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Discordance Rate in Estrogen Receptor, Progesterone Receptor, HER2 Status, and Ki67 Index Between Primary Unifocal and Multiple Homogenous Breast Carcinomas and Synchronous Axillary Lymph Node Metastases Have an Impact on Therapeutic Decision

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Background: We aimed to demonstrate that in breast carcinomas the tumor profile is not stable during the metastatic process, with impact on therapeutic decisions.

Materials and Methods: We analyzed the estrogen receptor (ER), progesterone receptor (PR), and HER2 status and Ki67 index in 41 primary unifocal (PU) and 37 primary multiple (PM) breast carcinomas with identical immunohistochemical profiles among multiple tumor foci and the matched axillary lymph node metastases. We defined as concordant cases in which the primary tumor (PU or PM) and lymph node metastases displayed identical positivity or negativity for ER, PR, HER2, Ki67 and as discordant cases in which there was a mismatch in at least 1 biological parameter among PU and PM tumor and lymph node metastases. Moreover, we defined as concordant cases in which the molecular profile (based on the immunohistochemical evaluation of ER, PR, HER2, and Ki67) was concordant among PU and PM tumors and lymph node metastases and mismatch cases as those in which the molecular profile of the primary tumor differs from one of the lymph node metastases in at least 1 lymph node.

Results: The positivity for the biological markers is not stable during the metastatic process. In this study the total rate of discordant cases was 92.7% in PU tumors and 75.7% in PM homogenous tumors ($P = 0.058$, odds ratio = 0.245, 95% confidence interval, 0.06-0.991). The total rate of shifted cases was 64.9% in PM tumors and 82.9% in PU tumors. The highest rate of shifting was encountered from Luminal B-like to Luminal A-like. In 11 out of 37 (29.7%) PM and in 17 out of 41 (41.5%) PU cases the subtype shifted to a poorer one with respect to prognosis.

Conclusions: The patients in whom the primary tumor is hormone receptor and/or HER2 negative but is positive for these markers in the axillary lymph nodes could become eligible for hormonal treatment and/or trastuzumab treatment, which may significantly improve the patient's outcome.

Key Words: breast carcinoma, lymph node, heterogeneity

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Breast cancer is the most common malignant tumor occurring in women worldwide and can present as a primary unifocal (PU) or as primary multiple (PM) lesions. Both PU and PM tumors can be very heterogenous genetically but also with respect to the morphology (histologic type and grade) within the same tumor or among multiple tumor foci.[1,2] As a routine practice, during the histopathologic examination, the pathologist establishes not only the morphologic classification of each tumor but also the molecular classification, based on the estimation of the estrogen receptor (ER), progesterone receptor (PR), HER2 status, and Ki67 index, using immunohistochemical markers as surrogates. Moreover, clinical and therapeutic decisions are based on the molecular profile of the primary tumor.[3] Previous data revealed the instability of the tumor cell proliferation index throughout the metastatic process, which could have clinical consequences and can result in therapeutic changes. Moreover, on comparing the ER, PR, and HER2 status in the primary tumor and paired lymph node metastases, several studies found a variable rate of instability.[4–7]

We read with great interest the paper published by Fulga et al[8] in which they analyzed 43 cases of PU grade 2

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TABLE 1. Specifications of Various Antibodies Used in the Study

Antibody	Clone	Vendor	Dilution
Estrogen receptor	6F11	Novocastra	1:100
Progesterone receptor	312	Novocastra	1:100
Ki67	MM1	Novocastra	1:200
HER2	CB11	Novocastra	1:200

infiltrating breast carcinomas of no special type (NST). Immunophenotypic profile including ER, PR, and HER2 status and Ki67 index was assessed in the PU tumor and its ipsilateral axillary lymph node metastases. The authors demonstrated that the tumor profile is not stable during the metastatic process; the total rate of shifted cases was 23.3%, the highest rate of shifting (6.9%) being encountered from Luminal B-like/Ki67 to Luminal A-like subtype. Furthermore, in 5 cases the subtype shifted to a poorer one with respect to prognosis.

Another previous study demonstrated that the molecular subtype was discordant between PU (NST and non-NST types, G1-3) tumors and axillary metastases in 85 patients (11% of cases), with a shift to a more aggressive subtype in the metastases.[9] Moreover, Ieni et al found a discordance rate of 4.7% of cases in the HER2 status between PU G1-3 NST breast carcinomas and synchronous axillary lymph node metastases in 148 cases of unifocal tumors.[10]

We have also previously demonstrated that in PM breast carcinomas, the histologic features (type and grade) of axillary lymph node metastases can differ from those of primary tumors and usually correspond to the histologic type with unfavorable prognosis and/or highest histologic grade, which is not necessarily of the largest tumor focus.[11]

However, none of the previous publications demonstrated whether there is a discordance rate in the ER, PR, and HER2 status and KI67 index between primary tumors and axillary lymph node metastases in multiple breast carcinomas.

MATERIALS AND METHODS

In this study, we analyzed ER, PR, Ki67 index, and HER2 status in 41 PU and 37 PM breast carcinomas with identical immunohistochemical profiles among multiple tumor foci and consecutively diagnosed between 2007 and 2012, and we compared them with the matched axillary lymph node metastases. We excluded from this study cases of multiple breast carcinomas in which the tumor foci were molecular heterogenous (because we expect these tumors to be associated with heterogenous lymph node metastases from a molecular point of view). To define PM cases, we used the definitions by Tot et al[12] and Boyages et al.[13] ER, PR, Ki67, and HER2 testing was performed by immunohistochemistry according to international recommendations[14–17] on each tumor focus and each axillary lymph node containing metastases despite its size (Table 1). We interpreted tumor foci as ER/PR positive if expression was observed in at least 1% of the tumor cell nuclei (in the total area of the tumor, regardless of staining intensity, with positive internal control) and ER/PR negative when $<1\%$ of the tumor cells were positive. Ki67 index was defined as low (when 14% of all tumor cell nuclei were positive) or high (14%) by assessing the whole section and recording the overall average score based on the local laboratory values.[18] HER2 expression was scored as follows: 0 (no staining), 1+ (weak incomplete membrane positivity in at least 10% of the tumor cells), 2+ (weak/moderate complete membrane positivity in at least 10% of the tumor cells), and 3+ (strong complete membrane positivity in at least 30% of the tumor cells). For statistical analysis, 0 and 1+ HER2 scores were considered negative, and scores 2+ [confirmed with chromogenic in situ hybridization (CISH) test] and 3+ were considered positive. Cases that were HER2-IHC(2+) but CISH(−) were considered as negative. In this study, CISH was performed in all cases with a 2+ score. We used surrogate definitions of intrinsic subtypes of breast cancer according to Goldhirsch et al.[18] We defined Luminal A-like cases as those that were ER and/or PR positive, HER2 negative, and Ki67 low ($<14\%$) both in tumor and in lymph nodes, Luminal B-like proliferative (HER2 negative) (Bp) cases as those that were ER and/or PR positive, HER2 negative, and Ki67 high, Luminal B-like (HER2 positive) (Bh) cases as those that were ER and/or PR positive, any Ki67, and HER2 positive, HER2-enriched (H) cases as those with HER2 overexpression and ER and PR absent, and triple-negative (TN) cases as those that were ER, PR, and HER 2 negative.[18,19]

Concordant cases were defined as those in which the primary tumor (PU and PM) and lymph node metastases

TABLE 2. Discordance Rate in the Molecular Profiles Between Primary Tumors and LN Metastases in Unifocal Breast Carcinomas

	ER Expression (41 Patients)		PR Expression (41 Patients)		HER2 Expression (41 Patients)		Ki67 Expression (41 Patients)	
	LN Metastasis		LN Metastasis		LN Metastasis		LN Metastasis	
Primary tumor	+	−	+	−	+	−	+	−
+	27 (65.9)	6 (14.6)	19 (46.3)	11 (26.8)	2 (4.9)	9 (21.9)	4 (9.7)	26 (63.4)
−	7 (17.1)	1 (2.4)	6 (14.6)	5 (12.2)	1 (2.44)	29 (70.7)	6 (14.6)	5 (12.2)
Total discordance	13 (31.7)		17 (41.4)		10 (24.4)		32 (78)	

Values are represented as n (%). The "+" or "−" represents positivity or negativity for ER, PR, HER2 in the primary tumor and lymph node metastases respectively.
ER indicates estrogen receptor; LN, lymph node; PR, progesterone receptor.

TABLE 3. Discordance Rate in the Molecular Profiles Between Primary Tumors and LN Metastases in Homogenous Multiple Breast Carcinomas

	ER Expression (37 Patients)		PR Expression (37 Patients)		HER2 Expression (37 Patients)		Ki67 Expression (37 Patients)	
	LN Metastasis		LN Metastasis		LN Metastasis		LN Metastasis	
Primary tumor	+	−	+	−	+	−	+	−
+	19 (51.3)	13 (35.1)	19 (51.3)	11 (29.7)	0	5 (13.5)	0	20 (54)
−	4 (10.8)	1 (2.7)	6 (16.2)	1 (2.7)	0	32 (86.5)	2 (5.4)	15 (40.5)
Total discordance	17 (45.9)		17 (45.9)		5 (13.5)		22 (59.5)	

Values are represented as n (%). The "+" or "−" represents positivity or negativity for ER, PR, HER2 in the primary tumor and lymph node metastases respectively.
ER indicates estrogen receptor, LN, lymph node, PR, progesterone receptor.

displayed identical positivity or negativity for ER, PR, HER2, and Ki67 and discordant cases in which there was a mismatch in at least 1 biological parameter (ER, PR, HER2, and Ki67) among PU and PM tumors and lymph node metastases. Moreover, we defined as concordant cases those in which the molecular profile (based on the immunohistochemical evaluation of ER, PR, HER2, and Ki67 as for financial reasons we did not use molecular tests in this study) was concordant among PU and PM tumors and lymph node metastases and as mismatch cases those in which the molecular profile of the primary tumor differed from that of the lymph node metastases in at least 1 lymph node. We used MedCalc, Belgium, and the Fisher exact test for statistical analysis when comparing frequencies between groups, and a P-value < 0.05 was considered statistically significant. The Ethical Committee of the University of Medicine and Pharmacy of Tîrgu Mureş approved this study.

RESULTS

The results are presented in Tables 2–6.

Of the 41 cases of PU carcinomas with lymph node metastases, we found ER discordance in 13 cases (31.7%), of which the primary tumor was ER positive and the lymph nodes were negative in 6 cases; in 7 cases (17.1%), although ER was not expressed at all in the breast tumor, they were positive in the lymph nodes. As far as PR was concerned, the proportion of discordance was even higher: 17 cases (41.5%). In 11 cases, the tumor was PR positive and the lymph nodes were negative, whereas in 6 cases (14.6%) the tumor was PR negative but the lymph nodes expressed PR. The fewest discordances were recorded when analyzing HER2 expression: 10 cases (24.4%); in only 1 case (2.4%) the tumor was HER2 negative and the lymph nodes were positive, and in all the other 9 cases (21.9%) the tumor was HER2 positive and the lymph nodes negative. The 14% cutoff value for the Ki67 proliferation index led us to the highest number of discordant cases (32 cases, 78%) between the breast tumor and the axillary lymph nodes. In 6 cases (14.6%) of tumors with a low Ki67 index, we noticed an increased proliferation index in the lymph nodes (Table 2 and Fig. 1).

Among the 37 PM multiple breast carcinomas we found discordance between ER expression in the breast tumors and in the axillary lymph nodes in 17 cases (45.9%); in 13 of these cases the breast tumors were ER positive and the lymph nodes were negative, and in 4 cases (10.8%), although the breast tumors were not hormone sensitive, ER was positive in the lymph nodes. The same percentage was found in PR: 45.9%, with 6 cases (16.2%) in which the breast tumors were PR negative and lymph nodes were positive. We noticed discordance in HER2 expression in only 5 cases (13.5%), all of which involved positive breast tumors and negative lymph nodes. The proliferation index presented discordance in 22 cases (59.4%). In only 2 of the latter cases the proliferation index was lower in the breast tumors than in the lymph nodes (Table 3).

In general, the discordance rate was higher for ER and PR and lower for KI67 and HER2 in the PM tumors compared with PU tumors (Table 4). However, no statistical significance was found when the Fischer test was applied.

DISCUSSION

We have demonstrated that the positivity for the biological markers is not stable during the metastatic

TABLE 4. Rate of Discordance Between the Positivity of ER, PR, Ki67, and HER2 in 41 PU Tumors and 37 PM Tumors and Their Lymph Node Metastases

	ER (%, No.Cases)	*P*	PR (%, No. Cases)	*P*	Ki67 (%, No. Cases)	*P*	HER2 (%, No. Cases)	*P*
PU	31.7/13	0.246	41.5/17	0.819	78/32	0.09	24.4/10	0.261
PM	45.9/17		45.9/17		59.4/22		13.5/5	

ER indicates estrogen receptor; PM, primary multiple; PU, primary unifocal; PR, progesterone receptor.

TABLE 5. Concordances (Yellow) and Discordances (Blue) Rate in the Molecular Profile Between 37 Homogenous PM Breast Carcinomas and Axillary LN Metastases

Primary tumor	No. Cases	LN Metastases	No. Cases	%
Luminal A-like	13		13	35.1
Luminal A-like	3	TN	2	64.9
		TN, A	1	
Luminal Bh-like	5	TN	2	
		A	1	
		Bp, A	2	
Luminal Bp-like	11	A	7	
		TN	1	
		TN, A	2	
		TN, A, Bp	1	
TN	5	A, TN	3	
		A	1	
		Bp, TN	1	

A indicates luminal A-like; Bh, luminal B-like HER2 positive; Bp, luminal B-like proliferative; LN, lymph node; PM, primary multiple; TN, triple negative.

process. In this study the total proportion of discordant cases was 92.7% (38 out of 41 cases with discordances in at least 1 of the markers between the primary tumor and lymph node metastases) among PU tumors and 75.7% (28 out of 37 cases) among PM homogenous tumors ($P = 0.058$, odds ratio = 0.245, 95% confidence interval, 0.06-0.991). To our knowledge, this is the highest rate of shift for ER and PR status so far and the only confirmation in the shift of Ki67. Moreover, according to St Gallen 2011 intrinsic subtypes definition,[18] the total proportion of shifted cases was 64.9% in PM tumors and 82.9% in PU tumors (Tables 5, 6). The highest rate of shifting was encountered from Luminal B-like to Luminal A-like (7 out of 11 in PM and 12 out of 17 in PU), the same as in a paper by Fulga et al.[8]

In 11 out of 37 cases (29.7%) of PM and in 17 out of 41 cases (41.5%) of PU, the subtype shifted to a poorer one with respect to prognosis.

Previous studies revealed the instability of the ER, PR, HER2, and Ki67 status between the primary tumor and recurrence or distant metastases in breast cancer, with great impact on overall survival.[20] The data from this study support the heterogeneity of the primary breast tumors and the unstable molecular profile through the axillary lymph node metastases process in all breast carcinomas but especially in PU tumors. The guidelines recommend that the molecular profile should be performed only on the primary tumors and in case of multiple tumors only on the largest tumor focus to decide the patient's management. However, these data are in favor of a routine evaluation of the primary tumor and axillary lymph node metastases. This evaluation should be carried out not only in the PU but also in the PM tumors that are homogenous from a molecular point of view. The results of this evaluation would not only help to establish tailored therapies but also to predict the behavior and prognosis of these patients.

CONCLUSIONS

Synchronous axillary lymph node metastases may represent the potential of metastatic breast cancer better than the primary tumor.[21] Especially those patients in whom the primary tumor is hormone receptor and/or HER2 negative but positive for these markers in the axillary lymph nodes could become eligible for hormonal treatment and/or trastuzumab treatment, which may significantly improve the patient's outcome.

TABLE 6. Concordance (Marked With Yellow) and Discordance (Blue) Rate Between Molecular Profile of 41 PU Breast Carcinomas and Axillary LN Metastases

Primary Tumor	No. Cases	LN Metastases	No. Cases	%
Luminal A-like	5	Luminal A-like	5	7/41 = 17.1 concordant
Luminal Bp-like	1	Luminal Bp-like	1	
Luminal Bh-like	1	Luminal Bh-like	1	
Luminal A-like	4	A, Bp	4	34/41 = 82.9 discordant
Luminal Bh-like	6	A, Bp	3	
		A	1	
		A, H, Bh	1	
		Bp	1	
Luminal Bp-like	17	A	12	
		A, Bp	3	
		TN, Bp	1	
		A, Bp, Bh	1	
HER2 enriched	4	Bh	1	
		TN	1	
		TN, H, Bh	1	
		TN, H, Bh, A	1	
TN	3	A	1	
		Bp, TN	1	
		Bp, A	1	

A indicates luminal A-like; Bh, luminal B-like; HER2 positive; Bp, luminal B-like proliferative; LN, lymph node; PU, primary unifocal; TN, triple negative.

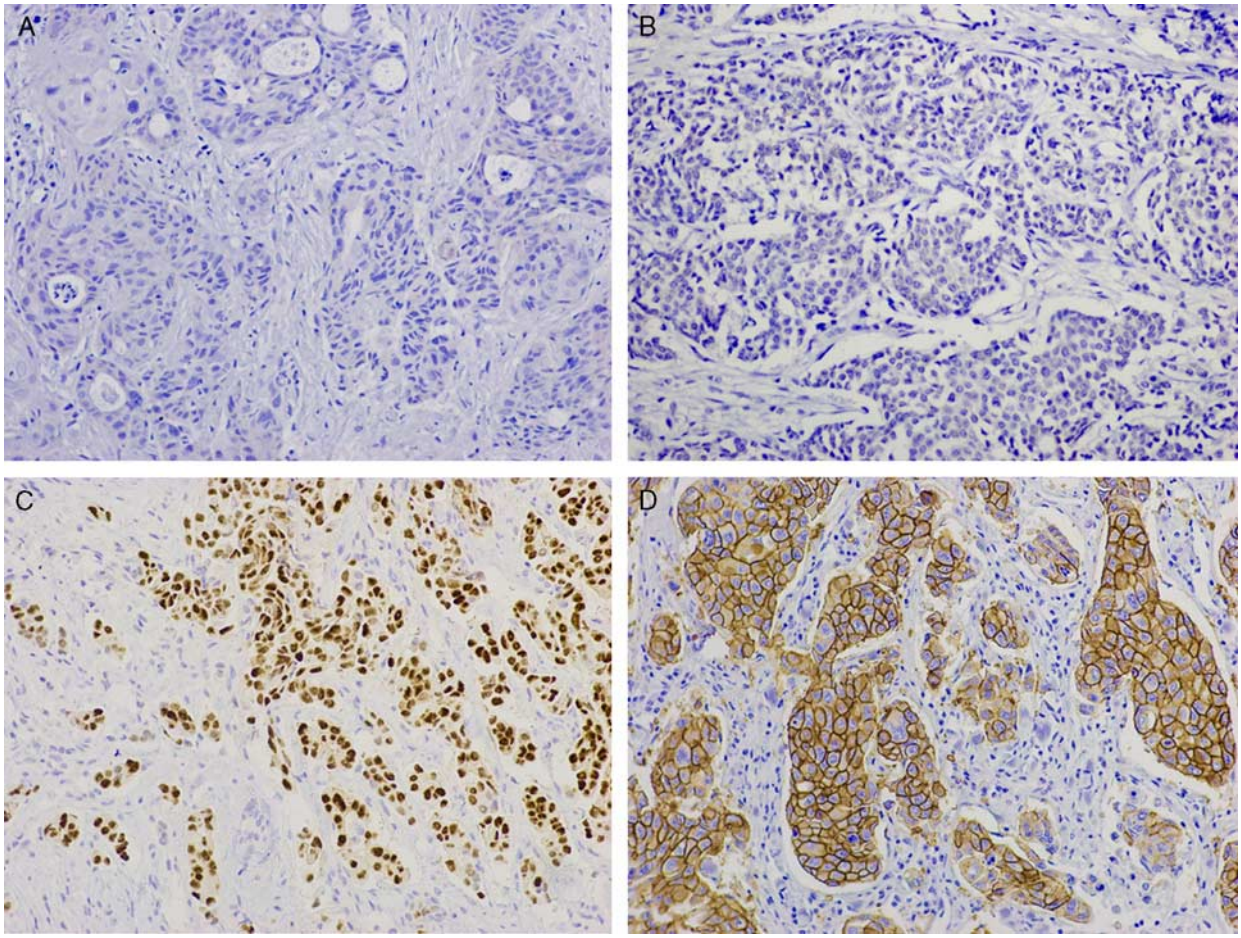

FIGURE 1. Triple-negative primary unifocal tumor (infiltrating carcinoma of no special type) showing estrogen receptor (ER) negative (A) and HER2 negative (B), which was associated with axillary lymph node metastases positive for ER (C) and HER2 (D); progesterone receptor negativity of the primary tumor is not shown in this picture.

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