, chemotherapy and radiotherapy. PNET is not very sensitive to routine chemotherapy, so a serial of aggressive combined chemotherapy regimen are recommended. The most representative one of chemotherapy is alternating the use of the CAV protocol (cyclophosphamide: CTX; adriamycin: ADM; and vincristine: VCR) and the IE protocol (Ifosfamide: IFO; and etoposide: ETO) [16]. In this cohort, 3 patients received CAV regimen and 1 patient received CAV/IE regimen in adjuvant setting; and 1 patient received CAV regimen and 2 patients received CAV/IE regimen in the first-

Table 4. Univariate prognostic analysis between clinical pathological characteristics and overall survival in patients with PNET of breast (12 cases)

	Factors	N	OS	
			Univariate analysis (months)	*p* value
Age	≤30 years	3 (25.00%)	15.67±8.38 (0-32.10)	0.314
	>30 years	9 (75.00%)	34.33±6.98 (20.66-48.01)	
LN Metastasis	Negative	8 (66.70%)	44.00±5.37 (33.48-54.52)	0.001
	Positive	4 (33.30%)	7.75±3.30 (1.28-14.22)	
Faraway Metastasis	Negative	8 (66.70%)	43.00±6.39 (30.48-55.53)	0.008
	Positive	4 (33.30%)	10.75±4.52 (1.90-19.60)	
Surgery	No	4 (33.30%)	10.75±4.52 (1.90-19.60)	0.008
	Yes	8 (66.70%)	43.00±6.39 (30.48-55.53)	

line chemotherapy. In our case, in addition to a radical surgery, the patient received 6 cycles of chemotherapy of CAV regimen. 51 months after surgery, the patient is with persistent stable disease without distant metastasis. So far, she is the longest survival of all patients reported.

Median follow-up duration was 18.08±14.58 (3-50 mo) months in the present study (**Table 4**). 1 case presented relapsed disease and 4 patients died. The 1- and 3-year survival rate of the whole group was 75.0% and 58.3%, respectively. Univariate analysis revealed that lymph node status, faraway metastasis and surgery were significant prognostic factors ($P<0.050$). Therefore, PNET of breast is an extremely rare disease, and so far only 11 cases have been reported before, and the present case is the first one in China reported. Diagnosis of PNET is based on morphologic, immunohistochemical and genetic analyses. Surgery in conjunction with systemic chemotherapy and radiotherapy are recommended. Close follow-up shoud be carried out in order to find recurrent or metastatic disease as soon as possible.

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Disclosure of conflict of interest

None.

Authors' contribution

YCX and FCZ designed the clinical protocol; BSY, LT, HYX and YJY were involved in treating patients and collecting data; BC and NNY were involved with laboratory diagnosis; LT and YCX wrote the paper with contributions from the other authors. All authors read and approved the final manuscript.

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