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Original article

Clinicopathological characteristics of metaplastic breast cancer – analysis of the basic immunohistochemical profile and comparison with other invasive breast cancer types

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ABSTRACT

Introduction: Metaplastic breast cancer (MpBC) is a rare but aggressive type of breast cancer accounting for 0.25–1% of all diagnosed invasive breast cancers. Morphologically, it is characterized by differentiation of the neoplastic epithelium into squamous cells and/or mesenchymal-looking tissue.
Material and methods: We analyzed 13 MpBCs selected from the group of 1122 invasive breast cancers. Histopathological examination and analysis of estrogen (ER), progesterone (PR) and HER2 receptors expression in MpBC patients and their comparison to other types of invasive breast cancer has been performed.
Results: 13 MpBC cases represented 1.16% of the 1122 invasive breast cancers. The MpBC group presented with a significantly larger tumor size (≥T2, 69% versus 49%, $p < 0.001$) and with higher grade of histological malignancy (G1-G3) ($p < 0.001$). MpBC group had significantly more cases with no hormone receptors (ER, PR) and HER2 overexpression/gene amplification compared with the other invasive breast cancer types group (ER-, 69% versus 23%, $p < 0.001$; PR-, 69% versus 28%, $p < 0.001$; HER2 0/1+, 93% versus 82%, $p = 0.019$). Most MpBCs (62%) were triple-negative. We found a correlation between hormone receptors expression and lymph node metastasis ($p < 0.001$). The analysis of the HER2 expression allowed us to find correlation between its expression and tumor histological grade (G1-G3) ($p < 0.001$), tumor size (T1a-T4) ($p < 0.001$) and lymph node metastasis (pN0-pN4) ($p < 0.001$) in MpBCs.
Discussion: MpBCs are usually larger at primary diagnosis and most of MpBCs present with other poor prognostic indicators and show lack of steroid hormone receptors expression as well as HER2. Hormone receptor status and HER2 expression seems to correlate with histological grade of malignancy (G1-G3), tumor size (T1a-T4) and regional lymph node involvement (pN0-pN4) and these features are directly related to MpBC malignancy.

1. Introduction

In practice, breast cancer is a heterogeneous group of lesions containing a wide range of tumors that vary in clinical course, imaging appearance and biology. The most common types are invasive ductal and invasive lobular breast cancers. Together, they account for over 70% of all breast cancer cases. Metaplastic breast cancer (MpBC), on the other hand, is very rare and is a heterogeneous group of tumors that primarily display non-glandular differentiation.

MpBC is a rare but aggressive type of breast cancer. It was described for the first time by Huvos et al. (1973) as a breast cancer that simultaneously contains epithelial and sarcomatoid elements [1]. However, it was not until 2000 when it was qualified by the World Health Organization (WHO) as a separate type of breast

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Table 1
WHO classification of metaplastic breast cancer (MpBC).

Metaplastic carcinomas (pure epithelial types)
Adenosquamous carcinoma (called 'low grade')
Squamous cell carcinoma
Spindle cell carcinoma
Metaplastic carcinomas with mesenchymal differentiation (mixed epithelial/mesenchymal carcinomas)
Carcinoma with chondroid metaplasia
Carcinoma with osseus metaplasia
Myoepithelial carcinoma
Carcinosarcoma

cancer. Morphologically, it is characterized by differentiation of the neoplastic epithelium into squamous cells and/or mesenchymal-looking tissue. Therefore, the MpBC classification is based mainly on the microscopic characteristic of tumors and is concerned with identifiable cell types and histological patterns seen by light microscopy. On this basis purely epithelial tumors (squamos, adenosquamos and spindle cell carcinomas) and mixed tumors with epithelial and mesenchymal elements (carcinoma with chondroid/osseus metaplasia and carcinosarcoma) can be recognized (Table I).

In mammography, MpBCs show many similarities to invasive ductal breast cancer (IDC) as well as benign lesions, which delays and makes it difficult to make a definitive diagnosis. The MpBC treatment regimen is not clearly defined due to the rarity of this cancer, however, studies suggest that tumor removal and the use of adjuvant radiotherapy brings the most benefits. In recent years, there has been an increase in the number of reported cases of MpBC, however, this may be associated with increased recognition of MpBCs by pathologists. Currently, the MpBC constitutes on average 0.25–1% of diagnosed breast cancers [2].

The etiology and pathogenesis of MpBC is unknown. Most likely, these tumors originate directly from the epithelium of the breast glands, but a competitive theory claims that the tumor is formed on the basis of squamous metaplasia present in pre-existing breast adenocarcinoma [3]. Another theory defended by Guerriero et al. (2005) says that squamous metaplasia is a disease of varying degrees of severity, and metaplastic cancer represents an extreme form of squamous metaplasia within adenocarcinoma [4].

Usually, MpBCs do not express estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (HER2) receptor. As suggested by Mourad et al. (1998), it is caused by the absence of extensive areas of glandular tissue in the tumor mass [5]. These tumors are considered as a subgroup of basal like breast cancers when classified by gene expression [6] and carry poor prognosis due to lack of response to hormonal therapy as shown by previous reports [7].

2. Aim of the study

The present work aims at revision of the histopathological features and analysis of estrogen, progesterone and HER2 receptors expression in MpBC patients and their comparison to other types of invasive breast cancer presented to the Military Institute of Medicine in Warsaw.

3. Material and methods

The material consisted of histological preparations derived from 1122 female patients treated for invasive breast cancer. Metaplastic carcinoma of the breast was found in 13 out of 1122 patients that had a diagnosis of invasive breast cancer within the period of 3 years (2009–2011) and were treated in Military Institute of Medicine in Warsaw. Demographic and clinical characteristics of patients were retrieved from the patient files. The material for the study came from biopsies, excisional biopsies, and modified radical mastectomies. Histological and immunohistochemical studies were performed at the Department of Pathology, Military Institute of Medicine in Warsaw. Samples of tumors were fixed in 10% phosphate buffered formalin. After 24 h, fixation tissues were dehydrated in alcohol of gradually increasing concentration: 50%, 60%, 70%, 80%, 90%, 96%, followed by pure alcohol and xylene, and subsequently embedded in paraffin. Paraffin blocks were cut into sections with a thickness of 4 μm each. The obtained sections were stained with different methods for diagnostic purposes. Preparations stained with hematoxylin and eosin (H&E) were used to identify tumor type (WHO classification), histological grade of malignancy, intensity of division, and degree of mitotic index of neoplastic cells as the mean number of mitoses in neoplastic cells counted in 10 fields of vision at an objective magnification of 400x (surface field 0.17 mm^2).

Routinely, patients had a basic immunohistochemical profile assessed, i.e. expression of estrogen (ER) and progesterone receptor (PR) and HER2. Immunohistochemical (IHC) methods employed paraffin sections attached onto glass slides covered with 2% silane/acetone solution (Merck, Darmstadt, Germany) and dried for 24 h at 42 °C. Before commencing the immunohistochemical procedure, sections were dewaxed by inserting them in a series of alcohols of gradually decreasing concentrations, followed by washing in distilled water. Immunohistochemical assays were performed using the En-Vision TM + complex HRP DakoCytomatic (DAKO, Santa Clara, United States) (En-Vision™ Dual Link System-HRP, DAB+, Code: K4065). In order to determine the expression of steroid receptors, monoclonal antibodies against estrogen (Monoclonal Mouse Anti-Human Estrogen Receptor alpha, 1: 50 dilution, Clone: 1D5, Code: IR654, DAKO, Santa Clara, United States) and progesterone (Monoclonal Mouse Anti-Human Progesterone Receptor, 1: 400 dilution, Clone: PgR636, Code: IR068, DAKO, Santa Clara, United States) were used. The study was conducted as follows: sections were incubated in an incubator at 60 °C overnight and then dewaxed. The next step was to reveal the epitope by heating slides in a buffer for 40 min. Subsequently, preparations were left at room temperature for 20 min. Preparations were rinsed in buffer, and then endogenous peroxidase was blocked in 3% H_2O_2. In the next step, preparations were incubated with an appropriate antibody. After incubation, preparations were rinsed in a buffer for 10 min and then incubated with the reagent (Visualization Reagent) for 30 min. After incubation with the reagent the preparations were washed in TBS (Tris-Buffered Saline, Code: S1968) pH 7.6 for 10 min, and then preparations were incubated with 3,3′-diaminobenzidine (DAB) (Substrate-Chromogen Solution) for 10 min to visualize the color reaction. At the end of the procedure hematoxylin preparations were stained and preparations were sunk in Canadian balm. Subsequently, color reactions were evaluated according to a scale that takes into account the extent and intensity of staining of nuclei in cancer cells. Nuclear staining in > 10% of tumor cells was considered positive (+) for ER and PR.

HER2 expression was determined using the HerceptTest™ Dako test (Code: K5204). In enabled the detection of HER2 expression using a polyclonal antibody against this protein (Rb A – Hu HER2 – Rabbit Anti-human HER2 Protein). HER2 status was determined by assessing protein expression on the membrane of tumor cells using IHC or by assessing the number of HER2 gene copies using fluorescence *in situ* hybridization (FISH). HER2 results were determined based on the maximum area of staining intensity according to the instruction in the package insert and the ASCO/CAP guidelines as follows: strong circumferential membranous, staining >30% of invasive carcinoma cells was graded 3+, moderate, circumferential membranous staining in ≥10% of invasive tumor cells or strong

circumferential membranous staining in ≤30% of cells was designated as 2 + staining, weak and incomplete membranous staining in invasive tumor cells was scored as 1 + and no staining was marked 0. Score 0 and 1 + were considered negative for HER2 amplification. Score 3 + was considered positive. Score 2 + was considered equivocal and FISH was ordered for confirmation. HER2 was considered to be amplified if the average HeER2 copy number was ≥6 signals/cells or ER2/CEP17 ratio ≥2 [8]. Positive and negative control preparations were previously determined.

All statistical analyses were performed with SPSS software v. 12.0 for Windows. The χ^2 and Fisher's Exact Tests were used appropriately. Differences were considered statistically significant at $p \leq 0.05$.

4. Results

We analyzed 13 MpBC (Table I) cases representing 1.16% of the 1122 invasive breast cancer cases. All patients were female with a median age of 58.54 years (range: 35–79 years). Patients were divided into seven age groups: under 30 years; from 31 to 40 years; from 41 to 50 years; from 51 to 60 years; from 61 to 70 years; from 71 to 80 years; and over 81 years (Fig. 1). Among 1122 cases of invasive breast cancer, there were 856 (76.29%) invasive ductal carcinomas, 158 (14.08%) invasive lobular carcinomas, 41 (3.65%) mixed ductal and lobular carcinomas, 13 (1.16%) metaplastic carcinomas, 19 (1.69%) mucinous carcinomas, 14 (1.25%) tubular carcinomas, 12 (1.07%) carcinomas with medullary features and 9 (0.81%) invasive micropapillary carcinomas (Table III). Clinical and histological findings of the patients with MpBC are summarized in Tables II and IV (Fig. 2 A – C). The median size of the primary MpBC tumor was 2.78 cm (range: 1.2–5.2 cm). The left breast was involved in seven patients and the right breast in six. All cases were unifocal, except for one multifocal case. After the examination of the H&E preparations, 10 patients (77%) were demonstrated to have a squamous epithelial type of metaplastic cell differentiation, 1 myoepithelial, 1 low-grade adenosquamous and 1 mixed epithelial/mesenchymal (chondroid type). Most patients (63%) had T2 disease (tumor size: 2–5 cm). Presence of distance metastases haven't been assessed. Postoperative microscopic examination disclosed metastases to the axillary lymph nodes in 5 cases (39%). Depending on the histological grading, the largest group of MpBC consisted of grade III cancers (G3) (93%), while the largest group of other invasive breast cancer types consisted of grade II cancers (G2) (60%).

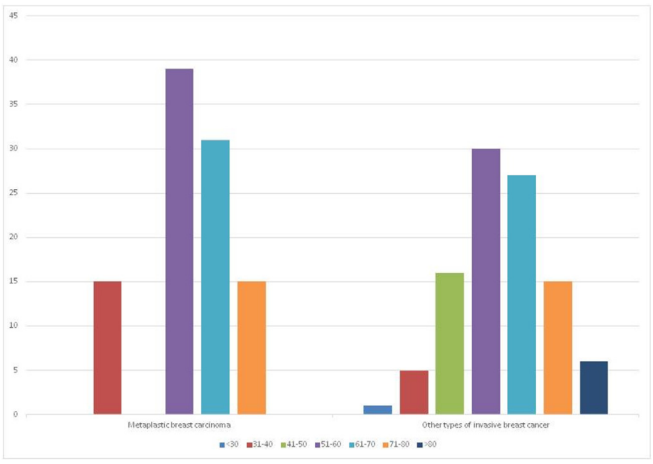

Fig. 1. Age distribution of patients with metaplastic breast cancer and other invasive breast cancer types.

Table 2
Comparison of the clinicopathological features between MpBC and other invasive breast cancer histological subtypes.

	Metaplastic breast cancer No. (%)	Other invasive breast cancer subtypes No. (%)	p-value
Age			
Median	58.54	60.04	<0.001*
range	35–79	27–91	
<=30	0 (0)	9 (1)	
31–40	2 (15)	54 (5)	
41–50	0 (0)	179 (16)	
51–60	5 (39)	334 (30)	
61–70	4 (31)	300 (27)	
71–80	2 (15)	162 (15)	
>=81	0 (0)	71 (6)	
Side			
Right breast	6 (46)	532 (48)	0.777
Left breast	7 (54)	577 (52)	
Median size	2.78 cm	1.91 cm	
Tumor size (T-stage)			
T1a	0 (0)	22 (2)	<0.001*
T1b	0 (0)	89 (8)	
T1c	4 (31)	454 (41)	
T2	8 (62)	468 (42)	
T3	1 (7)	24 (2)	
T4	0 (0)	52 (5)	
Lymph nodes (N-stage)			
pN0	8 (62)	699 (63)	0.963
pN1	3 (24)	265 (24)	
pN2	1 (7)	89 (8)	
pN3	1 (7)	56 (5)	
Metastasis (M-stage)			
M0	0 (0)	657 (59)	<0.001*
Mx	13 (100)	452 (41)	
Tumor grade			
G1	0 (0)	101 (9)	<0.001*
G2	1 (7)	668 (60)	
G3	12 (93)	319 (29)	
Can not be assessed (necrosis/autolysis)	0 (0)	21 (2)	
Estrogen receptor status			
ER-ER+	9 (69) 4 (31)	256 (23) 853 (77)	<0.001*
Progesterone receptor status			
PR-PR+	9 (69) 4 (31)	312 (28) 797 (72)	<0.001*
HER2 status			
HER2 0/1+	12 (93)	908 (82)	0.011*
HER2 2+	0 (0)	67 (6)	
HER2 3+	1 (7)	134 (12)	

The MpBC group presented with a significantly larger tumor size (≥T2, 69% versus 49%, $p < 0.001$; median size, 2.78 cm versus 1.91 cm) and with higher grade of histological malignancy (G1-G3) ($p < 0.001$). 93% of MpBC versus 29% of other invasive breast cancer types were given G3 tumor grade. Among other invasive breast cancer types, the largest groups were T2 (42%) and T1c (41%)

Table 3
Distribution of histological types in the group of 1122 patients with invasive breast cancer.

Type	No.	%
Invasive ductal carcinoma	856	76.29
Invasive lobular carcinoma	158	14.08
Mixed ductal and lobular invasive carcinoma	41	3.65
Metaplastic carcinoma	**13**	**1.16**
Mucinous (colloid) carcinoma	19	1.69
Tubular carcinoma	14	1.25
Carcinoma with medullary features	12	1.07
Invasive micropapillary carcinoma	9	0.81
	1122	100.00

Table 4
Clinicopathological features of 13 cases of metaplastic breast carcinoma.

No.	Age	Material	Side	Pathological diagnosis	Max diameter (cm)	Necrosis	pT	pN	M	Grade	Lymph nodes	ER	PR	HER2
1.	56	Postoperative material	R	Myoepithelial carcinoma	2.5	–	2	0	x	3	0/15	+	+	0
2.	62	Postoperative material	R	Squamos cell carcinoma	3.5	–	2	0	x	3	0/16	–	–	0
3.	79	Postoperative material	L	Squamos cell carcinoma	1.5	–	1c	2a	x	3	6/15	–	–	0
4.	55	Postoperative material	L	Squamos cell carcinoma	4.0	–	2	0	x	3	0/33	–	–	0
5.	77	Postoperative material	L	Squamos cell carcinoma	1.2	–	1c	0	x	3	0/13	–	–	0
6.	59	Postoperative material	L	Low-grade adenosquamous carcinoma	3.0	–	2	3a	x	3	30/33	+	+	0
7.	63	Postoperative material	L	Squamos cell carcinoma	2.4	+	2	0	x	3	0/10	+	+	0
8.	68	Postoperative material	R	Mixed epithelial/mesenchymal carcinoma	4.5	+	2	1	x	3	3/21	–	–	0
9.	51	Postoperative material	R	Squamos cell carcinoma	2.5	–	2	0	x	3	0/16	–	–	1+
10.	38	Postoperative material	L	Squamos cell carcinoma	5.2	–	3	0	x	3	0/5	–	–	1+
11.	35	Postoperative material	L	Squamos cell carcinoma	2.5	–	2	1	x	3	1/12	–	–	0
12.	63	Postoperative material	R	Squamos cell carcinoma	1.4	–	1c	1	x	3	3/17	+	+	0
13.	55	Postoperative material	R	Squamos cell carcinoma	2.0	–	1c	0	x	2	0/10	–	–	3+

R – right breast L – left breast.

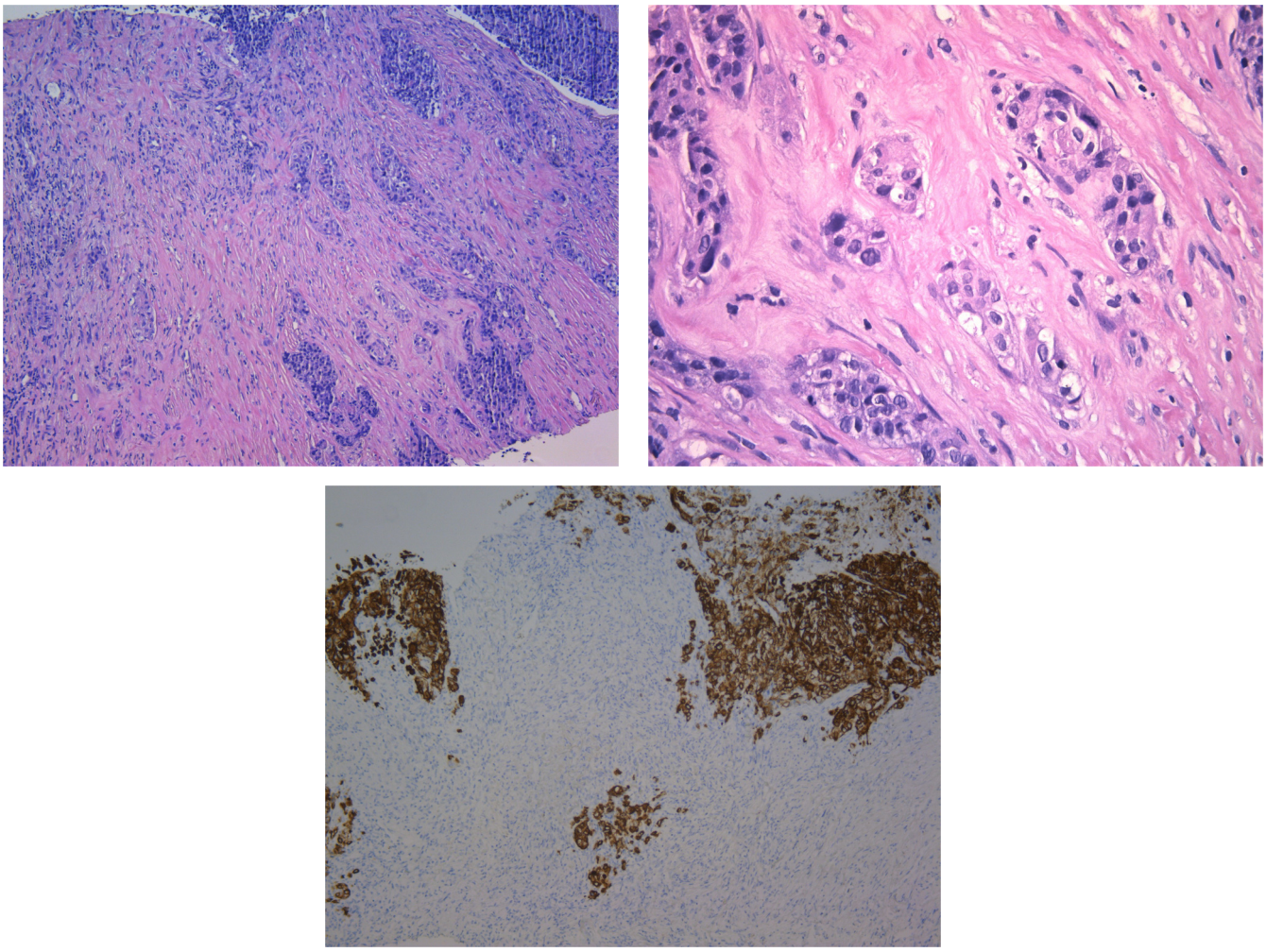

Fig. 2. A – Histopathological image of metaplastic breast carcinoma (MpBC) (grade 3, H&E, original magnification, 100 ×), B – Histopathological image of metaplastic breast carcinoma (MpBC) (grade 3, H&E, original magnification, 400 ×), C – Immunohistochemical analysis of CK 5/6 expression in metaplastic breast carcinoma with lymph node metastases. Positive staining image, original magnification, 100 ×.

tumors. During the study we also assessed the status of the lymph nodes, noting that in all investigated breast cancers (MpBC, other invasive types) women without metastasis to regional lymph nodes (pN0) (62% versus 63%) constituted the largest group. There was no statistically significant difference in the presence of nodal metastasis in studied groups ($p = 0.963$).

Most MpBC tumors (62%) were triple-negative, 31% were ER/PR positive, and 7% were HER2 positive. The MpBC group had significantly more cases with hormone receptors (ER, PR) negativity and no HER2 overexpression/gene amplification compared with the

Table 5
Relationship between histological type of invasive breast cancer and the basic immunohistochemical profile (ER, PR, HER2).

Receptor profile	Metaplastic breast carcinoma No. (%)	Other invasive breast cancer types No. (%)	p-value
ER-/PR-/HER2 0/1+	**8 (62)**	126 (11)	
ER-/PR+/HER2 0/1+	0 (0)	41 (3)	<0.001*
ER+/PR-/HER2 0/1+	0 (0)	71 (6)	
ER+/PR+/HER2 0/1+	4 (31)	**670 (60)**	
ER-/PR-/HER2 2+	0 (0)	21 (2)	
ER-/PR+/HER2 2+	0 (0)	0 (0)	
ER+/PR-/HER2 2+	0 (0)	3 (1)	
ER+/PR+/HER2 2+	0 (0)	43 (4)	
ER-/PR-/HER2 3+	1 (7)	68 (7)	
ER-/PR+/HER2 3+	0 (0)	0 (0)	0.148
ER+/PR-/HER2 3+	0 (0)	23 (2)	
ER+/PR+/HER2 3+	0 (0)	43 (4)	
	13 (100)	1109 (100)	

*Statistically significant results $p < 0.05$.

other invasive breast cancer types group (ER-, 69% versus 23%, $p < 0.001$; PR-, 69% versus 28%, $p < 0.001$; HER2 0/1+, 93% versus 82%, $p = 0.019$) (Table VI). Analysis of data shows that the highest percentage of patients with other invasive breast cancer types showed a positive response to both steroid receptors and negative to HER2 (ER+, PR+, HER2 0/+) (60%).

We found statistically significant correlation between steroid hormone receptors profile (ER, PR) and group of invasive breast cancer (MpBC versus other invasive breast cancer types) but only in the subgroup with negative HER2 status (HER2 0/1+) ($p < 0.001$) (Table V). The MpBCs group showed statistically significant correlation between the lack of HER2 expression (HER2 0/1+) and the lack of steroid hormone receptors expression (ER-/PR-), while other invasive breast cancer types showed predominant tendency to demonstrate HER2 negativity (HER2 0/1+) correlated with the expression of both steroid hormone receptors (ER+/PR+).

The obtained results allowed to evaluate the relationship between lymph node metastasis and steroid receptors expression in MpBC. We found a correlation between ER expression and lymph node metastasis ($p < 0.001$) and between PR expression and lymph node metastasis ($p < 0.001$). No statistically significant differences between steroid receptors expression and tumor histological grade (G1-G3) (ER, $p = 0.096$; PR, $p = 0.096$) and between steroid receptors expression and tumor size (T1a-T4) (ER, $p = 0.059$; PR, $p = 0.059$) in MpBC were found. The analysis of the HER2 expression allowed us to find correlation between its expression and tumor histological grade (G1-G3) ($p < 0.001$), between HER2 expression and tumor size (T1a-T4) ($p < 0.001$) and between HER2 expression and lymph node metastasis (pN0-pN4) ($p < 0.001$) (Table VII) in MpBC (Table VII).

5. Discussion

Our study was carried out in a group of 13 patients with MpBC isolated from 1122 patients with invasive breast cancer. In our study, a detailed analysis of basic histopathological features of MpBC, such as the histological grade, primary tumor size, lymph node status and the basic immunohistochemical breast cancer profile (ER, PR, HER2) were carried out.

Table 6
The percentage of invasive breast cancers negative or positive for the presence of ER, PR and HER2 depending on the histological type.

	ER No. (%)			PR No. (%)			HER2 No. (%)		
	Expression of steroid receptors			Expression of steroid receptors			Expression of HER2		
	negative	positive	p-value	negative	positive	p-value	negative	positive	p-value
Metaplastic breast carcinoma	9 (69)	4 (31)	<0.001*	9 (69)	4 (31)	<0.001*	12 (93)	1 (7)	0.019
Other invasive breast cancers	256 (23)	853 (77)		312 (28)	797 (72)		908 (82)	201 (18)	

Statistically significant results $p < 0.05$.

Table 7
Relationship between the expression of estrogen (ER), progesterone (PR), HER2 receptors and histological degree of tumor malignancy (G1-G3), tumor size (T1a-T4) and regional lymph-node status (pN0-pN3) in metaplastic breast cancer.

	ER No. (%)			PR No. (%)			HER2 No. (%)		
	Expression of steroid receptors			Expression of steroid receptors			Expression of HER2		
	negative	positive	p-value	negative	positive	p-value	negative	positive	p-value
Tumor grade									
G1	0 (0)	0 (0)	0.096	0 (0)	0 (0)	0.096	0 (0)	0 (0)	<0.001*
G2	1 (7)	0 (0)		1 (7)	0 (0)		0 (0)	1 (7)	
G3	8 (62)	4 (31)		8 (62)	4 (31)		12 (93)	0 (0)	
Tumor size (T-stage)									
T1a	0 (0)	0 (0)	0.059	0 (0)	0 (0)	0.059	0 (0)	0 (0)	<0.001*
T1b	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
T1c	3 (24)	1 (7)		3 (24)	1 (7)		3 (24)	1 (7)	
T2	5 (39)	3 (24)		5 (39)	3 (24)		8 (62)	0 (0)	
T3	1 (7)	0 (0)		1 (7)	0 (0)		1 (7)	0 (0)	
T4	0 (0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Lymph nodes (N-stage)									
pN0	6 (46)	2 (15)	<0.001*	6 (46)	2 (15)	<0.001*	7 (54)	1 (7)	<0.001*
pN1	2 (15)	1 (7)		2 (15)	1 (7)		3 (24)	0 (0)	
pN2	1 (7)	0 (0)		1 (7)	0 (0)		1 (7)	0 (0)	
pN3	0 (0)	1 (7)		0 (0)	1 (7)		1 (7)	0 (0)	

Statistically significant results $p < 0.05$.

MpBC is a rare subtype of invasive breast cancer that accounts for between 0.02% and 5% of invasive breast cancer. In our study, the incidence rate of MpBC was 1.16%. What is interesting, we found that increasing number of patients with MpBC were reported each year (years 2009–2011). The increased incidence we noted may represent an actual increase in disease or may be a result of improved awareness and recognition by pathologists [7,9–11]. Clinically, the usual presentation of patients with MpBC was with palpable breast mass in women more than 50 years of age [12,13], which trend was also noticed in our study.

In our study the age of the patients ranged from 35 to 79 years, with a mean age of 58.54 years. As it was found in other studies, MpBC is more commonly seen in postmenopausal women, and the mean age at diagnosis was reported around 54.5 years [14–16], however some studies reported significantly younger age at MpBC diagnosis [17,18]. The data showed, that the mean age of patients with MpBC is lower than the mean age of patients with other invasive breast cancer types (60.04) which tendency is also confirmed by other studies.

Most of previous studies showed that the tumor was large at the time of MpBC diagnosis. The median MpBC tumor size in our study was 2.78 cm (range, 1.2–5.2 cm), larger then median size of other invasive breast cancers (1.91 cm). Pezzi et al. (2007) reported that the larger size of MpBC at clinical presentation appeared to result from a more rapid growth [9]. In our study only 3 MpBCs (24%) were found to be < 2 cm in size. In the population based study by Pezzi et al. (2007) 29.5% of MpBCs were found to be < 2 cm in size compared with 65.2% of invasive ductal breast carcinomas [9]. A connection between tumor size, recurrence and survival rate in MpBC has been suggested [19], although there are studies that indicate that there is no such a relationship [15,20].

MpBC is characterized by the presence of divergent cellular differentiation including spindle, squamous, adenosquamous, myoepithelial, sarcomatoid and rarely chondroid or osseous elements with or without an accompanying conventional *in situ* or invasive mammary carcinoma elements (Table I) [21]. The spindle cell carcinoma contains cells forming poorly cohesive sheets of predominant spindle cell morphology and often resembles a low grade sarcoma or reactive process. The squamous cell subtype contains polygonal cells with eosinophilic cytoplasm and possible keratin pearl formation. The adenosquamous carcinoma contains glands which display varying degrees of squamous differentiation, characterized by pavement-like architecture, prominent intracellular bridges and, to a lesser extent, keratin formation. The carcinosarcoma is built of both malignant epithelium and malignant stroma. MpBC with mesenchymal differentiation contains overt carcinoma with a transition to osseous and/or cartilaginous stromal matrix. The myoepithelial carcinoma of the breast is extremely rare tumor, composed entirely or almost entirely of malignant spindle cells with myoepithelial differentiation [19,22].

Molecularly, MpBC is distinct from other breast cancers. It might arise from earlier, more chemoresistant breast epithelial precursor than basal-like or luminal breast cancers [23]. Weigelt et al. (2009), suggested that MpBC are part of the spectrum of basal-like breast cancers and demonstrate a myoepithelial and epithelial to mesenchymal transition molecular make up [24]. Further immunohistochemical and genomic studies characterized MpBC as a member of either the basal-like or the claudin-low molecular subtype with features of an epithelial to mesenchymal transition (EMT) and stem cell-like characteristics, including increased presence of CD44+/CD24-tumor stem cells [24,25]. According to some reports MpBC tends to originate from cancer stem cells or myoepithelial progenitors [26], but others adopted the theory of transformation of the carcinomatous component into the sarcomatous component through epithelial to mesenchymal transition [23]. This theory is supported by the overexpression of genes linked to motility, adhesion, migration and extracellular matrix formation [23]. On the other hand, stem cell theory may be supported by high CD44/CD24 and CD29/CD24 expression in MpBCs, consistent with a high level of stem-like cells in these tumors [27]. Despite its molecular classification, MpBC shows inferior clinical outcome including poorer response to chemotherapy [28] compared with other invasive breast cancer types. The observation that MpBCs represent tumors enriched in EMT and stem-like cells may account for their resistance to therapy and propensity to metastasize [23].

Due to its heterogeneity, accurate diagnosis of MpBC may be a challenge in preoperative core needle biopsies [29]. Therefore, surgical excision is the necessary procedure to achieve proper final diagnosis. Rakha et al. (2014) found that the most common subtype of MpBC in Western countries was spindle cell carcinoma (34%) [30], while squamous cell carcinoma (34%) was the most common subtype in patients from Singapore and Hong Kong. Lai et al. (2013) reported that squamous cell carcinoma (35.6%) was the most common subtype in Taiwan [31]. Luini et al. (2007) showed that MpBC with matrix-production was the leading subtype (45.9%) in European patients, followed by carcinosarcoma (24.3%) and squamous cell carcinoma (18.9%) [22]. Study by Zhang et al. (2015) showed that spindle cell carcinoma (34.4%) is a major MpBC subtype in Chinese population [32]. Using the current WHO classification, we found that squamous cell carcinoma (77%) is the most common subtype of MpBC in Polish population. The different frequencies of thoughtful MpBC subtypes in different populations may have resulted from a small number of patients in most studies and variation in classification [19,32].

Lymph node metastasis was observed in the minority of cases, and no difference in the rate of lymph node metastasis was observed with respect to other histologies (38% versus 37%) ($p = 0.963$). In previous studies lymph node metastasis has been shown to be less frequent in MpBC [10,31], but not all of them have confirmed this remark [7,33]. MpBC presented with axillary nodal involvement with similar frequency to other invasive breast cancers. 5 of the 13 patients with MpBC (38%) had nodal involvement in our study, what reports slightly higher incidence of axillary nodal involvement than in previous studies, where incidences at diagnosis of MpBC were between 6% and 28%. MpBC is observed to metastasize to the bones and lungs with hematogenous rather than lymphatic spread [34].

In our study, there was a very low incidence of hormone receptors (ER, PR) positivity in MpBC compared to other invasive breast cancers. As it was previously noted, the lack of ER and PR expression might be due to the absence of prominent glandular epithelial compartment in these tumors [5]. Previous studies have found HER2 overexpression ranging from 0% to 25% [26,35]. In our study, one of 13 MpBC patients (7%) had HER2 overexpression, what is consistent with previous studies. Triple-negative breast cancer cases accounted for 62% of MpBCs, within the range of previous studies, where 6%–96% of patients with MpBC showed triple-negativity [7,10,21,36,37].

So far, there is no available detailed analysis of data regarding the association of expression of steroid receptors (ER, PR) and HER2 with the above-described histopathological features of MpBCs. In our study, we observed a statistically significant relationship between the expression of ER and PR and the presence of metastasis to regional lymph nodes (pN0-pN4) and the relationship between HER2 expression and histological grade of malignancy (G1-G3), tumor size (T1a-T4) and involvement of axillary lymph nodes (pN0 -pN4). Despite interesting observations, due to the small group of patients with MpBC diagnosis, further study on a larger group of patients is necessary to confirm observed relationships. However, as lack of the expression of any receptor ER, PR or HER2 shows the

correlation with the presence of metastasis to the regional lymph nodes, a highly likely conclusion arises, that simultaneous lack of all these receptors expression additionally increases the probability of axillary lymph nodes involvement. It means, that triple-negativity (ER-/PR-/HER2 0/1+) may be considered as an independent risk factor for regional lymph nodes metastasis, which is confirmed by recent studies [30,32,37].

In general, MpBC is considered to be associated with poor prognosis compared with invasive ductal or invasive lobular carcinomas [10,22,31]. In the study by Wright et al. (2014) overall 5-year survival for MpBC was 62.2% compared with 81.2% for invasive ductal (IDC) ($p < 0.001$) and 80.2% for invasive lobular breast cancers (ILC) ($p < 0.001$) [38]. Metaplastic histology was found to be an independent risk factor for worsened prognosis for both hormone receptor-negative and -positive disease. Contrary to other invasive breast cancer subtypes, hormone receptor positivity (ER+, PR+) seems to not improve prognosis in MpBCs [38]. However, as mentioned above, hormone receptor status and HER2 expression seems to correlate with histological grade of malignancy (G1-G3), tumor size (T1a-T4) and regional lymph node involvement (pN0-pN4) and these features are directly related to MpBC malignancy. Taking all these facts into account, it is necessary to analyze the expression of ER, PR and HER2 in MpBCs on a larger scale. As it is a routinely assessed protein panel in breast cancers, it is highly probable it may bring us new information about MpBC biology and prognosis.

6. Conclusion

In conclusion, MpBC is a rare entity among breast carcinomas in Poland, which is similar to the reports of MpBC from all over the world. It comprises of a heterogeneous and histologically diverse group of cancers consisting of epithelial and mesenchymal elements. Most of MpBCs present with poor prognostic indicators and show lack of steroid hormone receptors expression (ER, PR) as well as HER2. Due to the rarity of the disease, poorer prognosis and suboptimal response to systemic therapies further research is needed for formulating comprehensive treatment plans. Also more data on the biologic characteristic of MpBC may allow us to understand the reason why it presents differently from other invasive breast cancer types in the term of clinicopathology.

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