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The molecular profile of breast cancer: primary tumor *versus* corresponding lymph node metastases

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Abstract

Breast cancer (BrCa) is the most frequent malignancy in female, and lymph node metastases (LNM) is an important prognostic and therapeutic parameter. The molecular classification is nowadays largely applied to characterize the primary tumors, but few studies focused on the comparison between the molecular profiles of the primary with corresponding LNM. In the current work, we investigated the expression of conventional markers used by molecular classification in both primary tumors and axillary LNM. A series of 156 patients with BrCa was investigated, and from these 80 cases showed LNM. After routine pathological investigation, including the histopathological form and grade, we performed additional step sections from the primary and lymph nodes for immunohistochemistry. All procedures for hormone receptors, human epidermal growth factor receptor 2 (HER2), Ki67, cytokeratin 5 (CK5), epidermal growth factor receptor (EGFR), p53, E-cadherin, and B-cell leukemia/lymphoma-2 (Bcl-2) were performed using the standard automated procedures. We found significant differences between the primary tumors and corresponding LNM in luminal A, luminal B, and basal-like carcinoma. No phenotypical interconversions were noticed in HER2 and unclassified BrCa. Our data demonstrate that in almost 20% of the cases the molecular profile of the primary does not overlap with aspects found in the lymph nodes. Our results strongly suggest performing the molecular classification in both primary tumors and in LNM. Current data suggest that the application of this diagnostic procedure will significantly influence the therapeutic strategy.

Keywords: breast cancer, immunohistochemistry, molecular classification, lymph node metastases, prognosis, targeted therapy.

Introduction

Breast cancer (BrCa) is a common disease that includes many molecular types. Although their common source, these neoplastic diseases are different in their histopathological appearance, molecular profile, prognosis, and overall survival. To define each tumor type, most studies focused on the detailed evaluation of the primary and based on the findings it is established the adjuvant and/or neoadjuvant therapeutic strategy. As it was pointed out many years ago, an indicator of major importance is the status of axillary lymph nodes. Clinical trials have shown that micrometastases in the sentinel lymph node is not an indication for axillary dissection, and chemotherapy and/or radiotherapy are enough powerful to eliminate remnant tumor cells [1, 2]. This conclusion is also based on the finding of Perou *et al.* [3], who believe that the "molecular program" of the primary is strictly maintained in the lymph node and distant metastases. In other words, if the molecular profile of the primary shows a given predictive value, this will be automatically attributed to its metastasis.

Based on the data mentioned before, some clinical aspects were hard to be explained: why some cases that express hormone receptors do not respond to hormone therapy, and why some cases, although rare, which do not express the same receptors do respond to therapy. Moreover, it is not clear why in some cases with human epidermal growth factor receptor 2 (HER2) type it is found to be the so-called resistance to therapy. Is it a real resistance or some tumor cell clones do not express this marker and natural evolution continues by progression and metastatic spread, which are insensitive to Trastuzumab?

In the last years, there were accumulated a lot of data that demonstrate the instability of the molecular profile of malignant cells over time. Falck *et al.* [4] showed that the profile of carcinoma is not stable along with its evolution and shows differences between subtypes and also between the primary tumor and lymph node metastases (LNM) in 11% of the cases. Prat & Perou [5] describe BrCa as heterogeneous, and some cells, particularly with stem phenotype are more resistant to therapy and more active in their metastatic spreading. The molecular instability of the tumor seems to be induced in part by blocking estrogen receptors (ERs). This could be a change in the pathway of survival. There were reported series of cases with transfer from ER-positive to HER2-positive, and reversal, as a response to adjuvant therapy [6–9]. In all these three studies, only the molecular profile of the primary was evaluated at the initiation of the therapy, and the metastatic tumor was evaluated significantly later. The possible change

in the phenotype during the natural evolution of the disease, with or without therapy, is of practical importance. Therefore, we compared in the present study the profile of the primary BrCa and axillary lymph nodes at the moment of diagnosis.

In patients which received aromatase inhibitors, there were described mutations at the level of estrogen receptor 1 (*ESR1*) gene that encode the domain of fixation for estrogens and develop resistance to hormone therapy [10–12]. Maybe of importance, is the fact that these mutations were detected only in the lymph nodes metastases, and not in the primary tumors. Results concerning the molecular stability during the metastatic spread are controversial. Until now, there were not published large series of patients regarding the evolution of the molecular type of LNM in comparison with the primary. The potential existence of the molecular profile transfer could have not only prognostic value but also a major impact on therapeutic strategy.

Despite intensive research on BrCa to elucidate the heterogeneous nature of neoplastic proliferation, results are far to clarify the problem. For decades, the conventional morphological classification was considered the "golden standard" and remains today an important diagnostic procedure. However, using this classification, patients are stratified based on some common morphological criteria. Therefore, the prognostic and therapeutic impact of this information is limited.

An important moment in the therapeutic strategy was the introduction of hormone therapy and targeted therapy with humanized antibodies, like Trastuzumab. Detection of ER, progesterone receptor (PR), and HER2 became of major importance in the therapeutic strategy and opened the era of targeted therapy. The molecular classification of BrCa had an immediate impact on the response to therapy and is largely accepted for its predictive value on medium and long-term survival. On the other hand, overall survival did not improve as expected. The immunohistochemical (IHC) expression of ER, PR, and HER2 in tumor cells is a major indication for specific adjuvant therapy. Targeted therapies are based on the molecular evaluation of the primary tumor and in most of the cases, LNM and/or distant metastases are not investigated from this point of view. Therefore, the molecular profile of LNM is not a routine procedure at present time. Only a few studies showed the importance of such an evaluation [13–15]. All these studies are limited to discordances of ER, PR, HER2, and Ki67 expression in the primary tumors *versus* LNM. They do not bring information about the potential clinical impact of these discordances, and consecutively, they were not applied in current oncological therapeutic procedures.

In fact, currently, the routine molecular evaluation of BrCa is performed on specimens taken from the primary and it is supposed that the lymph node or distant metastases show the same molecular profile. Based on this hypothesis, it is possible to explain the failure of therapeutic strategy in several cases, and the expression of some markers in both primary tumors and metastases could be a documented reason to make therapeutic changes in individual patients.

Aim

In the current work, we investigated the expression of IHC markers used in molecular classification in both primary tumor and LNM.

Materials and Methods

For the present study, there were selected 156 patients with documented BrCa. From these, 80 (51.28%) cases showed LNM. The patients had between 34 and 84 years (average 58.9 years), and only five (3.2%) cases reported a history of familial BrCa. In 125 (80.12%) of the patients, we noticed menopausal status. The tumor size had an average of 3.8 cm, and lymphovascular invasion was noticed in 65 (41.68%) of the patients. Looking for the lymph node status, we found negative results in 76 (48.7%) cases, and LNM in 80 (51.3%) cases. Concerning the microscopic type of the tumor, we noticed ductal invasive carcinoma not-otherwise-specified in 130 cases, lobular invasive in six cases, medullary in seven, mucinous carcinoma in two, metaplastic in nine, and papillary in two cases. From these, 17 cases were evaluated as G1, 81 as G2, and 58 as G3. Local recurrences were noticed in 12 (7.69%) cases.

Primary processing

Specimens were fixed in 10% neutral buffered formalin for 24–48 hours, pH 7.2–7.4, and embedded in Paraplast High Melt (Leica Biosystems). Step sections, 3–5 μm thick (Shandon, HM355S Automatic Microtome, Thermo Scientific, USA), stuck on slides were stained with conventional Hematoxylin–Eosin (HE) for the histopathological diagnosis and grade. After staining, slides were mounted with Leica CV Mount (Leica Biosystem Newcastle, Ltd., New Castle Upon Tyne, UK). The grading was done in accordance with *World Health Organization* (WHO) recommendations.

Immunohistochemistry

All procedures (dewaxing, antigen retrieval, visualization) were performed using Leica Bond-Max (Leica Microsystems GmbH, Wetzlar, Germany). Briefly, slides were dewaxed in two baths of Bond Dewax Solution five minutes each, followed by rehydration with decreasing alcohols for two minutes each, and distilled water. Endogenous peroxidase was blocked with Dako REAL™ Peroxidase-Blocking Solution for five minutes. Nuclei were stained with modified Mayer's Hematoxylin (HMM500, ScyTek Laboratories, Inc.). Slides were then dehydrated, clarified, and mounted with Leica CV Mount (Leica Biosystems). Details on the primary antibodies, dilution, antigen retrieval, and working system are shown in Table 1.

Table 1 – ***Antibodies, working system, and expression of the final product***

Antibody	Clone	Dilution	Antigen retrieval	Incubation	Working system/ Chromogen	Expression
ER	1D5	RTU	MW, 30 minutes citrate buffer, pH 6	30 minutes, RT	LSAB+/HRP, DAB	Nuclear
PR	Pgr636	RTU	MW, 30 minutes citrate buffer, pH 6	30 minutes, RT	LSAB+/HRP, DAB	Nuclear
Ki67	MIB1	RTU	MW, 30 minutes citrate buffer, pH 6	30 minutes, RT	LSAB+/HRP, DAB	Nuclear

Antibody	Clone	Dilution	Antigen retrieval	Incubation	Working system/ Chromogen	Expression
HER2	Rabbit polyclonal	RTU	MW, 30 minutes, antigen retrieval solution HercepTest™	30 minutes, RT	HercepTest™ visualization reagent, DAB	Membrane pattern
p53	DO7	RTU	MW, 30 minutes citrate buffer, pH 6	30 minutes, RT	LSAB+/HRP, DAB	Nuclear
Bcl-2	124	RTU	MW, 30 minutes citrate buffer, pH 6	30 minutes, RT	LSAB+/HRP, DAB	Nuclear, cytoplasmic
E-cadherin	NCH 38	1:100	MW, 30 minutes citrate buffer, pH 6	30 minutes, RT	LSAB+/HRP, DAB	Membrane/cytoplasmic or both
CK 5/6	D5/16 B4	1:80	MW, 30 minutes citrate buffer, pH 6	30 minutes, RT	LSAB+/HRP, DAB	Cytoplasmic
EGFR	Polyclonal	RTU	MW, 30 minutes citrate buffer, pH 6	30 minutes, RT	EGFR pharmDx™ visualization reagent, DAB	Membrane, cytoplasmic

Bcl-2: B-cell leukemia/lymphoma-2; CK 5/6: Cytokeratin 5/6; DAB: 3,3'-Diaminobenzidine dihydrochloride; EGFR: Epidermal growth factor receptor; ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; HRP: Horseradish peroxidase; LSAB: Labeled Streptavidin–Biotin; MW: Microwave; PR: Progesterone receptor; RT: Room temperature; RTU: Ready-to-use.

Interpretation

Slides stained for nuclear markers, like ER, PR, and Ki67 were evaluated using the semi-automated method proposed by Suciu *et al.* [16], using NIS-Elements D2.30 (Nikon Instruments Europe BV) software and Nikon Eclipse 80i microscope, adjusted with Nikon DS-Fi1 (Nikon Instruments Europe BV) video camera. Hormone receptor expression was scored by applying the Allred score [17], which combines the percent of positive nuclei with the intensity of the final product of the reaction. HER2 status has been interpreted based on the *American Society of Clinical Oncologists*' recommendations, and only +2 and +3 cases were considered positive. E-cadherin-positive reaction was scored according to the system largely accepted in the literature [18]. Only cases scored as +2 and +3 were considered to be positive. Bcl-2 was scored according to the system proposed by Callagy *et al.* [19], and p53 was evaluated based on the recommendations of Yamashita *et al.* [20]. Cytokeratin 5 (CK5) was performed to characterize basal-like carcinoma, and CK8 and CK18 were done to identify micrometastases in the lymph nodes. EGFR was evaluated based on the recommendation of the Dako guide (EGFR pharmDx™, Dako, Denmark). The reaction was considered positive if more than 5% of tumor cells were positive.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 22.0 software (SPSS, Inc., Chicago, IL, USA). A Student's *t*-test was applied and a value of $p<0.05$ was considered significant.

Results

LNM were found in 80 of 156 patients, in most of the cases in the subcapsular space and more rarely occupying the whole parenchyma of the lymph node. Metastases were present regardless of the status of the lymph nodes, including here the atrophic lymph nodes. In most of the cases, the staining with HE was enough for the diagnosis, and only in five cases, it was necessary to demonstrate malignant cells with CK staining.

We analyzed first the molecular profile of primary breast tumors included in the present study. We defined the main molecular types of carcinomas based on the consensus from St Gallen, as follows. Luminal A cases (n=53) were defined based on the strong expression of ER and PR, a low expression for Ki67, and a negative reaction for HER2, CK5, and EGFR. Bcl-2 was positive in most of the cases from this group, and positive expression for p53 was slight and noticed in a minority of the cases. Luminal B carcinoma was demonstrated in 15 cases in the primary tumor. All these cases were characterized by a positive reaction for ER and PR (usually with lower intensity than in luminal A), a high rate of Ki67 proliferation index, Bcl-2 was positive in almost all cases, and p53 was positive in eight from 15 cases. All tumors with luminal B phenotype were consistently negative for HER2, CK5, and EGFR. The HER2 subclass (10 cases) of BrCa strongly expressed HER2 protein, and only cases noticed with +2/+3 were considered positive. Hormone receptors were negative in all these cases, and on occasion, CK5 (n=2), and EGFR (n=3) were positive. Basal-like carcinoma was found in only three cases in the primary tumor, all were negative for ER, PR, and HER2. These cases strongly expressed CK5, EGFR, and p53. The rate of Ki67 proliferation index was between 20% and 25% in the malignant cell population. We found four cases which did not express any of the markers used in this study except for Ki67 noticed in all cases, and E-cadherin in two of four cases. E-cadherin was not found to be helpful in the molecular classification, as it showed positive and negative cases in each group (Table 2).

Table 2 – ***Distribution of cases stratified based on the expression of the marker in the PT and corresponding LNM***

Molecular type (n=85)	Primary tumor	LNM
Basal-like	3 (3.52%)	1 (1.17%)
Luminal A	53 (62.35%)	48 (56.47%)
Luminal B	15 (17.64%)	22 (25.88%)
HER2	10 (11.76)	10 (11.76%)
Unclassified	4 (4.70%)	4 (4.70%)

HER2: Human epidermal growth factor receptor 2; LNM: Lymph node metastases; PT: Primary tumor.

To compare the molecular profile of the primary tumor with the corresponding LNM, we applied the same methods to sections from the lymph nodes, respectively, ER and PR, HER2, Ki67, CK5, Bcl-2, p53, E-cadherin, and EGFR. We applied the same criteria in interpretation as we used for the primary tumor. Ki67 was evaluated using the semi-automated method to avoid overinterpretation in LNM. For each marker mentioned before, it was appreciated if the reaction was similar in the primary *versus* metastases, and we showed positive and negative differences. The

values higher or smaller in the primary in comparison with corresponding LNM were not considered to reflect a major discordance, but we showed these values for each marker included in the study. It was considered important the analysis of the expression of all markers mentioned before because we noticed occasional co-expression in virtually all five major molecular types. The comparison between the molecular profile of the primary tumor and the corresponding LNM is shown in Table 3.

Table 3 – ***A comparison between the PT and LNM***

Immunomarker	PT=LNM	PT-/LNM+	PT+/LNM-	PT+>LNM+	PT+<LNM
ER (*n*=68)	59 (86.76%)	2 (2.94%)	7 (10.29%)	NS	NS
PR (*n*=68)	48 (70.58%)	4 (5.88%)	6 (8.82%)	5 (7.35%)	5 (7.35%)
HER2 (*n*=10)	9 (90%)	NS	NS	1 (10%)	NS
Ki67 (*n*=85)	54 (63.52%)	2 (2.35%)	1 (1.17%)	9 (10.58%)	19 (22.35%)
CK5 (*n*=25)	16 (64%)	2 (8%)	5 (12.5%)	3 (12%)	2 (8%)
Bcl-2 (*n*=40)	23 (57.5%)	NS	14 (35%)	2 (5%)	1 (2.5%)
p53 (*n*=37)	20 (54.05%)	2 (5.4%)	8 (21.62%)	4 (10.81%)	3 (8.1%)
EGFR (*n*=5)	3 (60%)	NS	2 (40%)	NS	NS

Bcl-2: B-cell leukemia/lymphoma-2; CK5: Cytokeratin 5; EGFR: Epidermal growth factor receptor; ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; LNM: Lymph node metastases; *n*: The total No. of cases positive for the respective marker; NS: Not significant; PR: Progesterone receptor; PT: Primary tumor. Percent was calculated separately for each immunomarker.

We then evaluated the expression of individual markers used in this study. ER IHC expression is a good indicator of response to hormone therapy, and ER status is a good prognostic factor to predict long-term disease-free interval and overall survival. In the lymph node, the only elements positive for ER are tumor cells, and therefore, the method is highly specific. Results concerning the expression of ER in the primary tumors and LNM are shown in Table 3 and Figure 1. As is noticed in Table 3, in only 80.19% of the cases, the expression is similar between the primary and LNM. We found major differences in six cases (two with PT-/LNM+, and four with PT+/LNM-). The immunoreaction for PR was nuclear-restricted and was positive in 48 of the cases. In four cases, the primary was negative, and the corresponding metastases were positive, and in six showed a reversal aspect. We noticed a higher number of discordances for PR than reported for ER. The most frequent aspect was related to the positive primary with negative axillary metastases (Table 3; Figure 1).

HER2 was found in 10 of 85 cases, and it was the most stable molecular subtype of BrCa. Only a minor discordance was noticed, with strong staining of the primary tumor and mild (+2) reaction in the metastases. Results on HER2 expression are shown in Table 3 and Figure 1. Ki67 was expressed in all tumors included in the present study, but we found significant differences between the primary tumor and the corresponding LNM (Table 3). The germ center of lymphoid follicles was considered internal positive control. As expected, minor differences were noticed more frequently than for other markers. Usually, the number of Ki67-positive is significantly higher in the metastases than in the primary. This could be explained by the cellular heterogeneity of the lymph nodes with metastases and the selection of some specific clones from the primary.

Additional markers used in the current study were CK5, p53, Bcl-2, EGFR, and E-cadherin. CK5 was expressed on all basal-like carcinoma and in addition in other 22 cases of luminal type. The distribution of CK5+ cells was different. In basal-like carcinoma, most tumor cells were positive, and in luminal types only scattered cells were noticed. Bcl-2 promotes cell survival, and its expression does not correlate with the pathological form. In this group of patients, Bcl-2 was positive in 40 of the primary tumors, but in 14 of them, the LNM were negative. We found a positive reaction for p53 in 37 cases (all basal-like carcinoma, eight HER2 cases, 22 cases with luminal profile, and two unclassified. The positive reaction for p53 was nuclear-restricted and limited to tumor cells. More primary tumors than corresponding LNM were positive for p53. Five cases were positive for EGFR, and in two cases the primary tumor was positive and the corresponding axillary LNM were negative. No significant differences were found between the primary and LNM concerning the expression of E-cadherin. Results for CK5, Bcl-2, p53, EGFR, and E-cadherin are shown in Table 3 and Figure 2.

There were not found phenotypical interconversions for HER2 and unclassified molecular types. Phenotypical interconversions were noticed particularly in luminal A, luminal B, and basal-like carcinoma. We found a significant number of cases with interconversion in the major markers, particularly hormone receptors (ER and PR), and the most constant profile in HER2-positive cases. Looking at the second group of markers (CK5, p53, EGFR, and Bcl-2), discordant results were reduced, except for CK5 and p53 (Table 3).

Discussions

Falk *et al.* [13–15] reported for the first-time significant discordances between primary BrCa and LNM, based on the molecular profile assessed by immunohistochemistry. In this research performed on a representative number of cases, there were reported concordances between the primary and the LNM in 93% for ERs, 84% for the PR, 97% for HER2, and 85% for Ki67. Our data confirm these observations and suggest that HER2 type is the most stable concerning the molecular profile. Simultaneous detection of the molecular profile of the primary tumor and LNM might have a major influence on therapeutic strategy. These results were confirmed by others [21]. Currently, the therapeutic decision is based strictly on the molecular analysis of the primary that in many cases has been already removed by surgery. In another study, it was shown a conversion of the molecular type in seven from 142 cases with particular reference to lymph nodes [22], and it seems that discordances in the molecular profile are even higher concerning distant metastases.

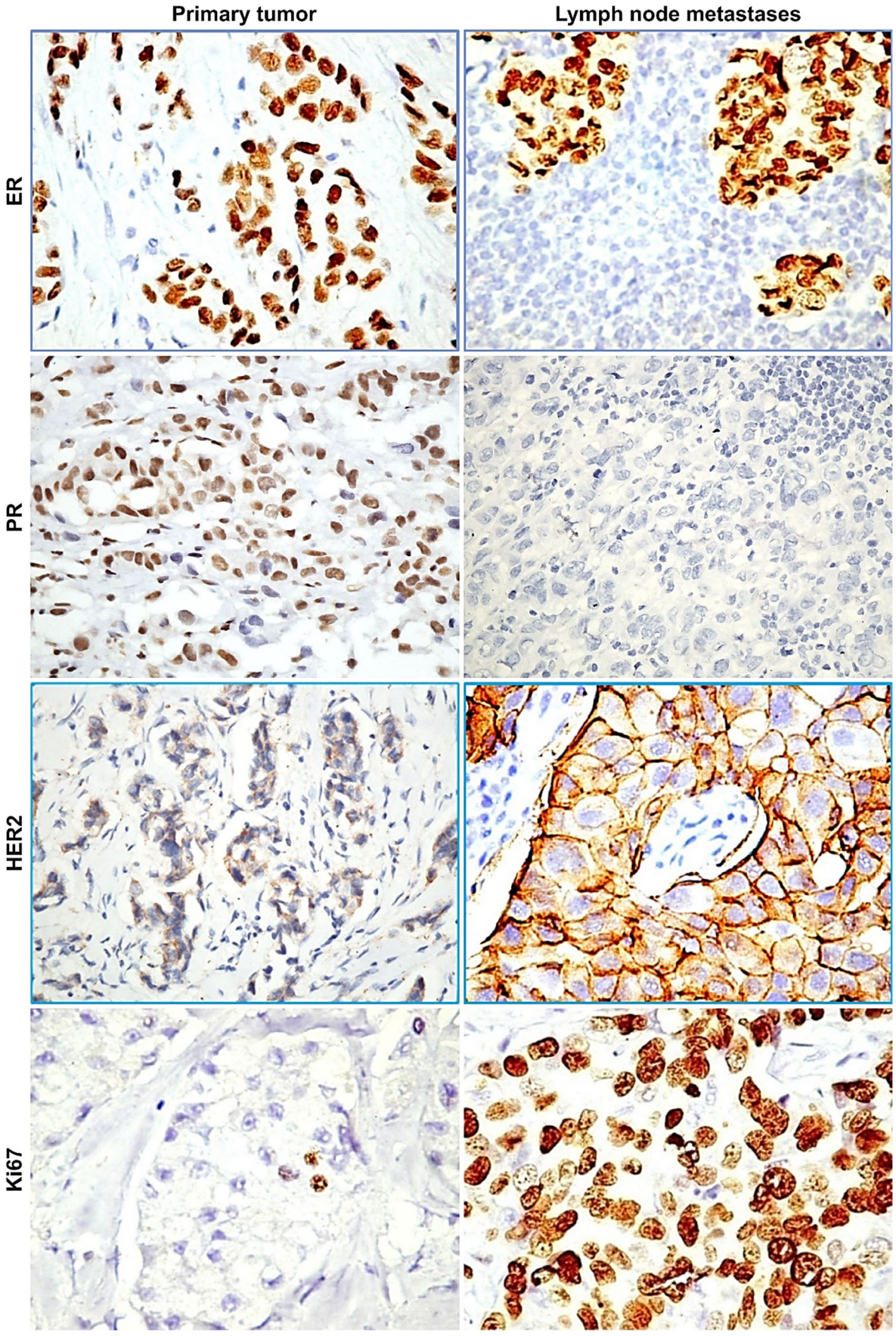

Figure 1 – ***Expression (×400) of main markers of the immunohistochemical profile in the primary tumor (left column) and lymph node metastases (right column). ER: Estrogen receptor; HER2: Human epidermal growth factor receptor 2; PR: Progesterone receptor.***

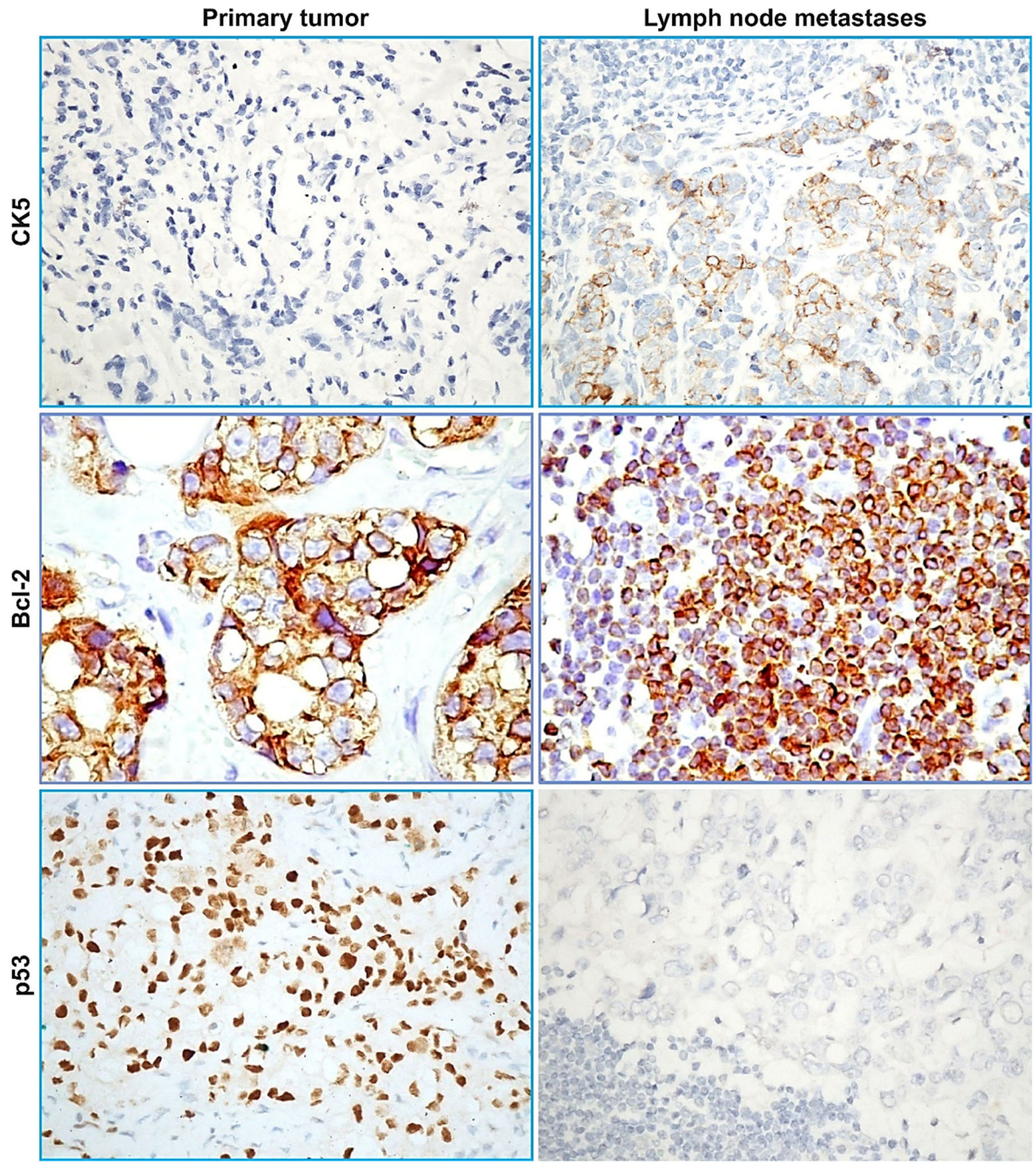

Figure 2 – ***Expression (×400) of additional markers in the primary tumor (left column) and lymph node metastases (right column). Note the lack of expression of CK5 in the primary tumor and becomes positive in lymph mode metastases. Conversely, p53 positive in the primary tumor was largely negative in the lymph mode metastasis. Bcl-2: B-cell leukemia/ lymphoma-2; CK5: Cytokeratin 5.***

A new concept was introduced early in this century concerning molecular classification. Studies published by Perou *et al.* [3] have an immediate impact a new stratification of patients with BrCa, recognizing a minimum of five different molecular types. Each type showed a typical molecular profile, growth characteristics, and invasive and metastatic potential. It was thought even from the beginning that this procedure will improve the response to specific therapy, prognosis, and survival. Despite all these efforts, the development of therapy resistance is still frequently reported, it is associated with recurrence, sometimes with a worst prognosis than the primary tumor [23].

A common characteristic of BrCa regardless of the histological form or the molecular profile is the ability of tumor cells to spread on the lymphatic route. Nowadays, most pathologists give a detailed description of the molecular profile of the primary, and this confirms the neoplasia, and it is the base for adjuvant therapy. The molecular profile of the LNM is largely neglected and considered to be identical to the primary. There were published few data about this topic, and results were not yet applied in oncological practice until now. We consider that elucidation of this aspect has crucial importance in a large number of cases, because approximately 20% may benefit from personalized therapy. It is expected that such an evaluation will lead to a significant improvement in prognosis.

Montel *et al.* [24] have demonstrated some differences concerning markers expression between metastatic and non-metastatic tumor cells. The authors mentioned before noticed that with the increased metastatic potential of tumor cells there occur some changes in the molecular profile, and in particular, a change in the expression of hormone receptors. These findings are also supported by our data because almost 20% of the cases included in our study showed this behavior. This is particularly true in the case of ER which seems to be the less stable marker during the metastatic cascade. All these data support a major change in the molecular classification of LNM in comparison with the primary tumor. It seems that the primary tumor consists of many different clones of tumor cells, which could be different in terms of the molecular profile, and with different metastatic potential. Our results demonstrated the heterogeneous distribution of ER, PR, and HER2, and support the existence of tumor cell lines with different metastatic potential. This demonstrates that the malignant phenotype is not static and pre-determined, but evolutive during the natural evolution of the tumor [25–28]. On the analysis of metastases, we have reported a significant increase in basal-like carcinoma in the LNM. This suggests the aggressive behavior of the tumor and it could be an explanation for resistance to therapy.

Significant discordances concerning HER2 status were reported by Santinelli *et al.* [29] who found major differences in 6.7% of cases with LNM, in 13.3% of local recurrences, and in 28.6% from distant metastases. Similar results were published by Niikura *et al.* [30] who investigated 40 untreated HER2-positive cases. In this short series, four cases were converted to HER2-negative. We cannot confirm these data in our series of 80 patients, and in our study the expression of HER2 was the most constant and stable, without significant molecular interconversion. On the other hand, the metastatic tumor cell heterogeneity from lymph nodes and a different micromedium could be an explanation for differences noticed in comparison with the primary [31, 32].

In the last years, there were accumulated a lot of data regarding the discordances between the primary tumors and corresponding axillary LNM [33–35]. These data are confirmed by our results in a homogeneous series of patients. In addition, we identified molecular types of BrCa which preserve their phenotype in LNM. On the other hand, we noticed a heterogeneous expression of basic molecular markers in luminal and basal-like carcinoma. In this study, we used an extended panel of antibodies, to better demonstrate that BrCa can change the phenotype during its natural evolution. Discordances between the primary tumor and distant metastases were even more dramatic [36].

Not only diagnostic IHC markers could be different in the primary BrCa in comparison with axillary LNM. Significant differences were noticed for markers that characterize the epithelial–mesenchymal transition [37], the expression of fibroblast growth factor [38], or for podoplanin-associated fibroblast in both tumors' micro-environment and LNM [39]. The examples mentioned before, support the heterogeneity of breast malignant tumors and still less predictable response to a specific therapy.

In the multimodal evaluation of malignant tumors, the identification of prognostic and therapeutic markers is a "gold standard" [40].

Our results suggest a higher rate of discordances in the molecular profile of primary BrCa and LNM. This means a comparison in the molecular profile between the primary, and metastases before the final therapeutic strategies are decided. The development of flexible and personalized strategies is now mandatory, to reduce the number of cases of resistance to therapy.

Conclusions

Our results suggest a major discordance in the molecular profile between the primary BrCa and the corresponding LNM. In this series, we found no significant changes in cases originally diagnosed as HER2 and non-classified. After the examination of the IHC expression of the markers selected for the current study, the number of luminal A carcinoma decreased by 3%, and basal-like carcinoma by 2%. On the other hand, luminal B carcinoma increased by 5%. The increase in the metastatic capacity of tumor cells induces major changes in the molecular profile. Our data support the synchronous examination of both primary tumor and LNM. The result may have a major impact on therapeutic strategy.

Conflict of interests

None to declare.

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