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PROGNOSTIC FACTORS AND CLINICAL-PATHOLOGICAL FINDINGS OF SENTINEL LYMPH NODE INVASION IN BREAST CANCER

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Abstract: ***Aims:*** *Breast cancer is particularly recognized as highly impredictible, even in early stages. The natural evolution of breast cancer is however determined by both clinical and histopathological characteristics. The aim of the study is to evaluate these characteristics from a lot of 93 patients with early stages of breast cancer and subsequently to determine which are the factors with statistical significance that are more likely linked to more aggressive forms of the disease, in which the invasion of the sentinel lymph node had already occurred.* ***Methods:*** *The study included 93 patients with breast cancer that underwent sentinel node biopsy and oncologic treatment at the Bucharest Insitute of Oncology. We assessed data that included histopathological findings such as tumor size, sentinel lymph node involvement, receptor status, and imunohistochemistry parameters ranging. Also, a comparisson to data obtained from similar published studies was made.* ***Results:*** *Cases with aggressive types of breast cancer, that had already spread to axillary lymph nodes, were characterized by early age, poor Bloom Richardson grading, the presence of peritumoral infiltration, and high levels of Ki67, PCNA and p53.*
Conclusions: *Daily practice has shown the need for personalised treatment in cancer patients. If possible, it is highly useful to establish the aggressiveness profile of a malignant breast tumor, in order for a specific patient with a particular set of tumor characteristics to benefit from a targeted and optimized treatment plan.*

Key words: *early breast cancer, sentinel lymph node biopsy, prognostic factors, agressiveness factors.*

1. Introduction

Recent years have witnessed a significant increase in the incidence of breast cancer. The evolution of the disease is unpredictable, some of the tumors having a high degree of aggressiveness and a poor prognosis. Despite the dreadful attributes of the disease, breast cancer can be kept under control provided it is detected early, appropriately treated and followed up.

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Until not very long ago, mastectomy accompanied by axillary lymphadenectomy represented the only surgical approach to breast cancer irrespective of the disease stage at the time of detection. This radical intervention, having a mutilating character and a major impact on the patient's life, does not however guarantee oncological results every time. Mastectomy avoidance in the early stages of the disease is possible, without impairing survival, by means of conservative surgery that has become an alternative to mastectomy in select cases as a consequence of the conceptual breakthrough with respect to breast cancer dissemination. The sentinel lymph node concept is an idea that has revolutionized oncological surgery. In breast cancer, the sentinel lymph node technique enables the selection of cases where full axillary lymphadenectomy is useful thus offering the chance to reduce morbidity associated with an extensive surgical act targeting the regional lymphatics.

Identification and biopsy of the sentinel lymph node, i.e. of the first lymph node where the primary tumour disseminates, is a thorough method of assessing the aggressiveness of the disease. A sentinel lymph node infiltrated by cancerous cells in an apparently incipient stage of the disease is a negative prognostic factor in breast cancer, as it points to a high degree of aggressiveness of the respective tumour.

This study has included a number of 93 patients with breast cancer, for whom surgery was proposed as the first treatment step, with tumours having a clinical diameter below 3 cm, with no suspicious locoregional lymph node enlargements. The purpose of the study was to identify the aggressiveness profile of a malignant breast tumour capable of lymph node metastasis even in the early stages of the disease.

2. Material and Method

The group included 93 patients with breast cancer in less advanced stages, with an initial indication for surgery, treated at the "Prof. Dr. Al. Trestioreanu" Institute of Oncology in Bucharest.

Excisional biopsy, accompanied by intraoperative histopathological examination, is a mandatory step in setting the malignancy diagnosis in incipient breast cancer. It should comply with strict rules as to the correct indication and surgical details, thus constituting the first stage in the surgical strategy.

Identification and excisional biopsy of the axillary lymph node using the radioactive tracer technique were performed in accordance with a European protocol, EUSOMA-ESSO, accompanied by an intraoperative histopathological examination of the sentinel lymph node meant to establish a possible lymph node invasion [1].

The appropriate surgical treatment of the primary tumour took into account the histopathological type, the ratio of the tumour diameter versus the breast size as well as the location of the tumour and consisted either in conservative treatment, i.e. limited mammary resection, or in mastectomy techniques. Surgical treatment of the axillary lymphatic basin consisted in full axillary lymphadenectomy particularly when the intraoperative histopathological examination of the sentinel lymph node revealed metastatic invasion.

Microscopic examination is based on malignant cells recognition, defining their degree of differentiation, identification of the histological type and sub-type of proliferation, assessment of local invasion, of intravascular invasion, host tissue reaction (presence of lymphocytic infiltration, fibrosis, necrosis, etc), assessment of sentinel node invasion as well as the invasion of other lymph nodes

that had to be removed taking into account the axillary station they belong to. The examination also serves in identifying tissular prognostic factors. [2]

Immunohistochemical examination of the primary tumour has determined the expression of a series of cell markers such as estrogen and progesterone receptors, presence of oncogenes and of cellular proliferation markers. In order to highlight the aggressiveness characters and the varying evolution of breast cancer in early stages we have examined the following markers:

Hormonal receptors: Estrogen receptors ER and progesterone receptors PR. Estrogen and progesterone receptors have an important prognostic value. It is mandatory to determine them in order to make a decision on adjuvant systemic and hormonal treatment.

Tumour proliferation markers: ki67and pcna. Ki67 recognizes a nuclear antigen which is present in the cells in the active phases of the cellular cycle G1, S, G2 and M, but which is absent in G0 (the static phase). Data in literature mention a correlation between a high ki67 activity and the other aggressiveness indicators in breast cancer such as mitotic activity, histological grade, absence of estrogen receptors and anneuploidy [3]. PCNA is a nuclear antigen of a polypeptidic type whose distribution in the cellular cycle is similar to that of ki67, but which has a different expression degree. PCNA level in the nucleus grows during the G1 phase and drops in the G2 and M phases [4].

Oncogenes, growth factors and tumour suppressor genes: c-erb-B2 (Her, neu) p53 protein, bcl2. C-erb-B2 (Her2 neu) is a proto-oncogene codifying an EGFR homologue protein and expressing tyrosine kinasic activity. Amplification of the gene is accompanied by a higher expression of the genic product - c-erb-B2 protein, that can be identified by immunohistochemistry of paraffin embedded sections. This amplification of the c-erb-B2 oncogene, identified in some breast cancers, is associated with a more aggressive evolution [5].

P53 protein is a tumour suppressor oncoprotein which, when mutating, is expressed in an increased amount at the nuclear level. Inactivation of the tumour-suppressing function determines an activation of tumoural cell growth [6].

Bcl2 is a tumour suppressor oncogene with a role in inhibiting cell apoptosis while bc12 protein is located at the mitochondrial level [7].

After going through the above-mentioned phases, every patient followed an individual treatment plan based upon the stage of the disease, aggressiveness factors and prognostic [Table 1].

The multimodal oncological treatment included administration of chemotherapy in an adjuvant setting, hormonal therapy and post-surgery radiation therapy.

Clinic and histopathological features assessed Table 1

Clinical Aspects	**Age** **Tumor size**		
Histological Features	**Type of cancer** **Tumor differentiation grade** **Peritumoral infiltrate** **Multicentricity** **Number of invaded lymph nodes** **Size of the metastasis**		
Imunohistochemistry Markers	**Hormone Receptors**	**Tumor Growth Markers**	**Oncogenes**
	ER **PR**	**Ki 67** **PCNA**	**c-erb B2** **p53** **bcl2** **EGFR**

3. Results

The studied group included patients between 31 and 69 years of age, mean age 53.21 years. Two age sub-groups included a larger number of cases: the 41-50 years age group and the 51-60 one. They accounted for 29.03% (27 patients) and, respectively, 38.70% (36 patients) of the total number of patients in the study.

Patients' distribution by hormonal status shows a higher percentage of post/menopausal patients (74.2% - 69 patients) compared to premenopausal patients (25.8% - 24 patients).

In compliance with the selection criteria for the sentinel lymph node technique, no suspicious axillary lymph node enlargement was present in any of the patients on pre-surgical examination.

The average tumour diameter was 17.5 mm (clinically determined), tumour size varying between 6 and 29 mm. Almost half of the patients in the study (44.08%) had tumours whose size varied between 21 and 29 mm. The average tumour diameter on intraoperative histopathological examination was 16 mm, with limits between 5 and 27 mm.

The most frequently encountered histopathological type of primary tumour was invasive ductal carcinoma, in 76% of the cases.

The distribution of tumour differentiation grade in the studied group was the following: G1 25.81% (24 patients) G2 – 34.4% (32 patients), G3 – 39.79% (37 patients).

Invasion of peritumoural tissues was noted on histopathological examination in 47 cases (50.53%). A number of 60 tumours (64.51%) showed peritumoural lymphocytic infiltration on histopathological examination.

In the 93 procedures there were 76 patients in whom 1 axillary sentinel lymph node was found, 15 patients having 2 axillary sentinel lymph nodes, and 2 patients in whom 3 axillary sentinel lymph nodes were present. A total number of 112 sentinel lymph nodes were identified, i.e. 1.25 sentinel lymph nodes per case on average.

Metastatic invasion of the sentinel lymph node was identified in 34 cases (36.55%) of all the patients included in the group. In 59 cases there was no invasion of the sentinel lymph node. All the sentinel lymph nodes identified were in an ipsilateral axillary location.

Estrogen and progesterone receptors were immunohistochemically analyzed in 90 cases. In 64.4% of the patients (58 cases), estrogen and progesterone receptors were positive.

The tumour proliferation markers evaluated through immunohistochemical examination were ki67 and pcna. Ki67 was determined in 90 cases and was found to be positive in 47 patients (52.22%). PCNA was considered to be positive in 33 patients (41.25%).

The c-erb B2 (Her-neu) oncogene was determined in 84 patients, and in 24 cases (28.57%) it was overexpressed.

Proteins p53 and Bcl2 were determined in 85 and, respectively, 73 cases, with 28 (32.94%) and, respectively, 36 (49.31%) patients found positive.

4. Establishing factors of aggressiveness

Correlation between patients age, T (TNM) category and sentinel lymph nodes status

Analysis of sentinel lymph node invasion and patients age in the studied group has shown the presence of 20 metastatic sentinel lymph nodes in the 36 patients under 50 years of age ($p < 0.03$). Sentinel lymph node invasion was statistically significant in the patients whose tumours were in stage T2 of the TNM classification of the UICC compared to patients with T1 tumours ($p < 0.04$) [7].

Correlation between tumour differentiation grade and sentinel lymph nodes status

In the group under study, a number of 19 patients with G1 differentiation grade tumours, 24 with G2 and 16 with G3 had a negative sentinel lymph node on histopathological examination (Graph 11). G3 (Bloom Richardson) differentiation grade associated more frequently with metastatic invasion of the axillary sentinel lymph node (in 21 cases) having a statistically significant value ($p <0.01$)

Correlation between peritumoural lymphocytic infiltration and sentinel lymph nodes status

A number of 22 tumours, in which peritumoural lymphocytic infiltration was present, associated the presence of cancerous invasion of the sentinel lymph node ($p < 0.01$). In 48 cases where no peritumoural lymphocytic infiltration was present the sentinel lymph node was disease free.

Correlation between ki67and pcna expression in the primary tumour and axillary lymph nodes status

Analysis of the tumour proliferation factors and sentinel lymph node status identified a direct correlation between ki67 and pcna and the sentinel lymph nodes with disease invasion.

Correlation between cerbB2 expression in the primary tumour and sentinel lymph nodes status

In the studied group, overexpression of the cerbB2 oncogene in the primary tumour did not associate to a statistically significant degree with the presence of axillary node metastases ($p > 0.3$)

Correlation between p53 and bc12 expression in the primary tumour and sentinel lymph nodes status

Expression of p53 protein in the primary tumour directly associated with the presence of positive sentinel lymph nodes, therefore of axillary metastatic disease ($p < 0.01$). Bc12 protein located at the mitochondrial level and expression of Bc12 tumour suppressor oncogene that plays a role in cellular apoptosis inhibition did not associate to a statistically significant degree with the presence of lymphatic node metastases.

Analysis of tumour histopathological differentiation grade function of Ki67 and p53 markers expression.

In the studied group, ki67 expression directly correlates with the histopathological grade of tumour differentiation , the marker's expression increasing to a statistically significant degree in the more poorly differentiated tumours ($p<0.01$). Protein p53 accumulation in the nucleus also directly correlated with the histopathological grade of tumour differentiation, p53 being mostly present in the poorly differentiated tumours belonging to G3. [Figure 1]

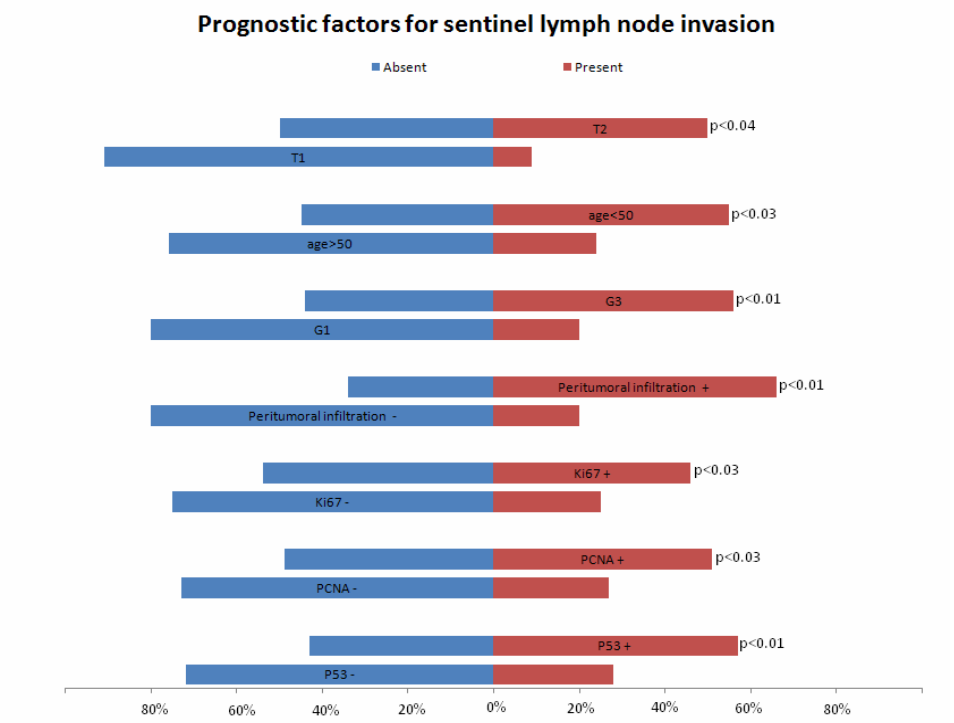

Fig. 1. *Main prognostic factors for the invasion of the axillary sentinel lymph node*

5. Discussions

The predictive factors of the invasion of other axillary lymph nodes, in case of positive sentinel lymph nodes, can be grouped into two categories: the primary tumour characteristics and the characteristics of the positive sentinel lymph node.

A tumoural diameter in excess of 1.5 cm is indicative of a high risk of axillary metastases in the non-sentinel lymph nodes according to a study published in *The Oncologist* that evaluated the prognostic factors of cancer evolution in less advanced breast cancer [8]. In our study too, the patients with tumours in excess of 2 cm more frequently presented a positive axillary status ($p<0.04$) compared to those whose tumour size was below 2 cm. Also, peritumoural lymphocytic infiltration correlated with metastases identification in the sentinel lymph node ($p<0.01$).

Incidence of axillary metastases in non-sentinel lymph nodes can be correlated with certain characteristics of the invaded sentinel lymph node: the number of positive sentinel lymph nodes and the size of metastases.

The number of invaded sentinel lymph nodes is a predictive factor for a possible invasion in the remaining axillary lymph nodes [9].

Viale states that even in patients with the most favourable combination of predictive factors (small number of invaded sentinel lymph nodes, absence of peritumoural lymphocytic infiltration, small size of metastases in the sentinel lymph node), the risk of metastases being present in the non-sentinel lymph nodes is not lower than 13% [10].

Performance of excisional biopsy of the suspicious axillary lymph nodes, in addition to the lymph nodes that bound the radioactive tracer (sentinel lymph nodes) reduces the rate of false negative results [11].

The ever-increasing role of systemic adjuvant treatment and of postoperative radiation therapy brought under scrutiny the benefits of full axillary lymphadenectomy as the axilla has been staged by means of the sentinel lymph node technique. The EORTC 10981-22023 (AMAROS) trial, a phase III randomized trial, demonstrates that as the success rate in identifying the sentinel lymph node is over 97%, with invasion being present in 18% of all cases, one can safely go for postoperative axillary radiation therapy rather than for full axillary lymphadenectomy.[12]

In brief, age, breast tumour diameter, its histopathological type, clinical suspicion of lymph node invasion, lymphatic and vascular invasion, differentiation grade, tumour location and multifocality are more frequently associated with sentinel lymph node metastases.

Estrogen receptors are present in approximately 80% of women with breast cancer. In most cases they associate with positive progesterone receptors (PR) [13].

Several authors have demonstrated however that association of positive estrogen receptors with negative progesterone receptors correlates with a positive axillary status in women below 50 years of age [14]. Positive progesterone receptors, tumoural grade of differentiation and tumour size are independent prognostic factors for lymph node invasion in women with estrogen receptor positive breast cancer below 50 years of age. Also it was found that efficiency of aromatase inhibitors is higher than that of tamoxifen in women with positive estrogen receptors when the progesterone ones are not expressed. [15] Breast cancers with negative ER/positive PR are rather well differentiated, occur more especially in young women and express HER-2neu more frequently compared to the negative ER/negative PR phenotype [16].

Tumours with a high grade of differentiation and with no axillary metastases associate with improved survival.

Fiets et al. report a significantly lower overall survival in patients with G3 tumours compared to those with G1 [17].

Breast cancer occurring in young women (below 35 years of age) is more likely to have a low differentiation grade (G2,G3), to be ER(-) and to have a positive sentinel lymph node, thus expressing a high degree of aggressiveness and a reserved prognosis [18]. In our study, G3 grade of tumour differentiation more frequently associated with metastatic invasion of the axillary lymph nodes (in 21 cases), having a statistically significant value ($p<0,01$).

The grade of differentiation and Ki67 expression can be predictive factors with respect to adjuvant treatment as their high degree presence associates with a good response to adjuvant polychemotherapy. Tumour proliferation rate is a biological parameter that needs to be considered both in establishing the indication and in assessing the efficiency of polychemotherapy. Mitotic index and Ki 67 have proved to be major predictive factors for the sensitivity of breast carcinomas to anthracycline based therapy. Also Ki 67 and mitotic index more frequently associate with negative ER and negative PR, consistent with poorly differentiated, highly proliferative tumours.

Analysis of tumour proliferation markers depending on sentinel lymph node status identified a direct correlation between Ki-67, PCNA and metastatic invasion of the sentinel lymph node ($p<0.03$ and, respectively, $p<0.03$). In the studied group, Ki67 expression directly correlates with the histopathological grade of tumour differentiation, the marker's expression increasing to a statistically significant degree in the more poorly differentiated tumours ($p<0,01$).

The high grade of differentiation, positive Ki 67 and ER(-), PR (-) are predictive factors for a full histopathological response to the adjuvant treatment. In the future, molecular biology techniques would enable treatment individualization depending on the expression of tumour aggressiveness markers. At present there are even methods of selection based on an analysis of the genetic profile of patients for whom adjuvant therapy is indeed necessary [19].

C-erbB 2 is an independent prognostic factor for disease free survival and for overall survival as well as for the presence of axillary lymph node metastases. Linderholm et al. , noting the association between c-erbB 2 and VEGF (vascular endothelial growth factor), state that c-erbB 2 is at least partially involved in inducing angiogenesis in breast cancer. Expression of the genes in the EGF family is induced by the proinflammatory cytokines. Association of c-erbB 2 with the angiogenic factors explains the aggressiveness of the c-erbB 2 positive phenotype [20]. In the studied group, over expression of the c-erbB 2 oncogene in the primary tumour did not associate to a statistically significant degree with the presence of axillary lymph node metastases ($p>0.3$).

Expression of c-erbB 2 is undoubtedly a predictive factor in the case of response to trastuzumab treatment. Also, overexpression of HER 2 associates with a low response to tamoxifen and alkylating agents and a high sensitivity to anthracycline treatment. Increased expression of p53, EGFR and HER-2 associates with a modest response to hormonal treatment. Polychemotherapy is the most important method of treatment in patients with negative ER and negative HER-2 tumours. Herceptin (Trastuzumab) is the therapy of choice in patients with negative ER and positive HER-2 [21].

About 30% of breast carcinomas express EGFR; in these cases the patients have a more reserved prognosis if the tumours have no estrogen receptors either. EGFR expression correlates with tumours having a high grade of differentiation and can identify tumours that respond poorly to hormonal therapy. Recent studies demonstrate that tumours with the most unfavourable prognosis are those showing a positive reaction for EGFR and for c-erbB 2 [22].

Patients with negative sentinel lymph nodes, that however overexpress c-erbB 2, have a reserved prognosis when this associates with tumours over 2 cm in size, G3 and lympho-vascular invasion. Tumours expressing both p53 and c-erbB 2 have an aggressive behaviour. Evaluation of these two latter parameters in patients with breast cancer can assure a better therapeutic plan as well as a better prognosis.

Alteration of p53 protein in breast tumours frequently associates with: absence of estrogen and progesterone receptors, presence of a high proliferative index, poorly differentiated histological grade and a more unfavourable prognosis.

This association is even more significant in the case of tumours of smaller size (1-3 cm) as it is an indicator of unfavourable tumour behaviour. In the studied group protein p53 accumulation at a nuclear level also correlated directly with the histopathological grade of tumour differentiation, p53 being represented especially in the poorly differentiated tumours belonging to G3 ($p<0.01$). More than half of the solid breast tumours express mutant p53. This enables them to avoid apoptosis and to disrupt the p53-induced cellular cycle. Restoration of the wild p53 genotype function would induce massive apoptosis in the tumoural cells and thus the tumour would be eradicated. At present there are attempts to identify several molecules (ELIPTICIN, PRIMA 1, WR 1065) meant to restore the native

conformation of p53 and to represent the basis for developing new adjuvant treatments [23].

Immunofluorescence studies and RNA hybridization studies have launched the hypothesis according to which the ability of breast cancer to metastasise has a well determined genetic profile at the very onset of the disease and is not based on aggressive clones selection [24].

6. Conclusions

The clinical aggressiveness factors we studied and which significantly correlated with the presence of metastatic invasion in the sentinel lymph node are: age below 50 years ($p<0.03$) and tumour size in excess of 2 cm ($p<0.04$).

The histological aggressiveness factors considered in the study that significantly correlated with positive sentinel lymph nodes are: peritumoural lymphocytic infiltration ($p<0.01$)and differentiation grade G3 ($p<0.01$).

The immunohistochemically evaluated aggressiveness factors that correlated with disease infiltrated sentinel lymph nodes are: presence of tumour proliferation markers Ki 67 ($p<0.03$) and PCNA ($p<0.04$) as well as p53 expression in the primary tumour ($p<0.01$). In our study we found that poorly differentiated tumours belonging to G3 correlate to a statistically significant degree both with the expression of Ki 67 ($p<0.01$) and with the accumulation of p53 ($p<0.01$).

Even in clinically less advanced stages, breast cancer in patients below 50 years of age, G3 graded, with peritumoural lymphocytic infiltration present, Ki67-positive, PCNA- positive, p53 - positive, is more aggressive and associates with an increased frequency of locoregional lymph node metastases.

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