Association Between Tumor Size and Immunohistochemical Expression of Ki-67, p53 and BCL2 in a Node-negative Breast Cancer Population Selected from a Breast Cancer Screening Program

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Abstract. *Background/Aim: Breast cancer is the most common type of cancer among women. Breast infiltrating ductal carcinoma (IDC) is the most common type of breast cancer, approximately 80% of all breast carcinomas. The aim of this study was to analyze the association of tumor size, evaluated after histopathological analysis, with different clinical and biological parameters in IDC. Materials and Methods: The study group included 251 women with IDC without axillary lymph node involvement, aged between 27 and 81 years. Analyzed parameters were: age, histological grade, menopausal status, menarche, pregnancy, abortion, breastfeeding, contraceptive use, hormone replacement therapy, estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), Ki-67, p53 and BCL2. Results: Pathological tumor size was between 0.2 and 5.1 cm (1.43±0.86 cm). Tumors in 45 cases exceeded 2 cm, in eight 3 cm and only in one 5 cm. Pathological size was significantly associated with age >70 vs. <50 years (p=0.054), histological grade III vs. I (p=0.0003), positivity for Ki-67 (p=0.0003) and for p53 (p=0.0032). Conclusion: Tumor size was significantly associated with age >70 years, histological grade 3 and immunohistochemically-augmented expression of Ki-67 and p53.*

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Breast cancer is the most common type of cancer among women, accounting for 23% of the total cancer cases and 14% of the cancer-related deaths worldwide (1). Breast infiltrating ductal carcinoma (IDC) is the most common type of breast cancer. About 80% of all breast carcinomas are IDCs.

Tumor size is an important parameter in the biology of malignant tumors. In this regard, we know that tumors detected during screening and the luminal A subtype usually have a smaller size (2, 3), whilst conversely, the triple-negative subtype have a larger size (4). Size is associated with axillary lymph node involvement and distant spread, setting the classic prognostic TNM classification. Histological grade, lymphovascular invasion and hormone receptors status are also important prognostic factors (5-7). In young women, prognostic value is found to be associated with the axillary and molecular subtype, whereas in patients without axillary lymph node involvement, the only prognostic factor was the molecular subtype (8).

Among patients with node-negative disease, increasing tumor size has been associated with increased breast cancer-specific mortality. In addition, increasing tumor size has been correlated with a higher risk of axillary lymph node involvement. Although larger tumor size and increasing lymph node involvement have traditionally been considered independent predictors of higher mortality, tumors which metastasize to lymph nodes early in the disease process (at a small tumor size) may reflect a more biologically-aggressive phenotype, and thus a smaller tumor size may paradoxically be associated with a higher risk of distant spread (9).

Endocrinologically, it is noteworthy that an increase of body-mass index (BMI) and dehydroepiandrosterone sulfate (DHEAS) levels have been associated with larger tumors by

partial correlation, whereas higher androstenedione levels corresponded with smaller tumors (10). Numerous imaging techniques have been used to define tumor size, with no total agreement about its practical usefulness (11, 12), although the recent introduction of breast tomosynthesis appears to be the most effective technique (13, 14).

Less well-known is the association between tumor size and tissue-based tumor markers, including hormone receptors, and new prognostic factors such as p53, BCL2 or Ki-67. These are tumor markers frequently expressed in breast cancer (16, 17).

The aim of this work was to analyze tumor size in patients with IDC without axillary lymph node involvement, in relation to: i) clinicopathological parameters, ii) hormonal receptors and iii) tissue-based tumor markers.

Materials and Methods

Patients. Two hundred and fifty-one women affected by breast IDC without axillary lymph node involvement, aged between 27 and 81 years (mean age=60.1±8.4 years, median 60 years) who had undergone no prior treatment were studied at the Breast Unit at the Monte del Naranco Hospital, Oviedo, Spain. They were selected from a breast cancer screening program from 2000 to 2007. All patients signed informed consent for the sampling and analysis of their tissue for research purposes.

Methods. Pathological tumor size was considered between 0.2 and 5.1 cm (mean size=1.43±0.86 cm; median=1.3 cm). Parameters analyzed were: age, histological grade, menopausal status, menarche, pregnancy, abortion, breastfeeding, contraceptive use, anticonception, hormone replacement therapy (HRT). We also considered immunohistochemical expression of estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), Ki-67, p53 and BCL2.

Immunohistochemical staining of tissue sections of 4-5 microns was performed by the EnVision method with a heat-induced antigen retrieval step. Sections were immersed in boiling 10 mmol/l sodium citrate at pH 6.5 for 2 min in a pressure cooker. ER and PR were determined using monoclonal antibodies to ER and PR phramDx (clones 1D5 and ER-2123 respectively), 1294 for the PR, p53 (DO-7, dilution 1/50; Dako), Ki-67 (MIB-1, dilution 1/200; Dako), BCL2 (Biogenex, dilution 1/150) and androgen receptor (AR441, dilution 1/150; Dako) were used in this study. ER and PR were assessed according to the Allred score as negative (scores 0-2) and positive (score 3-8), and positivity thresholds for p53 and Ki-67 were 20% and 15% , respectively. AR was classified as positive or negative without any score, and BCL2 as negative (–), weakly positive (+) and strongly positive (+ +).

The Windows SPSS software was employed for statistical analysis. Continuous variables with a normal Gaussian distribution are expressed as the mean and standard deviation, while non-parametric variables are expressed by the range and median. We used the Chi-square test with Yates correction, if necessary, for comparison of qualitative variables, and Mann Whitney test for continuous ones. The criteria on for differences to be considered as significant was $p<0.05$.

Results

In the study group analyzed, pathological tumor size ranged from 0.2 to 5.1 cm (1.43±0.86 cm). Tumors in 45 cases exceeded 2 cm; in eight, 3 cm and only in one, 5 cm.

Table I shows tumor size according to the clinicopathological parameters analyzed. Pathological size was significantly associated with age >70 *vs*. <50 years ($p=0.054$), and histological grade III *vs*. I ($p=0.0003$).

Table II shows the relationship between tumor size and the tissue-based tumor markers analyzed. There were significant differences for Ki-67 ($p=0.001$) and p53 positivity ($p=0.006$).

Table III shows the relationship between tumor size and the hormonal receptors analyzed. There were no significant differences when the expression of ER, PR and AR were considered.

Discussion

Tumor size is a classical parameter of tumor biology and is directly related to a greater chance for regional axillary involvement, a greater number of invaded nodes and greater probability of recurrence and death. Its prognostic value can be seen in cases with and without axillary lymph node involvement, being very important in the absence of regional spread because it may help identify patients with high or low risk of recurrence (15-17).

The Surveillance, Epidemiology and End Results (SEER) database included 13,464 women with node-negative breast cancer and patients with tumors less than 1 cm which had a 5-year overall survival (OS) close to 99%, compared to 89% for those with tumors between 1 and 3 cm and 86% for tumors between 3 and 5 cm (18). This association persists with longer follow-up. Rosen *et al*. examined the relationship between tumor size and 20-year recurrence-free survival and found a significant association, with a 20-year recurrence-free survival of 88% for these with tumors ≤1 cm, 72% for those with tumors 1.1 to 3 cm, and 59% for those with tumors between 3.1 and 5 cm (19). Furthermore, the median time-to-development of metastatic disease also shortens when tumor size increases (20, 21).

The more remarkable fact, from a practical point of view, is the relationship between tumor size and axillary lymph node involvement, so both determine different survival. Size is an important prognostic factor, especially in N0 cases, with differences between pT1a-b and pT1c (14). Size was a poor prognostic factor of tumors with HER2/ERBB2 overexpression (22). It is interesting to note that the size of a contralateral tumor is associated with the size of the primary tumor (23). Many relationships have been described between tumor size and different clinical and biological factors, including: BAX, cathepsin D, aneuploidy, Ki-67,

Table I. *Relationship between tumor size and clinicopathological parameters. A p-value of 0.05 was considered to be significant.*

	Tumor size (cm)				
	n	Range	Mean±SD	Median	*p*-Value
Age					
<50 years	32	0.2-3.5	1.6±0.8	1.5	0.054
>70 years	48	0.7-1.3	2.4±1.3	2.0	
HG					
I	71	0.4-4.3	1.2±0.6	1.0	<0.001
III	61	0.3-3.4	1.5±0.7	1.5	
Menarche					
<14 years	154	0.3-8.0	1.5±0.9	1.4	ns
≥14 years	97	0.3-6.0	1.6±1.0	1.4	
Menopause					
Pre-menopausal	33	0.2-8.0	1.2±0.7	1.2	ns
Post-menopausal	212	0.3-8.0	1.5±0.9	1.3	
Pregnancy					
Yes	205	0.3-8.0	1.4±0.9	1.3	ns
No	46	0.2-4.3	1.3±0.8	1.2	
Abortion					
Yes	42	0.4-5.0	1.4±0.9	1.2	ns
No	209	0.2-8.0	1.4±0.0	1.3	
Lactating					
Yes	162	0.3-8.0	1.4±0.9	1.4	ns
No	89	0.3-4.3	1.5±0.8	1.3	
Contraceptive use					
Yes	13	0.6-15	1.2±0.3	1.2	ns
No	238	0.2-8.0	1.4±0.9	1.3	
HRT					
Yes	25	0.5-8.0	1.5±1.5	1.2	ns
No	226	0.2-5.0	1.4±0.8	1.3	

HG: Histological grade; HRT: hormone replacement therapy.

Table II. *Relationship between tumor size and and tissue-based tumor markers. A p-value of 0.05 was considered to be significant.*

	Tumor size (cm)				
	n	Range	Mean±SD	Median	*p*-Value
Ki67					
–	126	0.4-8.0	1.4±0.9	1.2	0.001
+	125	0.2-4.0	1.5±0.7	1.4	
p53					
–	205	0.3-8.0	1.4±0.9	1.2	0.006
+	46	0.2-4.0	1.6±0.8	1.6	
BCL2					
–	94	0.3-4.0	1.5±0.9	1.2	ns
++	157	0.4-8.0	1.5±0.9	1.3	

Table III. *Relationship between tumor size and and hormonal receptor expression. A p-value of 0.05 was considered to be significant.*

	Tumor size (cm)				
	n	Range	Mean±SD	Median	*p*-Value
ER					
–	38	0.3-4.0	1.5±0.9	1.3	ns
+	213	0.3-5.0	1.4±0.8	1.3	
PR					
–	108	0.2-5.0	1.4±0.9	1.3	ns
+	143	0.3-4.3	1.4±0.8	1.3	
AR					
–	90	0.2-4.0	1.6±0.9	1.4	ns
+++	161	0.3-5.5	1.4±0.9	1.3	

ER: Estrogen receptor, PR: progesterone receptor, AR: androgen receptor.

cyclooxygenase 2, mean nuclear area, FOXP3 and immunosuppressive regulatory T-cells. No relation with lymphovascular invasion (FDG), cell-regulatory proteins, matrix metalloproteinases, mammaglobin, hTERT, Wilms gene, tumor vascularity, vimentine, mitotic figure counts and apolipoprotein D has been described. In relation to ERBB2, described results are inconsistent (23).

In the present study, we analyzed possible associations between tumor size and clinicobiological factors commonly used in daily clinical practice in patients with breast IDCs without axillary lymph node involvement, that is, focusing exclusively on the size. Following analysis of 251 cases, a statistically significant association was found between tumor size and age over 70 years, advanced histological grade, high cell proliferation and immunohistochemical expression of p53. With regards to age, we found larger tumor sizes in women over 70 years, of borderline statistical significance, a finding also described by other authors (24). In a previous study, we found that women older than 70 years had an increased tumor size when were from a hospital breast unit, but not if they were from screening campaigns, and this was also confirmed in women between 60 and 70 years, which highlights the importance of the patient's origin and supports the possible cause of greater tumor size being a delayed diagnosis (25).

We found larger tumors with histological grade 3, a fact consistent with that described by other authors (26, 27). Histological grade is a classic parameter of breast tumor biology and is associated with survival and TNM classification (15, 28-30), being of particular relevance in cases without axillary lymph node involvement, which allows patients to be stratified for certain therapies (26). Although histological grade has long been considered a prognostic factor in breast cancer, it was not included in the American Joint Committee on Cancer (AJCC) staging criteria (31). We note, in addition, an association between

increased tumor size and immunohistochemical expression of Ki-67 and p53.

Cell proliferation is a prognostic factor in cases without axillary lymph node involvement (32) and correlates with various biological factors, including p53. Recently, Silvestrini *et al*. demonstrated that tumor cell proliferation is an important predictor of axillary relapse in elderly patients with ER-positive breast cancer and could help to identify patients who should undergo axillary surgery (33). Association between larger tumor size and p53 has been described by others, but not by Temmin *et al*. (34) and Al Joudi *et al*. (35), who found that this behaves as a prognostic factor for some groups. As for histological grade, it could be useful to distinguish risk subgroups in cases without axillary lymph node involvement. By itself, it is useful in T2N0 cases, but not inT1N0 cases (36).

Tumor size is, therefore, associated with certain clinical and biological factors which, at the same time, relate to each other and several other factors that determine a more undifferentiated and aggressive phenotype such as lymphovascular invasion, aneuploidy and loss of hormone-dependence (27).

Conclusion

These results led us to the following conclusion: in breast IDCs without axillary lymph node involvement, tumor size was significantly associated exclusively with age over 70 years, histological grade 3 and increased immunohisto-chemical expression of Ki-67 and p53, all of which support its prognostic value.

Conflicts of Interest

The Authors declare that they have no competing interests.

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