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Mammaglobin A Immunohistochemical Expression in Molecular Types of Breast Cancer

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ORIGINAL RESEARCH ARTICLE

Abstract

Mammaglobin A is a specific marker of the normal and neoplastic mammary tissue that usually is detected by RT-PCR, and more rarely by immunohistochemistry. Few data are available about the immunohistochemical expression of this marker in molecular types of breast cancer and to its potential contribution in stratification of patients based on molecular analysis. Our purpose was to investigate the sensitivity of the mammaglobin expression in breast cancer and to determine its correlations with already well-defined molecular types, namely luminal A and B, HER2, basal-like, and unclassified. There were investigated 94 patients with breast carcinoma, and slides from paraffin blocks were stained with an antibody against hormone receptors, HER2, cytokeratin 5, epidermal growth factor receptor, p53, and mammaglobin A. The immunohistochemical reaction was scored based on the percentage of positive tumor cells. Mammaglobin A expression was found in 72 from 94 cases (74.22%). A significant correlation was found between the mammaglobin A expression in the primary tumor, grade, and lymph node status, but not with the age of the patient, pathologic subtype of carcinoma and stage of the tumor. The expression of Mammaglobin A strongly correlated with hormone receptor positive cases, and partial correlation was noticed with HER2 type. Most of the cases with basal-like and unclassified specimens were consistently negative for Mammaglobin A. Our results suggest that mammaglobin A is a sensitive marker of breast carcinoma and defines particularly hormone sensitive patients with better prognosis.

Keywords: breast cancer, immunohistochemistry, mammaglobin A, molecular classification, diagnosis, prognosis

I. INTRODUCTION

Breast cancer is the most frequent neoplasia in female, and the number of cases and specific mortality continue to increase, although there were introduced many new methods in the early diagnosis and therapeutic strategy. Patients with breast cancer diagnosed with the same pathological form and similar clinico-pathologic features frequently have different clinical outcome. This difference is mainly due to different molecularly distinct tumors, usually classified as ductal invasive carcinoma based on their microscopic appearance [1]. Twenty years ago, Perou et al. [2] classified breast cancer based on the gene expression profile and therefore by the immunohistochemical expression of cytokeratin (5/6 and 8/18), estrogen receptors, and HER2. This classification, which includes at present time minimum five different types (luminal A and B, basal, unclassified and HER2), has been adopted soon for clinical practice and it was shown that a given molecular type responds differently to preoperative chemotherapy [3] and to adjuvant postoperative therapy [4]. These publications strongly contributed to a better knowledge of the natural evolution and biology of breast cancer, but the characterization of the molecular profile of individual patients is not yet fully understood [5]. This is why it remains mandatory the identification of potential biomarkers that could bring new information on carcinogenesis on one hand, and could be potential targets for therapy on the other [6]. A good and promising biomarker is represented by the discovery of mammaglobin, originally used in the diagnosis and prognosis of breast carcinoma, and soon after that, as a potential target for therapy.

Mammaglobin A is a glycoprotein that belongs to secretoglobin superfamily and is specifically expressed by normal mammary tissue and overexpressed by breast carcinoma [7, 8]. The functional significance of mammaglobin A remains unknown, but the immunohistochemical expression restricted to the mammary tissue suggested that this molecule is a possible breast cancer marker [9]. The role of

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mammaglobin A in the diagnosis of primary breast cancer, lymph node, circulating tumor cells, and distant metastases has been already demonstrated [10.11. 12. 13].

Current available results are based on gene analysis and with lesser extent, from immunohistochemistry. Immunohistochemistry is simpler as routine method, associated with good sensitivity. Testing the immunohistochemical method it was shown a sensitivity of about 80% in the case of ductal invasive carcinoma, and positive reaction in all specimens of normal mammary gland [14]. The method using anti-mammaglobin A does not stain other tissue and cells, excepting for sweat glands of the skin.

Immunohistochemistry is commonly used today to detect macro-/micro metastases in axillary lymph nodes but this investigation strongly depends on antigens expressed by all epithelial cells. Mammaglobin A was detected by RT-PCR in 66% of the lymph nodes involved by metastases and in 8 to 13% of cases with histologically negative sentinel nodes [15, 16]. These findings suggest that mammaglobin may be useful as a marker of micro metastases. Few and controversial data are available about the diagnostic value of mammaglobin immunohistochemistry in the detection of lymph node metastasis, and virtually, there are no data regarding the correspondence between the expression in the primary and metastases. Because of these reasons, we have been examining the expression of mammaglobin A for its potential application in the pathologic diagnosis of breast cancer in terms of expression in different molecular types of breast cancer, and its value as prognostic biomarker. The relationship between mammaglobin A immunohistochemical expression and molecular types of breast cancer was investigated by few studies and the significance of this association is still unclear.

II. PATIENTS,MATERIAL AND METHODS

Patient's data. There were investigated 94 patients admitted with breast cancer, aged between 24 and 81 years. Primary tumors were T1 to T4 with or without prior chemotherapy or radiotherapy. Partial or radical mastectomy together with or without lymph node dissection was performed in all cases. Lymph node metastases were found in 58 cases (72.34%). Distribution of cases based on the tumor stage and lymph node metastasis are shown in Table 1.

Table 1. Distribution of cases based on tumor stage and lymph node metastases (n=94)

Tumor stage/N	N-	N+	Total
T1	3	0	3 (3.19%)
T2	15	15	30 (31.91%)
T3	22	37	59 (62.76)
T4	2	6	8 (8.51%)

Legend: N, lymph node status; N-, without lymph node metastases; N+, with lymph node metastases

Tissue processing. Specimens from the primary tumors and lymph nodes were fixed in buffer formalin and embedded in paraffin. 5µm thick sections were stained with haematoxylin-eosin for the pathological diagnosis and grading of the tumors. The grade was assessed using the Scarff-Bloom-Richardson system.

Immunohistochemistry. Additional slides from the primary tumors and lymph nodes were stained for mammaglobin A. Deparaffinized and hydrated slides were treated with hydrogen peroxide 3% for 5 minutes, and antigen retrieval was performed at microwave in buffer solution pH9 for 40 minutes. Slides were than incubated for 30 minutes with the antibody against mammaglobin A, clone 304-1A5, ready-to use. The working system was EnVision, and 3,3'-diaminobenzidine was used as chromogen. Nuclei were stained with Lillie's modified hematoxylin. All reagents used in the present study were from Dako Cytomation (Denmark).

Molecular classification was based on the immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR), HER2 protein, cytokeratin 5, EGFR, and p53, Evaluation was based on Allred system for hormone receptors, and Herceptest for HER2 expression. Cytokeratin 5, EGFR, and p53 were considered positive if more than 10% of tumor cells were stained at the level of the nucleus (p53), membrane (EGFR), and cytoplasm (cytokeratin 5). Luminal A was defined as a ER-PR-expressing tumor, without other positive markers and low rate of proliferation detected by Ki67 expression. Luminal B was defined as a tumor with ER and PR expression with high rate of proliferation. Basal-like carcinoma was characterized by lack of ER-PR and HER2 expression, and positive reaction for cytokeratin 5 with or without EGFR expression. Unclassified carcinoma was the only penta-negative tumors, lacking expression for all markers used in this study.

Evaluation was performed with Eclipse 80i Nikon microscope and the reaction for mammaglobin was scored in five microscopic fields (x200 magnification) in the tumor area and in three fields of adjacent normal mammary tissue. The same procedure was applied for slides from lymph node metastasis. Results for mammaglobin detection in the primary tumors were compared with those found in corresponding lymph nodes. Finally, we performed a correlation between mammaglobin immunohistochemical expression and stage of the tumor, grade of differentiation, and lymph node status.

Mammaglobin scoring. The scoring of the mammaglobin reaction was based on the number of positive epithelial cells, as follows: negative (0), weak positive with less than 10% positive cells (+1), moderate positive with 11 to 50% stained cells (+2), and strong with over 50% stained cells (+3). The intensity of the final product of reaction was not taken into account, based on the fact that all stained cells showed a strong reaction.

Statistical analysis. Statistical analysis was performed using Stat Plus 2007 program with Spearman's correlation tests. A significant correlation was reported for a value of $p<0.05$. We tried to find if there is any correlation between age, histopathology, tumor stage, grade, association

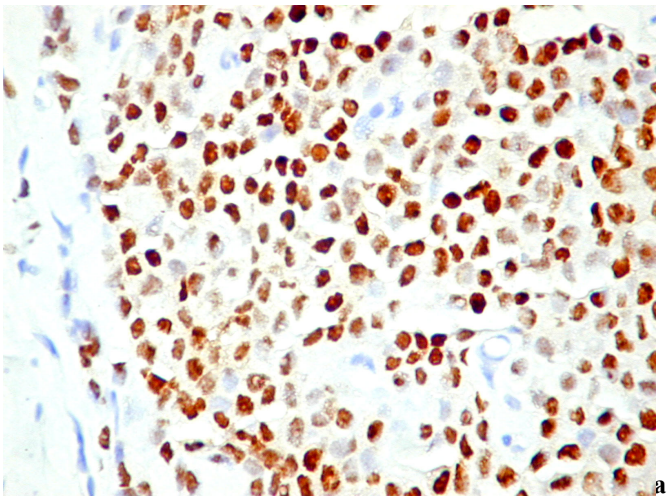
of DCIS with invasive carcinoma, and lymph node status with the expression of mammaglobin.

III. RESULTS AND DISCUSSION

A. Results

Pathologic findings. Ductal invasive carcinoma (not otherwise specified) was diagnosed in 84 cases, lobular invasive carcinoma was found in 5 cases, papillary carcinoma in 3 cases, and mucinous carcinoma in 2 cases. Eleven cases were well differentiated (G1), 57 cases were moderately differentiated (G2), and 26 were undifferentiated (G3). Normal mammary tissue surrounding the tumor was evaluated in 54 patients.

Molecular classification. We stratified first the cases of this series (n=94) according to the accepted definitions for molecular types of breast cancer. The most numerous patients fall in the group of hormone sensitive breast cancers, n=64. There were 38 cases (40.42%) with luminal A tumors, with strong expression of estrogen (ER) and progesterone receptor (PR), and low rate of proliferation, shown by Ki67. We found lower level of expression for ER and PR, associated with high rate of proliferation in 26 cases (27,65%) with luminal B type. HER2 type was found in 11 cases (11.70%), with strong reaction with membrane pattern, regardless the incidental expression of epidermal growth factor receptor in 3 cases. Both basal-like and unclassified carcinoma did not express hormone-receptors and HER2 protein. Basal-like (n=12, 12.76%) cases were positive with cytoplasmic pattern for cytokeratin 5 and/or epidermal growth factor receptor. Unclassified type included 7 cases (7.44%) and were consistently negative with all five basic markers mentioned before, but with high rate of proliferation shown by Ki67 index. We found no correlation between the molecular types, histological for, grade of differentiation, and clinico-pathologic factors of prognosis. Examples of positive immunohistochemical reaction for main markers are shown in figure 1.



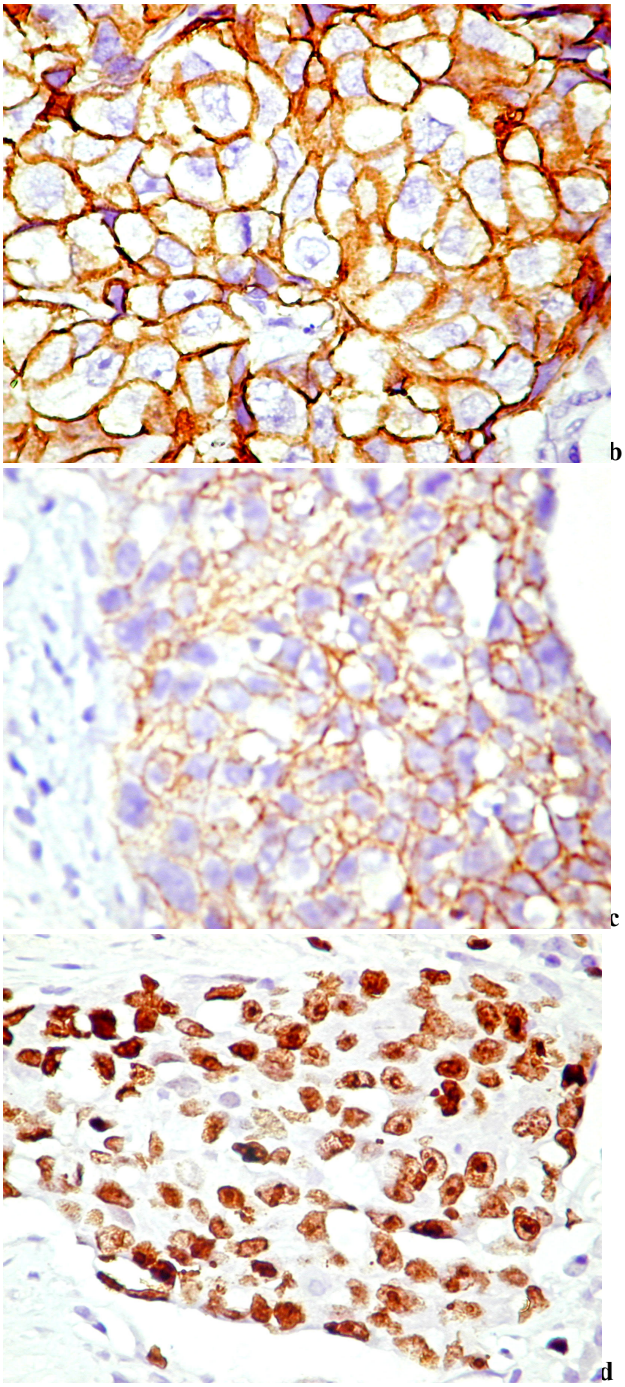


Fig.1. Immunohistochemical expression of estrogen receptor (a, x200), HER2 (b,x400), epidermal growth factor receptor (c, x400), and Ki67 (d, x400).

Mammaglobin expression. The final product of reaction for mammaglobin was intensely stained, with cytoplasmic granular pattern, and restricted to epithelial cells. Normal mammary tissue adjacent to the tumor was positive in all 54 cases, but the final product of reaction was heterogeneous and found in less than half of glandular cells.

In apocrine metaplasia almost all cells were intensely stained, and weak or moderate reaction was noticed in ductal atypical hyperplasia. DCIS associated to the ductal invasive carcinoma was positive in all cases (n=31) in which this neoplastic lesion was present in the specimen.

In the current series of patients with ductal invasive carcinoma, we found 72 from 94 cases (74.22%) positive with mammaglobin A. In most of the positive cases the immunohistochemical reaction was heterogeneous, and frequently less than 10% of tumor cells were intensely stained. Based on the score mentioned above, 22 cases were noticed with "0", 31 with "+1", 24 with "+2", and 17 with "+3". We found no statistic significant relationship between the expression of mammaglobin A, histological type, and grade. On the other hand, we found a strong correlation between mammaglobin A expression and hormone-sensitive types ($p<0.00031$), and an inverse correlation with triple negative cases ($p<0.0004$). we found an interesting relationship with lymph node metastases. From 48 cases with lymph node metastases included in this series, only 29 cases showed positive reaction for mammaglobin in the primary tumor. This observation significantly reduces the sensitivity of the method in detecting lymph node and distant metastases. Models of distribution of the final product of reaction for mammaglobin A are shown in figure 2.

B. Discussion

In the present work we demonstrated that immunohistochemical expression of mammaglobin correlates with hormone sensitive cases of breast cancer. This could be an additional indicator for therapeutic strategy in doubtful cases. Watson and Fleming [10] identified a gene called mammaglobin that encodes a 93-amino acid protein, which nowadays is called mammaglobin A. This substance belongs to the group of human secretoglobins, and is highly specific for the normal ant malignant mammary tissue. This is supported by this finding because we found positive reaction in 72 from 94 patients. More important is the relationship with the molecular types of breast cancer. Until now, after the first report on the relationship between mammaglobin A and estrogens [17], only one team showed the link between mammaglobin A expression and hormone receptor positive breast cancer [18]. Besides its role in diagnosis, particularly in carcinoma of unknown origin, mammaglobin A reaction may interfere with the therapeutic strategy. Mammaglobin A was detected by RT-PCR and immunohistochemistry in 55.4 to 81% of the cases with breast carcinoma [19, 20, 21] and in only 43% of the cases of nonmalignant breast by RT-PCR [22]. There were accumulated a lot of data that support the use of mammaglobin A as a biomarker for the diagnosis and prognosis of breast cancer. Nowadays is largely accepted that strong expression of mammaglobin correlates with better prognosis and good response to hormone therapy. Mammaglobin is a better marker than gross cyst disease fluid protein-15 (GCDFP-15) for breast carcinoma but lacks the specificity of GCDFP-15. Only very few cases are negative for both markers, and therefore, the combination is extremely helpful for the diagnosis.

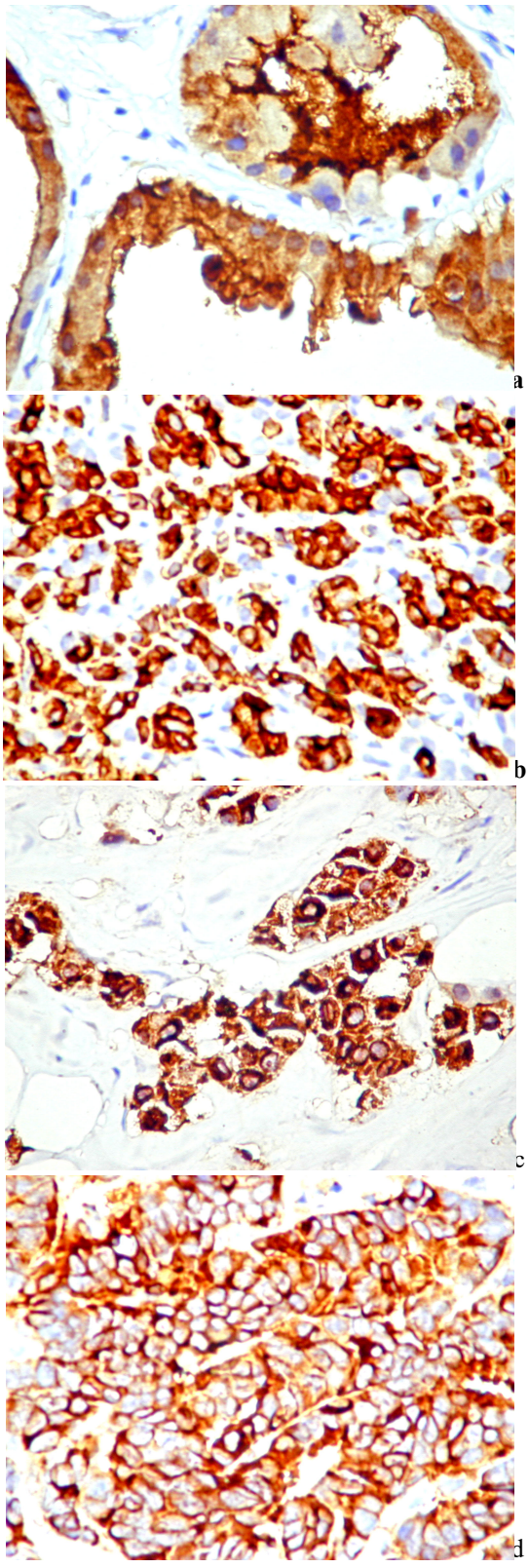


Fig.2. Immunohistochemical expression of Mammaglobin A. Normal mammary gland and apocrine metaplasia (a). Ductal invasive carcinoma with tumor cells arranged as small islands

(b), cords (c), and solid proliferation (d). Original magnification x200.

Our results confirm previous studies that demonstrated that high mammaglobin expression reflects a better differentiation, a low proliferation rate and high hormone dependence [24, 25]. It has been shown that mammaglobin protein exists in two main forms in breast tissue, and only the high molecular weight form is found i in receptor-positive cancers. The high molecular weight expression of mammaglobin correlated positively with hormone receptors and negatively with tumor grade and seems to be associated with better prognosis. The relationship with grade is not supported by our findings, but on the other hand, most of our cases were G2. All together, these results demonstrate that the assessment of mammaglobin expression could be useful to stratify patients for adjuvant therapy.

Could be mammaglobin of help in the planning the therapeutic strategy? This idea is not new, it was advanced almost 15 years ago [26, 27]. Some recent findings indicated that azurin-mammaglobin A recombinant vector plays an essential role against the formation and expansion of breast tumors in the animal model. In addition, this recombinant vector is safe and has the proper ability to stimulate the immune system [28]. Targeted doxorubicin delivery and release within breast cancer environment is already investigated in cell cultures using anti-human mammaglobin A [29]. Moreover, it seems that some epitopes of Mammaglobin significantly enhance the response of tumour tissue to specific therapy [29].

IV. CONCLUSION

In summary, the immunohistochemical expression of mammaglobin was found in 74.22% of the cases with breast cancer, and strong expression is frequently correlated with the lack of lymph nodes metastases. We found no correlation between the expression of mammaglobin A and clinico-pathologic factors of prognosis but correlates with hormone receptor positive primary tumors. Our findings suggest the mammaglobin expression defines a subgroup of patients with better prognosis and is a useful method to detect breast cancer metastases.

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Conflict of interest. None to declare.

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