Infiltrating Breast Carninomas Multifocality: Clinical and Biological Features

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Abstract: In order to know the associations between multifocality (MF), without related multicentricity (MC), and common clinical and biological parameters, and posterior influence in breast carcinoma behavior, we have developed this study. 816 successive women affected from invasive breast carcinomas, of which 96 were multifocal and 720 non-multifocal were included in the study. We considered age, size, lymph node involvement, distant metastasis, histological grade, ploidy, cellular synthesis phase, as well as expression of estrogen receptor (ER), progesterone receptor (PgR), androgen receptor (AR), p53, bcl2 and Ki67 by immunohistochemical assays.

Taken as a whole, multifocal invasive carcinomas (11%) showed exclusively more distant metastasis and more tumor-related-deaths. However, when tumors were classified according to histological type, in ductal carcinomas MF courses exclusively with greater lymph node involvement, while in non ductal carcinomas MF showed higher percentage of distant metastasis, higher proliferation and higher number of recurrences. Also, there were NO differences between axillary lymph node involvement and tumor size in multifocal tumors regardless of histology.

Our results suggest: 1) MF was found in 11% of invasive breast carcinomas and was associated with higher distant metastasis and number of tumor-related-deaths. 2) in invasive ductal carcinomas, MF was associated, exclusively, with increased axillary node involvement, whereas in other histological types, with a predominance of lobular, it did with higher distant metastasis, cell proliferation and recurrence number, suggesting, in this subtype of tumors where there is higher prognostic/diagnostic value has the MF presence.

Keywords: Infiltrating, breast carninomas, multifocality.

INTRODUCTION

Breast carcinoma is one of the most common tumors in daily practice and is usually associated with multifocality and multicentricity, mainly due to the use of new imaging technology for diagnosis. While some useful prognostic factors in these tumors are clinically well established [1-7], less known and controversy is the multifocality, which is not always well defined from a conceptual point of view [8-9] and often analyzed and associated with multicentricity. Also, tumor size is still under discussion [10] which one considered, the larger lesion or the sum of all lesions; it seems that the first option shown has superior clinical utility [11-12]. Multifocality seems to be genetically linked to chromosome 2 gains and losses on chromosome 15 [13]. Also, correct diagnosis is important for surgical approach and, although it is associated with an increased risk (20%) of lymph node involvement, prognostic value is not fully defined, so further studies are needed to precise it [14].

We define multifocality as "existence of two or more invasive tumor foci separated by benign tissue in the same quadrant", using the larger size and not the sum of the lesion sizes [15, 16]. In this study, we aimed to present our experience, comparing multifocality, separated from multicentricity, with other clinical and biological parameters, and analyzed the influence on breast carcinoma patients through monitoring, classified according to the major histological subtypes

MATERIAL AND METHODS

The cohort included 816 women affected from invasive breast carcinomas where 96 were multifocal (74 ductal carcinomas, 15 lobular, 3 cystosarcoma, 3 mucinous and 1 medullary) and 720 non-multifocal (605 ductal carcinomas, 46 lobular, 5 cystosarcoma, 30 mucinous, 15 medullar, 2 adenocystic, 6 apocrine, 17 tubular and 2 inflammatory). All were diagnosed between 1992 and 2009, in the Hospital Monte de Naranco (Oviedo, Spain), where the breast pathology unit and breast screening unit were located.

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We considered age, lymph node involvement (N), distant metastasis (M), histological grade (HG), ploidy and cellular synthesis phase (SP), determined by flow cytometry in fresh samples (Fascam. Beckton-Dikinson. USA), and expressions of estrogen receptor (ER phramDx, 1D5/ER-2-123, Dako, Denmark), progesterone receptor (PR; 1294, Dako) androgen receptor (AR; 441, Dako, dilution 1/150), p53 (D0-7, Dako, dilution 1/100), bcl-2 (124; Dako, dilution 1/150) and Ki67 (MIB1, Dako, dilution 1/200), evaluated by immunohistochemical assays, using monoclonal antibodies. Immunohistochemical staining on tissue sections of 4-5 microns was done by the EnVision method with a heat-induced antigen retrieval step. Sections were immersed in boiling 10 nmol/L sodium citrate at pH 6,5 for 2 minutes in a pressure cooker.

ER and PR were assessed according to the *Allred* score, negatives (score: 0-2) and positives (score: 3-8). Cut-offs for p53 and Ki67 positivity were 30% and 15%, respectively. AR was classified as positive or negative without any score, and bcl-2 as negative (-), weak positive (+) or strong positive (++).

Performed treatment was surgery, preferably conservative, followed by chemotherapy, radiotherapy and hormone therapy. Checkups by the Surgery Department began when the treatment was completed. Follow-ups were quarterly or half yearly the first two years, depending on the severity, type of tumor and prognosis. After the second until the fifth year, checkups were six-monthly, and, thereafter, annually with exceptions.

Windows SPSS software was employed for statistical analysis. Continuous variables with a Gaussian distribution were expressed as the range and the mean +/- SD (standard deviation), while, if not, were expressed as the range and the median. To validate any statistically significant difference, *Chi-square* distribution was used (with *Yates* correlation, if necessary). Survival curves were analyzed by Kaplan-Meier method and their difference by the log-rank test. The criteria to be considered as significant was $p < 0.05$.

RESULTS

a) Taken as a whole, and presented in Table **1**, multifocal invasive carcinomas showed, exclusively, more distant metastasis (p=0.032). During the follow-up period, ranged from 1 to 196 months (51.5 +/-38.6, median 44 months), multifocal carcinomas showed more tumor-related-deaths (p=0.002).

b) When tumors were classified according to histological type, we found in ductal carcinomas (see Table **2**), multifocality courses exclusively with higher lymph node involvement (greater than 3), close to statistical significance, but there were NO differences in recurrence number and/or tumor-related-deaths. By contrast, in non-ductal carcinomas (see Table **3**), multifocality showed higher distant metastasis percentage (4/22 *vs* 4/115, p=0.021) and higher recurrence number (4/16 *vs* 3/102, p=0.0025).

c) Analyzing axillary lymph node involvement (N) *versus* multifocality, tumor subtype and tumor size as described in Table **4** we observed NO difference between axillary lymph node involvement and tumor size in multifocality breast carcinomas regardless of histology. By contrast, in non-multifocal tumors, infiltrating ductal carcinomas coursed with more axillary involvement are independent of the size. Multifocal non-ductal carcinomas, smaller than 2cm, showed higher axillary involvement (p=0.043) than non-multifocal.

Table 1. Clinical and Biological Differences of *Invasive Carcinomas* with or without Multifocality

Parameter	Multifocality	Non Multifocality	p
N+	45/96	284/720	ns
N+>3	20/96	113/720	ns
M+	16/96	69/720	0,032
GH3	31/96	192/720	ns
Aneuploidy	14/33	152/364	ns
ER+	56/67	281/344	ns
PR+	42/66	207/342	ns
AR+	43/55	209/257	ns
P53+	14/59	63/300	ns
Bcl-2++	43/57	214/277	ns
Bcl-2+++	34/57	185/277	ns
Ki67+	38/69	176/345	ns
>2cm	41/96	299/720	ns
>5cm	5/96	27/720	ns
SP>7%	16/31	162/340	ns
Age	34-86 (60,4+/-10,6)	27-88 (61,0+/-10,4)	ns
Recurrences	13/83	56/541	ns
Deaths	9/79	19/525	0,002

N: lymph node involvement.
M: distant metastasis.
HG: histological grade.
ER: estrogen receptor.
PR: progesterone receptor.
AR: androgen receptor.
SP: cellular synthesis phase.
ns: non significant.

d) Analyzing distant metastasis (M) *versus* multifocality, histological subtype and tumor size, multifocal carcinomas, as showed in Table **5**, spread increased with size in non-ductal while the same happen with ductal carcinoma in non-multifocal. Multifocal non-ductal carcinomas with size greater than 2 cm, showed higher distant metastasis than those without multifocality (p = 0.00015).

e) Comparing multifocal carcinomas with each other, 74 ductal showed, exclusively, more axillary lymph node involvement >3 (1/22 *vs* 19/74, p=0.048), whereas 22 non-ductal showed a trend toward more recurrences and tumor-related-deaths, but without statistical significance, possibly by the small number of patients, (4/16 *vs* 6/67 and 4/16 *vs* 5/63).

Table 2. Clinical and Biological Differences of *Invasive Ductal Carcinomas* with or without Multifocality

Parameter	Multifocality	Non Multifocality	p
N+	36/74	254/605	ns
N+>3	19/74	102/605	0,061
M+	12/74	65/605	ns
HG3	27/74	182/605	ns
Aneuploidy	9/23	138/306	ns
ER+	45/54	234/285	ns
PR+	33/53	169/283	ns
AR+	37/46	176/218	ns
P53+	14/49	55/254	ns
Bcl-2+	35/47	182/234	ns
Bcl-2++	26/47	159/234	ns
Bcl-2+++	18/37	104/171	ns
Ki67+	38/69	176/345	ns
SP>7%	13/22	140/285	ns
>2cm	33/74	246/605	ns
>5cm	4/74	18/605	ns
Age	34-86(60,4+/-10,6)	27-88(61,1+/-10,0)	ns
Recurrences	9/67	53/439	ns
Deaths	5/63	18/425	ns

N: lymph node involvement.
M: distant metastasis.
HG: histological grade.
ER: estrogen receptor.
PR: progesterone receptor.
AR: androgen receptor.
SP: cellular synthesis phase.
ns: non significant.

Table 3. Clinical and Biological Differences of *Invasive Non-Ductal Carcinomas* with or without Multifocality

Parameter	Multifocality	Non Multifocality	p
N+	9/22	30/115	ns
N+>3	1/22	11/115	ns
M+	4/22	4/115	0,021
HG3	4/22	10/115	ns
Aneuploidy	5/10	14/58	ns
ER+	11/13	47/59	ns
PR+	9/13	38/59	ns
AR+	6/9	33/39	ns
P53+	0/10	8/46	ns
Bcl-2+	8/10	32/43	ns
Bcl-2++	8/10	26/43	ns
Bcl-2+++	4/6	19/35	ns
Ki67+	6/13	20/56	ns
SP>7%	3/9	22/55	ns
>2cm	8/22	53/115	ns
>5cm	1/22	9/115	ns
Age	46-85 (63,5+/-10,1)	27-85 (60,4+/-11,8)	ns
Recurrences	4/16	3/102	0,0025
Deaths	116	1/100	ns

N: lymph node involvement.
M: distant metastasis.
HG: histological grade.
ER: estrogen receptor.
PR: progesterone receptor.
AR: androgen receptor.
SP: cellular synthesis phase.
ns: non significant.

DISCUSSION

There are different definitions for multifocality, but one of the most accepted is the existence of two or more foci in the same quadrant. We have found this situation in 96 of 818 invasive breast carcinomas (11%). Meanwhile positive levels reported in the literature goes between 13 and 75% [13], our value is similar to Joergensen *et al*. (13.5%) [9] and Coombs *et al*. (11%) [10], higher than Jürgensen *et al*. (6.6%) [17] and slightly lower than Pedersen *et al*. (17%) [18]. Our rate is at the low limits, probably because our hospital is the reference center for breast cancer screening, so it can prevail over the initial stage tumors, as happens to other authors [10]. Multifocality percentage is closely related to the imaging techniques used to detection, from 15% with mammography to higher values (34%) when ultrasound (19) or MRI [19-21] were used, although according to Bendifallah *et al*. [14], MRI could reflect many false positives, therefore, it is not recommended as the first-line technique. In women below 35 years, multifocality percentage is 19% [22].

Table 4. Distribution of Axillary Lymph Node Involvement in Invasive Carcinomas Classified According to Multifocality, Size and Histological Subtype

Multifocal		
	Ductal	**Non-Ductal**
Size	**N+**	**N+**
< 2cm	14/36 (A)	6/15 (B)
> 2 cm	22/38 (C)	3/7 (D)
Non Multifocal		
	Ductal	**Non-Ductal**
Size	**N+**	**N+**
< 2cm	77/298 (E)	8/57 (F)
> 2 cm	169/307 (G)	22/58 (H)

B *vs* F: p:0,043.
E *vs* F: p:0,060.
G *vs* H: p:0,017.
E *vs* G: <0,0001.
F *vs* H: p:0.003.

Table 5. Distribution of Distant Metastasis in Invasive Carcinomas Classified According to Multifocality, Size and Histological Subtype

Multifocal		
	Ductal	**Non-Ductal**
Size	**M+**	**M+**
< 2cm	3/34 (A)	1/16 (B)
> 2 cm	9/40 (C)	3/6 (D)
Non Multifocal		
	Ductal	**Non-Ductal**
Size	**M+**	**M+**
< 2cm	16/331 (E)	2/43 (F)
> 2 cm	49/277 (G)	2/72 (H)

D *vs* H: p:00015.
B *vs* D: p: 0,046.
E *vs* G: p: <0,0001.
G *vs* H: p: 0,001.

When we analyzed clinical and biological differences of invasive breast carcinomas as a unique group, multifocal carcinomas showed, exclusive and statistically, increased distant metastasis and higher death rate. We found no association with size, especially those higher than 5 cm, lymph node involvement, especially more than 3 nodes, premenopause, age <40 years, advanced histological stage and grade, as described by other authors [9, 10, 17]. There is no association between multifocality, tumor location and age, but only with bilaterality [17].

Joergensen *et al*. [9] explain association between multifocality and estrogen receptor (ER) expression. We found no differences when ER using immunohistochemical assays were analyzed, until it is determined in the cytosol by an enzyme immunoassay in 429 cases, 33 multifocal (range 1-605, median 31 fmol/mg prot.) *versus* 396 non-multifocal carcinomas (range 1-1240, median 18.2 fmol/mg prot.), p=0.043. These differences were not observed with Progesterone Receptor (PR). In this regard, Garimella *et al*. [23] using immunohistochemical assays, described small differences in ER expression between different tumor foci, which do not affect the positive/negative final result, while PR showed higher variability.

About relationship between multifocality and disease prognosis, we found bigger number of tumor-related deaths in multifocal, but not the recurrences. In the literature, there is much disagreement, so, some authors relate it with disease-free interval (DFI), but not with overall survival (OS) [9, 24], some found no association with DFI or OS in 5 years monitoring [17, 25], or women age below 35 years [22]; and other authors found positive association with a worse survival [26], both in terms of recurrence and distant metastasis, being a prognostic factor after multivariate analysis [10, 27].

Boyages *et al*. [12], with a 10-year follow-up study, verify that multifocal carcinomas had higher number of tumor-related deaths (p=0.022); however, 10-year survival was not affected by tumor multifocality </= 2cm, but only in those higher than 2 cm, where 10 years survival was 72.1% in unifocal and 54. 7% in multifocal using the higher tumor size (p=0.008), and 69.5% was and not significant, respectively, when tumors were classified adding all malignant foci sizes, the fact that supports the practical relevance of the first criterion.

However, these discrepancies could be due to histology that had not been validated separately, but together. To address this possibility we have analyzed the role of multifocality on histological subtypes. We have seem that in ductal carcinomas, multifocality is exclusively associated with increased lymph node involvement, but not with prognosis and/or evolution; however, in other subtypes, multifocality is related to distant metastasis, increased proliferation, higher recurrence number (See Fig. **1**) and tumor-related deaths. Analyzing the other subtypes, lobular predominated (15 of 22), they were more frequently multifocal, multicentric and with higher size at the time of diagnosis, than the ductal carcinoma, which is frequently associated with positive boundaries after conservative surgery and radiotherapy [28], which may reflect higher biological aggressiveness and validate our findings.

In multifocal carcinomas there were no differences in regional axillary involvement when histological subtype and size were considered. However, in non-multifocal, percentage of axillary node involvement increase with tumor size and was higher in ductal carcinomas than in other histologies. Moreover, infiltrating non-ductal tumors below 2 cm, showed higher axillary lymph node involvement, when they were multifocal.

All this reflects the fact that multifocal carcinomas have "*per se*" better capability to invade lymph nodes, which means could be "more aggressive" independently of tumor size, these details were also suggested by Cabioglu *et al*. [24]. When distant metastatis ability was measured, there were NO differences in terms of multifocal carcinoma histology, increased according to the size of non-ductal; however, in non-multifocal, ductal carcinomas were found to be associated with greater spread and size increasing, which is an opposite behavior observed in the multifocal cases.

Comparing multifocal carcinomas with each other, ductal are associated with increased lymph node involvement, whereas non-ductal with higher number of recurrences and tumor-related deaths. Furthermore, 33 ductal carcinomas had higher cytosolic ER concentrations than 14 non-ductal (range 1-605, median 31 *vs* 1-59, median 13.7 fmol/mg prot., respectively. p=0.004).

Our results suggest: 1) MF was found in 11% of invasive breast carcinomas and was associated with higher distant metastasis and number of tumor-related-deaths. 2) In invasive ductal carcinomas, MF was associated, exclusively, with increased axillary node involvement, whereas in other histological subtypes, with a predominance of lobular, it did with higher distant metastasis, cell proliferation and recurrence number, suggesting in this tumor subtype, where there is higher prognostic/diagnostic value, has the MF presence.

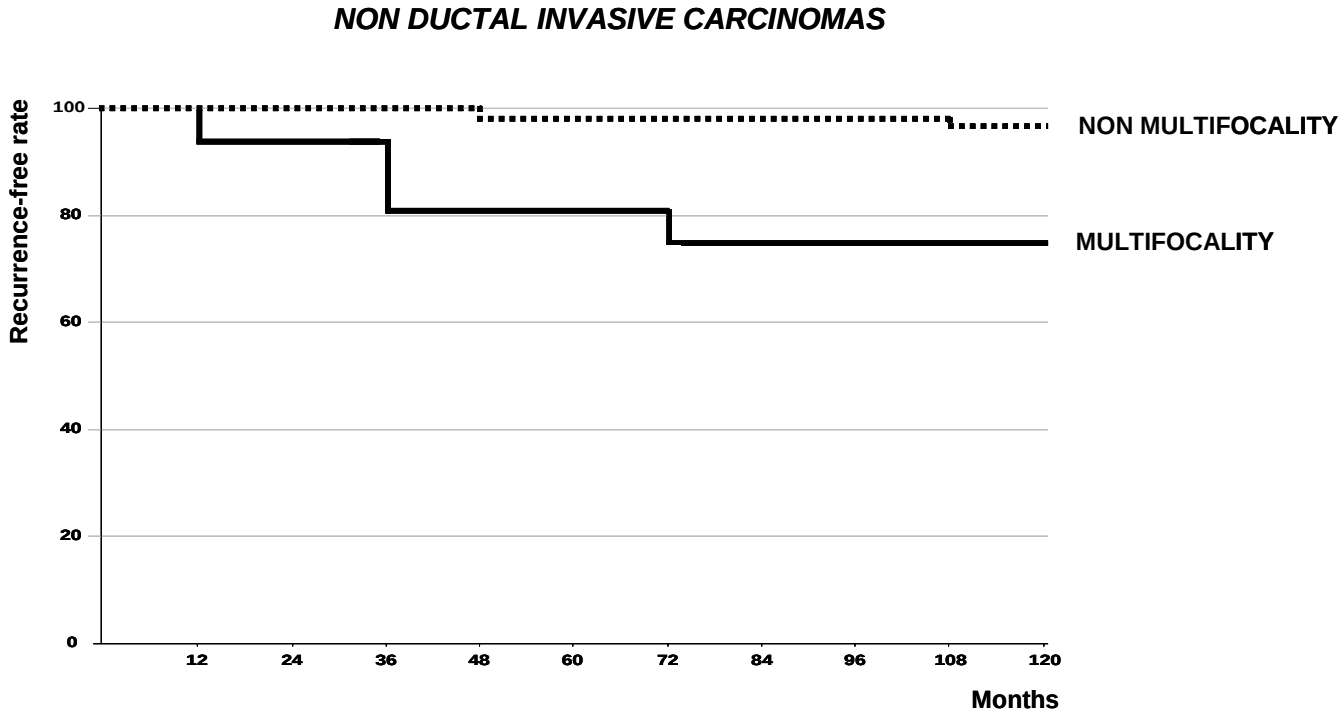

Fig. (1). Recurrence–free survival rates with and without multifocality in patients with non-ductal invasive carcinoma. A significant association between multifocality and recurrence free-rate was shown.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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