Primary Neuroendocrine Carcinoma of the Breast and Invasive Carcinoma of No Special Type: A Case-Control Study on Clinicopathological Features and Factors Associated with Lymph Node Metastasis (Postprint)

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Abstract

Objective: To investigate the clinicopathological differences between primary neuroendocrine carcinoma of the breast (NEBC) and invasive carcinoma of no special type (NST), as well as the relevant factors influencing lymph node metastasis. Results: There were no statistically significant differences between the two groups in terms of patient age, tumor diameter, histological grade, number of dissected lymph nodes, and TNM stage, indicating relatively consistent baseline levels. Among 50 cases of primary neuroendocrine carcinoma of the breast, 15 patients had lymph node metastasis. The positive expression rates were 96% for estrogen receptor, 84% for progesterone receptor, 8.5% for HER-2, 58% for chromogranin A, and 97.9% for synaptophysin. In the analysis of factors related to lymph node metastasis, T stage showed a significant difference in the NST control group (P=0.006) but not in the study group (P=0.872). No clear differences were found between lymph node metastasis rate and T stage, histological grade, or immunohistochemical indicators in the study group (P>0.05). Conclusion: In primary neuroendocrine carcinoma of the breast, estrogen receptor and progesterone receptor show high positive expression rates, HER-2 shows a low positive expression rate, and synaptophysin shows a higher positive expression rate than chromogranin A. Although prognostic criteria for evaluating NEBC remain controversial, combined detection of the aforementioned immunohistochemical indicators and lymph node status should still be emphasized.

Full Text

Clinicopathological Features and Lymph Node Metastasis-Related Factors in Primary Neuroendocrine Breast Cancer and Invasive Breast Cancer of No Special Type: A Case-Control Study

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Abstract

Objective: To investigate the clinicopathological differences between primary neuroendocrine breast cancer (NEBC) and invasive breast cancer of no special type (NST), as well as the factors influencing lymph node metastasis.

Results: No statistically significant differences were observed between the two groups in age, tumor diameter, histological grade, number of dissected lymph nodes, or TNM stage, indicating comparable baseline characteristics. Among the 50 NEBC patients, 15 had lymph node metastasis. The positive expression rates were 96% for estrogen receptor, 84% for progesterone receptor, 8.5% for HER-2, 58% for chromogranin A, and 97.9% for synaptophysin. In the analysis of factors affecting lymph node metastasis, T stage showed significant differences in the NST control group (P=0.006) but not in the NEBC study group (P=0.872). No significant associations were found between lymph node metastasis rate and T stage, histological grade, or immunohistochemical markers in the study group (P>0.05).

Conclusion: Primary neuroendocrine breast cancer exhibits high positive expression rates of estrogen and progesterone receptors, low HER-2 positivity, and higher synaptophysin than chromogranin A expression. Although prognostic criteria for NEBC remain controversial, combined detection of these immunohistochemical indicators and assessment of lymph node status warrant significant attention.

Keywords: Neuroendocrine breast cancer; Estrogen receptor; Progesterone receptor; Human epidermal growth factor receptor 2; Chromogranin A; Synaptophysin; Lymph node metastasis

Introduction

Neuroendocrine breast cancer shares similar pathological features with small intestinal neuroendocrine tumors, with the earliest records dating back to 1963[1]. It remains a rare histological subtype of breast cancer with distinct biological behavior and prognostic characteristics[1,2]. Depending on diagnostic and classification criteria, neuroendocrine breast cancer accounts for 1% to 5% of all breast cancers[3]. Cubilla and Woodruff[4] established a histological classification for neuroendocrine breast cancer in 1977, leading to gradual recognition of this rare entity. The 2003 WHO classification[5] defined neuroendocrine breast cancer, establishing that expression of neuroendocrine markers in more than 50% of cells on immunohistochemical staining is the sole mandatory diagnostic criterion. The 4th edition of the WHO classification (2012)[6,7] categorized breast cancers with neuroendocrine features into three types based on morphological characteristics: well-differentiated neuroendocrine tumors, poorly differentiated neuroendocrine carcinoma or small cell carcinoma, and invasive carcinoma with neuroendocrine differentiation. Current prognostic studies on primary neuroendocrine breast cancer are limited. This study compares immunohistochemical marker positivity and lymph node metastasis rates between primary neuroendocrine breast cancer and invasive breast cancer of no special type to explore the biological characteristics and provide evidence for treatment and prognostic evaluation.

Subjects and Methods

Subjects

We selected 50 patients with primary neuroendocrine breast cancer treated at Peking Union Medical College Hospital between 2004 and 2016 as the study group, and randomly selected 200 patients with invasive breast cancer of no special type who underwent surgery during the same period as the control group. The NEBC group had a mean age of 56.4 years (range 31-80), while the NST group had a mean age of 54.9 years (range 28-82). The randomization method involved selecting NST patients from the same years and randomly matching them by clinical stage. Inclusion criteria required patients to be newly diagnosed breast cancer patients who underwent surgery at our hospital with subsequent treatment and regular follow-up; patients lost to follow-up were excluded.

Methods

We collected detailed pathological and immunohistochemical data as well as postoperative follow-up results for both groups. All pathological diagnoses were confirmed by paraffin sectioning according to the 4th edition of the WHO Classification of Tumours of the Breast, with interpretation by at least two pathologists. Comparative analysis of immunohistochemical positivity rates, lymph node metastasis status, postoperative comprehensive treatment, and follow-up outcomes was performed to identify differences between the two groups.

Statistical Analysis

SPSS 22.0 software was used for statistical analysis of clinical characteristics and factors influencing lymph node metastasis rate. Chi-square test or Fisher' s exact test was applied, and Pearson correlation analysis was used for correlation assessment. P<0.05 was considered statistically significant.

Results

Pathological Characteristics

The pathological features of primary neuroendocrine breast cancer include: (1) microscopic appearance of tubular or trabecular structures, with some tumor cells showing larger nuclei and fine chromatin; (2) high positive expression rates of estrogen and progesterone receptors and low HER-2 positivity; and (3) positive expression of at least one of chromogranin A or synaptophysin in more than 50% of cells. In our study group, 18 patients (36%) had type 1 well-differentiated neuroendocrine tumors, 3 patients (6%) had type 2 poorly differentiated neuroendocrine carcinoma or small cell carcinoma, and 29 patients (58%) had type 3 invasive carcinoma with neuroendocrine differentiation.

[Figure 1: see original paper] A. HE staining showing numerous cancer cells with tubular and trabecular structures $×$150; B. Immunohistochemical staining for ER showing strong positivity in tumor cells $×$150; C. Immunohistochemical staining for PR showing moderate positivity in tumor cells $×$150; D. Immunohistochemical staining for HER-2 showing no significant staining in tumor cells $×$150; E. Immunohistochemical staining for chromogranin A showing diffuse positivity in tumor cells $×$150; F. Immunohistochemical staining for synaptophysin showing diffuse positivity in tumor cells $×$150.

Patient Demographics and Clinical Features

We reviewed pathological information from 9,442 breast cancer patients treated at Peking Union Medical College Hospital between November 2004 and October 2016, identifying 50 cases (0.5%) of primary neuroendocrine breast cancer. In the study group of 50 NEBC patients, the mean age was 56.4 years (range 31-80) and the mean maximum tumor diameter was 2.16 cm (range 0.2-10 cm). In the control group of 200 NST patients, the mean age was 54.9 years (range 28-82) and the mean maximum tumor diameter was 1.98 cm (range 0.3-7 cm).

Among NEBC patients, 30 (60%) underwent modified radical mastectomy, 6 had simple mastectomy plus sentinel lymph node biopsy, and 5 had breast-conserving surgery (local excision plus sentinel lymph node biopsy or axillary lymph node dissection). Nine elderly patients (over 70 years) underwent only local tumor excision without axillary lymph node staging, and 2 patients with stage IV disease at initial diagnosis underwent modified radical mastectomy. Forty-one patients had complete pathological staging, predominantly stage I and II: stage 0 (n=1, 2.4%), stage I (n=14, 34.1%), stage II (n=18, 43.9%), stage

III (n=6, 14.6%), and stage IV (n=2, 4.9%). In the NST control group, stage distribution was: stage I (n=68, 41.2.1%), stage II (n=65, 39.4%), and stage III (n=32, 19.4%). No statistically significant differences were found between the two groups in clinical staging parameters including tumor diameter, number of dissected lymph nodes, or number of metastatic lymph nodes.

Comparison of clinical characteristics between NEBC and NST groups

The comparison shows no statistically significant differences in age, tumor diameter, histological grade, number of dissected lymph nodes, or TNM stage between the selected patients, indicating comparable baseline characteristics.

Immunohistochemical Findings

Among the 50 NEBC patients, ER positivity was 96% (48/50), PR positivity was 84% (42/50), and HER-2 positivity (immunohistochemical staining 3+++ or FISH-positive) was 8.5% (5/48). Two patients with HER-2 2++ staining did not undergo FISH testing and were excluded from the HER-2 analysis. Chromogranin A positivity was 58% (29/50) and synaptophysin positivity was 97.9% (47/48) in all NEBC patients. Ki-67 index was available for 48 NEBC patients: 22 had Ki-67 <14% and 26 had Ki-67 $ $14%.

In the NST control group, ER positivity was 78.5% (157/200), PR positivity was 78% (156/200), and HER-2 positivity was 14.5% (29/200). Ki-67 index was available for 190 NST patients: 52 had Ki-67 <14% and 138 had Ki-67 $ $14%. Molecular subtyping was possible in 37 NEBC patients and 154 NST patients; notably, no HER-2 overexpression subtype was identified among NEBC patients.

Postoperative treatment data showed that 48% (24/50) of NEBC patients received intravenous chemotherapy, 34% (17/50) received radiotherapy, and 94% (47/50) received endocrine therapy. In the NST group, 62% (124/200) received chemotherapy, 40% (80/200) received radiotherapy, and 81% (162/200) received endocrine therapy.

Lymph Node Metastasis Analysis

Factors influencing lymph node metastasis rate in NEBC

Factors influencing lymph node metastasis rate in NST

In the analysis of factors affecting lymph node metastasis, T stage showed significant differences in the NST control group (P=0.006) but not in the NEBC study group (P=0.872). Stratified analysis by histological grade, estrogen receptor, progesterone receptor, HER-2 positivity, Ki-67 index, and molecular subtype revealed no statistically significant differences (P>0.05).

Follow-up and Survival

The mean follow-up duration was 44.2 months for NEBC patients. Two patients died from distant metastasis (brain and liver metastases), one died from

heart failure, two developed lung metastasis, two had local recurrence, and one had bone metastasis. Two patients with stage IV disease at initial diagnosis were excluded from overall survival analysis to avoid bias. In the NST control group, mean follow-up was 45.76 months, with two deaths (one from multiple visceral metastases, one from myocardial infarction), two liver metastases, nine bone metastases, three lung metastases, two brain metastases, two chest wall metastases, one local recurrence, and one lymph node metastasis.

[Figure 2: see original paper] Blue curve represents the NEBC group, red curve represents the NST group. No significant differences were observed between the two groups in overall survival or disease-free survival.

Figure 2A. Overall survival curve (OS). Figure 2B. Disease-free survival curve (PFS).

Discussion

Primary neuroendocrine breast cancer is a rare and distinct subtype of breast cancer. Since the 2003 WHO definition, this rare entity has gradually gained clinical recognition, with chromogranin A and synaptophysin serving as reliable diagnostic markers. Histologically, neuroendocrine carcinoma is characterized by uniform round or spindle cells with palisading nuclei and abundant eosinophilic granules in the cytoplasm[8-10]. Literature reports indicate that primary neuroendocrine breast cancer accounts for 1% to 5% of all breast cancers depending on classification criteria[2-3]; in our study, it represented 0.5% of all breast cancers treated during the same period, slightly lower than reported rates. The mean age at onset in our study was 56.4 years, later than the average for breast cancer overall[11] and similar to the 53.2 years reported by Li et al.[12] for Chinese patients. Notably, 38% (19/50) of our patients were over 60 years old, and 20% (10/50) were over 70.

The overall expression rates of ER and PR in all breast cancers are approximately 50%, whereas neuroendocrine breast cancer shows significantly higher expression[7,12]. In our 50 patients, ER positivity was 96% (48/50) and PR positivity was 84% (42/50), substantially higher than other breast cancer types, with 94% (47/50) receiving endocrine therapy postoperatively. PR positivity correlated negatively with primary tumor size (P=0.027), though literature on this relationship is limited and its clinical significance requires further investigation. The high hormone receptor expression in neuroendocrine breast cancer suggests suitability for endocrine therapy and may reflect prognostic implications.

The HER-2 gene, also known as HER-2/neu, plays a crucial role in breast cancer development, regulating cell growth, proliferation, and differentiation, with overexpression indicating poor prognosis. In our NEBC patients, HER-2 positivity was 8.5% (4/50), lower than the 15.1% reported in Chinese patients by Li et al.[12-13] but similar to the 9.5% reported by Feki et al.[14]. The NST control group showed 11.5% (23/200) HER-2 positivity. All four HER-2-positive

NEBC patients received anti-HER-2 therapy; during follow-up, two developed lymph node metastasis and one had bone metastasis. Whether HER-2 positivity indicates poorer prognosis in neuroendocrine breast cancer requires further investigation with larger case series; current case numbers are insufficient for statistical analysis.

Neuron-specific enolase (NSE) is a commonly used neuroendocrine marker, expressed in 16% to 50% of all breast cancers[15], including breast cancer cells without argentaffin granules, making it unsuitable as a sole diagnostic criterion for neuroendocrine breast cancer. Synaptophysin and chromogranin A are considered reliable markers, with positivity rates of 58% and 97.9% respectively in our study, consistent with literature reports[16,17]. We did not identify distinct expression patterns of these two antibodies across neuroendocrine carcinoma types, and their significance requires further investigation.

Lymph node metastasis status is crucial for guiding adjuvant therapy and predicting prognosis in breast cancer. Given the rarity of NEBC, studying its lymph node metastasis patterns has important clinical significance. Our comparative analysis of NEBC and NST patients revealed no significant differences in lymph node metastasis rates when stratified by T stage, ER, PR, HER-2, Ki-67 index, and molecular subtype. While T stage was significant in the NST group (P=0.006), it was not in the NEBC group (P=0.872). Unlike NST, NEBC lymph node metastasis does not appear to correlate clearly with T stage. Literature on prognosis comparison between NEBC and NST remains controversial[4,12,18,19]; our results suggest that NEBC lymph node metastasis may not correlate strongly with primary tumor size and may occur earlier in disease course.

Neuroendocrine breast cancer can metastasize to multiple sites including liver, bone, lung, pancreas, brain, and soft tissue, with corresponding cases observed in our study, emphasizing the importance of long-term postoperative follow-up. Our mean follow-up of 44 months is a limitation, as it falls short of 5 years; we will continue long-term follow-up to strengthen our data. Evaluating the biological characteristics of metastases has clinical significance for salvage therapy[20-21]; however, such data are limited in our study.

Although primary neuroendocrine breast cancer is rare, recent reports have identified PIK3CA mutations and vascular endothelial growth factor receptor 2 (VEGFR2) mutations[22-23], potentially providing therapeutic targets.

In summary, primary neuroendocrine breast cancer is a rare histological subtype with high ER and PR positivity and low HER-2 expression. Its prognosis remains controversial[17,18,24]. Unlike NST, NEBC lymph node metastasis does not show clear correlation with T stage. Combined detection of ER, PR, HER-2, synaptophysin, and chromogranin A expression, along with analysis of their relationship with clinicopathological data, helps evaluate the biological behavior of primary neuroendocrine breast cancer and provides valuable reference for treatment planning.

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Note: Figure translations are in progress. See original paper for figures.