Original Research

ONCOLOGY

Mammographic assessment of breast density as a tool for predicting the response to neoadjuvant therapy in breast cancer patients

Lorena Alexandra Lisencu[1], Andrei Roman[2], Andrei Pașca[1,3], Alexandru Irimie[1,3], Cosmin Lisencu[1,3], Mircea Negrutiu[4], Bogdan Fetica[5], Andrei Cismaru[6], Ovidiu Balacescu[7,8], Oana Tudoran[7,8], Carmen Lisencu[2]

1) Department of Oncological Surgery and Gynecological Oncology, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

2) Department of Radiology, "Prof. Dr. Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania

3) Department of Surgical Oncology, "Prof. Dr. Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania

4) Department of Dermatology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

5) Department of Pathology, Genomics and Experimental Pathology, "Prof. Dr. Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania

6) Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

7) Department of Genetics, Genomics and Experimental Pathology, "Prof. Dr. Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania

8) Department of Medical Oncology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

DOI: 10.15386/mpr-2554

Manuscript received: 06.07.2022
Received in revised form: 08.09.2022
Accepted: 02.10.2022

Address for correspondence:
Oana Tudoran
oana.tudoran@iocn.ro

Abstract

Background and aims. Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer-related death among women worldwide. For locally advanced diseases and high-risk tumors, neoadjuvant therapy (NAT) is the treatment of choice. Some studies show that mammographic density (MD) tumor margins and the presence of microcalcifications play a prognostic role in BC patients. Hence, the objective of this retrospective study was to assess if MD could predict the response to NAT among different molecular subtypes of BC patients undergoing NAT at The "Prof. Dr I. Chiricuta" Oncology Institute of Cluj-Napoca, Romania (IOCN). Furthermore, the association between MD, tumor margins and the presence of microcalcifications with clinico-pathological data was analyzed.

Methods. Eighty-four breast cancer patients diagnosed and treated at IOCN were included in this study. The morphological characteristics of the tumors were framed according to the BIRADS lexicon. The presence or absence of microcalcifications was also assessed. First, the significance of associations between breast density, margins and microcalcifications and clinico-pathological parameters of the patients were tested with Fisher or Fisher-Freeman-Halton Exact Test. Next, using multinomial logistic regression, we modelled the associations between the pathological response measured by Miller Payne and Residual cancer burden (RCB) systems and the BI-RADS. Variables having significant univariate tests were selected as candidates for the multivariable analysis (adjusted model).

Results. Breast densities were significantly associated with the age of the patients ($p=0.01$), number of positive lymph nodes ($p=0.037$), margins ($p=0.002$) and combined categories of Miller-Payne ($p=0.034$) and RCB pathological response ($p=0.021$). Margins was significantly associated with ki67 proliferation index ($p=0.029$), estrogen receptor (ER) ($p=0.007$), progesterone receptor (PR) ($p=0.019$), molecular subtype ($p<0.001$) and the number of clinically observed positive lymph nodes at diagnosis ($p=0.019$).

Conclusions. In our cohort, BC patients with lower MD had higher odds of achieving pCR following NAT, suggesting the role of MD as a clinical prognostic marker. Larger multicenter studies are warranted to validate the prognostic value of MD, which could aid in patients stratification based on their likelihood to respond to NAT.

Keywords: breast cancer, neoadjuvant therapy, mammogram, pathologic complete response, margins, microcalcifications

Background and aims

Breast cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer-related death among women worldwide [1]. The diagnosis of BC is established by clinical examination, imaging, and histopathological examination [2]. Molecular classification of BC is essential as the prognosis and treatment options differ among the molecular subtypes. BC is divided based on the expression of hormone receptors (HR) such as estrogen (ER) and progesterone (PR) receptors, the human epidermal growth factor receptor 2 (HER2) and the expression of proliferation index Ki67 into five molecular subtypes [3]: Luminal A (ER-positive, PR positive, Her2 negative, Ki67 <14%), Luminal B (ER-positive, PR positive or negative, HER2 negative, Ki-67 >14%), HER2 positive (overexpression of HER2) and basal-like/triple-negative (TN) subtype (negative for ER, PR and HER2) [3-5]. BC treatment options include surgery, radiotherapy and chemotherapy [6]. For locally advanced diseases and high-risk tumors, neoadjuvant therapy (NAT) is the treatment of choice. NAT has been used for downstaging and downsizing tumors before surgery since 1970 [7,8] NAT is also helpful in monitoring the treatment response and identifying the patients that do not respond to therapy, as the primary tumor remains intact [9,10]. NAT includes chemotherapy, endocrine therapy (for HR-positive tumors), and HER2 targeted therapy (for HER2-positive tumors) [8].

The main goal of NAT is pathologically complete remission (pCR) without evidence of malignant disease in the breast or axillary lymph nodes [8]. The response to NAT is monitored using imaging techniques like ultrasound (US), mammography and magnetic resonance imaging (MRI). However, currently, no standard imaging method is used to monitor the response to NAT, pathological evaluation still being essential [8,11].

Many systems have been developed in order to assess the response to NAT. The most frequently used systems are Miller-Payne (MP) [12] and residual cancer burden (RCB) [13]. The role of pCR in the prognostic of BC patients has been proven in extensive studies [9,10] PCR can predict the outcome in BC patients, as pCR is associated with improved disease-free survival and overall survival [8,14,15]. Patients that do not show pCR, especially those with a high Ki-67 index after NAT, have an unfavorable prognosis [10]. Some studies show other features that could be useful in assessing the prognosis of BC patients undergoing NAT. For example, increased mammographic density (MD), a known risk factor for BC [16], has been previously investigated as a therapy predictive biomarker [17,18]. Other reported mammographic findings that seem to play a role in the prognosis of BC are tumor margins [16] and the presence of microcalcifications [19]. Thus, in this retrospective study we assessed whether these mammografic features could predict NAT response in a cohort of BC patients treated at The "Prof. Dr I. Chiricuta" Oncology Institute of Cluj-Napoca (IOCN). Furthermore, we explored for associations between MD, tumor margins and microcalcifications and the clinical and pathological data of the patients.

Materials and methods

Patient cohort

Eighty-four breast cancer patients diagnosed and treated at IOCN, Romania were included in this study. Patients who had mammographic evaluations in other institutions were excluded from the analysis. All patients gave their consent for the study according to the Declaration of Helsinki. The study was approved by the IOCN ethical committee (Approval No. 59/29.11.2016) and the Iuliu Hatieganu University of Medicine and Pharmacy , Cluj-Napoca, Romania (290/09.09.2020). Clinical and pathological data of the patients were retrieved from digital medical charts.

Mammographic measures

All patients underwent 2D full-field digital mammography on Selenia Dimension Mammography System from Hologic (year of production: 2012). The same highly trained radiologist performed the interpretation of the mammograms under similar conditions. Mammograms acquired at the time of diagnosis, prior to systemic therapy, were gathered retrospectively.

The mammographic assessment was performed following the latest edition of the American College of Radiology (ACR)-Breast Imaging Reporting Data System (BIRADS) lexicon [19]. Glandular density and mammographic signs suggestive of cancer: masses (their margins) and glandular density asymmetries were analyzed. The assessment of glandular density was subjective and four categories have been described: a - almost entirely fat; b - scattered fibroglandular density; c - heterogeneously dense, and d - extremely dense. In order to increase the coherence of the statistical analysis, the morphological characteristics of the mammographic masses and of the asymmetries were framed according to the BIRADS lexicon and divided into four categories. These categories were constituted by grouping some descriptors according to their significance in predicting the malignancy as follows:

1. Spiculated margins
2. Microlobulated or indistinct
3. Obscured margins or asymmetries or tumor margins that cannot be assessed
4. Circumscribed margins

The presence or absence of microcalcifications was also assessed. Only microcalcifications with suspicious morphology were included according to BIRADS lexicon.

Statistical analysis

We summarized the clinico-pathological data of the patients according to the BI-RADS classification of breast density (see Table I). Categorical variables were summarized by counts and continuous variables by median values. First, the significance of associations between breast density, contour and microcalcifications and clinico-pathological parameters of the patients were tested with Fisher or Fisher-Freeman-Halton Exact Test.

Table I. Clinico-pathological data of the patients included in the study.

Variable	Patients characteristics
n = 84	
Age (median=59.5)	
≤ 50	23 (27.38%)
> 50	61 (72.62%)
Grading	
G1	15 (17.86 %)
G2	41 (48.81%)
G3	28 (33.33%)
KI67 (median=25)	
≤ 20	36 (42.86%)
> 20	48 (57.14%)
Molecular Subtype	
Luminal A	28 (33.33%)
Luminal B	27 (32.14%)
TN[1]	15 (17.86%)
HER2+ [2]	10 (11.90%)
NA[3]	4 (4.76%)
Clinical tumor size (cT)	
cT1	6 (7.14%)
cT2	39 (46.43%)
cT3	9 (10.71%)
cT4	22 (26.19%)
NA	8 (9.52%)
Clinical lymph nodes (cN)	
cN0	17 (20.24%)
cN1	24 (28.57%)
cN2	30 (35.71%)
cN3	5 (5.95%)
NA	8 (9.52%)
Clinical metastasis (cM)	
cM0	66 (78.57%)
cM1	5 (5.95%)
NA	13 (15.48%)
Clinical stage	
S-I	4 (4.76%)
S-II	25 (29.76%)
S-III	38 (45.24%)
S-IV	7 (8.33%)
NA	10 (11.9%)
Pathological tumor size (pT)	
pT0	10 (11.9%)
pT1	29 (34.52%)
pT2	21 (25%)
pT3	2 (2.38%)
NA	22 (26.19%)
Pathological lymph nodes (pN)	
pN0	34 (40.48%)
pN1	19 (22.62%)
pN2	8 (9.52%)
pN3	2 (2.38%)
NA	21 (25%)
Pathological lymphatic Invasion (L)	
L0	38 (45.24%)
L1	25 (29.76%)
NA	21 (25%)
Survival status	
Alive	74 (88.15)
Deceased	10 (11.9%)
n=71	

Table I. Clinico-pathological data of the patients included in the study (continuation).

Variable	Patients characteristics
Neoadjuvant therapy	
Only CT[4]	34 (47.89%)
Only HT[5]	20 (28.17%)
CT+Her2	7 (9.86%)
CT+HT	5 (7.04%)
CT+HT+Her2	1 (1.41%)
CT+RTE	2 (2.82%)
CT+HT+RTE[6]	2 (2.82%)
Miller-Payne System	
Grade 1	15 (21.13%)
Grade 2	6 (8.45%)
Grade 3	18 (23.35%)
Grade 4	7 (9.86%)
Grade 5	11 (15.49%)
NA	14 (19.72%)
RCB[7]	
RCB 0	9 (12.68%)
RCB-I	6 (8.45%)
RCB-II	32 (45.07%)
RCB-III	12 (16.90%)
NA	12 (16.90%)

1. TN- triple negative; 2. Her2- human epidermal growth factor receptor 2; 3. NA- not applicable; 4. CT- chemotherapy; 5. HT- hormonal therapy; 6. RTE- external radiotherapy; 7. RCB- residual cancer burden.

Next, using multinomial logistic regression, we modelled the associations between the pathological response measured by Miller Payne and RCB systems and the BI-RADS. Raw relationships between the pathological responses and clinico-pathological variable were first tested by univariate analysis (unadjusted model). For the Miller Payne system, unexpected singularities in the Hessian matrix were encountered; thus, we combined Grades 1 and 2 (Low response) and Grades 4 and 5 (high response) and maintained Grade 3 (intermediate). Variables having significant univariate tests were selected as candidates for the multivariable analysis (adjusted model). All analyses were carried out using SPSS (IBM SPSS Statistics for Macintosh, Version 28.0; IBM Corp Armonk, NY, USA).

Results

The clinico-pathological data of the patients included in the study are summarized in table I. The median age of the patients is 59.5 (35-82), with more than 70% of the patients being over 50 years old. Most patients had moderate to poorly differentiated carcinomas, and less than half of the patients presented low proliferative tumors (ki67≤ *20)*.

The predominant molecular subtype was Luminal, A and B, which were relatively equally distributed. According to the clinical TNM classification, more than half of the patients were diagnosed with advanced disease (III, IV). Seventy-one patients received an indication for

neoadjuvant therapy (NAT), distributed as follows: 34 received only chemotherapy (CT), 20 only hormonal therapy (HT), while the rest received combinatory regimens with or without additional radiotherapy (RTE). Eight out of the ten patients that had Her2+ tumors received also Her2 targeted therapy. Pathological TNM staging was retained for prognostic information (primary and post-NAT surgery), while Miller-Payne and RCB systems were used to evaluate the NAT pathological response. According to the Miller-Payne evaluation, around 30% of the patients showed no to minor response (Grade 1 and 2), 23% had an intermediate response (Grade 3), and 35% had an almost complete pathological response. According to the RCB classification system, 12.7% of the patients reached complete pathological response, 17% were therapy-resistant, and around half had a partial response.

The frequency distributions of the clinico-pathological data according to breast density, margins and microcalcifications categories are presented in tables II-IV. Breast densities were roughly uniformly distributed among the patients between categories a, b and c; only one patient presented highly dense breasts (category d) and thus could not be included in the statistical analysis, 31% of the patients presented microcalcifications alone or in combination with other mammographic signs. Microcalcifications have been identified more frequently in type (a) breast density.

Most frequently, tumor margins that could not be assessed or described as asymmetries were associated with type (c) density. In types (a) and (b) of MD, mostly spiculated, microlobulated or indistinct margins were found; furthermore, circumscribed margins were more commonly found in type (b) MD.

Breast densities were significantly associated with the age of the patients; with increasing age, a decrease in breast densities was observed (p=0.01). A significant association was also observed between breast densities and the number of positive lymph nodes (p=0.037), margins (p=0.002) and combined categories of Miller-Payne (p=0.034) and RCB pathological response (p=0.021) (Table II). Margins were significantly associated with ki67 proliferation index (p=0.029), ER (p=0.007), PR (p=0.019), molecular subtype (p<0.001) and the number of clinically observed positive lymph nodes at diagnosis (p=0.019) (Supplementary Table III). No significant associations were observed between microcalcifications and clinic-pathological data (Supplementary Table IV).

Table II. Patients' clinico-pathological data according to mammographic density at baseline>.

Density / Clinico-pathological	a	b	c	P value (Fisher exact test)
n = 84	31 (36.9%)	25 (29.76%)	27 (32.14%)	
Age				
≤50	4	6	13	0.01 **
>50	27	19	14	
ki67				
≤20	13	8	14	0.246
>20	18	17	11	
Biopsy Grading				
I	5	2	8	0.157
II	13	13	14	
III	13	10	5	
Molecular Subtype				
Luminal A	11	7	9	0.701
Luminal B	8	9	10	
TN[1]	6	6	3	
Her2+[2]	6	2	2	
ER[3] status				
ER-	11	6	4	0.2179
ER+	20	19	22	
PR[4] status				
PR-	14	13	8	0.307
PR+	17	12	18	
Her2 status				
Her2-	25	22	22	0.516
Her2+	6	2	2	
cT[5]				
1	3	2	1	0.264
2	9	14	15	
3	6	2	1	
4	10	6	6	

Table II. Patients' clinico-pathological data according to mammographic density at baseline> (continuation).

Density / Clinico-pathological	a	b	c	P value (Fisher exact test)
cN[6]				
N0	4	5	8	0.359
N1	9	8	7	
N2	11	11	7	
N3	4	0	1	
Lymph node status				
Negative	4	5	8	0.208
Positive	24	19	15	
C stage*				
I	3	1	0	0.142
II	5	9	11	
III	13	13	11	
IV	5	1	1	
pT[7]				
0	4	4	2	0.734
1	10	7	11	
>2	6	8	9	
pN[8]				
0	12	8	14	0.037*
1	5	5	8	
>2	3	7	0	
Pathologic Lymph nodes status				
N negative	12	8	14	0.331
N positive	8	12	8	
pL[9]				
L0	15	9	14	0.171
L1	5	11	8	
Miller Payne				
Grade 1	8	2	5	0.087
Grade 2	2	3	1	
Grade 3	3	7	8	
Grade 4	2	4	0	
Grade 5	5	5	1	
Miller Payne				
Low response (1+2)	10	5	6	0.034*
Intermediate response (3)	3	7	8	
High response (4+5)	7	9	1	
RCB[10]				
0	3	5	1	0.021*
I	2	3	1	
II	12	5	14	
III	3	8	1	
Margins				
Obscured/ Asymmetries/ Tumor cannot be assessed	3	1	13	0.002**
Circumscribed	1	3	1	
Microlobulated/Indistinct	9	8	4	
Spiculated	18	13	9	
Microcalcifications				
Present	12	6	8	0.505
Absent	19	19	19	

1.TN- triple negative; 2. Her2- human epidermal growth factor receptor 2; 3. ER- estrogen receptor; 4. PR-progesteron receptor; 5.cT- clinical tumor size; 6. cN- clinical lymph nodes; 7. pT- pathological tumor size; 8. pN- pathological lymph nodes; 9.pL- pathological lymphatic invasion; 10. RCB- residual cancer burden.

Multiple univariate logistic regression analyses with pathological response as outcome were used to systematically test for significant covariates (Table V) as predictors. Breast density was a significant predictive factor for both Miller-Payne and RCB evaluation systems. Significant covariates for both systems were biopsy grading at diagnosis, ER, PR, Her2 receptors, molecular subtype and NAT type. The ki67 proliferation index was also found to be significant for the Miller Payne system and pT, pN and pL for the RCB system.

Table V. Univariate logistic regression between pathological response and clinico-pathological variables.

Clinico-pathological	Univariable MLR	
	Miller-Payne (low vs. intermediate vs. high)	RCB[1]
Density	0.024	0.021
Margins	0.469	0.355
Microcalcifications	0.992	0.99
Age	0.631	0.223
ki67	0.029	0.321
Biopsy Grading	0.002	0.04
Molecular Subtype	<0.001	<0.001
ER[2] status	0.012	0.025
PR[3] status	0.003	0.001
Her2[4] status	0.007	0.016
cT[5]	0.992	0.681
cN[6]	0.313	0.840
C stage	0.445	0.801
pT[7]	<0.001	<0.001
pN[8]	0.308	<0.001
pL[9]	0.361	<0.001
NAT[10] Type		
Only CT[11]	0.054	0.714
Only HT[12]		
Her2		
Combined		

1. RCB - residual cancer burden; 2. ER- estrogen receptor; 3. PR- progesterone receptor; 4. HER2- human epidermal growth factor receptor 2; 5. cT- clinical tumor size; 6.cN- clinical lymph nodes; 7. pT- pathological tumor size; 8. pN- pathological lymph nodes; 9. pL- pathological lymphatic invasion; 10. NAT- neoadjuvant therapy; 11. CT- chemotherapy; 12. HT- horomoanl therapy

A total of 56 patients had complete data and were included in the logistic regression analysis. Next, we tested different models by adjusting the logistic regression analysis with the significant variables (Tables VI, VII). As expected, high co-linearity was observed between the ER, PR and HER2 individual variables and molecular subtype variable; thus, only the molecular subtype was considered for subsequent analysis. We also excluded the pT, pN and pL variables from the model, as these have no predictive value; they are evaluated post-NAT and are taken into account when calculating the pathological response.

In the Miller-Payne regression model, biopsy grading, NAT type and density-independent variables were significant predictors in the final model. As expected, biopsy grading and NAT type significantly impacted both low and intermediate responders compared to the high responder patients (Table VI). The odds ratio of reaching a higher pathological response decrease with increasing tumor grading and the need for therapy combination. Regarding breast densities, the odds of having a low or intermediate response rather than a high response are lower in patients with almost entirely fatty breasts (density a) compared to more dense breasts (density c). This model can predict high responders with 82.4% probability.

Table VI. Miller-Payne model adjusted for ki67 proliferation index, biopsy grading and NAT type.

Miller-Payne[a] (low vs. intermediate vs. high)	Sig.	Odds Ratio	95% CI
Low response			
ki67	.769	1.008	.955-1.064
Biopsy Grading	.012*	.053	.005-.520
NAT[1] Type	.026*	.275	.088-.857
[Density=a]	.255	.145	.005-4.032
[Density=b]	.079	.041	.001-1.443
[Density=c][b]	.	.	.
Intermediate			
ki67	.117	.939	.868-1.016
Biopsy Grading	.045*	.090	.009-.947
NAT Type	.003*	.091	.019-.438
[Density=a]	.047*	.026	.001-.954
[Density=b]	.095	.043	.001-1.717
[Density=c][b]	.	.	.

[a] The reference category is: Miller Payne (4+5) (>90% response)
[b] This parameter is set to zero because it is redundant.
1 NAT- neoadjuvant therapy

The odds of having a residual disease (RCB II and RCB III) rather than a complete pathological response decrease from luminal to TN and Her2+ tumors. They are increasing with tissue density (Table VII). In the RCB-adjusted model, besides the breast density, the other significant predictor was the molecular subtype at diagnosis. However, the specificity of this model is lower than the Miller Payne model; only 44.4% of the patients were correctly predicted to reach complete pathological response.

Table VII. RCB model adjusted for Biopsy grading and molecular subtype.

RCB[a]	Sig.	Exp(B)	95% CI
RCB-I			
Biopsy Grading	.151	.160	.013-1.954
Molecular Subtype	.793	.807	.163-3.992
[Density=a]	.887	1.304	.034-49.761
[Density=b]	.852	.710	.019-26.212
[Density=c][b]	.	.	.
RCB-II			
Biopsy Grading	.869	1.213	.122-12.034
Molecular Subtype	.021*	.175	.040-.765
[Density=a]	.404	.295	.017-5.170
[Density=b]	.053	.054	.003-1.044
[Density=c][b]	.	.	.
RCB-III			
Biopsy Grading	.936	1.108	.090-13.638
Molecular Subtype	.010*	.083	.012-.558
[Density=a]	.974	.940	.024-37.105
[Density=b]	.873	1.345	.035-51.112
[Density=c][b]	.	.	.

[a] The reference category is: RCB-0.
[b] This parameter is set to zero because it is redundant.

Discussion and conclusions

The possibility of reducing the size of the tumor and improving the prognosis of the disease by using NAT is a major advantage in BC patients with high-risk tumors. Several systems have been proposed to assess the pathological response to NAT. The MP system has 5 grades: G1- no change, G2 - <30% reduction in tumor cells, G3 - 30-90% reduction in tumor cells, G4 - >90% reduction in tumor cells and G5- pCR [12]. The most frequently used index for assessing residual disease after NAT is RCB, which combines the size of the primary tumor, the cellularity and the size of the largest affected lymph node [8]. It has 4 classes: RCB0- pCR, RCB1- minimal residual disease, RCB2- moderate residual disease, RCB3- extensive residual disease [13].

About 5% of patients show progression under NAT, so imaging assessment is critical in treatment planning [18,21]. There are many imaging methods, and this field is constantly developing. Assessment of residual tumor may be obtained by US, digital mammography (DM), digital breast tomosynthesis, MRI, positron emission tomography/computed tomography (PET/CT), futhermore, MRI diffusion-weighted imaging (DWI) and MRI perfusion-weighted imaging (PWI). The most frequently used techniques for NAT monitoring are US, DM or MRI. One of their roles is to monitor the tumor diameter, considered a parameter for the responsivity to NAT. When using DM and digital breast tomosynthesis, the evaluation is dependent on tumor characteristics, as calcifications and spicules are challenging to be interpreted. However, it seems that neither calcifications nor spicules represent indicators for residual disease. The downside of DM is that of a possible underestimation of tumor size. US seems more accurate than DM [22], but the downside is that it is operator-dependent [21]. It has been reported that the combination of US with DM highly correlates with pCR [21-23].

MRI is the most accurate and adequate available imaging method for monitoring NAT response [21], but it does not replace the pathological evaluation [21,22,24]. Moreover, MRI is limited in predicting the response to NAT in luminal tumors. A new promising method, called positron emission mammography (PEM), has been tested for pre-operative assessment of BC, and it seems more accurate than MRI. However, its role in monitoring the response to NAT has not been studied [22]. Currently, the standard of care in monitoring patients undergoing NAT remains breast US and mammography [22].

It is known that high breast density is a risk factor for BC [20]. The risk is four times higher in women with a high MD compared to those with fatty breast tissue [20]. Moreover, it seems that MD is a more substantial risk factor than family history or reproductive risk factors [25].

Consistent with literature reports, our patients present decreasing MD with increasing age [26,27]. It is thought that these changes are in close relationship with hormonal changes and are in coherence with the fact that after menopause, the rate of ER-negative tumors versus ER-positive tumors increases [27].

The literature data are controversial regarding the role of MD in response to NAT. There have been reports in the literature that MD is associated with pCR after NAT- it was shown that the higher the MD, the lower the odds of obtaining pCR following NAT [17,18]. Conversely, some studies state that BC patients with higher MD are more likely to obtain pCR [28]. A possible explanation could be that the more aggressive the tumor, the better the response to chemotherapy [28]. In our study, according to both MP and RCB systems, MD was a predictor for pCR as lower odds of reaching pCR were noticed in patients with higher MD. Some studies [29-31] did not find any association between MD and pCR to NAT- in a prospective study on 200 BC patients, even if a decrease in MD was noticed during NAT, it was not found to be associated with pCR in the neoadjuvant setting [30].

Regarding margins, spiculated margins on mammograms are associated with lower grade and HR-positive BC (luminal A), while TNBC tumors are most frequently circumscribed [32]. Consistently, we found spiculated margins to be most prevalent among Luminal A patients, while 4 out of 5 cases of the TNBC subtype presented circumscribed margins. Moreover, lower Ki-67 value and lower MD were associated with the presence of spiculated margins. Microlobulated margins are more characteristic of HER2-positive tumors [32]. Another type of margins frequently seen in mammography in HER2 tumors and TNBC is the one with indistinct margins. It is

known that both of these molecular subtypes are associated with a poor prognosis [33].

Another essential aspect that might be seen on mammograms is the presence of microcalcifications, which are tiny calcium deposits. These could represent the only finding suggestive of breast tumors in many cases. In general, their presence is associated with invasive behavior, high tumor grade, higher risk of recurrence and lymph nodes metastasis and a worse prognosis [19,34]. However, further studies are needed to clarify the prognostic role of microcalcifications. For example, several studies did not find significant associations between microcalcifications and lymph node metastasis [35,36]. Moreover, some studies found microcalcifications presence as being a predictor of larger tumors [37], others reported associations with decreased tumor size [38], while others did not find any association between the two [35]. In our study cohort, we did not find any association between the presence of microcalcifications and any of the clinico-pathological data.

One of the most significant limitations when it comes to mammography is in the cases of dense breast, as digital mammography sensitivity decreases from almost 100% in type (a) of MD to around 50% in type (d) MD; possible explanations for these are that cancers can be masked by the normal dense tissue and also, „white" masses are similar to the normal fibroglandular tissue, so they are difficult to be distinguished [39,40]. Microcalcifications play an essential role in diagnosing BC, especially when the primary tumor is not evident. Microcalcifications identification is less influenced by the MD compared to the masses [41].

An effective NAT increases pCR and decreases RCB in BC patients [42]. pCR is a highly significant endpoint in HER2+ [14] and triple-negative [15] BC patients, while in luminal subtypes, its role is not so well proven [10,43]. Some studies show that pCR is highest in HER2 positive tumors and TN subtype, followed by luminal B subtype, and is the lowest among luminal A BC patients [5,10].

We noticed similar trends, as according to RCB classification, the odds of reaching pCR were the highest in the TNBC subtype and the lowest in the luminal subtypes.

The literature reports have not reached a consensus regarding the role of pCR in the survival of BC patients. Some studies found that pCR improves neither disease-free nor overall survival [44]. On the other hand, other studies show that pCR is an independent prognostic factor for disease-free survival, especially for luminal B, TN and HER2 positive subtypes of BC. However, it is not significant regarding the OS [42,43]. Studies also show that pCR is associated with improved disease-free survival and overall survival in BC patients [45,46].

In **conclusion**, our results suggest that BC patients undergoing NAT presenting lower MD have higher odds of achieving pCR. Consistent with previous reports [17,18] including image-based biomarkers. Breast cancer (BC, these findings highlight the role of MD in predicting the patients that are more likely to benefit from NAT. Therefore, large multicenter studies would be justified to explore the role of MD as a clinical prognostic marker.

The strong points of our study are represented by the fact that all the mammographic interpretations and the histopathology reports were performed in the same institution; this way, the inter-observer variation decreases. All the mammograms were obtained using the same mammography device. Another strong point is that patients were treated in the same hospital following the same guidelines. Our study has some limitations, such as the small size of our group and the fact that the mammographic interpretation was performed by one radiologist, so a subjective bias is possible, even if it is a highly trained radiologist with more than 20 years of experience. The interpretation of density is device-dependent and operator-dependent.

Funding

This work was supported by the Competitiveness Operational Programme 2014–2020, Contract no. 41/02.09.2016, MySMIS 103557, Project title: Genomic and microfluidic approaches towards blocking breast cancer cell invasion and metastasis—BREASTMINCROGENOMICS and Knowledge Transfer of Biogenomics in Oncology and Related Domains in clinical applications grant—BIOGENONCO, MySMIS code: 105774, financing contract no: 10/01.09.2016.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee of the "Ion Chiricuță" Oncology Institute, Cluj-Napoca, Romania (Approval No. 59/29.11.2016) and the University of Medicine and Pharmacy Iuliu Hatieganu, Cluj-Napoca, Romania (Approval No. 290/09.09.2020.)

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Supplementary Tables

Supplementary Table III. Patients clinico-pathological data according to margins evaluations at baseline.

Margins / Clinico-pathological	Obscured/ Asymmetries/ Tumour cannot be assessed	Circumscribed	Microlobulated/ indistinct	Spiculated	P value (Fisher exact test)
n=84	18 (21.42%)	5 (5.95%)	21 (25%)	40 (47.61%)	
Age					
≤50	6	3	7	7	0.126
>50	12	2	14	33	
ki67					
≤20	6	0	7	23	0.029*
>20	11	5	14	16	
Biopsy Grading					
I	2	0	3	10	0.212
II	12	1	9	19	
III	4	4	9	11	
Molecular Subtype					
Luminal A	3	0	5	20	<0.001***
Luminal B	11	0	7	9	
TN[1]	0	4	5	6	
Her2+[2]	3	1	3	3	

Supplementary Table III. Patients clinico-pathological data according to margins evaluations at baseline (continuation).

Margins / Clinico-pathological	Obscured/ Asymmetries/ Tumour cannot be assessed	Circumscribed	Microlobulated/ indistinct	Spiculated	P value (Fisher exact test)
ER[3] status					
ER-	1	4	7	9	0.007**
ER+	17	1	14	30	
PR[4] status					
PR-	5	5	11	14	0.019*
PR+	13	0	10	25	
Her2 status					
Her2-	14	4	17	35	0.456
Her2+	3	1	3	3	
cT[5]					
1	1	0	1	4	0.976
2	9	2	9	19	
3	1	1	3	4	
4	4	1	7	10	
cN[6]					
N0	4	1	4	8	0.019*
N1	5	0	2	17	
N2	5	2	14	9	
N3	1	1	0	3	
Clinical stage					
I	0	0	1	3	0.135
II	8	1	2	14	
III	6	2	14	16	
IV	0	1	2	4	
pT[7]					
0	3	1	3	3	0.538
1	7	0	10	12	
>2	7	1	4	11	
pN[8]					
0	10	1	10	13	0.195
1	7	0	5	7	
>2	0	1	2	7	
pL[9]					
L0	11	1	10	16	0.974
L1	6	1	7	11	
Miller Payne					
1	3	1	5	6	0.792
2	0	0	1	5	
3	6	0	4	8	
4	1	0	3	3	
5	2	1	4	4	
Miller Payne					
1+2	3	1	6	11	0.692
3	6	0	4	8	
4+5	3	1	7	7	
RCB[10]					
0	1	1	3	4	0.326
I	1	0	3	2	
II	10	0	9	13	
III	1	1	2	8	

1.TN- triple negative; 2.Her2- human epidermal growth factor receptor 2; 3.ER- estrogen receptor; 4.PR-progesteron receptor; 5.cT- clinical tumor size; 6.cN- clinical lymph nodes; 7.pT- pathological tumor size; 8.pN- pathological lymph nodes; 9.pL- pathological lymphatic invasion; 10.RCB- residual cancer burden

Supplementary Table IV. Patients clinico-pathological data according to microcalcification presence at baseline.

Microcalcifications / Clinico-pathological	Absent	Present	P value (Fisher exact test)
N=84	58 (69%)	26 (31%)	
Age			
≤50	18	5	0.302
>50	40	21	
ki67			
≤20	27	9	0.469
>20	30	16	
Biopsy Grading			
I	9	6	0.082
II	33	8	
III	16	12	
Molecular Subtype			
Luminal A	20	8	0.075
Luminal B	17	10	
TN[1]	14	1	
Her2+[2]	5	5	
ER[3] status			
ER-	17	4	0.186
ER+	40	22	
PR[4] status			
PR-	27	8	0.231
PR+	30	18	
Her2 status			
Her2-	51	19	0.157
Her2+	5	5	
cT[5]			
1	3	3	0.453
2	29	10	
3	5	4	
4	14	8	
cN[6]			
N0	13	4	0.431
N1	13	11	
N2	21	9	
N3	4	1	
Clinical stage			
I	2	2	0.874
II	17	8	
III	27	11	
IV	5	2	
pT[7]			
0	6	4	0.494
1	17	12	
>2	17	6	
pN[8]			
0	26	8	0.090
1	9	10	
>2	6	4	
pL[9]			
L0	26	12	0.592
L1	15	10	

Supplementary Table IV. Patients clinico-pathological data according to microcalcification presence at baseline (continuation).

Clinico-pathological \ Microcalcifications	Absent	Present	P value (Fisher exact test)
Miller Payne			
1	10	5	0.992
2	4	2	
3	12	6	
4	4	3	
5	7	4	
Miller Payne			
1+2	14	7	0.942
3	12	6	
4+5	11	7	
RCB[10]			
0	6	3	1
I	4	2	
II	20	12	
III	8	4	
Margins			
Obscured/ Asymmetries/ Tumour cannot be assessed	12	6	0.504
Circumscribed	5	0	
Microlobulated/indistinct	13	8	
Spiculated	28	12	

1.TN- triple negative; 2.Her2- human epidermal growth factor receptor 2; 3.ER- estrogen receptor; 4.PR-progesteron receptor; 5.cT- clinical tumor size; 6.cN- clinical lymph nodes; 7.pT- pathological tumor size; 8.pN- pathological lymph nodes; 9.pL- pathological lymphatic invasion; 10.RCB- residual cancer burden