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Diagnostic utility of mammaglobin and GCDFP-15 in the identification of primary neuroendocrine carcinomas of the breast

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In addition to the presence of morphological neuroendocrine (NE) features, expression of the NE markers chromogranin A and synaptophysin has been considered the most sensitive and specific immunohistochemical feature in the diagnosis of pure, solid NE breast carcinomas [NEBC; 1]. Following these criteria, the existence of NEBC can be accurately confirmed in <2% of all primary breast cancers when using adequate cytological specimens including fine needle aspiration smears [1-4]. Within a working model for a breast cancer molecular taxonomy [5], recent gene expression profiling studies have confirmed a distinct nature of NEBC from conventional invasive ductal carcinomas as well as their belonging to the good-prognosis (being positive for hormone receptors and HER2-negative) subgroup of luminal A breast tumors [6]. NEBC may occur, however, as a first presentation of an occult metastatic non-mammary neuroendocrine tumor or as a *bona fide* primary breast tumor. Therefore, the clinical effect of the occurrence of neuroendocrine differentiation in breast cancer should be carefully examined to reduce or avoid the risk of a misdiagnosis or under-diagnosis. In this regard, Richter-Ehrenstein et al. have recently evaluated a new diagnostic tool to distinguish between primary NEBC from a metastatic disease of other sites [7]. Using the immunohistochemical markers for epithelial of breast origin mammaglobin and Gross Cystic Disease Fluid Protein (GCDFP-15), they compared a subset of pure NEBC (n=9) with a cohort of non-mammary neuroendocrine tumors. In their hands, mammaglobin was positive in 4 out of 9 tumors while GCDFP-15 was expressed in 6 out of 9 cases. In three patients, both markers were negative. Remarkably, they did not find any expression of mammaglobin or GCDFP-15 in immunohistochemical evaluation of chromogranin A/synaptophysin-positive non-mammary neuroendocrine tumors [7].

We have recently determined the incidence, clinico-pathological features and immunohistochemical profiling of primary pure NEBC in a large series of infiltrating breast tumors collected from 1989 to 2010 in our institution (Dr. Josep Trueta University Hospital, Girona, Catalonia). From the entire series of more than 1500 carcinomas, only 8 cases fully satisfied the primary pure NEBC criteria by the WHO (i.e. the presence of > 50% tumor immunoreactivity for one of neuroendocrine markers including chromogranin, synaptophysin and CD56), which constitutes an incidence of solid NEBC as low as 0.5% [data updated from ref. 8]. To definitely confirm the primary nature of these endocrine tumors, we recently tested the

hypothesis that mammaglobin staining can help, in combination with GCDFP-15 (a sensitive and specific marker of breast carcinoma), to establish the correct diagnosis of breast carcinomas [9]. **Table 1** summarizes the immunohistochemical profile for the expression of estrogen receptor (ER), progesterone receptor (PR), HER2, mammaglobin, and GCDFP-15 in tumor specimens from NEBC patients. Our findings largely recapitulate those presented by Richter-Ehrenstein et al [7]. All NEBC expressed high levels of estrogen and progesterone receptors. Mammaglobin was positive in 4 out of 9 samples. In contrast, GCDFP-15 was expressed in 7 out of 9 specimens. We did not observe discordant expression of mammaglobin and GCDFP-15 markers between primary and metastatic tissues in a patient that debuted with metastasis in the soft tissue of the cheek prior to diagnosis of primary NEBC. It was intriguing that HER2 2+ staining (but absence of gene amplification) was found in the sole NEBC that was negative for both markers. Richter-Ehrenstein et al similarly reported that HER2 overexpression was found in only one NEBC, which was negative for both markers [7]. Although the very small number of cases obviously limits the relevance of the correlation between HER2 positivity and mammaglobin/GCDFP-15 double-negative NEBC patients, additional studies with longer follow-up and more cases need to be further pursued to establish whether this “combimarker” (i.e. detecting HER2 overexpression in the absence of mammaglobin/GCDFP-15 markers) may delineate a distinctive subgroup of luminal B-like (i.e. ER-positive or PR-positive and HER2-positive [10]) among the well-established ER/PR-positive HER2-negative status of NEBC. Although GCDFP-15 has classically been considered a secretory marker of apocrine differentiation in breast cancer [11, 12], the fact that the GCDFP-15 positivity is transient (i.e. breast cancer cases which have apocrine features but lack GCDFP-15 expression suggest advanced apocrine carcinomas [13]) together with the pre-clinical finding that the prolactin-inducible protein *PIP/GCDFP-15* gene was the mostly up-regulated mRNA in luminal B (ER-positive/HER2-positive) BT-474 breast cancer cells treated with the insulin-like growth factor-I receptor (IGF-IR) antagonist NVP-AEW541 and the anti-HER2 monoclonal antibody trastuzumab [13], reinforce the suggestion that yet to be established regulatory relationship between GCDFP-15, HER2, and apocrine differentiation would take place in subsets of NEBC [14, 15].

In summary, our findings strongly support Richter-Ehrenstein's conclusion that mammaglobin and GCDFP-15 as markers of epithelial breast origin may efficiently work as a new reliable diagnostic tool to distinguish endocrine tumors of the breast from a metastatic non-mammary neuroendocrine disease. Given that the diagnosis of NEBC cases has been made mostly following surgical intervention including mastectomy and that most studies have confirmed that NEBC show a less aggressive clinical behavior when compared with unselected breast cancers, these findings strongly suggest that when suspecting neuroendocrine differentiation, preoperative pathological diagnosis by core biopsies can be employed to immunohistochemically establish the primary nature of mammary NEBC to guide in their conservative medical and surgical management. Further research effort using large clinically annotated series of breast tumors is warranted to definitely establish whether NEBC defined by two- (chromogranin A and synaptophysin) or five (chromogranin A, synaptophysin, HER2, mammaglobin and GCDFP-15)-biomarker surrogate panels might explain a recent suggestion of invasive NEBC as a distinctive subtype of biologically aggressive breast cancer[17]

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FIGURE LEGEND

Figure 1. Immunohistochemical staining of different NEBC tissues for mammaglobin and GCDFP-15. Representative slides of primary (and metastatic) lesions of solid NEBC including surgical specimens and trucut core biopsies were analyzed for mammaglobin (FLEX Monoclonal Mouse Anti-Human Mammaglobin Clone 304-1A5, Ready-to-Use [Link]. DAKO. Code IR074) and GCDFP-15 FLEX Monoclonal Mouse Anti-Human Gross Cystic Disease Fluid Protein-15 Clone 23A3 Ready-to-Use [Link]. DAKO. Code IR077) using standardized immunohistochemical techniques and robotic autostainers (Dako Autostainer Plus system; DAKO, DakoCytomation, Inc. Glostrup, Denmark). Binding results were visualized with the Envision® System (DakoCytomation, Inc.), which uses horseradish peroxidase-labelled polymer that is conjugated with secondary antibodies. 3,3'-diaminobenzidine tetrahydrochloride (DAB) was used for the visualization of the antibody/enzyme complex. Slides were counterstained for nuclei with Meyer's hematoxylin and examined by light microscopy. Tumor immunoreactivity (negative, weak positive, moderate/strong positive) was scored in combination with the percent of cells showing positive staining. Figure shows typical examples of negative (Neg) or positive (Pos) strong staining of some tumor cells for mammaglobin (*A, B, C*) and moderate to strong cytoplasmic expression for GCDFP-15 (*D, E, F*)

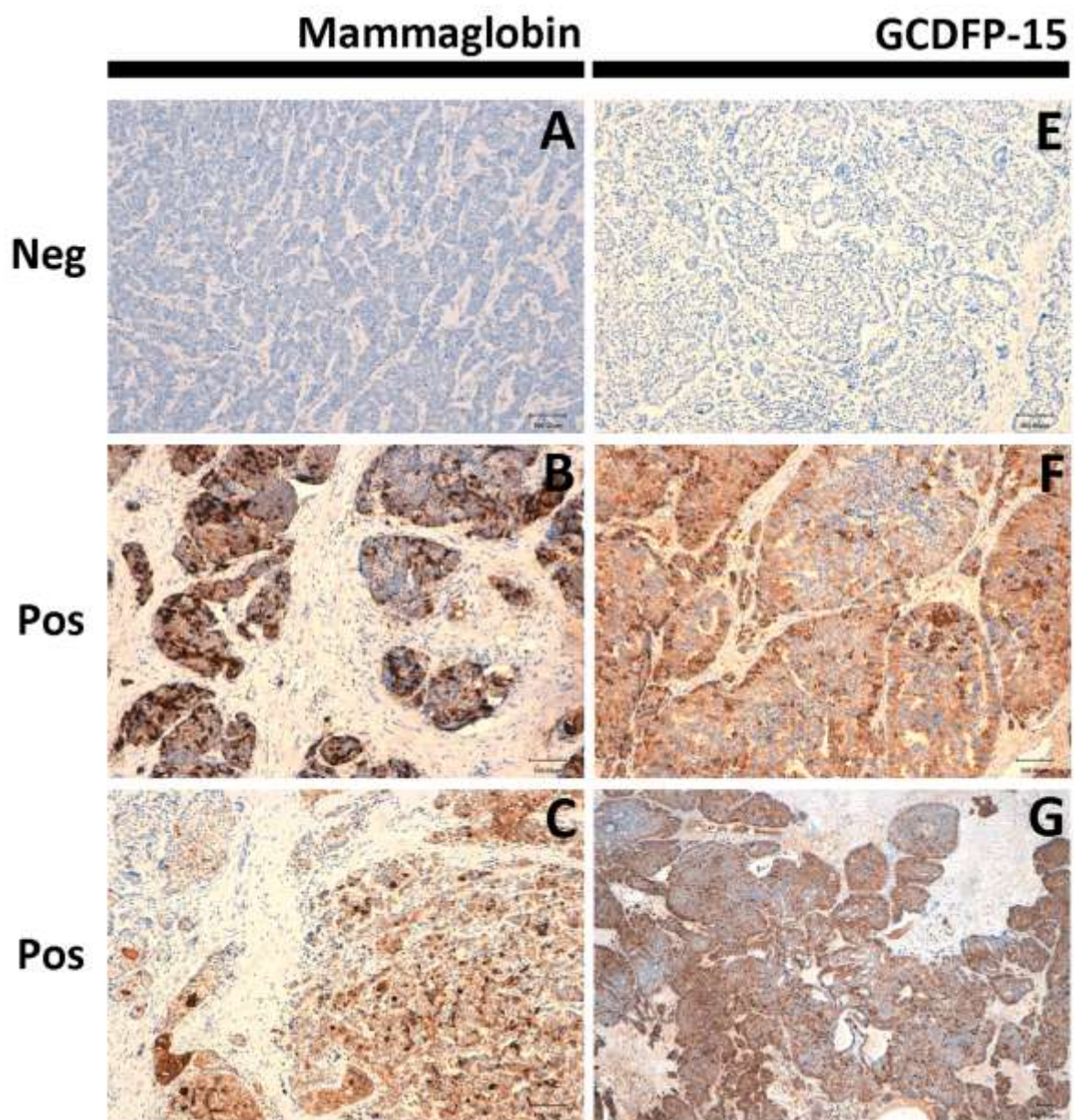


Table 1. Immunohistochemical expression profiles of primary pure neuroendocrine breast carcinomas

Patient Number	Estrogen Receptor	Progesterone Receptor	HER2	Mammaglobin	GCDFP-15
1	Positive	Positive	-	Positive	Positive
2	Positive	Positive	-	Negative	Positive
3	Positive	Positive	-	Positive	Positive
4	Positive	Positive	-	Negative	Positive
5	Positive	Positive	-	Positive	Positive
6M ^a	Positive	Positive	-	Negative	Positive
6P ^b	Positive	Positive	-	Negative	Positive
7	Positive	Positive	2+ (HercepTest™) ^c	Negative	Negative
8	Positive	Positive	-	Positive	Negative

- ^a This woman debuted with metastasis in the soft tissue of the cheek (6M)
- ^b Extensive staging revealed a primary neuroendocrine breast carcinoma (6P)
- ^c HER2 immunohistochemistry was performed using a Dako HercepTest (Dako) kit according to the designated procedure