Neuroendocrine Carcinoma of the Breast - Diagnostic and Clinical Implications

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Abstract. *Background: Neuroendocrine breast carcinomas (NEC) are rare. Carcinomas with mixed composition often behave differently from 'pure' histological types, and the prognosis is determined by the proportion of the more aggressive tumour. The molecular classification helps in making therapeutic decisions. Case Report: A 56-year-old Caucasian woman with palpable and preoperatively biopsied breast tumour was treated with breast-conserving surgery. The histological specimen revealed a 17-mm invasive carcinoma with an equal proportion of neuroendocrine and invasive-ductal differentiation, accompanied by peritumoural ductal carcinoma in situ. TNM classification was pT1c(is), pN0 (0/1sn), G3, L0, V0, Pn0, R0. The diagnosis was enhanced by immunohistochemistry: high positivity for synaptophysin, neuron-specific enolase (NSE), neural cell adhesion molecule (CD56), Ki-67 (proliferation index 46%), estrogen receptor (ER) and progesterone receptor (PR), negative for Her-2-neu and cytokeratin 5/6, resulting in diagnosis of the molecular 'luminal B' subtype. Radiation and adjuvant chemotherapy with six cycles of 5-fluorouracil, epirubicin and cyclophosphamide, followed by tamoxifen and subsequent exemestane for five years, were recommended. Conclusion: Immunohistochemistry plays a crucial role in the diagnosis of rare cancer subtypes. NEC is characterized by high biological aggressiveness. Molecular classification facilitates therapeutic decisions.*

Rare cancer types are difficult to diagnose and treat due to lack of individual experience and of reliable large studies. Carcinomas with mixed composition often behave differently from the 'pure' histological types, and the prognosis is determined by the proportion of the more aggressive tumour. Neuroendocrine tumours of the breast represent 0.5-1% of all breast carcinomas (1, 2). Their clinical behaviour and therapeutic outcomes are reported to be contradictory in literature (2-4). Recently, the need for exploring therapeutic approaches for this newly-defined, unique entity was emphasized (4).

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Case Report

A 56-year-old woman, gravida 2, para 2, body mass index 37, non-smoker, after hysterectomy and unilateral adnexectomy for endometriosis 15 years earlier, palpated two bean-sized tumours in both her breasts. She immediately sought help at our department. The right-sided tumour appeared suspicious on ultrasound (a hypoechogenic lesion 15×12×11 mm, with angular margins, spiculation, microlobulation, posterior shadowing). Both lesions were ultrasound-guided core-biopsied. On the right side, the malignancy was confirmed as poorly-differentiated, solid-growing, 'probably invasive ductal' breast carcinoma, with a high expression of estrogen receptor (ER) in ca. 90% tumour cells, progesterone receptor (PR) in 60-70%, and human epidermal growth factor receptor 2 (c-ERBB2) score +2 [chromogenic *in situ* hybridization (CISH)- negative], with fibrous mastopathy as an additional finding. On the left side, a benign fibroadenoma was diagnosed. Serum levels of the tumour markers carcinoma antigen 15-3 (CA15-3) and carcinoembryonic antigen (CEA) were within normal range (15.2 U/ml and 0.9 ng/ml, respectively). We performed a segmental breast resection of the right side. The radionuclide-guided (20 MBq ^{99}mTc-nanocolloid) sentinel lymph node biopsy was negative. Additionally, a needle-guided simple tumour excision was performed on the left side. The final histology revealed a poorly-differentiated, equally mixed (50%:50%) neuroendocrine and invasive ductal carcinoma (Figure 1) with disseminated peritumoural ductal carcinoma *in situ* (DCIS) on

Figure 1. *Haematoxylin and eosin staining, ×200. Solid-growing, poorly-differentiated tumour with clusters of cells with moderate to abundant cytoplasm, nuclei with vesicular to finely granular chromatin and a high number of mitotic figures, 33/10 high power fields.*

Table I. *Immunohistochemical characteristics of the tumour.*

Marker	Reaction
Synaptophysin	Positive (ca. 50% of tumour cells)
Neuron-specific enolase	Positive (ca. 50% of tumour cells)
Neural cell adhesion molecule (CD56)	Positive (ca. 50% of tumour cells)
Ki-67	Positive 46%
Estrogen receptor	Positive 90% (100% in the NEC part)
Progesterone receptor	Positive 60-70%
Tumor protein p63	Partial positive
Cytokeratin 5/6	Mostly negative
CD34 molecule	Uncertain
Monoclonal M2A antibody (D2-40)	Negative/non-specific

the right side. The maximal tumour diameter was 17 mm. Because of narrow resection margins of the DCIS part, a secondary resection was carried out. The final TNM classification was pT1c(is), pN0 (0/1sn), G3, L0, V0, Pn0, R0, ER> 90%, PR 60-70%, c-ERBB2 negative, proliferation index (Ki-67) 46%. This resulted in diagnosis of the molecular subtype luminal B. On the left side, a benign fibroadenoma was confirmed. The diagnosis of neuroendocrine tumour was supported by extensive immunohistochemical phenotyping (Table I, Figure 2). About 50% of all tumour cells revealed a high expression of the neuroendocrine markers synaptophysin and neuron-specific enolase (NSE). One hundred percent of the neuroendocrine carcinoma cells expressed ER, and most of them also expressed PR (Figure 2). All staging investigations (chest X-ray, abdominal ultrasound, bone scintigram) were negative. Based on 'luminal B' breast cancer diagnosis, the interdisciplinary tumour board recommended chemotherapy of six cycles of 5-fluorouracil, epirubicin and cyclophosphamide (FEC), followed by radiotherapy and tamoxifen at 20 mg/day, switching to exemestane at 25 mg/day after 2-3 years for a total of 5 years. Currently, the patient is alive and free of relapse, 15 months after the first diagnosis.

Discussion

Pure NEC of the breast is extremely rare; about 40 cases have been so far reported in the literature. The WHO definition from 2003, based on Sapino *et al*'s study, divides

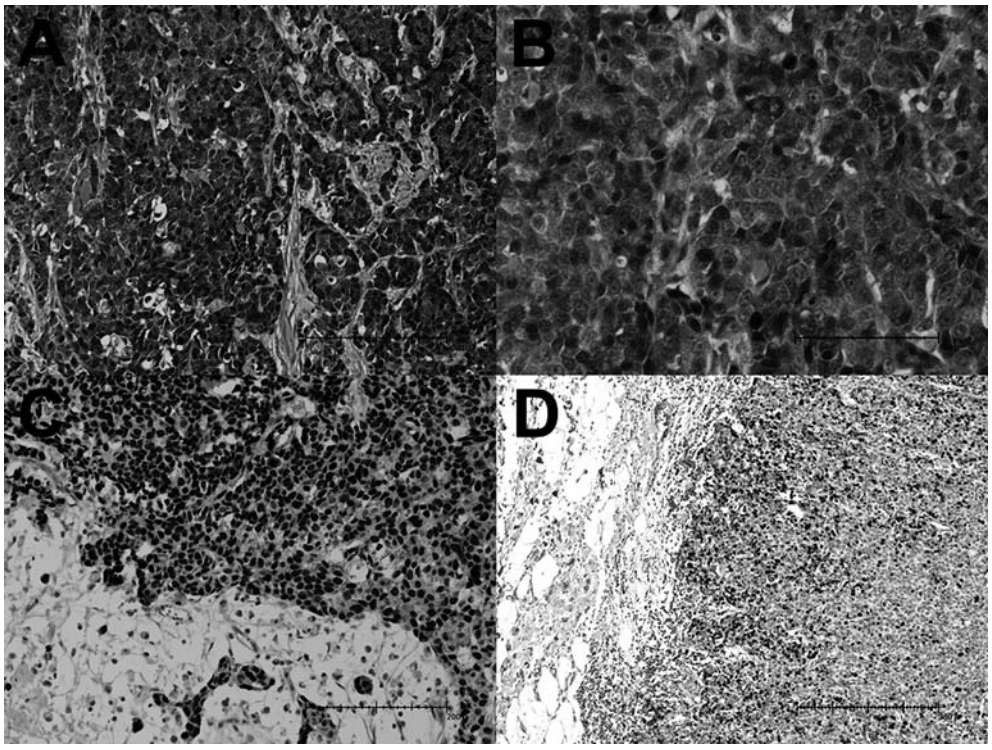

Figure 2. *High positivity of neuroendocrine tumour for synaptophysin (A), neuron-specific enolase (B), Ki-67 (C), and estrogen receptor (D).*

breast cancer with neuroendocrine differentiation into 'neuroendocrine' and 'partial neuroendocrine' tumours, depending on whether more or less than 50% of the tumour cells express immunohistochemical neuroendocrine markers (5, 6). A newer analysis of 1,368 infiltrating breast tumours found only 7 (0.5%) fulfilling the strong WHO criteria for NEC. Remarkably, these tumours belonged to the luminal A subtype (1). The most frequent feature is the partial neuroendocrine differentiation (fewer than 50% of tumour cells). This feature should be seen in up to 2-5% of all breast carcinomas (5). In addition, the WHO definition describes three subtypes of NEC: the solid, the small-cell, and the large-cell NEC. Since strong criteria were established in 2003, only a few studies could be considered as reliable for real diagnostic and therapeutic purposes (4). The diagnosis of NEC is based on morphological features and neuroendocrine markers. Chromogranin A and synaptophysin show the best sensitivity and specificity as immunohistochemical markers (2). In contrast to NEC of the intestinal type, most neuroendocrine breast carcinomas exhibit a high expression of ER and PR proteins (4). This is a relevant therapeutic target. The therapeutic decision should be supported by molecular subtyping. Gene expression profiling of breast cancer has identified two biologically-distinct ER-positive subtypes of breast cancer: luminal A and luminal B. Luminal B tumours have a higher proliferation index (measured as Ki 67 expression in >13% cells) and a poorer prognosis than luminal A tumours (7). The luminal B subtype is associated with a higher metastatic potential (particularly bone metastases) (8). In the present case, the poorly-differentiated tumour with a high Ki 67 proliferative index of 47%, ER/PR-positive, and Her-2-neu-negative belonged to the luminal B subtype. The recommendation for chemotherapy is based on the risk assessment. According to traditional criteria, grading and age were unfavourable risk factors in the presented case, whereas negative nodal status, small tumour diameter, ER and PR positivity and Her-2-neu negativity were favourable risk factors. A decision as to whether the case represented a 'pure' or 'partial' NEC would be an academic discussion, as almost exactly 50% of tumour cells exhibited immunohistochemical neuroendocrine features. The therapeutic decisions were determined through stage, grading, and proliferation index. Taking the molecular classification as "luminal B" into account made the recommendation for chemotherapy easier. The latest data have consistently indicated that NEC is a clinicopathological entity with a worse outcome than invasive ductal carcinoma (4, 9). Wei *et al*. compared 74 patients with NEC with 142 age- and stage-matched invasive ductal controls. NEC was more likely to be ER/PR-positive and Her-2-neu-negative.

Despite similar age and disease stage at presentation, NEC had a more aggressive course than invasive ductal carcinoma, with a higher propensity for local and distant recurrence and poorer overall survival. High nuclear grade, large tumour size, and regional lymph node metastasis were significant negative prognostic factors for distant recurrence-free survival (4). Using the same patient population, Tian *et al*. concluded that the overall survival of patients with NEC is determined significantly by tumour size, lymph node status, and proliferation rate (Ki-67). They postulated routine use of the proliferation index in the diagnosis of breast NEC (9). In one case report, serum CEA was recommended for diagnosis and follow-up of breast NEC (10). As the initial concentration of CEA and CA15-3 were, in our case, normal, we did not use them for follow-up. Two small studies (11, 12) dealt with partial (<50%) neuroendocrine differentiation in breast cancer. They showed no impaired prognosis compared to invasive ductal carcinoma. Importantly, both reports were submitted before publication of the strict WHO criteria. The small number of cases in (11) and (12) (11 and 9 cases, respectively), their heterogeneity (neuroendocrine differentiation in 5-50% of cells), the expression of neuroendocrine markers (NSE and/or chromogranin A and/or synaptophysin) as the only analysed criterion (no separate analysis by grading or proliferation rate), and other surprising findings – *e.g*. the majority of cases in (11) were ER-negative - indicate caution in the interpretation of those results. Sapino *et al*. stated that the histological grade is more important than the immunophenotype in determining the prognosis of NEC of the breast (6). Furthermore, Tian *et al*. concluded that the proliferation rate measured as Ki-67 (that is, indirectly, the molecular subtyping of NEC) and nodal status are the only significant and independent prognostic factors for disease-free survival in NEC (9). Generally, the nature of the more aggressive part is considered as crucial for therapeutic decisions. In the present case, the pathological statement that 'almost' 50% of the tumour cells expressed neuroendocrine markers showed, on the one hand, the difficulty of such 'gray zone' findings, and on the other, played practically no role in the therapeutic strategy, as the molecular classification allowed a clear classification as the luminal B type.

Conclusion

Immunohistochemistry plays a crucial role in the diagnosis of rare cancer subtypes. This case (proliferation index 46%, poor differentiation) provides further evidence that NEC is a biologically aggressive tumour type. The typical positivity for ER and PR provides a relevant therapeutic target. The molecular classification facilitates the therapeutic decision.

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