ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Multiple *versus* unifocal breast cancer: clinicopathological and immunohistochemical differences

ANDRIAN PANUŢA[1,2], IULIAN RADU[1,2], BOGDAN GAFTON[3,4], NICOLAE IOANID[1], CRISTINA TERINTE[5,6], DAN FERARIU[6], MIHAELA BUNA-ARVINTE[1,2], DRAGOŞ VIOREL SCRIPCARIU[1,2], VIOREL SCRIPCARIU[1,2]

[1] *No. 1 Department of Surgical Oncology, Regional Institute of Oncology, Iaşi, Romania*

[2] *Department of Surgery, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[3] *Department of Oncology and Radiotherapy, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[4] *Department of Medical Oncology, Regional Institute of Oncology, Iaşi, Romania*

[5] *Department of Morphofunctional Sciences, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania*

[6] *Department of Pathology, Regional Institute of Oncology, Iaşi, Romania*

Abstract

Multiple breast cancer (MBC) is a controversial topic due to the lack of a consensus regarding its definition, classification issues and imprecise management recommendations in current reference guidelines. In four years, 756 patients with breast cancer (BC) were surgically treated in our Unit, 91 (12.03%) of them being pathologically diagnosed as MBCs. We present the results of our retrospective case-control study that performed a comparison between the clinicopathological characteristics and immunohistochemical (IHC) profiles of our MBC group *versus* a control group, represented by a sample of 184 cases randomly chosen from those with unifocal breast cancer (UBC). Starting from the premise of increased biological aggressivity of MBC, showed by several reports, we proposed to research the possible differences between these groups and to highlight their potential predictive and/or prognostic value. We found that MBC patients have a poorer prognosis than UBC ones – younger age at diagnosis [more cases less than 50 years old (p=0.03)], a lower frequency of T_1 and a higher rate of T_3 tumors [when using aggregate tumor size measuring method (p<0.001)], fewer node-negative (N_0) cases (p=0.046) and a higher frequency of mucinous breast carcinoma (p=0.026). It worth mentioning that we obtained lower rates of poorly differentiated (G_3) tumors (p=0.022) in the MBC group, this result being opposite to those found by other researchers. Our study also revealed a higher rate of human epidermal growth factor receptor 2 (HER2/neu)-type cases in MBC group (p=0.022), these patients having the chance to benefit from treatment with monoclonal antibodies, with a better outcome than patients with triple-negative type. We registered significantly lower progesterone receptor (PR) positivity rates in patients with MBC, thus having a negative predictive value by showing a worse response to hormone-based therapies. Besides, we found heterogeneity of IHC features among tumor foci in MBC that may influence the therapeutic decisions. Our results sustain that MBC is biologically a more aggressive type of mammary neoplasia requiring a more particular therapeutic approach.

Keywords: multicentric breast cancer, multifocal breast cancer, immunohistochemistry, prognostic factors, predictive factors.

Introduction

Multiple breast cancer (MBC) accounts for approx. 11–16% of all breast cancers (BCs) [1]. However, the incidence of this disease reported in the literature varies widely – between 9% and 75% [2]. The definitions of multicentric breast cancer (MCBC) and multifocal breast cancer (MFBC), respectively, have not been standardized yet [3]. The majority of the available reports are providing similar but not universally applicable definitions for multifocality, and multicentricity in BC is based on different principles. Bendifallah *et al.* [4] have highlighted this issue since 2010, but it does not seem to have been fully resolved until now. Several studies consider multiple synchronous ipsilateral foci of *in situ* carcinoma or the coexistence of a single invasive focus associated with multiple foci of *in situ* carcinoma as MBC [5]. This fact is generating difficulties in conducting meta-analyses and, consequently, is slowing down the development of new guidelines for the management of MBC [4]. Within this context, we consider it appropriate to underline the main characteristics of unifocal breast cancer (UBC), MFBC, MCBC, and MBC that set up the currently accepted definitions.

UBC refers to the presence of a single, primary, epithelial, malignant, invasive tumor in the breast [1]. The term of MFBC indicates the presence of at least two simultaneous, ipsilateral, primary, epithelial, malignant, invasive tumors separated by minimum 5 mm of non-malignant breast tissue within the same quadrant of the breast, less than 5 cm apart [6]. MCBC implies the presence of at least two, simultaneous, ipsilateral, primary, epithelial, malignant, invasive tumors, separated by minimum 5 mm of nonmalignant breast tissue within different quadrants of the breast or in the same quadrant but more than 5 cm apart [7]. The term of MBC is used to define the presence of at least two, simultaneous, ipsilateral, primary, epithelial, malignant, invasive tumors within the breast separated by minimum 5 mm of nonmalignant breast tissue (regardless the location of the foci in the breast quadrants) [4].

Based on our experience in the surgical management of breast carcinoma, the main objective of our study was

to evaluate the differences between clinicopathological and immunohistochemical (IHC) features in MBC *versus* UBC, in order to establish possible specific characteristics of MBC cases with an independent prognostic and/or predictive value.

Patients, Materials and Methods

Of the 756 female patients who underwent surgery for BC between May 2012 and May 2016 in our Unit, 665 (87.97%) patients were diagnosed as UBC and only 91 (12.03%) cases being represented by MCBC and MFBC (62 and 29 cases, respectively). For practical purposes, we have merged MFBC with MCBC groups and used the common term of MBC for both of these entities.

Written consent regarding the usage of clinical and pathological data of the patients included in our study was obtained and approved by the Ethics Commissions of our Hospital (Regional Institute of Oncology, Iaşi) and the "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania.

All our MBC and UBC patients underwent Madden-type modified radical mastectomy, a surgical technique that implies the complete removing of the breast (including the suprajacent skin and nipple-areolar complex) and ipsilateral level I, II axillary lymphadenectomy [8].

We performed a comparative analysis of the biological and IHC features in MBC cases *versus* a representative sample of 184 (27.66%) patients with UBC (control group). The inclusion criteria in the current study were a biopsy-confirmed diagnosis of UBC or MBC, a known pathology-measured tumor size(s) and no personal history of or simultaneous other malignancies.

The following tumor characteristics were evaluated: tumor size, multifocality, multicentricity, histological type, molecular subtype, histological grade, lymphovascular and perineural invasion, nodal status, systemic metastases. All of these features were assessed according to the 4th edition of *World Health Organization* (WHO) Classification of Breast Tumours [9] and the 8th edition of the *American Joint Committee on Cancer*/*Union for International Cancer Control* (AJCC/UICC) Tumor-Node-Metastasis (TNM) Classification [10]. Besides the dominant tumor size (maximal linear diameter of the largest tumor focus), as an alternative method of measurement, we used aggregate tumor size – the sum of maximal diameters of all tumor foci within the breast.

Also, we assessed the following immunomarkers: estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu), E-cadherin and Ki67, in order to establish the subtypes defined into the framework of molecular classification of breast carcinoma [11]. The immunoexpression of these markers was semi-quantitatively analyzed by using suitable scores reported in the literature. ER and PR were quantified according to Allred score [12]; HER2/neu status was evaluated as per the standard criteria, on a scale from 0 (negative), 1+ (negative), 2+ (equivocal) to 3+ (positive) [13], and for equivocal results the silver-enhanced *in situ* hybridization (SISH) method was applied; E-cadherin immunoreaction was categorized into positive or negative [14]; Ki67 was classified as high (>20%) or low (≤20%) [15].

The IHC exams were performed using an automatic method, in particular the ultraView Universal 3,3'-Diaminobenzidine (DAB) Detection Kit from Ventana. All the (prediluted) reagents used for immunostaining are presented in Table 1.

Table 1 – ***Reagents used in our study for immuno-staining***

IHC marker	Reagent
ER	Anti-ER (SP1) rabbit monoclonal antibody
PR	Anti-PR (1E2) rabbit monoclonal antibody
Ki67	Anti-Ki67 (30-9) rabbit monoclonal antibody
HER2/neu	Anti-HER2/neu (4B5) rabbit monoclonal antibody
HER2/neu equivocal (2+)	SISH – Ventana Benchmark XT automatic technique
E-cadherin	Anti-E-cadherin (EP700Y) rabbit monoclonal antibody

IHC: Immunohistochemical; ER: Estrogen receptor; PR: Progesterone receptor; HER2/neu: Human epidermal growth factor receptor 2; SISH: Silver-enhanced *in situ* hybridization.

The main limitations of our study include its retrospective nature, a relatively reduced number of patients (in comparison to other similar studies) and possible errors caused by human factor. Biases may also occur due to the lack of standard definitions of MCBC and MFBC, which creates difficulties in performing meta-analyses on this type of BC.

In order to compare the clinicopathological characteristics of the patients in the two study groups (MBC *versus* UBC) the χ^2 (*chi*-square) test (with Yates correction when the compared values were ≤5) was used for evaluating the differences between proportions and the Student's *t*-test for comparing continuous data. A p-value <0.05 was considered statistically significant. The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows software package (ver. 19.0; SPSS Inc., Chicago, IL, USA).

Results

By comparing the data strings, namely the age of each patient in MBC and UBC groups, we obtained negligible differences (p=0.167). Also, the mean (59.1 *vs.* 61.3 years) and median (60 *vs.* 63 years) age of the patients were found similar. When patients were further categorized in two age subgroups – younger (<50 years) and older (≥50 years) – we found a statistically significant difference between groups (p=0.032); more MBC patients were younger than 50 years as opposed to patients with UBC (Table 2). When considering the largest size (maximal diameter) of the largest tumor (dominant tumor size), as it is used in the current TNM classification, no significant differences between UBC and MBC groups were found (T_4 tumors were not compared).

A statistically significant difference was found when using aggregate tumor size (the sum of the largest dimension of all tumors). T_1 tumors were more common in the UBC group (p<0.001), T_2 tumors were equally common in MBC and UBC groups (p=0.332), while MBC tumors were more likely to be T_3 (p<0.001) (Table 3).

The comparison of lymph node involvement revealed a statistically significant difference (p=0.046) between

UBC and MBC groups. UBC was found to be more likely associated with a node-negative status. No difference in N+ cases was found between the two groups (Table 4).

The rates of distant metastases were similar in the study groups: 12 (6.52%) cases in the UBC group and five (5.49%) cases in the MBC group (Table 5). The frequency of lymphovascular and perineural invasion was similar in the two groups (Table 6).

The analysis of the tumor's differentiation grade (or Nottingham grade) revealed a statistically significant difference between the study groups (p=0.022), G_3 tumors being more frequent in the UBC group. Four (4.4%) of the MBC cases had divergent histological grading among foci (Table 7).

Similar proportions of all histological types, including cases with mixed types of breast carcinoma, were found in both groups except for the mucinous type, which was significantly more common in the MBC group (p=0.026) (Table 8).

Table 2 – ***Age groups in the MBC* versus *UBC patients***

Age [years]	MBC [number (%)]	UBC [number (%)]	*p*-value
Age group	34–81	35–90	0.167
Median	60	63	
Mean	59.1	61.32	
<50	26 (28.57%)	32 (17.39%)	0.032
≥50	65 (71.43%)	152 (82.61%)	

MBC: Multiple breast cancer; UBC: Unifocal breast cancer.

Table 3 – ***Primary tumor status in the MBC* versus *UBC cases. T_4 cases were not compared***

T status	MBC [number (%)]	UBC [number (%)]	*p*-value
Dominant tumor size			
T_1	22 (24.72%)	51 (30.91%)	0.577
T_2	59 (66.29%)	88 (53.33%)	0.195
T_3	8 (8.99%)	26 (15.76%)	0.159
Aggregate tumor size			
T_1	2 (2.25%)	51 (30.91%)	<0.001
T_2	56 (62.92%)	88 (53.33%)	0.332
T_3	31 (34.83%)	26 (15.76%)	<0.001

MBC: Multiple breast cancer; UBC: Unifocal breast cancer; T: Tumor.

Table 4 – ***Lymph node status in the MBC* versus *UBC cases***

N status	MBC [number (%)]	UBC [number (%)]	*p*-value
N_0	22 (24.18%)	72 (39.13%)	0.046
N_1	35 (38.46%)	52 (28.26%)	0.157
N_2	17 (18.68%)	26 (14.13%)	0.369
N_3	13 (14.29%)	16 (8.7%)	0.179
N_x	4 (4.4%)	18 (9.78%)	0.137

MBC: Multiple breast cancer; UBC: Unifocal breast cancer; N: Node.

Table 5 – ***Systemic metastases status in MBC* versus *UBC cases***

M status	MBC [number (%)]	UBC [number (%)]	*p*-value
M_1	5 (5.49%)	12 (6.52%)	0.739
M_0	86 (94.51%)	172 (93.48%)	

MBC: Multiple breast cancer; UBC: Unifocal breast cancer; M: Metastasis.

Table 6 – ***Lymphovascular invasion and perineural invasion in the MBC* versus *UBC cases***

Feature	MBC [number (%)]	UBC [number (%)]	*p*-value
Lymphovascular invasion			0.444
Negative	45 (49.45%)	82 (44.57%)	
Positive	46 (50.55%)	102 (55.43%)	
Perineural invasion			0.952
Negative	61 (67.03%)	124 (67.39%)	
Positive	30 (33.97%)	60 (32.61%)	

MBC: Multiple breast cancer; UBC: Unifocal breast cancer.

Table 7 – ***Nottingham grades in the MBC* versus *UBC cases***

Nottingham grade	MBC [number (%)]	UBC [number (%)]	*p*-value
G_1	21 (23.08%)	34 (18.48%)	0.422
G_2	27 (29.67%)	56 (30.43%)	0.914
G_3	8 (8.79%)	49 (26.63%)	0.002
G_X	31 (34.07%)	45 (24.46%)	0.154
Intertumoral heterogeneity	4 (4.4%)	–	

MBC: Multiple breast cancer; UBC: Unifocal breast cancer.

Table 8 – ***Histological types in the MBC* versus *UBC cases***

Histological type	MBC [number (%)]	UBC [number (%)]	*p*-value
NST	57 (62.64%)	141 (76.63%)	0.198
Lobular	5 (5.49%)	12 (6.52%)	0.747
Apocrine	1 (1.1%)	2 (1.08%)	0.993
Mucinous (colloid)	4 (4.4%)	1 (0.54%)	0.026
Micropapillary	1 (1.1%)	5 (2.71%)	0.393
Tubular	1 (1.1%)	4 (2.17%)	0.534
Medullary	1 (1.1%)	3 (1.63%)	0.731
Mesenchymal	0	1 (0.54%)	0.482
Cribriform	0	1 (0.54%)	0.482
Squamous cell carcinoma	1 (1.1%)	1 (0.54%)	0.611
Mucinous + NST	3 (3.3%)	4 (2.17%)	0.583
Micropapillary + NST	4 (4.4%)	3 (1.63%)	0.176
Squamous cell carcinoma + NST	3 (3.3%)	1 (0.54%)	0.201
Lobular + NST	1 (1.1%)	0	0.155
Signet cell carcinoma + NST	2 (2.2%)	1 (0.54%)	0.216
Cribriform + NST	2 (2.2%)	1 (0.54%)	0.216
Micropapillary + Mucinous	1 (1.1%)	0	0.155
Tubular + NST	1 (1.1%)	1 (0.54%)	0.611
Apocrine + NST	2 (2.2%)	1 (0.54%)	0.216
Medullary + NST	1 (1.1%)	0	0.155

MBC: Multiple breast cancer; UBC: Unifocal breast cancer; NST: No specific type.

We found no difference between the ER expressions in the two groups. Five (5.5%) cases in the MBC group presented divergent (positive/negative) ER status between foci.

The PR expression was significantly higher in the UBC than in the MBC group (p=0.002), six (6.6%) cases in the MBC group having different PR status between foci. No significant differences between groups were found in the expression of HER2/neu protein in tumor cells tested either by IHC or, in equivocal cases, by SISH.

In two (2.2%) MBC cases HER2/neu expression differed between foci. The rate of cell proliferation index – Ki67 protein expression labeled as high (≥20%) or low (<20%) was higher in the MBC group, but the difference was not statistically significant (p=0.078).

In six (6.6%) MBC cases, Ki67 protein expression differed between foci. E-cadherin expression was similar in the two groups. In three (3.3%) MBC cases, its expression differed between foci (Table 9).

Table 9 – *IHC features in the MBC* versus *UBC cases*

IHC marker	MBC [number (%)]	UBC [number (%)]	*p*-value
ER			0.732
Positive	72 (79.12%)	157 (85.33%)	
Negative	14 (15.38%)	27 (14.67%)	
Intertumoral heterogeneity	5 (5.49%)	–	
PR			0.002
Positive	52 (57.14%)	145 (78.8%)	
Negative	33 (36.26%)	39 (21.2%)	
Intertumoral heterogeneity	6 (6.59%)	–	
HER2/neu			0.254
Positive	14 (15.38%)	20 (10.87%)	
Negative	75 (82.42%)	164 (89.13%)	
Intertumoral heterogeneity	2 (2.2%)	–	
Ki67			0.078
High	50 (54.95%)	87 (47.28%)	
Low	35 (38.46%)	97 (52.72%)	
Intertumoral heterogeneity	6 (6.59%)	–	
E-cadherin			0.617
Negative	71 (78.02%)	153 (83.15%)	
Positive	17 (18.68%)	31 (16.85%)	
Intertumoral heterogeneity	3 (3.3%)	–	

MBC: Multiple breast cancer; UBC: Unifocal breast cancer; IHC: Immunohistochemical; ER: Estrogen receptor; PR: Progesterone receptor; HER2/neu: Human epidermal growth factor receptor 2.

The molecular subtypes of BC were analyzed in both groups (MBC *versus* UBC). A statistically significant difference was found for HER2/neu-type, which was more common in the MBC group (p=0.029). No significant differences in the frequencies of luminal A, luminal B, and triple-negative subtypes were present. Nine (9.89%) of all MBC cases showed intertumoral heterogeneity – different molecular subtypes between distinct tumor foci (Table 10).

Table 10 – *Molecular subtypes found in distinct foci in MBC cases*

Molecular subtype	MBC [number (%)]	UBC [number (%)]	*p*-value
Luminal A	32 (35.16%)	79 (42.93%)	0.34
Luminal B	36 (39.56%)	79 (42.93%)	0.684
HER2/neu-type	8 (8.79%)	5 (2.72%)	0.029
Triple-negative	6 (6.59%)	21 (11.41%)	0.23
Intertumoral heterogeneity		–	–
Luminal A / Luminal B	4 (4.4%)	–	–
Luminal A / HER2/neu-type	1 (1.1%)	–	–
Luminal A / Triple-negative	1 (1.1%)	–	–
Luminal B / HER2/neu-type	1 (1.1%)	–	–
Luminal B / Triple-negative	2 (2.2%)	–	–

MBC: Multiple breast cancer; UBC: Unifocal breast cancer; HER2/neu: Human epidermal growth factor receptor 2.

Examples of highly expressed immunomarkers in tumor cells (PR, ER, HER2/neu, Ki67 and E-cadherin, respectively) found in our MBC cases are presented in Figures 1–5.

Discussions

The current study evaluated the differences in the clinicopathological characteristics and immunophenotypes in patients with MBC *versus* UBC, in order to estimate their potential predictive and prognostic values. We found that, compared to the UBC group, more patients with MBC were younger than 50 years. Kanumuri *et al.* obtained similar results for the MCBC group, but he found no significant age differences for patients with MFBC [5]. The fact that BC in young patients has worse outcomes is well documented [16, 17]. Therefore, our findings may explain, at least partially, the more aggressive biological behavior of MBC.

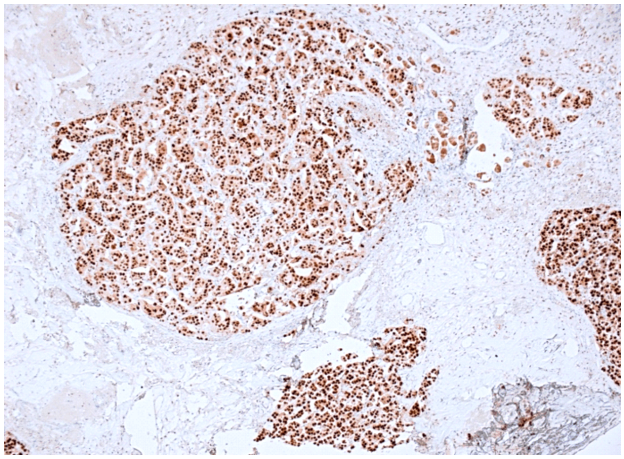

Figure 1 – *Positive immunomarker for ER in 90% of tumor cells (invasive mammary carcinoma). Anti-ER (SP1) rabbit monoclonal antibody immunostaining, ×100. ER: Estrogen receptor.*

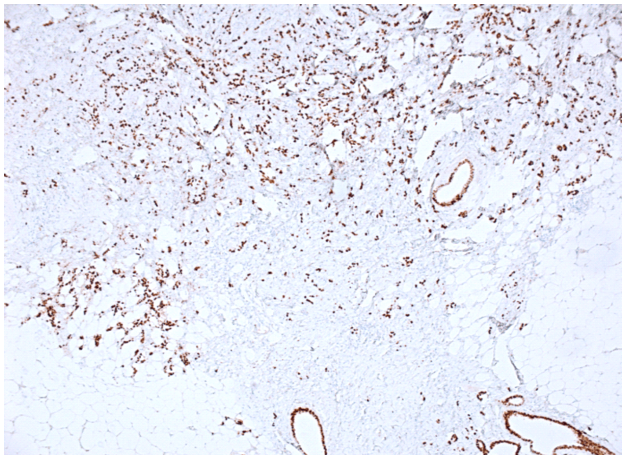

Figure 2 – *Positive immunomarker for PR in 90% of tumor cells (invasive mammary carcinoma). Anti-PR (1E2) rabbit monoclonal antibody immunostaining, ×100. PR: Progesterone receptor.*

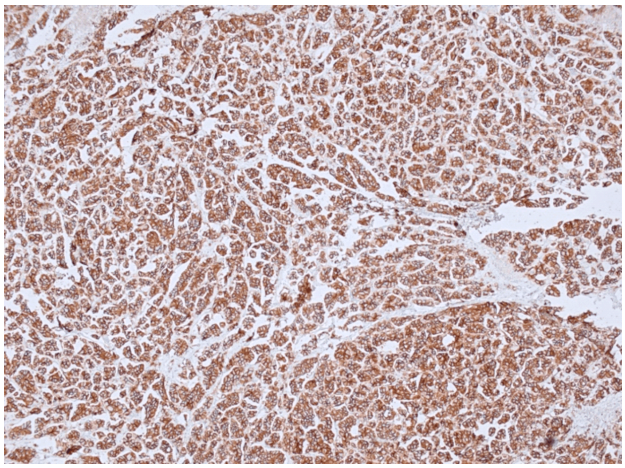

Figure 3 – ***Positive (+++) immunomarker for HER2/neu in tumor cells (invasive mammary carcinoma). Anti-HER2/neu (4B5) rabbit monoclonal antibody immunostaining, ×100. HER2/neu: Human epidermal growth factor receptor 2.***

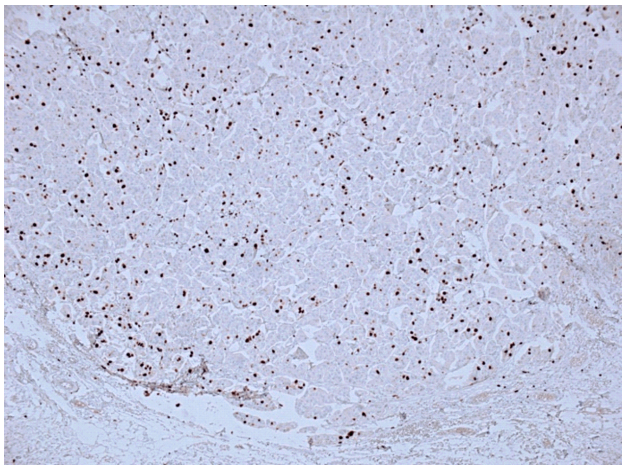

Figure 4 – ***Highly expressed (60%) Ki67 immunomarker in tumor cells (invasive mammary carcinoma). Anti-Ki67 (30-9) rabbit monoclonal antibody immunostaining, ×100.***

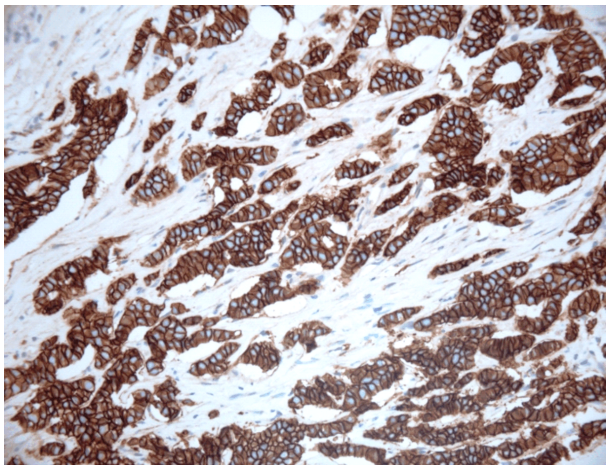

Figure 5 – ***E-cadherin diffusely positive in tumor cells (invasive mammary carcinoma). Anti-E-cadherin (EP700Y) rabbit monoclonal antibody immunostaining, ×100.***

In our current practice, the postoperative pathology reports contain T status established by macroscopically measuring the maximal diameter of the primary breast tumor in accordance with the current TNM classification system [10]. Additionally, *AJCC*/*UICC* recommends that, in cases of MBC, only the maximal diameter of the largest tumor focus (dominant tumor) should be considered [18, 19]. This last guidance has generated controversies from the sustainers of the hypothesis of a higher "tumor burden" in MBC that may be associated with worse overall survival (OS) and disease-free survival (DFS). It is suggested that the multifocality/multicentricity status of BC justifies assigning the higher T status to these cases and must be adequately managed [20, 21].

In order to estimate the role of tumor burden, other researchers use alternative methods of tumor size measurement, such as combined/cumulative tumor diameter or aggregate tumor size [5, 16, 22] along with other modalities of tumor foci measurement, such as tumor volume or tumor area [23, 24].

The results of these studies have led us to evaluate the differences between dominant and aggregate tumor sizes in our two study-groups. When considering the dominant tumor size, we found no differences between the MBC and UBC groups. Instead, a statistically significant difference was established when using aggregate tumor size measurement method: T_1 tumors were more common in the UBC group, T_2 tumors were equally frequent in the MBC and UBC groups while MBC tumors were more likely to be T_3. It worth mentioning that lymphovascular and perineural invasion showed similar rates in our study groups.

Both tumor size and nodal status are major prognostic factors with a direct impact on OS and DFS in BC [25]. The direct proportional dependence between tumor size and lymph node involvement is supported by relevant published data [19]. These statements mostly refer to the "usual" UBC but their validity in MBC remains disputable [26, 27].

Certain studies suggest that, in 20% of cases, the nodal involvement is associated with MBC regardless of the tumor size [18, 28]. Furthermore, Moutafoff *et al.* [18] demonstrated that multifocality in BC is an independent risk factor for lymph-node invasion and thus confirming the results of earlier studies [22–24].

In our study, the comparison between UBC and MBC revealed a strong association between a node-positive status and the presence of multiple invasive breast tumors, confirming the already published data [18]. In other words, the UBC group was found to be more likely associated with a negative lymph-node status (N_0). This fact, along with a significantly lower rate of T_1 tumors (when using aggregate tumor size) among our patients with MBC, revealed in our study, demonstrates the aggressiveness and poorer prognosis of MBC.

Several studies have established that when compared with UBC cases, patients with MBC show higher rates of local recurrences and metastatic disease leading to lower DFS rates in this group [26, 27, 29]. Nevertheless, in our study, we found similar frequencies of metastatic disease when comparing MBC *versus* UBC group.

The predictive role of Nottingham grade in BC has been demonstrated and mentioned in international guidelines [30, 31]. In our study, the comparison of histological

grades showed a higher frequency of poorly differentiated tumors (G_3) in the UBC group. Our results are opposite to those obtained by other researchers who have established that G_3 tumors are more common in MBC cases [27, 32]. In our opinion, these results could be due to the large number of locally advanced cases included in the study groups, as a consequence of poor addressability and the lack of a national screening program.

Similar proportions of all histological types of breast carcinoma (mixed types included) were found in the MBC and UBC groups, except for the mucinous type – more frequently detected in the MBC group. This rare type of breast carcinoma is known to have a poor prognosis [33], but the small number of available cases does not allow us to draw a reliable conclusion on its association with MBC cases.

The predictive and prognostic role of IHC features of BC is indisputable. However, there is still no clear evidence proving that the MBC immunophenotype is more aggressive than that of UBC [34].

ER and PR overexpressions in breast carcinomas guide the selection of therapy towards an antiestrogen-based treatment [35]. Our findings revealed nearly equal rates of ER positivity in the two study groups. We have established significantly lower PR positivity rates in patients with MBC, which has a negative predictive value, pointing towards a lower response rate to hormone-based therapies.

HER2/neu overexpression is a known indicator of poor prognosis in BC [25, 36]. We found no significant difference between groups when assessing this marker alone. Additionally, we revealed an intertumoral heterogeneity in HER2/neu expression. HER2/neu-positive BC is sensitive to anthracyclines but resistant to Tamoxifen and Cyclophosphamide-based chemotherapies [36, 37]. On the other hand, patients with HER2/neu-positive BC have a chance to benefit from treatment with monoclonal antibodies (such as Trastuzumab), and thus improving their outcomes compared to patients with the triple-negative type.

Ki67 tumor proliferation index is an important prognostic factor. Its high value correlates with worse outcomes in BC patients [38]. Our results showed no statistically significant differences between the proliferative activities in the two study groups. This fact indicates that the tumor multiplication rate is not enough to estimate the tumor aggressiveness in MBC, which should be evaluated as an outcome of intermingled mechanisms involving cellular changes.

E-cadherin is an essential protein in cell biology, responsible for the regulation of intercellular adhesion mechanisms. Recent research suggests that the loss of E-cadherin expression is associated with enhanced MBC aggressiveness [39]. However, our results did not show significant differences between the two study groups. A possible explanation could be the large variability of the histological types, with a predominance of no special type (NST).

Few papers focus on the peculiarities of the molecular profile in MBC. It was shown that triple-negative molecular subtype within MBC patients is associated with a significantly increased frequency of metastatic disease in comparison with UBC cases with triple-negative subtype [40, 41]. Contrariwise, other researchers report no significant correlations between MBC and the molecular subtypes [42].

When comparing the molecular subtypes in our two groups, we found only a significantly higher rate of the HER2/neu-type in our MBC patients. Considering no similar findings in the literature, we cannot exclude that the small number of cases could bias this result. However, the HER2/neu positivity could be regarded as a potential predictive and prognostic marker for patients with MBC.

The intertumoral (interfocal) heterogeneity in MBC is currently a subject to controversy. Reported data show the presence of intertumoral heterogeneity in a range of 10–12.7% [43, 44], and a possible relationship with the applied classification system (Nielsen, St. Gallen 2011 or Sotiriou system). It seems that the phenotypic deviations were found most often in patients with microscopically homogenous foci [44].

Our results were similar to these data, namely nine (9.89%) MBC cases showing IHC heterogeneous molecular subtypes between tumor foci, while their histological appearance was the same. The practical significance of IHC testing of each focus in MBC cases is highly debated because its hypothetical predictive value is plagued by confronts cost-efficiency issues. Reports are showing that the assessment of IHC features of each focus would have resulted in different adjuvant treatments in only 12.4% of patients, concluding that only the biological status of the most massive tumor should be taken into account [45, 46]. Contrariwise, other studies [25, 32] recommend IHC testing of each tumor focus. We agree with the last statement and, in our current practice, we always perform the IHC assessment of all invasive carcinoma masses within the breast.

Conclusions

The comparison between MBC and UBC groups revealed significant differences among several clinico-pathological and IHC features. Although none of our findings can be considered an independent and specific negative prognostic or predictive factor for MBC, taken together, our results strongly suggest that MBC is biologically a more aggressive type of mammary neoplasia requiring a more particular therapeutic approach. Furthermore, we consider the pathological and IHC assessment of each tumor focus in MBC cases to be justified, due to the relatively frequent intertumoral heterogeneity which may influence the choice of treatment and hence the outcome of these patients.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] van la Parra RFD, de Roos WK, Contant CME, Bavelaar-Croon CDL, Barneveld PC, Bosscha K. A prospective validation study of sentinel lymph node biopsy in multicentric breast cancer: SMMaC trial. Eur J Surg Oncol, 2014, 40(10):1250–1255.

[2] Jain S, Rezo A, Shadbolt B, Dahlstrom JE. Synchronous multiple ipsilateral breast cancers: implications for patient management. Pathology, 2009, 41(1):57–67.

[3] Khan SA. The many questions that surround multicentric and multifocal breast cancer. Breast J, 2010, 16(3):219–221.
[4] Bendifallah S, Werkoff G, Borie-Moutafoff C, Antoine M, Chopier J, Gligorov J, Uzan S, Coutant C, Rouzier R. Multiple synchronous (multifocal and multicentric) breast cancer: clinical implications. Surg Oncol, 2010, 19(4):e115–e123.
[5] Kanumuri P, Hayse B, Killelea BK, Chagpar AB, Horowitz NR, Lannin DR. Characteristics of multifocal and multicentric breast cancers. Ann Surg Oncol, 2015, 22(8):2475–2482.
[6] Neri A, Marrelli D, Megha T, Bettarini F, Tacchini D, De Franco L, Roviello F. Clinical significance of multifocal and multicentric breast cancers and choice of surgical treatment: a retrospective study on a series of 1158 cases. BMC Surg, 2015, 15:1.
[7] Rosenkranz K. Surgical management of multifocal and multicentric breast cancers: can we achieve the same, with less? Ann Surg Oncol, 2015, 22(8):2464–2465.
[8] Madden JL, Kandalaft S, Bourque RA. Modified radical mastectomy. Ann Surg, 1972, 175(5):624–634.
[9] Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ. World Health Organization (WHO) classification of tumours of the breast. 4th edition, WHO Classification of Tumours, International Agency for Research on Cancer (IARC) Press, Lyon, France, 2014, 203–231.
[10] Brierley JD, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours. 8th edition, John Wiley & Sons, 2016.
[11] Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature, 2000, 406(6797):747–752.
[12] Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol, 1998, 11(2):155–168.
[13] Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H, Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL, Wolff AC. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Arch Pathol Lab Med, 2010, 134(6):907–922.
[14] Canas-Marques R, Schnitt SJ. E-cadherin immunohistochemistry in breast pathology: uses and pitfalls. Histopathology, 2015, 68(1):57–69.
[15] Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, Ellis M, Henry NL, Hugh JC, Lively T, McShane L, Paik S, Penault-Llorca F, Prudkin L, Regan M, Salter J, Sotiriou C, Smith IE, Viale G, Zujewski JA, Hayes DF; International Ki-67 in Breast Cancer Working Group. Assessment of Ki67 in breast cancer: recommendations from the international Ki67 in breast cancer working group. J Natl Cancer Inst, 2011, 103(22):1656–1664.
[16] Zhang X, Yang J, Cai H, Ye Y. Young age is an independent adverse prognostic factor in early stage breast cancer: a population-based study. Cancer Manag Res, 2018, 10:4005–4018.
[17] Hironaka-Mitsuhashi A, Tsuda H, Yoshida M, Shimizu C, Asaga S, Hojo T, Tamura K, Kinoshita T, Hiraoka N, Fujiwara Y. Invasive breast cancers in adolescent and young adult women show more aggressive immunohistochemical and clinical features than those in women aged 40–44 years. Breast Cancer, 2019, 26(3):386–396.
[18] Moutafoff C, Coutant C, Bézu C, Antoine M, Werkoff G, Benbara A, Uzan S, Rouzier R. Facteurs prédictifs et pronostiques des cancers du sein multifocaux. Gynecol Obstet Fertil, 2011, 39(7–8):425–432.
[19] Tirada N, Aujero M, Khorjekar G, Richards S, Chopra J, Dromi S, Ioffe O. Breast cancer tissue markers, genomic profiling, and other prognostic factors: a primer for radiologists. Radiographics, 2018, 38(7):1902–1920.
[20] Duraker N, Çaynak ZC. Axillary lymph node status and prognosis in multifocal and multicentric breast carcinoma. Breast J, 2013, 20(1):61–68.
[21] Kadioğlu H, Özbaş S, Akcan A, Soyder A, Soylu L, Koçak S, Cantürk NZ, Tükenmez M, Müslümanoğlu M. Comparison of the histopathology and prognosis of bilateral *versus* unilateral multifocal/multicentric breast cancers. World J Surg Oncol, 2014, 12:266.
[22] O'Daly BJ, Sweeney KJ, Ridgway PF, Quinn C, McDermott EWM, O'Higgins NJ, Hill ADK. The accuracy of combined versus largest diameter in staging multifocal breast cancer. J Am Coll Surg, 2007, 204(2):282–285.
[23] Hilton JF, Bouganim N, Dong B, Chapman JW, Arnaout A, O'Malley F, Gelmon KA, Yerushalmi R, Levine MN, Bramwell VHC, Whelan TJ, Pritchard KI, Shepherd LE, Clemons M. Do alternative methods of measuring tumor size, including consideration of multicentric/multifocal disease, enhance prognostic information beyond TNM staging in women with early stage breast cancer: an analysis of the NCIC CTG MA.5 and MA.12 clinical trials. Breast Cancer Res Treat, 2013, 142(1):143–151.
[24] Andea AA, Bouwman D, Wallis T, Visscher DW. Correlation of tumor volume and surface area with lymph node status in patients with multifocal/multicentric breast carcinoma. Cancer, 2004, 100(1):20–27.
[25] Pekmezci M, Szpaderska A, Osipo C, Erşahin Ç. Evaluation of biomarkers in multifocal/multicentric invasive breast carcinomas. Int J Surg Pathol, 2013, 21(2):126–132.
[26] Ustaalioglu BO, Bilici A, Kefeli U, Şeker M, Oncel M, Gezen C, Gumus M, Demirelli F. The importance of multifocal/multicentric tumor on the disease-free survival of breast cancer patients: single center experience. Am J Clin Oncol, 2012, 35(6):580–586.
[27] Lynch SP, Lei X, Chavez-MacGregor M, Hsu L, Meric-Bernstam F, Buchholz TA, Zhang A, Hortobagyi GN, Valero V, Gonzalez-Angulo AM. Multifocality and multicentricity in breast cancer and survival outcomes. Ann Oncol, 2012, 23(12):3063–3069.
[28] Desmedt C, Fumagalli D, Pietri E, Zoppoli G, Brown D, Nik-Zainal S, Gundem G, Rothé F, Majjaj S, Garuti A, Carminati E, Loi S, Van Brussel T, Boeckx B, Maetens M, Mudie L, Vincent D, Kheddoumi N, Serra L, Massa I, Ballestrero A, Amadori D, Salgado R, de Wind A, Lambrechts D, Piccart M, Larsimont D, Campbell PJ, Sotiriou C. Uncovering the genomic heterogeneity of multifocal breast cancer. J Pathol, 2015, 236(4):457–466.
[29] Weissenbacher TM, Zschage M, Janni W, Jeschke U, Dimpfl T, Mayr D, Rack B, Schindlbeck C, Friese K, Dian D. Multicentric and multifocal *versus* unifocal breast cancer: is the tumor-node-metastasis classification justified? Breast Cancer Res Treat, 2010, 122(1):27–34.
[30] Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E, Zackrisson S, Cardoso F; ESMO Guidelines Committee. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol, 2015, 26(Suppl 5):*v*8–*v*30.
[31] Gradishar WJ, Anderson BO, Balassanian R, Blair SL, Burstein HJ, Cyr A, Elias AD, Farrar WB, Forero A, Giordano SH, Goetz MP, Goldstein LJ, Isakoff SJ, Lyons J, Marcom PK, Mayer IA, McCormick B, Moran MS, O'Regan RM, Patel SA, Pierce LJ, Reed EC, Salerno KE, Schwartzberg LS, Sitapati A, Smith KL, Smith ML, Soliman H, Somlo G, Telli ML, Ward JH, Kumar R, Shead DA. Breast Cancer, version 4.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw, 2018, 16(3):310–320.
[32] Boros M, Podoleanu C, Georgescu R, Moldovan C, Molnar C, Stolnicu S. Multifocal/multicentric breast carcinomas showing intertumoural heterogeneity: a comparison of histological tumour type and Nottingham histological grade of primary tumour and lymph node metastasis. Pol J Pathol, 2015, 66(2):125–132.
[33] Naqos N, Naim A, Jouhadi H, Taleb A, Bouchbika Z, Benchakroune N, Tawfiq S, Sahraoui A, Benider N. [Mucinous carcinoma of the breast: clinical, biological and evolutive profile]. Cancer Radiother, 2016, 20(8):801–804.
[34] Tot T. Early and more advanced unifocal and multifocal breast carcinomas and their molecular phenotypes. Clin Breast Cancer, 2011, 11(4):258–263.
[35] Viale G, Regan MM, Maiorano E, Mastropasqua MG, Dell'Orto P, Rasmussen BB, Raffoul J, Neven P, Orosz Z, Braye S, Ohlschlegel C, Thürlimann B, Gelber RD, Castiglione-Gertsch M,

Price KN, Goldhirsch A, Gusterson BA, Coates AS. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. J Clin Oncol, 2007, 25(25):3846–3852.

[36] Press MF, Bernstein L, Thomas PA, Meisner LF, Zhou JY, Ma Y, Hung G, Robinson RA, Harris C, El-Naggar A, Slamon DJ, Phillips RN, Ross JS, Wolman SR, Flom KJ. *HER-2/neu* gene amplification characterized by fluorescence *in situ* hybridization: poor prognosis in node-negative breast carcinomas. J Clin Oncol, 1997, 15(8):2894–2904.

[37] De Laurentiis M, Arpino G, Massarelli E, Ruggiero A, Carlomagno C, Ciardiello F, Tortora G, D'Agostino D, Caputo F, Cancello G, Montagna E, Malorni L, Zinno L, Lauria R, Bianco AR, De Placido S. A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer. Clin Cancer Res, 2005, 11(13):4741–4748.

[38] Penault-Llorca F, Radosevic-Robin N. Ki67 assessment in breast cancer: an update. Pathology, 2017, 49(2):166–171.

[39] Weissenbacher T, Hirte E, Kuhn C, Janni W, Mayr D, Karsten U, Rack B, Friese K, Jeschke U, Heublein S, Dian D, Ditsch N. Multicentric and multifocal *versus* unifocal breast cancer: differences in the expression of E-cadherin suggest differences in tumor biology. BMC Cancer, 2013, 13(1):361.

[40] Moon HG, Han W, Kim JY, Kim SJ, Yoon JH, Oh SJ, Yu JH, Noh DY. Effect of multiple invasive foci on breast cancer outcomes according to the molecular subtypes: a report from the Korean Breast Cancer Society. Ann Oncol, 2013, 24(9): 2298–2304.

[41] Choi Y, Kim EJ, Seol H, Lee HE, Jang MJ, Kim SM, Kim JH, Kim S, Park SY. The hormone receptor, human epidermal growth factor receptor 2, and molecular subtype status of individual tumor foci in multifocal/multicentric invasive ductal carcinoma of breast. Hum Pathol, 2012, 43(1):48–55.

[42] Ilić IR, Petrović A, Živković VV, Randjelović PJ, Stojanović NM, Radulović NS, Randjelović D, Ilić RS. Immunohistochemical features of multifocal and multicentric lobular breast carcinoma. Adv Med Sci, 2017, 62(1):78–82.

[43] Pekar G, Gere M, Tarjan M, Hellberg D, Tot T. Molecular phenotype of the foci in multifocal invasive breast carcinomas: intertumoral heterogeneity is related to shorter survival and may influence the choice of therapy. Cancer, 2014, 120(1): 26–34.

[44] Duan YS, Mao QX, Li LF, Sun YD, Wang L, Cui SD. [Intertumoral heterogeneity of molecular phenotype and analysis of prognosis in multifocal and multicentric breast cancer]. Zhonghua Zhong Liu Za Zhi, 2016, 38(11):833–838.

[45] Buggi F, Folli S, Curcio A, Casadei-Giunchi D, Rocca A, Pietri E, Medri L, Serra L. Multicentric/multifocal breast cancer with a single histotype: is the biological characterization of all individual foci justified? Ann Oncol, 2012, 23(8):2042–2046.

[46] Grabenstetter A, Brogi E, Chou JF, Morrow M, Dickler M, Norton L, Wen HY. Multifocal/multicentric ipsilateral invasive breast carcinomas with similar histology: is multigene testing of all individual foci necessary? Ann Surg Oncol, 2019, 26(2): 329–335.

Corresponding author

Iulian Radu, Lecturer, MD, PhD, Department of Surgery, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi; Head of the No. 1 Department of Surgical Oncology, Regional Institute of Oncology, 2–4 General Henry Mathias Berthelot Street, 700483 Iaşi, Romania; Phone +40722–531 363, e-mail: raduiuli@gmail.com

Received: January 19, 2018

Accepted: June 1, 2019