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Research Paper

Biological Relevance of Clinical and Pathological Features in Breast Cancer Molecular Subtypes: Additional Insights for Personalized Decision-Making in Nodal Status Assessment

Medical Science

KEYWORDS : breast cancer, molecular subtypes, prognostic factors, sentinel node.

Buonomo O.C.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Orsaria P.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Pagnani G.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Scaggiante J.	Department of Diagnostic Imaging, Molecular Imaging, Interventional Radiology and Radiation Therapy, Tor Vergata University Hospital, 00133 Rome, Italy
Rossi P.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Venditti D.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Granai A.V.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Vanni G.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Varvaras D.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Esser A.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Bonanno E.	Department of Biopathology, Tor Vergata University Hospital, 00133 Rome, Italy
Caredda E.	Department of Biomedicine and Prevention, Tor Vergata University Hospital, 00133 Rome, Italy
Petrella G.	Department of Surgery, Tor Vergata University Hospital, 00133 Rome, Italy
Palombi L.	Department of Biomedicine and Prevention, Tor Vergata University Hospital, 00133 Rome, Italy

ABSTRACT

Management of the axilla in early breast cancer (BC) has evolved in the genomic era to highly and increasingly conservative approaches based on the assessment of the sentinel lymph nodes (SLNs) as well as intrinsic primary tumor biology and patients characteristics. This study aims to verify the definition of molecular subtypes (MST) of breast cancer with increased risk of nodal involvement, and validate the prognostic value of sentinel node assessment, even related to well established prognostic signatures. The authors enrolled 779 BC patients to perform a risk analysis in univariate and multivariate binary logistic models, comparing the baseline characteristics. Results revealed a strong significance in identifying the "Non Luminal" classes as a low-risk group for axillary metastatic involvement, with a risk decreased by 60% ($p<0.01$), and the "Luminal" classes as a high risk-group, nearly tripling the risk ($p<0.01$), also when these data were correlated with prognostic factors such as tumor size and histological grading.

INTRODUCTION

Recently classification of breast cancer (BC) based on key molecules and gene profiles has improved our understanding of the heterogeneity of this disease and revolutionized treatment concepts, resulting in improved survival and quality of life (1)

Several patterns of gene expression not only uncovered similarities and differences among the tumors, but in many cases pointed to a biological interpretation. Variation in growth rate, in the activity of specific signaling pathways, and in the cellular composition of disease were all reflected in the expression of specific subsets of phenotypes (2). Different molecular and clinical landscapes have been suggested as having distinct epidemiological risk factors, natural histories, and responses to treatment and prognosis.

Based on the presence of hormone receptors, including the Estrogen (ER) and Progesterone (PgR) receptors, human epidermal growth factor receptor 2 (HER-2) and other biomarkers like Ki-67, different pathological behaviors have categorized breast cancer into five major molecular subtypes (MST), providing new insights into the management of the disease (3).

In addition, the established risk factors compared to define guidelines, as tumor size (T) and histological grade (HG), provide foundations for treatment efficacy and individualized treatment. Genome-wide microarray-based expression profiling studies have provided further evidence that the biological features captured by the HG are important in determining growth pattern and outcome. Since the Nottingham Grading System (NGS) has independent but equally powerful prognostic value, it has been combined with LN stage and T to provide important informations for clinically relevant subgroups of patients (4-5-6).

Tumor size is one of the stronger prognostic indicators, even after 20 years of follow-up. A larger tumor has been related to more positive lymph nodes, thus their interaction further influences prognosis and therapeutic options. Furthermore, regional node involvement is a valuable predictor of worse prognosis, but the survival of node-positive patients improved due to better

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staging procedures and application of systemic treatment.

With regard to the outcome of the sentinel node (SN) procedure, the success rate is influenced by the method of detection and by several patient and tumor characteristics. As the nodal status still remains one of the primary prognostic discriminants in BC patients, appearing to be of great value as independent predictor of distant disease development, axillary staging is a mandatory step in surgical management. Although several institutional series differed in patient selection, follow-up, type of surgery and adjuvant therapies, they showed consistently that the percentage of positive lymph nodes is a significant indicator of survival in women with LN positive BC (7). The American Society of Clinical Oncology convened an Update Committee of experts to provide evidence-based recommendations to practicing oncologist, surgeons, and radiation therapy clinicians on the use of sentinel node biopsy (SNB) for patients with early-stage breast cancer (8). The need for performing axillary lymph node dissection (ALND) when SN is positive and contemporary adjuvant treatment including radiotherapy, chemotherapy and/or hormonal therapy is delivered has been questioned in recent years. On the other hand, ongoing trials are testing whether a node-positive patients can be spared chemotherapy, as clinicians increasingly rely on breast cancer biology for decision making regarding adjuvant systemic treatment (9). However, the inaccuracy of the imaging techniques allowed widening indications of sentinel node approach. Optimization of procedure could be implemented by dual mapping injection site skills, resection of all hot or blue nodes through tracer combination, and improvement in atypical drainage patterns mapping (10). It may also be useful for future studies to report diagnostic accuracy according to subgroups of patients with different stages and molecular subtypes of primary breast tumors in order to inform treatment decisions for these categories.

This study was designed to evaluate the interactions between the intrinsic biological properties of primary tumor, prognostic factors and the axillary status of BC patients, in order to decipher the probability of a positive sentinel node biopsy for each MST. Therefore, it is important to identify what are the appropriate inclusion's criteria for a customized surgery, taking into consideration whether the distinct molecular traits could be predictive for different nodal involvements and outcomes. Currently, given this apparent diversity of subgroups within BC subtypes and different genomic features, ongoing research strongly interacts for additionally characterizing what appears to be a distinct biologic behavior of disease in the complexities of internal interactions. Prospective database and improved molecular profiling techniques will also likely provide direction in the management of this increasingly frequent, clinical question.

PATIENTS AND METHODS

PATIENT SELECTION

This retrospective study was approved by the Tor Vergata University Hospital Institutional Review Board. Written consent was given by the patients for their information to be stored in the hospital database and used for research. Our dataset consisted of 779 breast patients treated between January 2008 and December 2012 and identified through the Institutional prospective BC database. We limited our study to women with unilateral or non metastatic T1-T2 invasive BC and no palpable axillary nodes, treated with conserving surgery or extended radical mastectomy plus sentinel node biopsies. Patients with prior malignancy, synchronous bilateral breast cancer, or treatment with preoperative systemic therapy were excluded. The following data were included in the analysis: patient age, pathological T stage, tumor grade (G) as well as lymph node status and the expression of immunohistochemically defined biological markers (ER, PR, Ki67, HER2).

TREATMENT CHARACTERISTICS

All patients underwent sentinel node biopsy (SNB) according standards concepts regarding different tumors and patients, and were stratified according to conventional prognostic factors and different molecular subtypes. The SNB procedure was performed with blue dye alone, or a combination with radioisotope. Preoperative SN imaging was done one day before surgery with radioisotope injected superficially, and this was followed by lymphoscintigraphy. On the day of surgery, the blue was injected and the area was massaged. Most SLNs in this group were found along sentinel lymphatic channels (SLCs). After the SNs were successfully dissected, they were sent to the pathology lab. The metastases of SNs were detected by imprint cytology, frozen section, hematoxylin-eosin (HE) staining or serial sectioning with immunohistochemistry (IHC). Tumor size was recorded on the largest tumor dimension which was measured by gross pathological evaluation.

Histological type, the tumor grade and prognostic factors (ER, PgR, Ki-67, HER-2 status) were assessed by pathologist, and used to classify patients in the molecular subclasses of the San Gallen International Expert Consensus Report 2013.

Monoclonal antibodies for ER (clone: SP1, dilution: 1:200; Dako, Glostrup, Denmark), Ki-67 (clone: MIB-1, dilution: 1:200; Dako, Denmark), PR (clone: 16SAN27, dilution: 1:200; Novocastra, DE), and HER2 (clone: 4B5; Ventana, Tucson, AZ, USA) were used for IHC analyses on a Ventana automated staining system. For the ER receptor, positive tumors were those containing ≥10% of cells with positively staining nuclei. According to ASCO guidelines (1-2) [13-21], all patients were tested for either HER-2 protein expression (IHC assay) or gene expression (FISH assay) using a validated HER-2 test. The HER-2 gene detection kit was purchased from Beijing GP Medical Technologies Co., Ltd. (Beijing, China). We considered an HER-2 positive status when (on observing within an area of tumor that amounts to > 10% of contiguous and homogeneous tumor cells) there is evidence of protein overexpression (IHC) or gene amplification (HER-2 copy number or HER-2/CEP17 ratio by FISH based on counting at least 20 cells within the area).

HER-2 tumors were scored as positive (3+) by immunohistochemistry if they contained strong and complete membrane staining in >10% of cells, low positive (2+) if weak to moderate complete membrane staining in >10% of cells or negative (0 or 1+) if there was no or partial staining or if it is complete in less than 10% of cells. We determined as "high Ki-67" labeling index a value from the threshold ≥ 20%, assessed with the MIB1 monoclonal antibody.

All patients with nodal metastases identified by SNB were randomized to undergo ALND and underwent dissection of 10 or more nodes. The use of adjuvant systemic therapy was determined by the treating physician and was not specified in the protocol.

CLASSIFICATION OF GROUPS

We identified the intrinsic BC subtypes, according to the recommended clinicopathologic criteria (2). Patients were categorized based on the receptor status of their primary tumor as follows: Luminal A (ER+ or PR+ and HER-2-), Luminal B HER-2- (ER+, HER-2 negative and at least one of: Ki-67 "high", PR "negative or low"), Luminal B HER-2+ (ER+, HER-2 over-expressed or amplified, any Ki-67, any PR), HER-2 (ER- or PR- and HER-2+), and Basal (ER- or PR- and HER-2-). ER and PR status was determined on the basis of immunohistochemistry staining. Tumors were considered HER-2 positive only if they were either scored 3+ by IHC and also HER-2 amplified (ratio>2) on the basis of fluorescence in situ hybridization (FISH). In the absence of positive FISH data tumors scored 2+ by IHC were considered negative for

HER-2. Tumors were also classified as Luminal and Non Luminal according to hormone receptor expression.

END POINTS

The first aim of our work was to assess the relation between the molecular subtype classification and the probability of a positive sentinel node biopsy, in order to identify, before surgery, a patient with a high risk of axillary metastasis and to assess if different intrinsic breast cancer subtypes are predictive of different nodal involvements of the axilla.

The second aim of this analysis was to decipher the interaction covariate between intrinsic BC subtypes, primary tumor size (T) and histological grading (G) in order to maximize the benefit from axillary staging procedures.

Our purpose was even to characterize whether these tests are indicated for a specific patient subpopulation, based on clinical staging and MST, and to discover whether the molecular traits of breast tumors provide more information regarding outcome compared to conventional histopathological features.

Finally, we aimed to develop and validate a predictive model for simultaneous consideration of multiple risk factors to calculate the overall probability of a specific outcome.

STATISTICAL ANALYSIS

All statistical analysis were carried out using SPSS Version V21 (SPSS, Chigago, IL). Differences between patients assigned to the breast cancer molecular subtypes were examinated using χ^2 tests. Risk analysis was performed in univariate and multivariate binary logistic models (Enter and Forward Stepwise models). Levin test and T-student test were performed to analyze the differences.

RESULTS

Among the 779 patients, 65 (8%) were pT1a, 163 (21%) pT1b, 386 (50%) pT1c, and 165 (21%) pT2, respectively. Tumor Grade was found as G1 in 112 patients (14%), G2 in 468 patients (60%) and G3 in 199 patients (26%). The median age of these patients was 60 years (range, 20-89 y).

Of the patients tested, 6% (n=50) had Basal-like triple negative subtype, 39% (n=301) were Luminal A-like, 28% (n=215) were Luminal B HER-2 negative, 23% (n=180) were Luminal B HER-2 positive, and 4% (n=33) were HER-2 enriched **(Table 1).**

All underwent a surgical excision of the primary tumor and a sentinel lymph node biopsy to define lymph-nodes involvement. The training set was composed of 220 patients with a positive sentinel node biopsy (28%) and 559 patients with negative SNB (72%).

Among the five BC subtypes, the incidence of SN involvement was 12% for Basal Like (pT1c n=2 and pT2 n=4, all G3); 27% for Luminal A (pT1a n=2, pT1b n=14, pT1c n=43 and pT2 n=22; G1 n=16, G2 n=51 and G3 n=15); 32% for Luminal B HER-2 negative (pT1a n=2, pT1b n=9, pT1c n=39 and pT2 n=19; G1 n=5, G2 n=44 and G3 n=20); 31% for Luminal B HER-2 positive (pT1a n=1, pT1b n=6, pT1c n=28 and pT2 n=21; G1 n=2, G2 n=36 and G3 n=19); and 18% for HER-2 (pT1a n=2, pT1c n=1 and pT2 n=3; G1 n=1 and G3 n=5) **(Table 2)**.

The association of molecular subtypes in the Luminal and Non Luminal classes, based on the prognostic and clinical management differences, is considered below, and identified 12 SN positive patients (14%) in the "Non Luminal" group (n=2 pT1a, n=3 pT1c and n=7 pT2; n=1 G1 and n=11 G3), and 208 SN positive patients (30%) in the "Luminal" group (n=5 pT1a, n=29 pT1b, n=110 pT1c and n=62 pT2; n=23 G1, n=131 G2 and n=54 G3).

The statistical analysis considered all 779 patients and described the risk of lymph-node metastases by tumor stage, tumor grade and biomolecular class, considering all the prognostic factors separately and associating all of them.

UNIVARIATE ANALYSIS

TUMOR STAGE

The analysis of the impact of pT on LN involvement, considering pT2 as reference, with an HR of 1, yielded significant results for all classes. pT1a shows an hazard ratio (HR) of 0.168 (95% CI: 0.072 – 0.390, p<0.001); pT1b shows an hazard ratio of 0.301 (95% CI: 0.181 – 0.500, p<0.001); pT1c shows an hazard ratio of 0.576 (95% CI: 0.394 – 0.841, p=0.004).

TUMOR GRADE

The analysis of the impact of tumor grade on lymph-node involvement, considering G3 as reference, with an HR of 1, G1 showed an hazard ratio of 0.545 (95% CI: 0.316 – 0.942, p=0.03); G2 produced a non significant result with an HR of 0.82 (95% CI: 0.572 – 1.175, p=0.279).

BIOMOLECULAR CLASS

The analysis of MST impact on lymph-node involvement yielded a non-significant result except for Basal Like tumors. The Luminal A (LUM A) class showed an hazard ratio of 1.091 (95% CI: 0.790 – 1.507, p=0.596); the Luminal B HER-2 positive class (LUM B+) described an HR of 1.218 (95% CI: 0.847 – 1.752, p=0.287). The Luminal B HER-2 negative (LUM B-) class showed an hazard ratio of 1.316 (95% CI: 0.935 – 1.853, p=0.115); the HER Type class reported a an HR of 0.56 (95% CI: 0.228 – 1.375, p=0.206). The Basal Like class indicated a significant result with an hazard ratio of 0.333 (95% CI: 0.140 – 0.792, p=0.013).

Associating subtypes in the "Luminal" and "Non Luminal" groups, we found a significant result for both the classes. The "Luminal" group produced an hazard ratio of 2.487 (95% CI: 1.321 – 4.685, p=0.005). The "Non Luminal" group was used as reference. **(Table 3)**

MULTIVARIATE ANALYSIS

Based on the lymph-node positivity, the multivariate analysis showed all significant results, except for the Basal Like subtype. On histopathological grading, G1 and G2 showed an hazard ratio of 0.534 (95% CI: 0.287 – 0.992, p=0.047) and 0.588 (95% CI: 0.389 – 0.888, p=0.012) respectively. G3 was used as a reference with a p=0.031. The relationship between tumor volume and nodal involvement produced an hazard ratio of 0.155 (95% CI: 0.066 – 0.366, p<0.001) for pT1a group, and 0.288 (95% CI: 0.170 – 0.489, p<0.001) for pT1b. In this cohort, pT1c breast cancer showed an HR of 0.537 (95% CI: 0.363 – 0.793, p=0.002) with pT2 as baseline (p<0.001). Among the five BC subtypes, Basal Like showed a non significant result (p=0.286) with an hazard ratio of 0.505 (95% CI: 0.144 – 1.772). In contrast, Luminal A demonstrated HR of 2.779 (95% CI: 1.035 – 7.462, p=0.043), Luminal B HER-2 negative 3.053 (95% CI: 1.143 – 8.156, p=0.026), Luminal B HER-2 positive 2.895 (95% CI: 1.082 – 7.746, p=0.034) and the HER-2 subtype was used as reference (p=0.002), **(Table 4; Fig.1)**.

DISCUSSION

Conventional and widely accepted prognostic factors may explain a significant proportion of early deaths among patients with breast cancer, but they were of limited value to explain late events, such as new primary malignancies and treatment complications (11). In early-stage BC, where the use of systemic therapy has to be determined for every patient, the three main prognostic determinants used in routine practice are tumor size, histological grade and lymph node status, in addition to the stratification by clinical relevant subtypes.

Among node-negative patients, increasing tumor size has been

associated with increased breast cancer-specific mortality (12). In addition the growth rate of the primary tumor has been correlated with a higher risk of axillary LN involvement (13). We identified a linear trend in the effect of tumor size for predicting nodal categories, but this conventional relationship seem to be unpredictable in a subset of small cancers with more aggressive phenotype and higher risk of invasion pathways (14). Although traditionally distant spread has been considered a late event in tumor progression, recent studies suggest that for some tumors, acquisition of metastatic potential may occur early in cancer development, even in the absence of detectable primary tumors (15-16). Current routine clinical management relies on robust clinical and predictive factors to support clinical and patient decision making in which potentially suitable treatment options are increasingly available. One of the best-established prognostic factors is histological grade, which represents the morphological assessment of tumor biological characteristics and has been shown to be able to generate important information related to the clinical behavior of breast cancers. To provide a consistent and uniform way of assessing histological grade and to improve its reproducibility, consensus criteria and guidelines have been published with critical evaluation of these issues and recommendations for good practice (17). Studies have also demonstrated that grade is an independent prognostic factor in specific subgroups of breast cancer, including ER-positive patients who have received neoadjuvant endocrine therapy and patients with LN negative or positive regardless of ER expression (18-19-20). Moreover, there is a compelling evidence to suggest that histological grade can accurately predict tumor behavior, particularly in earlier small tumors (stage pT1), more than other time dependent prognostic factors such as tumor size (21-22). However, the Nottingham Grading System might have limited prognostic value in HER-2 positive and triple-negative cancer as most of these tumors are of high grade (grade 3) and these tumors typically exhibit poor-prognosis gene signatures (23-24).

We sought to determine whether there was significant interaction between histological grade and LN involvement in predicting breast cancer specific outcome. We hypothesized that for high grade lesions and positive sentinel node, tumor degree of differentiation may be a surrogate for biologically aggressive disease and thus predict for worse relapse-free and overall survival. However, our observations suggest that univariate analyses should be avoided for these data; in fact most prognostic factors are interdependent, and multivariate analyses should be used in attempt to define the individual, independent value of each factor studied. In addition, the Nottingham Grading System is a validated alternative to molecular tests in parts of the world where access to new molecular technology is not currently available or likely to become available in the near future, and can be assessed relatively reliably whereas other well-established prognostic signatures, such as vascular invasion and tumor size cannot (25). Therefore we believe that treatment decisions based on TNM staging system, which measures the anatomic extent of the tumor, can be improved by the addition of histological grade, which measures the intrinsic biological features and reflects the potential of a carcinoma to metastasize or cause death.

The definition of BC molecular subtypes has increased our understanding of underlying tumor biology. This classification system is still evolving and has not been routinely incorporated into clinical practice. However, there are a number of clinically defined subtypes that have a distinct pattern and timing of recurrence that can provide a guide to tumor behavior. This additional information may assist in planning ongoing management and suggests that the more aggressive MST that have shorter recurrence times and most events occurring within 5 years should have more frequent breast imaging and follow-up (26). Several authors have underscored a strong relation between the molecular traits and the axillary status of breast cancer patients (27-28-29). As the nodal status is the most robust and strongest factor correlated to overall survival, and is one of the major determinants in therapeutic decisions, axillary staging is a mandatory step in decision making network. The aim of our work was to decipher the interaction between this classification and the probability of a positive sentinel node biopsy.

Subtyping of BC introduced a new qualitative approach in the clinical setting. Not yet used as a routine standard in clinical practice, it shows a different point of view about the natural history and the possibility of metastatic involvement in lymphatic axillary nodes. According to literature, the LN involvement was about 28%, but a different disease progression was found in different classes, especially between Luminal and Non Luminal profiles. Not only histological portrait of primitive tumor was considered in this analysis, describing ductal invasive histotype as the most common subtype, but was also possible to describe Luminal A as the most prevalent subgroup, with an higher significance for clinicians. However, the diversity and incidence of this tumor call for in depth genomic studies to explain its molecular heterogeneity and link it to clinical outcome (30).

Our current study has several inherent limitations. Classification according to ER, PR, and HER-2 and Ki-67 status and grade are only approximations of genotype based molecular BC subtypes, and our conclusion do not necessarily apply to genotype-based subtypes.

Detailed patient and tumor information that may have influenced treatment decisions were not available from the cancer registry data-base. Important factors regarding family history, genetic testing results, tamoxifen use, systemic chemotherapy, and mammographic findings were not available from this database. Another possible limitation relates to the lack of information about the extent of disease in the SN, and the association of isolated tumor cells and micrometastases in the regional nodes with the clinical outcome of breast cancer is unclear. Despite these potential limitations, we acquired data from a large number of cases, and the power of data appears high. Further study will be needed to confirm the findings based on these new definitions.

CONCLUSION

Clinic and pathological classification based on the analysis of tumor marker expression can help to distinguish between breast cancers subtypes, and provide a scientific basis to determine individualized treatment. In this study we showed for each MST a specific correlation pattern between tumor size and lymphatic disease progression, hypothesizing that nodal status is still a potential signature of the intrinsic biological properties of a primary tumor. The Luminal group was prevalent in the population and associated with a better prognosis. In particular, HER-2 and Basal like demonstrated to be rare and more aggressive, including the highest proportion of Grade 3.

The probability of having an axillary metastasis seemed to be more related to the Luminal BC group, with a three times higher risk for the Luminal B HER-2 negative, referring to HER-2 patients. These findings confirmed that Non Luminal BC, including HER-2 and Basal Like, was a "protective" factor for axillary node involvement with an high significance, lowing the risk by 60%. Although tumor subtype was of less predictive value, in our univariate analyses, than existing histopathological parameters, such as tumor size and LN status, it provided further information to complement these indices and may be useful in routine practice to help better inform both clinicians and patient about their anticipated outcome after BCT. Ideally, one would like to predict risk of axillary disease to stratify between ALND, SLNB, and potentially, a third group which requires no axillary surgical procedure (31).

This classification could lead to a different approach on breast cancer patients and should be incorporated in staging systems and algorithms to define therapy and predict treatment efficacy. Generally, completion ALND is still needed for the patients with a positive SN, and more morbidity would be carried including lymphoedema, seroma, arm weakness and so on (32-33). However, metastases in non sentinel lymph nodes were found in about 12% to 58% of the patients with positive SLNs, changing considerably the incidence with the disease extent (34-35-36). By demonstrating that prediction for axillary involvement at diagnosis might be augmented by the use of molecular subtype classification, our study demonstrates that such analysis might be of value in the decision-making process in BC management. Lower presentation in Non Luminal class could be interpreted as a metastatic involvement of other lymphatic stations, or as a different way of spreading of cancer cells. Prospective testing of the value of axillary node prediction with our simple IHC-based classification is warranted for the stratification of locoregional treatment approaches. Further work is required to establish whether there are reproducible organ-distinct patterns of distant metastatic spread across the different MST and the relationship with nodal involvement, its control, and ultimately, survival outcomes. This will be possible with the outcoming results of overall and disease-free survival, with emerging evidence regarding the relevance of biological profile classification to the subsequent behavior of breast cancer in routine clinical practice.

DISCLOSURE STATEMENT

The authors declares that there is no conflict of interests regarding the publication of this article.

TABLES

Table 1. Characteristics of breast cancer patients by tumor grade (G), tumor stage (T) and molecular subtype (MST), with number of "SLNB positive" patients and percentage

Tumor Grade	n	(slnb+)	(%)
G1	112	24	21.4
G2	468	131	28
G3	199	65	32.6
Tumor Stage	n	(slnb+)	(%)
T1a	65	7	10.8
T1b	163	29	17.8
T1c	386	113	29.3
T2	165	69	41.8
Biomolecular class	n	(slnb+)	(%)
Basal like	50	6	12
Luminal A	301	82	27.2
Luminal B-	215	69	32.1
Luminal b+	180	57	31.7
HER-type	33	6	18.2

Table 2. Distribution of tumor grade (G), tumor stage (T) and SNB among five molecular subtypes (MST).

	BASAL	LUM A	LUM B-	LUM B+	HER
Tumor Grade for biomolecular class					
G1	0	70	31	11	2
G2	7	200	143	114	7
G3	43	32	45	56	24
Tumor Stage for biomolecular class					
T1a	3	26	14	17	5
T1b	7	80	42	33	1
T1c	20	141	110	96	19
T2	20	54	49	34	8
SLNB for biomolecular class					
Positive	6	82	69	57	6
Negative	44	220	150	124	27

Table 3. Univariate analysis. Risk of lymph-node metastasis according to tumor stage (T), tumor grade (G) and molecular subtype (MST).

Binary Logistic Regression Analisys						
			UNIVARIATE ANALISYS			
	B	ES	HR	HR 95% CI		P*
TUMOR STAGE						
pT1a	-1,784	0,43	0,168	0,072	0,390	<0,001
pT1b	-1,2	0,259	0,301	0,181	0,500	<0,001
pT1c	-0,552	0,193	0,576	0,394	0,841	0,004
pT2	-0,33	0,158		REFERENCE		
TUMOR GRADE						
1	-0,607	0,279	0,545	0,316	0,942	0,03
2	-0,198	0,183	0,82	0,572	1,175	0,279
3	-0,746	0,152		REFERENCE		
BIOMOLECULAR AGGREGATE						
Luminal (Non aggressive)	0,911	0,323	2,487	1,321	4,685	0,005
Others (Aggressive)	-1,778	0,312		REFERENCE		
BIOMOLECULAR ANALITYC						
Luminal A	0,087	0,165	1,091	0,790	1,507	0,596
Luminal B +	0,197	0,185	1,218	0,847	1,752	0,287
Luminal B -	0,275	0,175	1,316	0,935	1,853	0,115
HER	-0,58	0,459	0,56	0,228	1,375	0,206
Basal	-1,101	0,433	0,333	0,140	0,792	0,013

Table 4: Multivariate analysis. Risk of lymph-node metastases by tumor stage (T), tumor grade (G) and molecular subtype (MST).

Binary Logistic Regression Analisys						
	MULTIVARIATE ANALISYS					
	B	ES	HR	HR 95% CI		P*
G1	-0,628	0,316	0,534	0,287	0,992	0,047
G2	-0,531	0,21	0,588	0,389	0,888	0,012
G3				REFERENCE		0,031
pT1a	-1,865	0,439	0,155	0,066	0,366	<0,001
pT1b	-1,244	0,27	0,288	0,17	0,489	<0,001
pT1c	-0,623	0,199	0,537	0,363	0,793	0,002
pT2				REFERENCE		<0,001
Basal	-0,683	0,64	0,505	0,144	1,772	0,286
Luminal A	1,022	0,504	2,779	1,035	7,462	0,043
Luminal B -	1,116	0,501	3,053	1,143	8,156	0,026
Luminal B +	1,063	0,502	2,895	1,082	7,746	0,034

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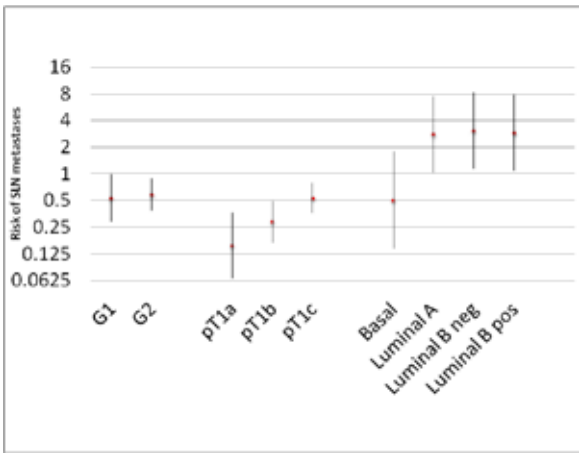
HER				REFERENCE		0,002

Fig 1: Risk analysis for tumor grade (G), tumor stage (T) and molecular subtype (MST). Grade 3, stage pT2 and HER-2 subtype considered as reference.



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