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Short Communication

Size In Breast Invasive Ductal Carcinoma Leads Changes in Important Biological Parameters

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Abstract

Introduction: We analyzed the behavior of certain clinical and biological aspects in breast invasive ductal carcinomas (IDC) when tumor size increased up to three centimeters

Material and Methods: We studied 627 women affected by IDC and aged between 29 and 88 years (median 62), classified into three groups: </ = 0.5 cm: 45; between 0.51 and 1 cm: 180 and 1.1 to 3 cm: 402 cases. The analyzed clinical and biological parameters were: axillary lymph node involvement (N), distant metastasis (M), immunohistochemical expression of estrogen receptors (ER), progesterone receptors (PgR), androgen receptors (AR), p53, Ki67, bcl2, and cytosolic concentrations of cathepsin D and hyaluronic acid (HA), as weel as the epidermal growth factor receptor (EGFR), HA, CD44v5 and CD44v6 in cell membranes

Results: Tumors <1 cm showed no statistical differences among all analyzed parameters. Tumors with size range between 1.1 and 3 cm presented, compared with size less than or between 0.5 and 1 cm, higher concentrations of cathepsin D (p = 0.001) increased expression of p53 (p <0.0001), ki 67 (p <0.0001), CD44v5 (p: 0.008), axillary lymph node involvement (p <0.0001), distant metastasis (p = 0.026), and decreased expression of bcl2 (p: 0.057) and membrane HA (p: 0.051).

Conclusions: Our results suggest that it is from one cm in size when is evidenced in invasive ductal breast carcinomas certain clinical and biological properties source of increased proliferation and tumor aggressiveness.

INTRODUCTION

Size is a key factor in the progress of breast cancer, because many of its invasive-metastatic properties appear as size increases. In addition, with lymph node involvement (N) and distant metastasis (M), set the TNM classification, widely used in daily practice [1]. We know that T1a carcinomas ((<0.5 cm) progress with axillary nodal involvement in 7.7% of cases, going to 12.5% in T1b (0,5-1cm), 29.2 % in T1c and 48.2% in T2 (> 2-5 cm) [2]. In addition, tumors less (or equal) than 1 cm have a very low risk of recurrence, and disease-free survival in cases without axillary lymph node involvement reaches 92-96%, surviving up to 10 years 90% of patients. Numerous studies have demonstrated the prognostic value of tumor size, evidenced in stages 1 with other clinical and biological parameters such as age, lympho vascular invasion and high proliferation [3-6].

We wanted to analyze the impact of size on the biology of breast IDC tumors analyzing the behavior of certain clinical and biological when tumor size was increased up to three centimeters.

MATERIAL AND METHODS

The study group included 627 women with breast invasive ductal carcinonas (IDC) and aged between 29 and 88 years (61.3 +/- 10.3; median 62), from the Breast Pathology Unit of Hospital Monte Naranco Oviedo (Spain). Depending on the tumor size, patients were classified into three groups: <0.5 cm: 45; between 0.51 and 1 cm: 180 and from 1.1 to 3 cm: 402 cases. The analyzed clinical and biological parameters were: axillary lymph node involvement (N), distant metastasis (M), estrogen receptor (ER), progesterone receptor (PgR), androgen receptor (AR), p53, Ki67, bcl 2 , cathepsin D, epidermal growth factor receptor (EGFR), hyaluronic acid (HA), CD44v5 positivity (> 3 ng / mg prt.) and CD44v6 (> 6ng / mg prot.) Breast carcinoma tissue samples were obtained at the time of surgery. Immediately after surgical resection, samples were processed for pathological examination while the remainder tissue was washed with cold saline solution, divided in a liquots, rapidly transported on ice to the laboratory (-70°C) pending biochemical studies. The specimens obtained from neoplastic tissues were pulverized with a microdismembrator

(Braun BiotechInternational, Melsungen, Germany) at -70°C and homogenized in TRIS-hydrochloride buffer (10 mM of TRIS, 1.5 mM of EDTA, 10% glycerol, 0.1% of monothioglycerol). Homogenates, kept at 4°C, were centrifuged at low speed (800 g for 10 min, at 4°C), and the supernatant was ultracentrifuged at 100.000 g for 60 min, at 4°C. We obtained a supernatant containing the cytosol and a precipitate with the membranes. Methods used were the following: CD44v6 and CD44v5 were assayed in cell surface membranes using an enzymoimmunoassay from Bender MedSystems (Vienna, Austria), epidermal growth factor receptor (EGFR) was assayed in cell surface membrane using a radiolig and method (ViennaLab, Austria), hyaluronic acid using a radiolig and method from Pharmacia (Sweden) and cathepsin D was assayed in cytosols using an immunoradiometric assays (CIS BioInternational. France). All results were referred to mg of protein measured by Bradford method [7]. Immunohistochemical expression was studied through the technique of tissue-matrix using Tissue Arrayer Device (Beecher Instruments, Sun Prairie, WI) to set up tissue blocks following conventional protocols [8]. The most representative areas were highlighted in the paraffin blocks and two pathologists performed case evaluation independently. Two selected 1-mm-diameter cylinders from two different areas were included in each case from the carcinomas as well as internal and external control areas. Each block was sectioned in 4 um and the immunohistochemical study was performed on 4 micron paraffin sections, using the Kit with universal secondary antibody that included a labelled-dextran polymer (DAKO EnVision Peroxidase/DAB; Glostrup. Denmark) to avoid false positive reaction due to endogenous biotin activity. Immunohistochemistry expression of Estrogen receptor (ER), progesterone receptor (PgR), androgen receptor (AR), Ki67, p53 and bcl2 were determined using mAbs ER/PR phramDx (clones 1D5 and ER-2-123 for ER and PgR1294 for the PR: Dako; Denmark), p53 (DO-7, Dako. Denmark), Ki67 (MIB-1, Dako; Denmark), bcl2 (124, Dako, Denmark) and androgen receptor (AR441, Dako; Denmark). ER and PgR were assessed according to the Allred score in negative (scores 0-2) and positive (score 3-8) and the thresholds of positivity for p53, Ki67 were 20% and 15 % respectively. AR were classified as positive or negative without any score, and bcl2 as negative (-: <10% stained cells), weakly positive (+: 10-20%) and strong positive (++: >30%).

Data obtained were evaluated using the SPSS 15.0 software for Windows (SPSS, Chicago, IL. USA). With the parameters that did not follow a normal distribution, values were presented as range, and median. We used the Chi square test with Yates correction, if necessary, for qualitative variables comparison and the Mann Whitney test for continuous ones. A p-value ≤ 0.05 was considered as statistically significant.

RESULTS

Analyzing the results, we found that tumors <1 cm showed no statistical differences among all analyzed parameters. However, tumors of size between 1.1 and 3 cm showed, versus those tumors size between 0.5 and 1 cm (Table 1) cathepsin D concentrations higher (p = 0.001), higher expression of p53 (p <0.0001), ki 67 (p <0.0001), CD44v5 (p: 0.008), axillary lymph node involvement (p <0.0001), distant metastasis (p = 0.026), and lower expression of bcl2 (p: 0.057) and HA membrane (p = 0.051).

DISCUSSION

Results show significant clinical and biological changes when tumor size exceeds one centimeter, reflecting:

Table 1: Clinical-biological differences between breast invasive ductal carcinomas with sizes between 0.51 and 1 cm vs size between 1.1 and 3 cm.

Parameter		0,51-1 cm			1,1-3,0 cm
	Nº	RANGE Median	Nº	RANGE Median	p
CATD*	31	1,6-126 (28,3)	294	1,5-1145 (45,2)	0,001
EGFR**	29	0,5-1030 (6,0)	278	0,1-2906 (4,7)	ns
HAc***	25	526-19074(3565)	236	591-430558(4642)	ns
HAcm***	16	449-4925 (2105)	117	50-6043 (1561)	0,051
ER+		147/180	327/402		ns
PgR+		112/180	223/402		ns
AR+		110/133	265/346		ns
p53+		13/149	71/370	<0.0001	
ki67+		18/155	153/402	<0,0001	
bcl2+		113/142	253/355		0,057
CD44v5+		14-Jun	86/110		0.008
CD44v6+		14-Apr	43/110		ns
N+		33/180	305/402		<0,0001
M+		6/180	36/402		0,026

*: pmol/mg prot.
CATD: cathepsin D
**: fmol/mg prot.
***: ng/mg prot.
EGFR: Epidermal Growth Factor Receptor N: lymph node involvement
M: distant metastasis; HAc: Cytosolic Hyaluronic Acid; HAcm: Cell Membrane Hyaluronic Acid

First, an increased tumor proliferation, as evidenced by the highest values of Ki67 [9]. We know that high K67is associated with higher nuclear grade, p53-positive and HER2-positive, being its value higher in triple negative than in other subtypes [10]; likewise it is an independent prognostic factor in invasive [11,12] and in situ carcinomas [13]. Some differences in Ki-67 in tumours of various receptor profiles have been described recently [14]. ER+/ Ki-67+ ratio can be used to differentiate invasive cancers from benign and proliferative breast tumours [15]. Second, an increased cell de-differentiation, reflected by lower concentrations of membrane hyaluronic acid, because we know that is inversely correlated with the histological grade and diploid [16]. Also we can find that the transition from HG1 to HG2 and from HG2 to HG3 was accompanied by a number of common features as global increase in size, greater number of tumor>2cm, decrease in membrane HA levels, increased cell proliferation (SF>7%) and a greater aneuploidy [17].

Third, amore invasive phenotype. We know that HA is an abundant extracellular matrix component and its synthesis is regulated by some growth factors (EGF, TGFbeta) and cytokines (IL 1beta). Increased synthesis of HA is often associated with malignancy in many different tumors including breast cancer [18] and likewise HA is involved in epithelia-mesenchimal transition (EMT) [19]. Increased HA production is able to stimulate the production of MMP2 and MMP9 leading to a more invasive phenotype in certain tumor cell lines [20]. CD44 is a transmembrane glycoprotein, widely expressed in almost all body cell types [21] and plays various functions in cell division, migration, adhesion, and signalling [22]. Its primary ligand is hyaluronic acid (HA). CD44 is an adhesion molecule and mediates the signal transduction of human epidermal growth factor receptor (HER) and other cell signalling pathways. We found increased expression of CD44v5 (> 3 ng / mg prot.) In Invasive ductal breast carcinomas (65.2%) vs DC in situ (25%) and the normal breast tissue (5.9%) [23]. We also found increased expression in tumors larger than 1 cm associated with the metastatic potential [24], with the highest expression of progesterone receptor and with tumor size> 2 cm [25]. In Invasive lobular carcinomas membranous staining of CD44v5 correlated with lymph node positive patients [26]. Also the concentration of soluble CD44v5 was associated with a greater capacity for dissemination [27]. We observed no difference in the expression of CD44v6 while XJ Wu et al., [28] describe a significant positive correlations between CD44v6 immuno positivity, tumour diameter and TNM stage. In relation to p53, we know that the expression of p53 and Ki67 are strong individual indicators of outcome [29]. TP53 mutations confer unfavourable prognosis in patients with Luminal A/B and TNBC tumors, while p53 immunopositivity may predict for trastuzumab benefit in the adjuvant setting [30]. Positive p53 status is associated with large tumors and lymph node metastases [31].

bcl2 is an interesting biological parameter in breast carcinomas. Immuno histo chemical expression of bcl2 in hormone-independent (ER and PgR negative) breast carcinomas is associated with greater axillary lymph node involvement and a greater number of deaths in the follow-up, being these data opposite to that observed in hormone-dependent tumors [32]. Also it is an independent predictor of outcome in basal-like triple-negative breast cancers treated with adjuvant anthracycline-based chemotherapy [33]. Significant and negative relations between bcl2 and Ki67 persisted during the progression of histological grade (HG), from HG1 to HG3, in invasive ductal carcinomas of the breast (IDC) <1 cm [34]. Bcl2/Ki67 combination phenotypes, together with PgR expression, can also refine luminal A cancers prognostic [35]. Ki67/BCL2 index correlated with shorter disease-free survival and overall survival in patients with early stage invasive ductal carcinoma (all $p<0.05$) [36]. Fourth, a greater invasive-metastatic ability, reflected by higher axillary lymph node invasion and distant metastasis, as well as the largest concentrations of cathepsin D. An important aspect is cathepsin D, a protease with important biological functions and involved in invasion -metastatización of mammary tumors [37-40]. We know that correlates with cell proliferation and histological grade III, and that age can influence their associations with hormone dependence [41]. Our results suggest that it is from one cm in size when is evidenced in invasive ductal breast carcinomas certain clinical and biological properties source of increased proliferation and tumor aggressiveness.

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