

ORIGINAL PAPER



Brain metastases originating in breast cancer: clinical-pathological analysis and immunohistochemical profile

ANCA SAVA^{1,2)}, CLAUDIA FLORIDA COSTEA³⁾, RUXANDRA VATAVU⁴⁾, MIHAELA GRIGORE^{5,6)}, MIHAELA DANA TURLIUC^{3,7)}, GABRIELA FLORENȚA DUMITRESCU²⁾, LUCIAN EVA⁷⁾, ANDREI GHEORGHE MARIUS MOTOC⁸⁾, CRISTINEL IONEL STAN¹⁾, LIVIU CIPRIAN GAVRIL¹⁾, SADIYE IOANA SCRIPCARIU⁶⁾

¹⁾Department of Morphofunctional Sciences I, Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy, Iași, Romania

²⁾Laboratory of Pathology, Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, Iași, Romania

³⁾Department of Surgery II, Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy, Iași, Romania

⁴⁾PhD Student, Doctoral School, Grigore T. Popa University of Medicine and Pharmacy, Iași, Romania

⁵⁾Department of Obstetrics and Gynecology, Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy, Iași, Romania

⁶⁾Clinic of Gynecology, Cuza Vodă Hospital of Obstetrics and Gynecology, Iași, Romania

⁷⁾2nd Clinic of Neurosurgery, Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, Iași, Romania

⁸⁾Department of Anatomy and Embryology, Victor Babeș University of Medicine and Pharmacy, Timișoara, Romania

Abstract

The incidence of brain metastases (BMs) originating in breast cancer (BC) is increasing due to advances in imaging techniques, which can detect such events early, and due to new therapies that can ensure longer survival. We performed a retrospective study on patients with BMs originating in BC and receiving surgical treatment in Neurosurgery Clinics of Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, Iași, Romania, from January 2018 to December 2019. We identified 10 consecutive patients who underwent a craniotomy for a BMs originating in a BC (either for diagnostic purpose or with therapeutic intent). Clinicopathological data were collected from the electronic medical record and included the patient demographics (age at diagnosis of their BM), morphological characteristics of BM [location, cytopathological features, histopathological (HP) subtype, and immunohistochemical features, *i.e.*, cytokeratin 5/6 (CK5/6), mammaglobin, estrogen receptor (ER), progesterone receptor (PR), and Ki67 labeling index (LI)], and time from BC diagnosis to BM diagnosis. Ninety percent of patients were in their sixth and seventh decades of life, with a median age of 57.9 years (47–65 years). Median time from BC diagnosis to BM was 34.42 months. Fifty percent of BMs were located in the parietal lobes, and 70% of all cases have multiple (≥ 2) BMs. All cases (100%) had a cytopathological examination, showing a hypercellular pattern, with poorly cohesive clusters of mild or pleomorphic cells, with nuclei with homogeneously distributed fine granular chromatin membrane, and with small or enlarged and irregular nucleoli. The dominant histopathology was invasive breast carcinoma of no special type (IBC-NST) (70%), but we also identified specific subtypes, *i.e.*, tubular carcinoma (TC) (20%) and invasive micropapillary carcinoma (IMPC) (10%). Correlating location with HP subtypes of BMs from BC, IBC-NST and IMPC were located mostly in parietal lobes, and TC developed only in the occipital lobe. We found three patterns of immunostaining: (i) CK5/6 +/-, mammaglobin+, ER+, PR-, which was much more characteristic for IBC-NST; (ii) CK5/6-, mammaglobin+, ER-, PR-, being identified in tubular breast carcinoma; (iii) CK5/6 +/-, mammaglobin-, ER-, PR-, which were revealed by invasive micropapillary breast carcinoma. Our study revealed the fact that BMs originating in BC show heterogeneity of hormone receptor status, although morphologically there is not so much diversity. We also found a very variable Ki67 LI, which correlated especially with the morphological subtype.

Keywords: breast cancer, brain metastases, mammaglobin, estrogen receptor, progesterone receptor, Ki67 labeling index.

Introduction

Brain metastases (BMs) originating in breast cancer (BC) represent 24% of all metastases located at this level and their percentage is increasing due to advances in imaging techniques, which can detect such events early, and due to new therapies that can ensure longer survival [1]. Lin *et al.* (2008) even reported a much higher percentage, which can range from 25% to 46% in patients with metastatic triple-negative BC (TNBC) [2].

BC is raising worldwide, this neoplasm being the second leading cause of cancer death worldwide after

lung cancer. If only female mortality from cancer is considered, then BC comes first [3].

According to *World Health Organization* (WHO) statistics, BC ranks first as the most common cause of death from a female malignancy, accounting for 17.0% of the 20 200 deaths in 2014. Among the female population in Romania, the incidence of BC is twice higher than that of colorectal or cervical cancer, registering a number of 8981 cases compared to 4496 and 4343 cases, respectively [4].

In the past, the brain was a relatively rare site of metastasis of a BC, probably due to lack of proper treatment.

However, with the refinement of the treatment, especially the chemotherapeutic one, there are estimations that 10–16% of women with BC can develop symptomatic BMs at one moment of the course of their neoplasia [5]. Symptoms and sign due to BMs depend on the location of the lesion. These patients can manifest various symptoms, such as seizures, visual disturbance, focal neurological dysfunction, altered mental state or stroke [6]. Rarely, BMs could be detected as the first site of metastasizing of a BC, but more often BM usually occurs late during BC, after first developing in lung, liver, or bones. The prognosis of these patients is poor, the average one-year survival being about 20%.

Aim

The aim of this study was to analyze the clinical-pathological features and the immunoprofile of BMs originating in BC to identify the risk factors for the occurrence of these neoplasias.

☐ Patients, Materials and Methods

A retrospective study on patients with BMs originating in BC and receiving surgical treatment in Neurosurgery Clinics of Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, Iași, Romania, from January 2018 to December 2019 was performed. We identified 10 consecutive patients who underwent a craniotomy for a BMs originating in a BC (either for diagnostic purpose or with therapeutic intent).

Clinicopathological data were collected from the electronic medical records and included the patient demographics (age at diagnosis of their BM), morpho-

logical characteristics of BM [location, cytopathological features, histopathological (HP) subtype, and immunohistochemical (IHC) features], and time from BC diagnosis to BM diagnosis. We only included women with pathologically diagnosed BMs from BC.

Cytopathological exam was realized on intra-operative smears using small specimens obtained during surgical intervention. We placed a small sample (1–2 mm³) of the biopsy specimen on one edge of a clean, dry and labeled slide and crushed it with the edge of another clean, dry and labeled slide with enough pressure to spread the bioptic tissue into a thin film on both slides. Then both slides were immediately stained with 1% Toluidine Blue staining. In all 10 (100%) cases the cytological diagnosis was consistent with a BM.

The remaining biopsy was submitted for formalin fixation. We used 4 μm HP sections, which were obtained from cutting at microtome the paraffin-embedded tissue blocks, and we stained them with Hematoxylin–Eosin (HE) staining. A pathologist established the diagnosis of a BM from a previous BC.

For this study, all slides and blocks from all these 10 BMs were retrieved. Two pathologists reviewed all HE-stained sections and tumors were classified according to the *WHO Classification of Tumors of the Breast* (2019) [7]. Representative sections of BM were used for IHC stainings, to identify the origin of the BM in a previous BC [anti-cytokeratin 5/6 (CK5/6) and anti-mammaglobin antibodies], the immunexpression of estrogen receptor (anti-ER antibody) and progesterone receptor (anti-PR antibody), but also the tumor proliferative activity (anti-Ki67 antibody) (Table 1).

Table 1 – Manufacturer descriptions of the antibodies we used in this study

Antibody	Manufacturer	Clone	Antigen retrieval	Class	Dilution	Cellular location
Anti-CK5/6	Bio SB, USA	EP24/EP67	Citrate, pH 6	Rabbit monoclonal	1:100	Cytoplasm
Anti-mammaglobin	Bio SB, USA	EP249	Citrate, pH 6	Rabbit monoclonal	Prediluted	Cytoplasm
Anti-ER	Bio SB, USA	RBT11	Citrate, pH 6	Rabbit monoclonal	Prediluted	Nucleus
Anti-PR	Dako, USA	PgR636	Citrate, pH 6	Mouse monoclonal	1:50	Nucleus
Anti-Ki67	Dako, Denmark	MIB-1	Citrate, pH 6	Mouse monoclonal	1:75	Nucleus

CK5/6: Cytokeratin 5/6; ER: Estrogen receptor; PR: Progesterone receptor.

IHC reactions were performed using the EnVision™+ Dual Link System–Horseradish Peroxidase (HRP) (Dako, Denmark) for mammaglobin, cytokeratin 5/6 (CK5/6) and ER, and UltraVision Quanto Detection System–HRP (Thermo Scientific, UK) for PR and Ki67 labeling index (LI), according to manufacturers' recommendations. Antigen retrieval was realized with sodium citrate buffer, pH 6, using a water bath at 95°C. In the end, 3,3'-Diaminobenzidine (DAB) was used as chromogen and Mayer's Hematoxylin for nuclear counterstaining against nuclear staining. Normal breast tissue was used as positive controls. IHC reactions were considered positive only when a brown staining of the cytoplasm (in case of anti-CK5/6 and anti-mammaglobin antibodies) or of the nucleus (in case of anti-ER, anti-PR, and anti-Ki67 antibodies) were obtained.

We calculated the mean Ki67 LI by counting both positive and negative nuclei in 10 different microscopic fields using a 40× objective. Firstly, we calculated the percentage of positive cells in each field, and then we used the arithmetic mean of the 10 previously recorded values to found out the mean Ki67 LI.

Data was first summarized using standard descriptive statistics and frequency tabulation.

☐ Results

Ten female patients with solitary or multiple BMs from BC underwent a craniotomy during the study period. Patients and tumor characteristics included in the study are summarized in Table 2. Ninety percent of patients were diagnosed with BMs from BC between the ages of 50–69 years (Figure 1a), with a median age of 57.9 years (47–65 years).

Median time from BC diagnosis to BM was 34.42 months, if we analyzed only the cases with BMs previously diagnosed with BC. For the whole series, however, because in three (25%) of all cases studied, BC was diagnosed synchronously with BMs, median time from BC diagnosis to BM was 24.1 months. Female patients older than 60 years at BM diagnosis had a BC diagnosis more than 20 months ago. Patients younger than 60 years were diagnosed with BM after less than 20 months since the BC diagnosis (Figure 1b).

Figure 1 – (a and b) Correlation between age of the patients and the period of time from breast carcinoma (BC) diagnosis to brain metastasis (BM) diagnosis.

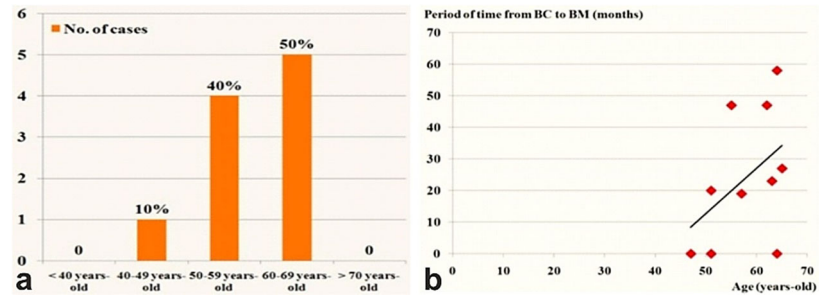


Table 2 – Clinical and pathological characteristics of patients with BMs from BC

Case No.	A [years]	nBMs	locBMs	Cytological features	HistoBM	pBC	TreatBC	tBMs [months]
1.	51	3	RPL	High cellularity, tumor pleomorphic epithelial cells with small nucleoli	TC	No	–	–
2.	55	1	RPL	Moderate cellularity, tumor epithelial cells with moderate pleomorphism	IBC-NST	Yes	M Ch, RT	47
3.	47	2	LCer	High cellularity pleomorphic epithelial cells, small nucleoli	IBC-NST	No	–	–
4.	64	1	RPL	Tumor epithelial cells with moderate pleomorphism	IBC-NST	Yes	Ch, RT	58
5.	63	2	ROL	High cellularity pleomorphic epithelial cells, small nucleoli	TC	Yes	M	23
6.	57	3	RCer	High cellularity pleomorphic epithelial cells, small nucleoli	IBC-NST	Yes	Ch, RT	19
7.	62	1	LPL	Markedly pleomorphic ductal cells with enlarged, irregular nuclei, large and prominent nucleoli and mitosis	IBC-NST	Yes	M Ch, RT	47
8.	65	3	LPL	Tumor cells with light cytoplasm and eccentric nuclei with fine granular chromatin and nucleoli	IMPC	Yes	M Ch, RT	27
9.	64	4	LOL	High cellularity moderate cellular pleomorphism, and small nucleoli	IBC-NST	No	–	–
10.	51	3	LPL	High cellularity pleomorphic epithelial cells, small nucleoli	IBC-NST	Yes	M Ch, RT	20

A: Patient's age at the time of admission to neurosurgery; BC: Breast carcinoma; BM: Brain metastasis; Ch: Chemotherapy; HistoBM: Histopathological subtype of brain metastasis; IBC-NST: Invasive breast carcinoma of no special type; IMPC: Invasive micropapillary carcinoma; LCer: Left cerebellum; locBMs: Location of brain metastases; LOL: Left occipital lobe; LPL: Left parietal lobe; M: Mastectomy; nBMs: No. of brain metastases; pBC: Previous known breast carcinoma; PL: Parietal lobe; RCer: Right cerebellum; ROL: Right occipital lobe; RPL: Right parietal lobe; RT: Radiotherapy; tBMs: Time to brain metastases (the period of time between the diagnosis of breast carcinoma and the diagnosis of brain metastases); TC: Tubular carcinoma; TreatBC: Type of treatment for breast carcinoma.

Our results showed that BC metastasized into the cerebellum (two cases, 20%), right parietal lobe (two cases, 20%), left parietal lobe (three cases, 30%), right occipital lobe (two cases, 20%), and left occipital lobe (one case, 10%) (Figure 2). Considering the area where BC metastasized, the supratentorial area was most often involved compared with infratentorial area (80% versus 20%).

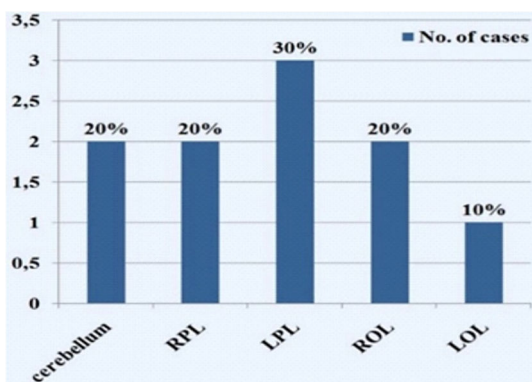


Figure 2 – Distribution of BCBM cases according to their location. BCBM: Breast cancer brain metastasis; LOL: Left occipital lobe; LPL: Left parietal lobe; ROL: Right occipital lobe; RPL: Right parietal lobe.

At the time of presentation to our Neurosurgery Clinics, 70% patients had multiple (≥ 2) BMs, and only 30% presented a single BM (Figure 3; Figure 4, a–d).

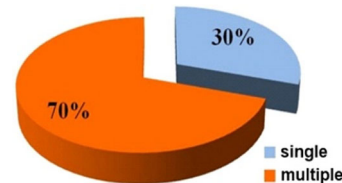


Figure 3 – Distribution of BCBM cases according to their number. BCBM: Breast cancer brain metastasis.

Thirty percent of patients were synchronously diagnosed with BMs and BC. Forty percent of patients were treated with mastectomy, chemotherapy, and radiotherapy, 20% were treated with chemotherapy and radiotherapy, and only one case suffered a mastectomy. Thirty percent of patients were not known as having BC until the diagnosis of their BM.

All cases (100%) with BM originating in a BC had a cytopathological examination, showing a hypercellular pattern, with poorly cohesive clusters of mild or pleomorphic cells, with nuclei with homogeneously distributed fine granular chromatin membrane, and with small or enlarged and irregular nucleoli (Figure 5, a–f). All cases (100%) could be intra-operative diagnosed as having a BM from a carcinoma.

The dominant histopathology of the BMs from BC was invasive carcinoma of no special type (IBC-NST) (70%), but we also identified specific subtypes, *i.e.*, tubular carcinoma (TC) (20%) and invasive micropapillary carcinoma (IMPC) (10%) (Figure 6; Figure 7, a–d).

Correlating location with HP subtypes of BMs from BC, 4/7 cases of IBC-NST were located in parietal lobes, 1/7 cases in occipital lobes and 2/7 cases in cerebellum; the two cases of TC developed only in the occipital lobe, and the only IMPC is identified only in the parietal lobe (Figure 8).

The IHC stainings are shown in Table 3. We found three immunostaining patterns: (i) CK5/6 +/-, mammaglobin+, ER+, PR-, which was much more characteristic for IBC-NST; (ii) CK5/6-, mammaglobin+, ER-, PR-, being identified in tubular breast carcinoma; (iii) CK5/6 +/-, mammaglobin-, ER-, PR-, which were revealed by invasive micropapillary breast carcinoma (Figures 9–11 / a–e).

Table 3 – Immunopositivity of tumor cells for antibodies used in this study

	Percent of cases	
	Positive	Negative
Mammaglobin	70%	3%
CK5/6	20% (focal)	80%
PR	20%	80%
ER	40%	60%
Ki67 LI	100% (its value ranges from 3% to 28%)	–

CK5/6: Cytokeratin 5/6; ER: Estrogen receptor; LI: Labeling index; PR: Progesterone receptor.

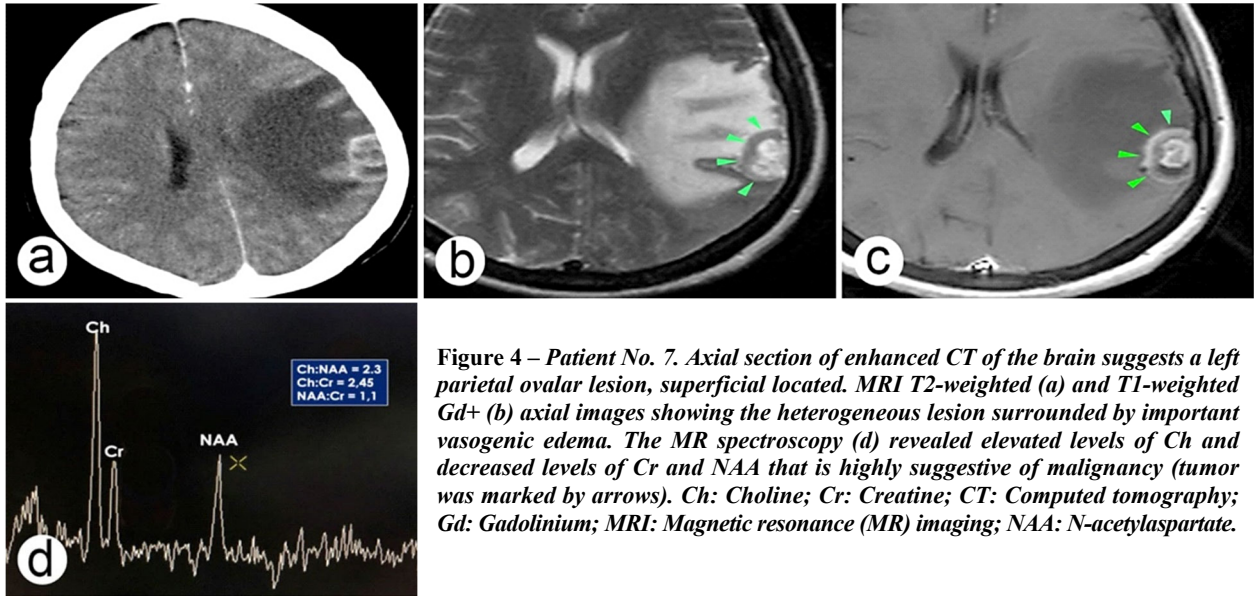


Figure 4 – Patient No. 7. Axial section of enhanced CT of the brain suggests a left parietal ovoid lesion, superficial located. MRI T2-weighted (a) and T1-weighted Gd+ (b) axial images showing the heterogeneous lesion surrounded by important vasogenic edema. The MR spectroscopy (d) revealed elevated levels of Ch and decreased levels of Cr and NAA that is highly suggestive of malignancy (tumor was marked by arrows). Ch: Choline; Cr: Creatinine; CT: Computed tomography; Gd: Gadolinium; MRI: Magnetic resonance (MR) imaging; NAA: N-acetylaspartate.

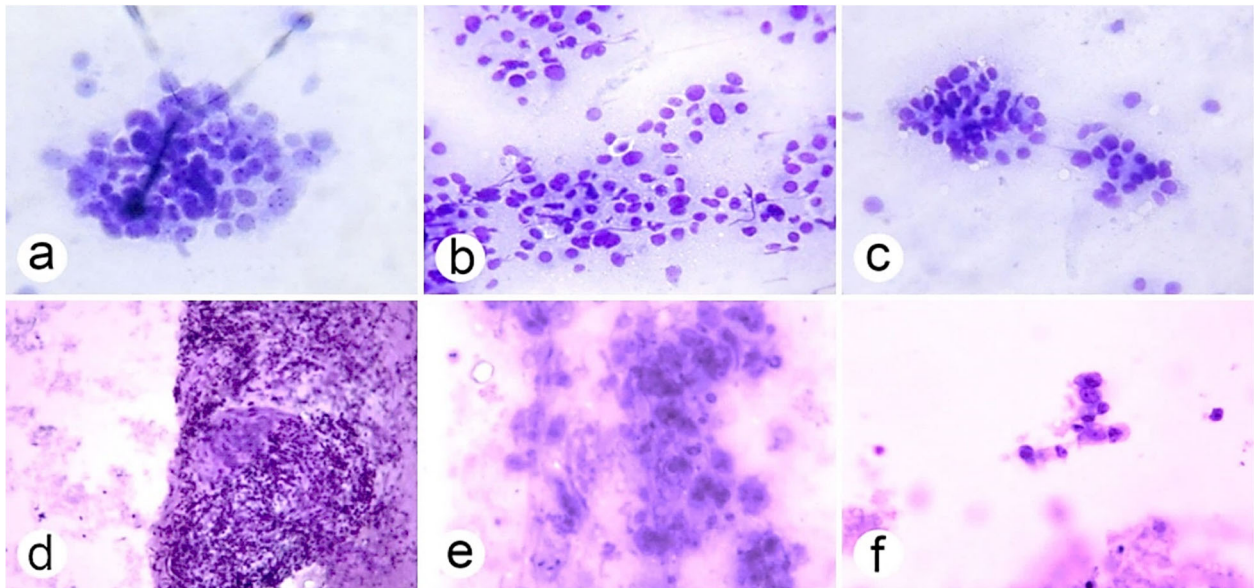


Figure 5 – Cytopathological features of brain metastases from breast carcinoma on intraoperative smear: (a) Patient No. 3 – high cellularity in a loosely cohesive cluster of mild pleomorphic ductal cells with nuclei showing homogeneously distributed fine granular chromatin membrane and small nucleoli; (b) Patient No. 9 – high cellularity with loosely cohesive clusters as well as single tumoral cells, with moderate pleomorphism, slightly irregular nuclear membrane, and small nucleoli; (c) Patient No. 1 – on a clear background, there are clusters and single tumoral ductal cells, with mild pleomorphism, light cytoplasm, regular nuclear membrane and indistinct nucleoli; (d) Patient No. 4 – a cluster of tumoral ductal cells amid the cells of the densely granular layer of the cerebellum; (e) Patient No. 7 – hypercellular pattern, with poorly cohesive clusters of markedly pleomorphic ductal cells with enlarged, irregular nuclei, presenting finely granular chromatin and large and prominent nucleoli and mitosis; the background showed granular debris; (f) Patient No. 8 – low cellular pattern with linear cell arrangements. Tumoral cells showed light cytoplasm and eccentric nuclei with fine granular chromatin and nucleoli. Toluidine Blue staining: (a–c, e and f) $\times 400$; (d) $\times 200$.

Figure 6 – Histological subtypes of brain metastases from breast cancer. IBC-NST: Invasive breast carcinoma of no special type; IMPC: Invasive micropapillary breast carcinoma; TC: Tubular breast carcinoma.

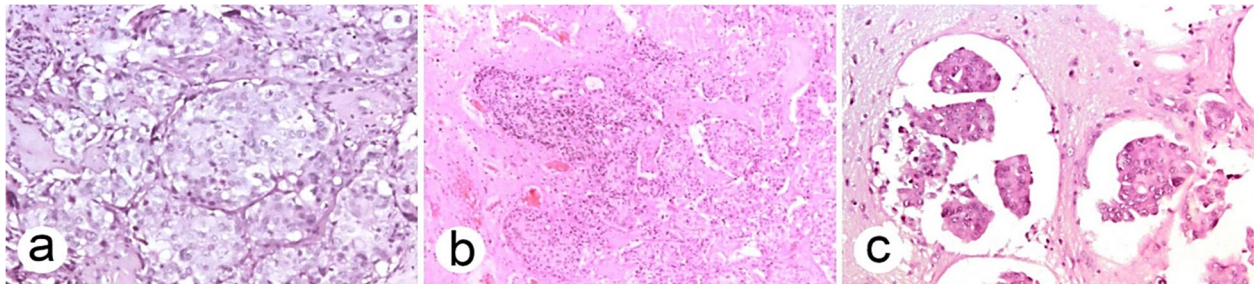
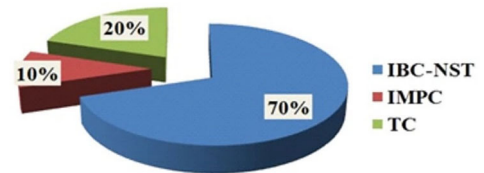


Figure 7 – Histopathological features of brain metastases from breast carcinoma: (a) Patient No. 6 – right parietal metastasis of an IBC-NST revealed solid clusters separated by little associated stroma; tumor cells showed abundant eosinophilic cytoplasm and nuclei with moderate pleomorphism, prominent nucleoli and mitoses; (b) Patient No. 9 – left parietal metastasis of an IBC-NST showed clusters of tumoral epithelial cells well delimited by the gliotic nervous tissue from its vicinity; glandular differentiation is apparent as few tubular structures with central lumina were identified in tumor clusters; (c) Patient No. 10 – left Rolandic metastasis of an IMPC showed angulated pseudopapillary clusters lacking a fibrovascular core made of medium-sized epithelial tumor cells, free floated in empty spaces surrounded by nervous glial tissue; (d) Patient No. 2 – right parietal metastasis of an IBC-NST showed a tumor that was well separated from the surrounding gliotic brain tissue and was made up of clusters of pleomorphic epithelial cells with well-defined limits and clear cytoplasm, similar with a “vegetaloid” appearance; some of the tumor nests revealed areas of comedo-type necrosis. HE staining: (a) $\times 400$; (b and d) $\times 100$; (c) $\times 200$. HE: Hematoxylin–Eosin; IBC-NST: Invasive breast carcinoma of no special type; IMPC: Invasive micropapillary breast carcinoma.

Figure 8 – Correlation between location and histological subtypes of brain metastases from breast cancer. IBC-NST: Invasive breast carcinoma of no special type; IMPC: Invasive micropapillary breast carcinoma; TC: Tubular breast carcinoma.

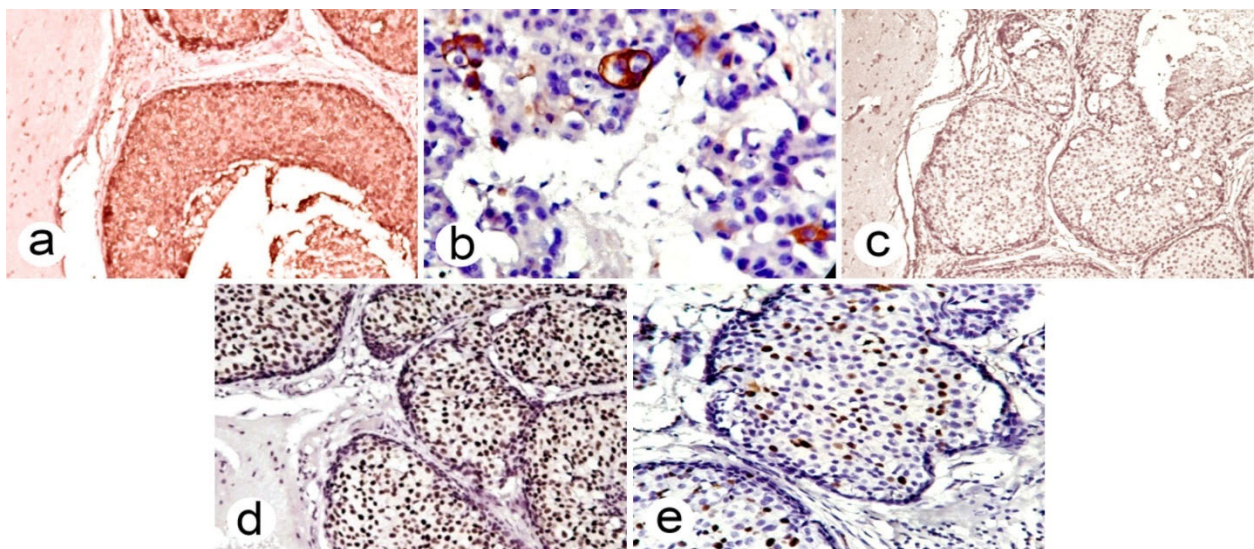
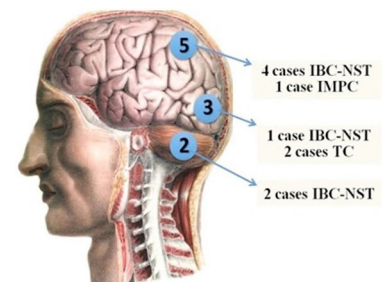


Figure 9 – Immunohistochemistry of invasive breast carcinoma of no special type: (a) Strong cytoplasmic positivity for anti-mammaglobin antibody ($\times 100$); (b) Focal strong cytoplasmic immunopositivity for anti-CK5/6 antibody ($\times 400$); (c) Negativity for anti-PR antibody in all tumoral cells and in the surrounding nervous tissue ($\times 100$); (d) Strong nuclear positivity for anti-ER antibody ($\times 200$); (e) Strong nuclear expression for anti-Ki67 antibody, with 16% Ki67 LI ($\times 400$). CK5/6: Cytokeratin 5/6; ER: Estrogen receptor; LI: Labeling index; PR: Progesterone receptor.

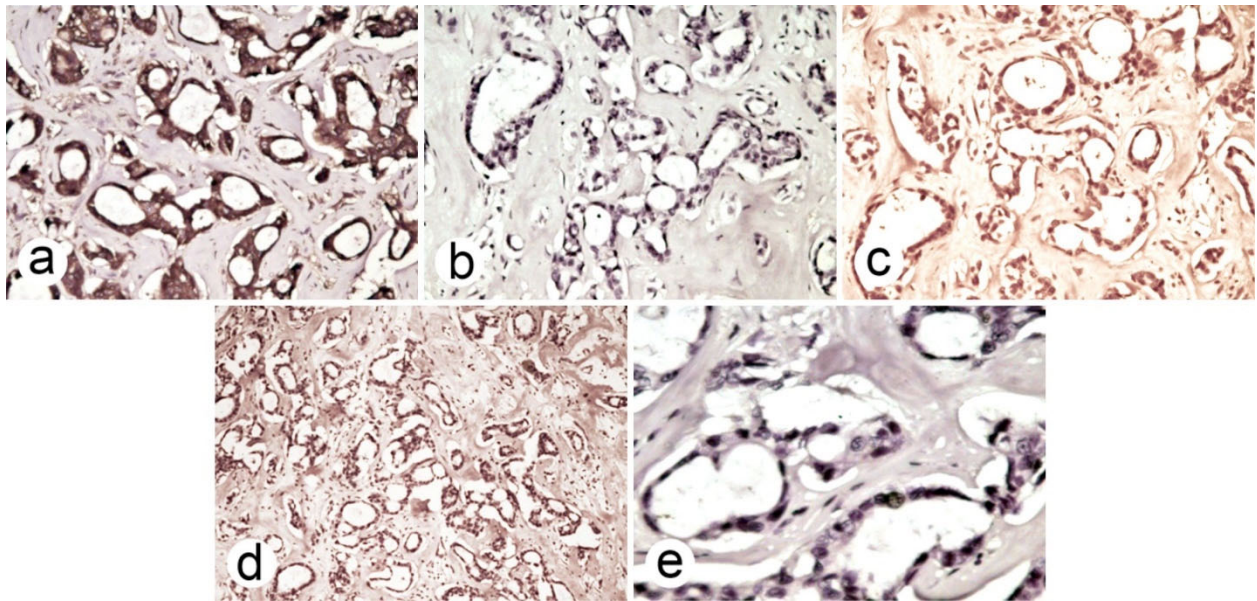


Figure 10 – Immunohistochemistry of tubular breast carcinoma: (a) Strong cytoplasmic positivity for anti-mamaglobin antibody ($\times 200$); (b) Negativity for anti-CK5/6 antibody in all tumor cells ($\times 200$); (c) Negativity for anti-PR antibody in all tumor cells and in the surrounding nervous tissue ($\times 100$); (d) Negativity for anti-ER antibody ($\times 200$); (e) Strong nuclear expression for anti-Ki67 antibody, with 3% Ki67 LI ($\times 400$). CK56: Cytokeratin 5/6; ER: Estrogen receptor; LI: Labeling index; PR: Progesterone receptor.

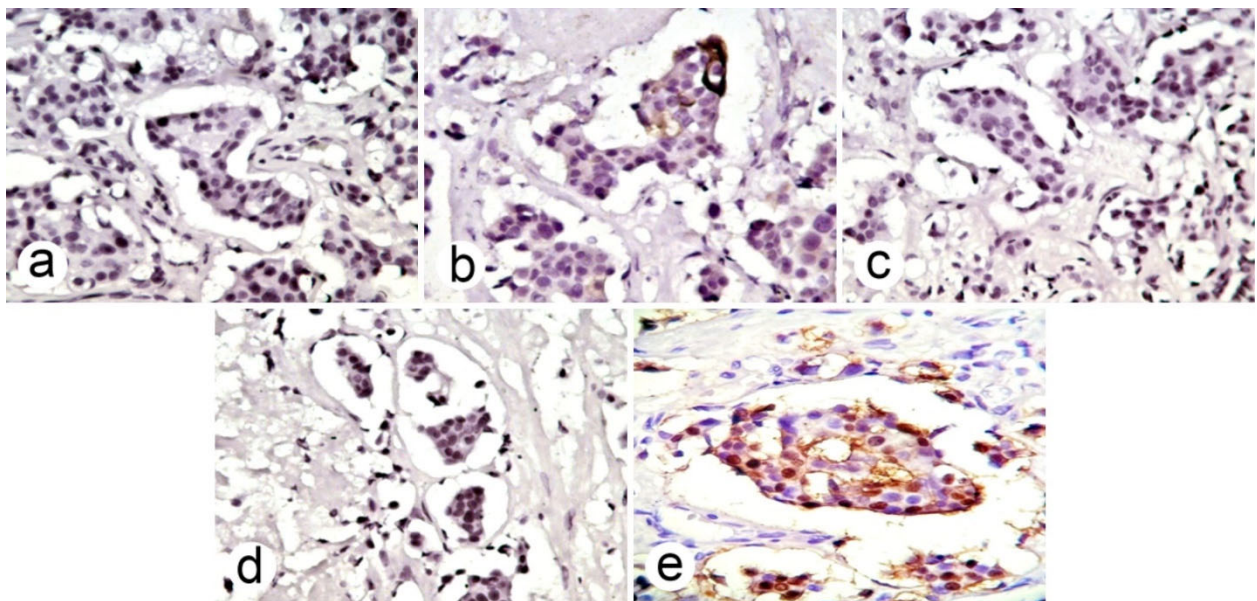


Figure 11 – Immunohistochemistry of invasive micropapillary breast carcinoma: (a) Negativity for anti-mamaglobin antibody ($\times 400$); (b) Strong focal cytoplasmic positivity for anti-CK5/6 antibody in all tumor cells ($\times 400$); (c) Negativity for anti-PR antibody in all tumor cells and in the surrounding nervous tissue ($\times 400$); (d) Negativity for anti-ER antibody ($\times 400$); (e) Strong nuclear expression for anti-Ki67 antibody, with 28% Ki67 LI ($\times 400$). CK56: Cytokeratin 5/6; ER: Estrogen receptor; LI: Labeling index; PR: Progesterone receptor.

☒ Discussions

The brain is the site of metastases originating in almost all organs of the human body, although some cancers with particular locations, such as mesothelioma [8], or colorectal carcinomas [9] are very rare at this level, but others, such as those originating in the lung or breast, are frequently diagnosed in this location.

Literature reported that the mean age of the patients at the time of BC diagnosis is 47 years. The average latency between the diagnosis of BC and the detection of BMs of BC is 2–3 years. However, BMs can occur even 20 years

after the diagnosis of BC [10]. Andreas Stark mentioned that BMs from BC are located more frequently in the supratentorial area than in the infratentorial space [10] and we identified the same distribution in our study.

BMs are associated with a poor prognosis because they cause sensory and cognitive neurological deficits [11]. The symptoms caused by the BMs depend on the exact size and location of the lesion. In cases with small tumors, neurological deficits are more obvious if the lesion is located in/near to eloquent brain areas. Large BMs can cause an increased intracranial pressure due to the blockage of the cerebrospinal fluid (CSF) pathways.

The most common symptoms when a patient with a BM from a BC is presenting to the hospital may be ataxia (23%), headache (21%), visual disturbances (15%), hemiparesis (11%) and vertigo (11%) [10].

In our study, one third of all patients were diagnosed with BM without a known BC, in these cases the brain being the primary site of metastasis. Although the breast is much more visible to both the patient and the doctor than the colon, it seems that patients do not see a doctor for early detection of their BC. A recent study showed that in the case of colorectal cancers (CRCs), more common in male patients, only 7.4% of patients admitted to a neurosurgery clinic for excision of their metastatic brain tumors presented neurological symptoms without a prior diagnosis of a CRC [9]. These data mean that early BC screening programs need to be implemented in Romania as quickly as possible.

In our study, we noticed that surgery was performed to excise metastases located especially in functionally important areas, such as “eloquent brain areas”, which are nervous regions that in case of an injury can result in loss of function [12], or in cerebellum, which is a major motor structure [13].

In the report published by Chow *et al.*, the mean time to develop BM from a BC was 29 months. The authors found that patients who developed BMs had BC with a high HP grade, a more advanced tumor (T)-stage and lymph node (N)-stage, an ER-, PR-, and c-erbB-2+ status [14].

In a recent review, Rostami *et al.* (2016) analyzed 106 articles, which have been published over the past three decades and included 14 599 patients with BMs from BC. These authors concluded that the median time between diagnosis of primary tumor and diagnosis of BM is 34 months. More than half (54.2%) of the patients had more than two intracranial metastases, most of them being supratentorial (33%), the cerebellar ones representing 16% [1].

In our study, we found that four-fifths of the BM from BC were located in the supratentorial region, affecting the parietal and occipital lobes, the rest being identified in the cerebellum. According to literature, there is a relationship between brain anatomy and BM location. Recently, Wang *et al.* (2019) [15] analyzed 335 patients with BMs from lung cancer and found out that this type of cancer metastasized mainly in the cerebellum (56%), right parietal lobe (54%), right frontal lobe (47%), and left frontal lobe (45%).

The spatial distribution of BMs from BC, according to their HP subtypes, showed that TC developed in the occipital lobe, IMPC was diagnosed in the parietal lobe, but IBC-NST could be found in parietal lobe, occipital lobe, and cerebellum.

As in the study by Kyeong *et al.* in 2017 [16], in our study BMs from BC tend to have a certain spatial distribution, with preferential involvement of the parietal and occipital lobes, as well as the cerebellum, an aspect that can be related to the biological characteristics of different HP and molecular subtypes. Compared to the mentioned study, our research did not identify any case located in the frontal lobe, the limbic region, but the number of cases was limited, and we analyzed only the location of metastases that were excised, often the patient

presenting more than two BMs. The above-mentioned authors [16] concluded in their article published in 2017 that the spatial distribution of BM is depending on the genetic composition of primary BC as TNBC can metastasize anywhere in the brain and human epidermal growth factor receptor 2 (HER2) subtypes positive and Luminal subtype is preferentially located in the territories irrigated by the posterior circulation, namely the occipital and cerebellum lobes.

Factors that increase the risk of developing a BM originating in a BC are young age (<35 years old), premenopausal status, poorly differentiated tumors (highly proliferative, and genomically unstable tumors), the negative status of hormone receptors (HRs) (ER- and PR- on immunohistochemistry), and higher number of non-central nervous system (CNS) metastatic sites [11, 17].

Although many studies considered that young patients (<35 years old) with BC have a particular risk of developing BMs [11, 17, 18], our patients were mostly in their seventh decade of life, and this feature could be particular for the BMs originating in BC diagnosed in our region.

Overall diagnostic accuracy of intra-operative smear technique achieved in the present study was 100%, which proves the advantages of this examination: relatively high speed, simplicity, and effectiveness of diagnosis. This technique is extremely useful in neurosurgery for excision of BMs located in important functional areas of the brain.

In our study, the same with other researches, IBC-NST was the most common HP subtype in BM originating in BC, but this fact could be correlated with the higher frequency of this HP type in the primary tumor, because most patients had invasive ductal histopathology (94%) and grade 3 tumors (72%) [19].

The 5th edition *WHO* Classification of Tumors of the Breast, published in December 2019 [7], reclassifies breast tumors and introduces new subtypes, while removing others. Regarding epithelial tumors of the breast, the group including invasive BC is maintained, but new subtypes are introduced.

Histologically, BM originating in BC from our study was well delineated by the surrounding gliotic nerve tissue, and mainly expressed the phenotype of IBC-NST, but rarer phenotypes such as TC and IMPC were also identified. We did not identify any case with an infiltrating lobular carcinoma (ILC). However, literature also admits that ILC is less likely than IBC-NST to be associated with BMs [20].

Hamilton *et al.* (2004) analyzed the demographic and pathological characteristics of patients with TC and compared them with those of patients with IBC-NST. They found that these patients were older at the time of diagnosis of their BM (63.9 years *versus* 59.6 years), the breast tumor evolved for a longer period of time until BM developed (87 months *versus* 34 months), and their survival after neurosurgical excision of BM/BMs was longer compared to those with IBC-NST [21].

A 2020 article published by De Souza *et al.* reported the fact that the predominant HP type among the phenotypes of their series with BMs originating in BC was the IBC-NST, which was diagnosed in 65.88% of cases [22], this value being similar to that obtained in our study.

Regarding the prognosis of IMPC, which is a rare and new HP variant of BC, there is controversial data. Some authors claim that IMPC has a worse prognosis than IBC-NST [23], but others affirm that there is no statistically significant difference between IMPC and IBC-NST, and therefore there is no need for proactive or radical clinical therapy [24].

Although in our series there is only one case of such a special type of BC, the time from the diagnosis of the primary tumor is 27 months, a period almost similar to the mean time to the onset of a BM for the entire series and this fact supports the hypothesis that the prognosis of this subtype of BC is not different from that of IBC-NST. If until recently the traditional classification of BC was based on morphological aspects, in 2000, considering that this classification system has certain limitations [25], a molecular classification of this type of cancer was proposed, which is still in the process of development and refining and represents the starting point for individualized therapies [26].

Based on molecular markers, BC can be classified into molecular subtypes [27, 28], as follows:

(1) Luminal type A is the most common subtype and is characterized by strong ER+ and/or PR+ immunostainings, but HER2-. From a HP point of view, it has a lower grade (low Ki67 LI) and is hormone responsive, having a good prognosis. Luminal type A disease generally requires only endocrine therapy.

(2) Luminal type B is characterized by ER+ and/or PR+ and HER2+ immunostainings. It correlates with tumors with higher grade (high Ki67 LI) and has a worse outcome compared to Luminal type A, but also like Luminal type A, this type B could be treated with endocrine therapy and chemotherapy.

(3) HER2+, but ER- is less common, being diagnosed at a younger age. Such cases present high-grade histopathology and an aggressive outcome, but this outcome could be improved with Herceptin.

(4) Basal-like type BC or TNBC is an aggressive subtype that is characterized by high grade histopathology, propensity for metastasis, and worse prognosis. Young females are at risk to develop this subtype of BC. Targeted therapies are currently under investigation, but chemotherapy seems to be indicated for most patients.

(5) Triple-positive BC with ER+/PR+ and HER2+ is a new subtype, which can be treated with anti-HER2 agents and chemotherapy [29].

In the study published by Laakmann *et al.*, in 2019, 16% of BC patients treated with neo-adjuvant chemotherapy were diagnosed with metastatic disease after a median monitoring time of five years. Only a small percent (3%) of patients were identified with BMs as the first metastatic site and factors significantly associated with this rare event were higher tumor stage before therapy, the presence of lymph node metastases, and HER2+ or triple-negative subtypes [30].

In another study, Gonzalez-Angulo & Hortobagyi (2010) also found a small percentage (5%) of patients who have developed BMs as the first site of metastatic disease. Negative HR status, nuclear grade 3, four or more positive lymph nodes and higher stage of the disease were factors considered to be associated with short time to BM in their series [31].

Kwon *et al.* (2010) found that the time period from the initial BC to the onset of BM was 52.9, 33.6, and 25.5 months for patients with HR+, HER2+, and triple receptor negative tumors [32], which can probably be extrapolated in our study, because there is a link between the presence of these molecular markers and the evolution of BC in our patients. The prognostic factors involved in the development of BM originating in BC are tumor phenotype (HER2, ER status, HP grade, proliferative rate), the degree of the extension of the disease outside the brain (the presence of lung, bone and/or BMs), patient's age, as well as patient performance status [33, 34]. The literature shows that young age, negative ER status, Ki67 LI are independent risk factors for the development of BM originating in BC [6]. A research group from Pakistan reported that ER positivity was visible in 87% of their cases of IMPC, but only 60% of IBC-NST showed ER positivity [35]. Another study, realized by some American researchers, showed that ER positivity ranged from 19.4% to 90.6% in IMPC [36]. We also found a strong, but focal cytoplasmic expression of basal CK5/6 only in 20% of BMs originating in invasive BC and/or IMPC. Positive CK5/6 (basal marker) expression was noted in 8% (12) cases of TNBC. As such, CK5/6 could serve as poor prognostic marker in TNBC [6].

There are some studies regarding the impact of depression on the overall survival (OS) of these patients. Using self-rating depression scale (SDS) questionnaires, Guo *et al.* (2017) analyzed the correlation between HR status and depressive symptoms in patients with metastatic BC [37]. They found no significant differences regarding median OS between the depressed and non-depressed patient groups, both groups having a median OS of approximately 20 months. However, these authors identified a statistically significant reduction in median OS for HR-positive BC patients, with a median OS of 25 months for those with depressive symptoms and 32 months for those without depression. In contrast, the depressive status did not affect OS in HR-negative BC, as groups of patients, *i.e.*, those with depression and those without depression had a median OS of about 14 months.

☐ Conclusions

Our study revealed the fact that BMs originating in BC show heterogeneity of HR status, although morphologically there is not so much diversity. In our study, the risk for the development of BMs seems to be correlated with the age over 50 years, BMs highlighting a negative status of PR and ER. We also found a very variable Ki67 LI, which correlated especially with the morphological subtype. We consider that our data could be useful for oncologists to apply some preventive or therapeutic measures.

Conflict of interests

The authors declare that they have no conflict of interests.

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Corresponding authors

Claudia Florida Costea, Associate Professor, MD, PhD, Department of Surgery II, Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy, 16 Universităţii Street, 700115 Iaşi, Romania; Phone +40744–972 648, e-mail: costea10@yahoo.com

Ruxandra Vatavu, MD, PhD Student, Doctoral School, Grigore T. Popa University of Medicine and Pharmacy, 16 Universităţii Street, 700115 Iaşi, Romania; Phone +40762–205 570, e-mail: ruxandravatavu@yahoo.com

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