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Primary neuroendocrine breast carcinoma, well differentiated

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ABSTRACT
Primary neuroendocrine tumor of breast is a rare tumor, with few cases reported each year and with an incidence less than 1% from all neuroendocrine tumors. In our report we present a case of a postmenopausal woman with a lump in her left breast whose histopathological aspect was strongly suggestive for primary solid neuroendocrine breast carcinoma. Immunohistochemical examination has been done using a panel of seven biomarkers in order to confirm our initial diagnose and both prognostic and predictive factors. We used cromogranin A, synaptophysin and neuron-specific enolase as neuroendocrine biomarkers. Diagnose was proved by a positive reaction in more than 50% of tumor cells for the first two antibodies. Immunophenotype of the tumor (estrogen and progesterone receptor positive, low Ki6 index and no supraexpression of HER2) is consisted with luminal A molecular subtype. The prognosis was good based on clinic-pathological features and immunohistochemical expression. The patient has a good clinical evolution after surgical treatment and adjuvant therapy, with no local recurrence or distant metastasis after 3 years of surveillance.

Keywords: Primary neuroendocrine breast carcinoma, Chromorganin A, Synaptophysin, Hormone receptors

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Introduction

Primary neuroendocrine breast carcinoma (NEBC) is a rare tumor, with few cases reported each year worldwide. First reference of BC with carcinoid-like features was made in 1963 [1], but the term "primary carcinoid tumor" was used for the first time fourteen years later [2]. The real incidence of this type of BC could not by exactly established for many years mainly due to lack of clear criteria of diagnosis. This deficiency was corrected in 2003 when World Health Organization (WHO) has proposed the following diagnostic criteria for NEBC: the morphological features have to resemble those of neuroendocrine tumors from lung and gastrointestinal tract and more than 50% of the tumor cells must be immunopositive for neuroendocrine markers [3]. Based on this rules the incidence of NEBC range from 0,5% [4] to <1 % [5] of all breast carcinoma.

The classification of NEBC has also changed as the years passed and new information emerged from larger studies. So, if in 2003 WHO were described three subtypes (solid neuroendocrine carcinoma, large cell neuroendocrine carcinoma and small cell/oat cell carcinoma), in 2012 this classification has changed and encompassed the following subtypes: neuroendocrine tumor well differentiated, neuroendocrine carcinoma poorly differentiated (small cell carcinoma) and invasive breast carcinoma with neuroendocrine differentiation [3,5]. NEBC is usually underdiagnosed both because its morphological features are similar

to those of other subtypes of breast carcinoma and because neuroendocrine markers are not frequently used in daily practice. In our report we present a case of a postmenopausal woman with a lump in her left breast which we proved to be a primary NEBC well differentiated with intermediate histological grade.

Materials and methods

Case report. A seventy eight years old woman was admitted in the surgical department of The Hospital County Constanta for a tumor mass in her upper-outer quadrant of the right breast. She was previously health with no remarkable medical history. Both mammography and ultrasonography showed a tumor nodule with features highly suggestive for malignancy and computer tomography or clinical examination revealed no other abnormalities. Invasive carcinoma was the diagnosis of frozen sections from biopsy of the lump and mastectomy with axillary node dissection was secondly performed. Macroscopic examination revealed a solid tumor mass measuring 2.3x2 cm, well-defined, firm and white-tan color. Histopathologically, tumor consisted of a malignant cell population with solid nests separated by a scant stroma and there was no axillary lymph node metastasis of the eleven examined (T2N0M0, stage IIA). Microscopic aspect was highly suggestive for primary NEBC and immunohistochemistry (IHC) was performed, using a panel of seven biomarkers in order to established the final diagnosis and both prognostic and predictive factors. The patient was treated with chemotherapy and hormonotherapy after surgery and has a good clinical evolution, free from metastases or local recurrence after three years of medical follow-up.

Methods.

Four μm thick sections of formalin fixed, paraffin-embedded tissue block of the tumour were stained with hematoxylin and eosin. The best representative slides were prepared for immunostains. After epitope retrieval, tissue sections were incubated with the following antibody from DakoCytomation – Denmark (ready to use): estrogen receptor (ER- monoclonal rabbit 1D5 clone), progesterone receptor (PR- monoclonal mouse PR 636 clone), HercepTest (rabbit immunoglobulin HercepTest), Ki-67 (monoclonal mouse MIB-1 clone) cromogranin A (polyclonal rabbit), synaptophysin (monoclonal mouse, SY38 clone) and neuron-specific enolase (NSE–monoclonal mouse BBS/NC/VI-H14 clone). We used as chromogen 3,3'diaminobenzidine (DAB), with brown staining of antigen concerned. Sections were finally counterstained with Mayer's Haematoxylin.

We assessed the antibody distribution pattern, percentage of positive cells and intensity of reaction for all biomarkers. A positive reaction for neuroendocrine biomarkers (cromogranin A, synaptophysin, NSE) was considered if cytoplasmic stain was observed in the tumor cells. Immunohistochemical expression of hormonal receptors (ER and PR) was assessed using the semiquantitative scoring method and a positive result was considered if at least 1% of cells show nuclear immunostain signal [6]. Evaluation of HER2 status (over-expression of the HER2/neu protein or amplification of the HER2 gene) was scored using the new recommendations of ASCO/CAP guidelines [7]. For Ki67 immunoexpression the absolute percentage of nuclear stained cell was recorded and a value of 14% was used as a cut-off value for low or high expression [8].

Results

The tumor breast was well defined, with pushing margins and consisted from solid nests with focal necrosis, separated by a thin fibrovascular stroma. The neoplastic cells were round to spindle or plasmacytoid, with eosinophilic cytoplasm and hypercromatic, round to oval nuclei, inconspicuous nucleoli, without mitotic figures. They tend to display a peripheral palisading and to form rare rosette-like structures within tumor islands (Figure 1). It was also

observed areas of solid intraductal carcinoma beside the invasive component of NEBC, proving its breast origin.

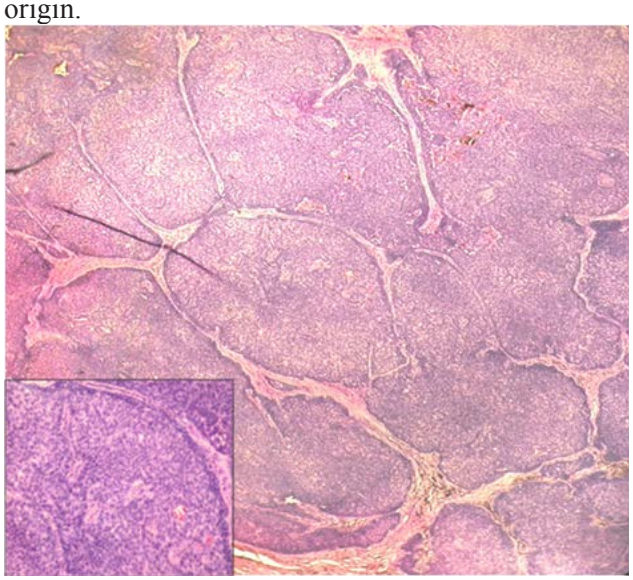

Figure 1 - Solid architecture with palisade arrangement of cells in the periphery of tumor islands and rosette-like structures (HE 40x; incase 100x).

Of the three NE biomarkers we obtained two positive in more than 50% of tumor cells: chromogranin A (Figure 2a) and synaptophysin (Figure 2b).

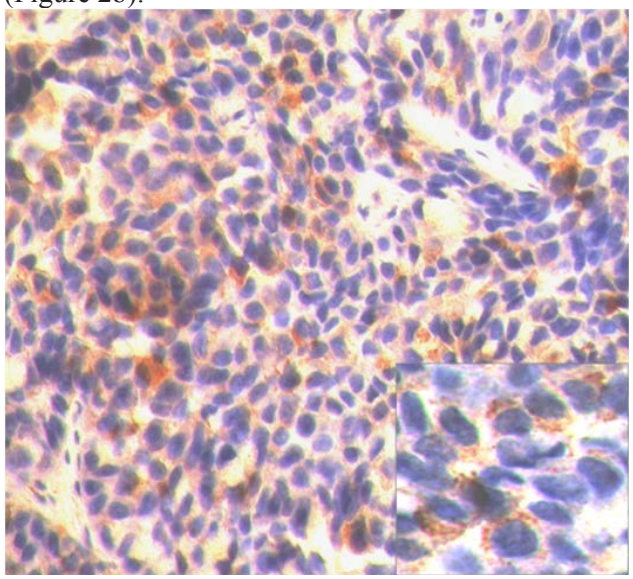

Figure 2a - Immunohistochemical expression of chromgranin A, a positive cytoplasm immunostain in more than half of tumor cells (IHC 100x, in case 200x).

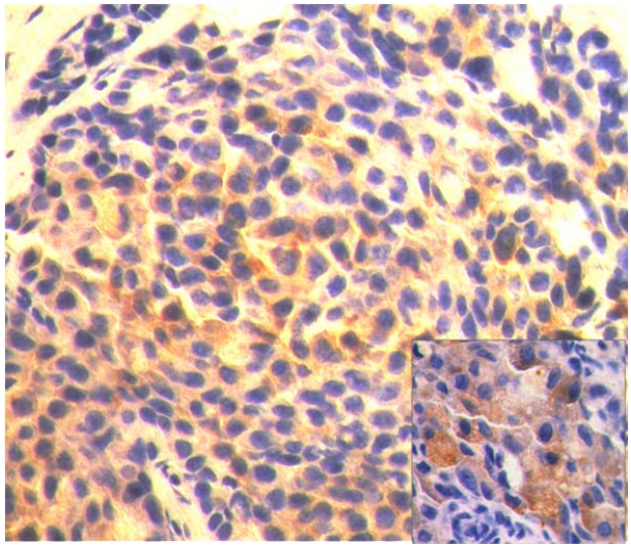

Figure 2b - Immunohistochemical expression of synaptophysin, a positive cytoplasm immunostain in more than half of tumor cells (IHC 100x, in case 200x)

NSE was focally positive (Figure 3). We also indentified an intense positive reaction for hormonal receptors, 75% for ER (Figure 4a) and 90% for PR (Figure 4b), low Ki67 index (<10%) and no supraexpression of HER2/neu oncoprotein.

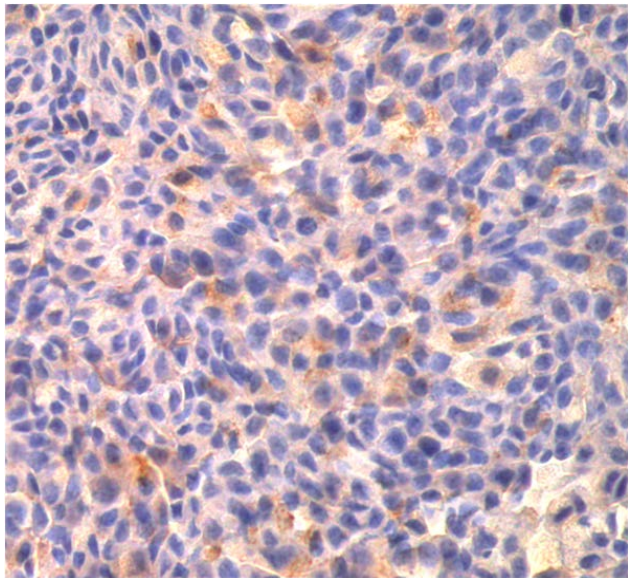

Figure 3 - NSE immunostain with focal cytoplasm positive reaction (IHC 100x).

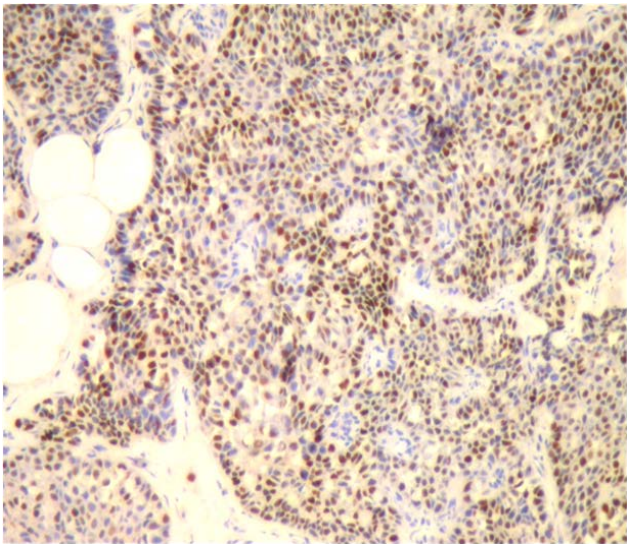

Figure 4a - Intense nuclear immunostain of estrogen receptor (IHC 40x).

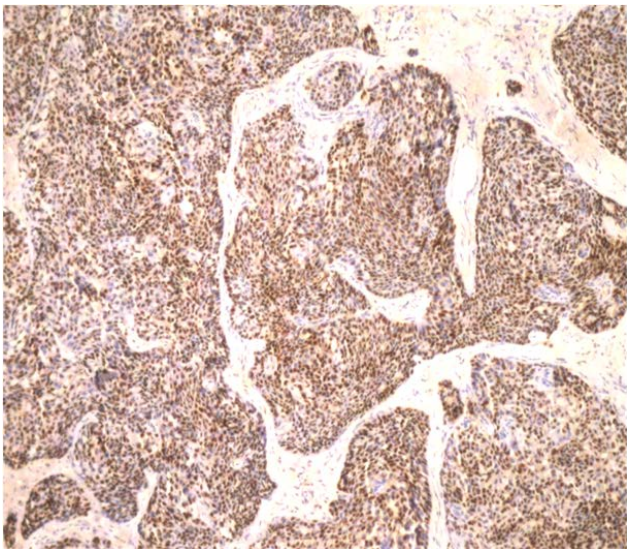

Figure 4b - - Intense nuclear immunostain of progesteron receptor (IHC 40x).

Based on morphological and immunohistochemical results, the final diagnose was primary solid NEBC according to 2003 WHO classification [3], but based on the latest classification from 2012 this case belong to neuroendocrine carcinoma, well differentiated sutype [5].

Discussions

Neuroendocrine carcinoma are extremely rare subtype of invasive mammary carcinoma with a propensity for sixth or seventh decade of life [9]. There are two important conditions for diagnosis of NEBC established in 2003 by WHO Classification of Tumor Series. Histopathologically, tumor must exhibit features of NE tumor of gut or lung and immunohistochemically, at least one of the NE biomarkers should be positive in more than 50% of the tumor cells [3]. The last condition is extremely important because many breast carcinomas may have a focal positive reaction for these biomarkers. Usually, these are invasive ductal carcinoma – no special type (NOS), but also it can be encountered in lobular or medullary carcinoma [10].

NEBC has no special macroscopic characteristics than IDC–NOS but there are some histopathological features suggestive for this diagnose [11]. Tumor architecture (nesting or alveolar pattern), palisade arrangement of cells in the periphery of tumor islands and rosette-like structures within the tumor aggregates are the most important features for diagnose [4, 12]. Although these morphological characteristics are highly suggestive for NEBC diagnose, they lack sometimes [13].

IHC is an extremely useful tool in established the diagnosis of NEBC. A positive reaction for at least one antibody against chromogranin, synaptophysin or NSE in more than half of the neoplastic cells is mandatory for diagnose. In our case we obtained an intense positive reaction for chromogranin and synaptophysin, but NSE was focally positive. These results were sufficient for diagnose of NEBC. IHC also plays an important role in differential diagnosis mainly with metastatic NE tumor in the breast. A positive hormonal status beside the presence of an intraductal component and no other primary tumor or metastasis at CT examination are consisted with diagnose of primary NEBC. The high rates of positivity for hormonal receptors are similar with results of other researchers [14] and represent a good predictive factor as these tumors can be treated with

endocrine therapy.

Since Perou et al [15] and Sorlie and colleagues [16] first published their results regarding molecular profile of breast cancer, based on gene expression analysis, intrinsic molecular classification is increasingly used in the field of breast pathology. Imunohistochemistry has been successfully used as a surrogate for intrinsic molecular classification with a high sensitivity and specificity. Nowadays, there are accepted four major molecular subtypes (Luminal A; Luminal B; HER2-positive and Triple negative), based on IHC expression of ER, PR, HER2 and KI67, with different prognosis and therapeutic recommendations [17]. Immunophenotype of our patient (ER+; PR+; Ki67 low; HER2-) corresponds to Luminal A molecular subtype. This result is in agreement with other studies [18] and predicts a good prognosis without a high risk for recurrence [19,20].

The prognosis for NEBC depends not only on IHC profile but some clinico-pathological features are also important, histological grade being the most important factor [21]. In our case the tumor was intermediate grade and may also explain the good clinical evolution. The study of Tian Z et al (2011) on 74 cases of NEBC demonstrates that over-all survival vary according to lymph node status, tumor size and Ki-67 index, both at univariate and multivariate analysis, but distant recurrence-free survival depends only by nodal status [22]. On the other hand, when compared with similar pathological stage of IDC-NOS, NEBC has a poor prognosis [22].

On these facts, we conclude a good clinical outcome for our patient as she had a low Ki67 index, a relatively small tumor size and no involvement of lymph node beside a luminal A phenotype. Our prediction may be validated by no local recurrence or distant metastasis after three years of surveillance. Also our case brings attention on a very rare subtype of invasive breast carcinoma, whose diagnosis is difficult and requires both a careful histopthological examination and positive immunostain for neuroendocrine markers.

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