Histological Grade (HG) in Invasive Ductal Carcinomas of the Breast of Less than 1 cm: Clinical and Biological Associations During Progression from HG1 to HG3

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Abstract. *Aim: To study the clinical and biological (cellular proliferation and hormone-dependence) associations during the progression of histological grade (HG), from HG1 to HG3, in invasive ductal carcinomas of the breast (IDC) <1 cm. Patients and Methods: The study group included 119 women with IDCs ≤1 cm, aged between 27 and 88 years (median=61 years). The parameters analyzed were: histological grade (HG1: 52; HG2: 45; HG3: 22); axillary lymph node involvement (N); distant metastasis (M); and immunohistochemical expression of estrogen (ER), progesterone (PR) and androgen (AR) receptors, and Ki67, p53 and B-cell lymphoma 2 (BCL2). Results: Compared to HG3 tumors, HG1s exhibited an increased expression of ER, AR and BCL2, as well as lower expression of p53 and Ki67. In HG1 tumors, significant (p<0.05) associations were found between ER and PR (positive), ER and p53 (negative), ER and Ki67 (negative), PR and AR (positive), PR and p53 (negative), AR and p53 (negative), p53 and BCL2 (negative), and between BCL2 and Ki67 (negative). HG3s only showed significant (p<0.05) associations between ER and Ki-67 (negative) and between BCL2 or Ki-67 (negative). Only two significant relationships (ER–Ki67 and BCL2–Ki67) persisted in all three grades. Conclusion: Our results lead us to the following conclusions: i) compared HG1, HG3 ductal carcinomas exhibited decreased expression of ER, AR and BCL2 and increased expression of p53 and Ki67; and ii) only two significant and negative relations (ER–Ki67 and BCL2–Ki67) persisted in all three grades.*

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Breast cancer is the most common tumor in women of the Western world (1). With advances in the sensitivity of mammographic screening and the broader population of women screened *via* national programs, presentation of these tumors has changed drastically. A high percentage (70-80%) of them has no axillary lymph node involvement and 25-30% of them are *in situ* carcinomas. Thus, the incidence of small tumors (<1 cm) has increased dramatically.

Histological grade (HG) is a classic parameter in breast cancer and is based on the evaluation of three morphological features (tubule formation, nuclear pleomorphic and mitotic count) essentially describing proliferation and differentiation in breast cancer (2). It is associated with the size and numerous clinico-biological factors, such as axillary lymph node involvement, distant metastases, increased cell proliferation and aneuploidy, and other biological parameters, such as p53,

hormone independence, breast cancer-overexpressed gene 1 (*BCOX1*), *JOXD10* mRNA, *etc*. (3-8). It is associated with prognosis in node-negative cases; likewise, together with the immunohistochemical expression of p53, it allows high-risk subgroups to be defined for systemic adjuvant therapy (9). It is closely associated with breast cancer-specific survival (BCSS) and disease-free survival (DFS), both overall and by stage (pT1a, pT1b, pT1c and pT2) and nodal status (pN0 , pN1 and pN2) (10-11). Recently, Merdad *et al*. identified 1,593 genes differentially expressed in different breast cancer grades in comparison to normal samples (12). Out of these genes, 429 were expressed throughout all grades along with tumor progression, while others were associated with specific grades (440 genes in HG1, 203 in HG2 and 394 in HG3 only). Likewise, matrix metallopeptidase 9 (MMP9) expression was significantly correlated with HG and overall survival. These findings open the possibility of being able to understand better the clinical and biological impact of the histological grade in clinical practice.

In the present study, we analyzed the possible associations of some clinical and biological parameters (cellular proliferation and hormone dependence) during the evolution from HG1 to HG3 in patients with invasive ductal carcinomas (IDC) sized <1 cm with the object of obtaining pathophysiological conclusions in order to confer a better understanding of the disease.

Materials and Methods

Patients. The study group included 119 women suffering from IDCs of ≤1 cm, aged between 27 and 88 years (mean=60.6±8.2 years, median=61 years) from the Breast Unit of the Monte del Naranco Hospital (Oviedo Spain). This study was cleared by our Institution Ethics Review Board for human studies and all patients signed an informed consent form.

Biological parameters. The parameters analysed were: HG (Nottingham-modified Scarff-Bloom-Richardson grading system: HG1: 52; HG2: 45; HG3: 22), axillary lymph node involvement (N) and distant metastasis (M) Immunohistochemistry expression of oestrogen (ER), progesterone (PR), androgen (AR) receptors; Ki67, p53, BCL2, ER and PR were determined by monoclonal antibodies (mAbs) ER/PR PhramDx (clones 1D5 and ER-2-123 for ER and PgR1294 for PR: prediluted), p53 (DO-7, prediluted; Dako, Glostrup, Denmark), Ki67 (MIB-1, prediluted; Dako), BCL2 (124, prediluted; Dako) AR (AR441, prediluted; Dako) and cytokeratin 5/6 (D5/16 B4, prediluted; 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 the thresholds of positivity for p53, Ki67 were 20% and 15%, respectively. AR was classified as positive or negative without any score, and BCL2 as negative (−), weakly positive (+) and strongly positive (++).

Statistical analysis. Windows SPSS software (IBM) was employed for statistical analysis. Continuous variables with a normal Gaussian distribution are expressed as the mean±standard deviation (SD), while non-parametric variables are reported as 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. Wilcoxon correlation test was also used. The criteria for differences to be considered as significant was $p<0.05$.

Results

Due to the importance of the observer at the time of tumor classification according to HG, we have chosen only HG1 and HG3, leaving out HG2. As shown in Table I, compared with HG3, ductal carcinomas of HG1 exhibited increased expression of ER ($p=0.001$), AR ($p=0.002$) and BCL2 ($p<0.001$), as well as lower p53 ($p<0.006$) and Ki67 ($p<0.001$).

We observed, as outlined in Table II, that in mammary carcinomas of HG1, there were significant associations between ER and PR (positive; $p=0.040$), ER and p53 (negative; $p<0.0001$), ER and Ki67 (negative; $p<0.001$), PR and AR (positive; $p=0.036$), PR and p53 (negative; $p=0.002$), AR and p53 (negative; $p<0.0001$), p53 and BCL2 (negative; $p<0.0001$) and between BCL2 and Ki67 (negative; $p<0.0001$). Significant associations between ER and K67 (negative; $p=0.029$) and between BCL2 or Ki67 (negative; $p=0.030$) was found in IDCs of HG3. When considering tumors with HG2, significant associations between ER and K67 (negative; $p=0.043$), PR and p53 (negative; $p=0.012$), AR and p53 (negative; $p=0.012$), p53 and BCL2 (negative; $p=0.002$) and between BCL2 and Ki67 (negative; $p=0.056$) were found. Only two significant relations (between ER and Ki67, and BCL2 and Ki67) persisted in all three grades, although with a decreasing statistical value. Another four associations (ER–Ki67, PR–p53, AR–p53 and p53–BCL2) existed only in HG1 and HG2 tumors, disappearing in HG3.

Discussion

In recent years, the incidence of small tumors (<1 cm) has increased dramatically. Being a group of breast carcinomas with an especially low risk of recurrence, some groups such as pT1a, pT1b and pT1cN0 do not require adjuvant treatment (13-14). Likewise, in pT1a and pT1b tumors with low grade and without vascular invasion, axillary dissection may be omitted. Factors determining the administration of adjuvant therapies (31.5% of the cases) are young age, histological grade 3, high proliferation index, ER negativity and $HER2^+$ status (15). It deserves to be noted that metastatic involvement increases significantly (odd ratios: 6.6) in progression from T1a to T2 tumors (16), Tumor size affects axillary lymph node involvement (17) and is a significant independent prognostic factor in breast cancer, especially in the absence of lymph node metastases, confirming its utility between subgroups pT1a –b and pT1c (18-21).

Table I. *Distribution of clinical and biological parameters according to histological grade (HG1 vs. HG3).*

Parameter	HG1	HG3	*p*-Value
N+	5/52	6/22	ns
N+>3	2/52	2/22	ns
M+	1/52	0/22	ns
ER+	48/52	13/22	0.001
PR+	35/52	11/22	ns
AR+	37/39	11/18	0.002
p53+	2/52	6/22	0.006
BCL2++	44/52	7/22	<0.001
Ki67+	9/52	19/22	<0.001

N: Lymph node involvement, M: distant metastasis, ER: estrogen receptor. PR: Progesterone receptor, AR: androgen receptor, BCL2: B-cell lymphoma 2. ns: No significant differences (p>0.05).

Table II. *Relationships between biological parameters according to the histological grade (HG).*

Association	HG1	HG2	HG3
ER *vs*. PR	(+) 0.040	ns	ns
ER *vs*. AR	ns	ns	ns
ER *vs*. p53	(−) <0.0001	ns	ns
ER *vs*. BCL2	ns	ns	ns
ER *vs*. Ki67	(−) <0.0001	(−) 0.043	(−) 0.029
PR *vs*. AR	(+) 0.036	ns	ns
PR *vs*. p53	(−) 0.002	(−) 0.012	ns
PR *vs*. BCL2	ns	ns	ns
PR *vs*. Ki67	ns	ns	ns
AR *vs*. p53	(−) <0.0001	(−) 0.012	ns
AR *vs*. BCL2	ns	ns	ns
AR *vs*. Ki67	ns	ns	ns
p53 *vs*. BCL2	(−) <0.0001	(−) 0.002	ns
p53 *vs*. Ki67	ns	ns	ns
BCL2 *vs*. Ki67	(−) <0.0001	(−) 0.056	(−) 0.030

(+)/(−): Positive/negative association. ns: No significant differences (p>0.05).

The most widely used histological grading system of breast cancer is the Nottingham grading system. Recently, Rakha *et al*. demonstrated that grade was associated with shorter BCSS and DFS in the whole cohort of patients with breast cancer, as well as in the different subgroups, including those with small-size tumors (T1a, T1b, and T1c), lymph node-negative and -positive tumors and this prognostic value of HG was independent of other prognostic variables, such as size or lymph node stage (17). Daveau *et al*. consider that HG in $HR^+/HER2^-$ group is important to identify patients with poor prognosis and start systematic therapy (22); Lin *et al*., using expression of survivin and histological grade, define, a subgroup of patients with locally advance breast cancer and a overall clinical response to docetaxel-based neoadjuvant chemotherapy (23). Purdom *et al*. suggest that HG does not impact clinical outcome in patients with T1N0 tumors, but in those with T2N0 tumors it might be prognostically significant and relevant, influencing decisions regarding the need for additional adjuvant therapy and optimal management (24). Niemiec *et al*. described the interest of tumor grade and matrix metalloproteinase 2 expression in stromal fibroblast in order to stratify the high-risk group of patients with early breast cancer (25), while Pathmanathan *et al*. observed that Ki67 expression was a prognostic factor in node-negative breast tumor, but not HG (26). A recent study, including 161,708 cases, shows the clinical value of HG as a prognostic factor regardless the number of positive lymph nodes and tumor size (27).

As there is great subjectivity in specifying the HG in such tumors, we compared only HG1 with HG3. We found compared with HG1 tumors, HG3 tumors exhibited reduced expression of ER, AR and BCL2, as well as increased expression of p53 and Ki67. We know that HG was inversely correlated with the expression of hormone receptors and, consequently, with luminal subtypes (4), which supports a similar behaviour for AR and BCL2, as these are associated with hormone dependence and are parameters of good prognosis (28-32). Furthermore, cell proliferation was inversely related to hormone dependence. In a previous study with 229 cases of IDC, considering all patients, we observed that the transition from HG1 to HG2 and from HG2 to HG3 was accompanied by a number of common features: a global increase in size, greater number of tumors greater in size than 2 cm, decreases in membrane hyaluronic acid concentrations, increased cell proliferation and greater aneuploidy (4). Other events observed during the transition from HG2 to HG3 were a decrease in ER, PR, tissue-plasminogen activator and cytosolic hyaluronic acid, parameters related to hormone dependence. This supports our current findings.

When we focused on pT1a and pT1b tumors, we noted that in HG1 tumors there were eight significant associations, being very important in the case of p53 and ER; ER and Ki67; AR and p53, p53 and BCL2, and BCL2 and Ki67. More important was the inverse association between hormonal parameters and cell proliferation, a phenomenon already described in literature (4, 33-34). In HG2 tumors, we found five significant associations (ER and Ki67, PR and p53, AR and p53, p53 and BCL2, and BCL2 and Ki67) existed in HG1 tumors, but with lower statistical significance. In HG3 tumors, there were only two significant associations (ER and Ki67, and BCL2 and Ki67). Only two associations (ER and Ki67, and BCL2 and Ki67) persisted in all three grades, and three (PR and p53, AR and p53, p52 and BCL2) existed in HG1 and HG2 cases and were lost in the progression to HG3.

With evolution from HG1 to 3, associations between different parameters are significantly reduced, and only the inverse associations between hormone dependence (ER and BCL2) and hormonal proliferation (Ki67) were preserved. These findings could contribute to the prognostic value of HG and pT1 tumor size, since hormone dependence and cell proliferation play a key role in the pathophysiology of the tumor (35-36).

Conclusion

Our results lead us to the following considerations: i) compared with HG1, HG3 ductal carcinomas exhibited decreased expression of ER, AR and BCL2, and increased expression of p53 and Ki67; ii) only two significant and negative relations (ER and Ki67, and BCL2 and Ki67) persisted in all three grades.

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