P-Cadherin as Prognostic Factor for Loco-Regional Relapse in Breast Cancer

Caderina-P: Valor Prognóstico na Recidiva Loco-regional do Cancro da Mama

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ABSTRACT

Background: Breast cancer is the most frequent malignant tumor and the leading cause of cancer death in women in Portugal. Due to its relation to an increase in distant metastasis and subsequent death, loco-regional relapse is one major concern in breast cancer women. Several classic prognostic factors as tumour size, nodal stage, histological grade, HER2 status and hormonal receptors have been identified as the most important factors for determining loco-regional relapse, disease free and overall survival. However, there is heterogeneity in prognosis and tumor behaviour in patients with identical disease staging and a similar pattern of expression of known molecular markers, hence the need to discover new prognostic factors. One of the possibilities is P-cadherin, already described by researchers as a possible independent marker of prognosis in breast cancer. The aim of this work was to study in a retrospective series of patients the correlation of P-cadherin expression with loco-regional recurrence in breast cancer women.

Material and methods: We analyzed the clinical records of 1432 consecutive patients with breast cancer and treated in a University Hospital over a 10 year period. Patients with loco-regional relapse (n=101) without prior or simultaneous distant disease were selected as case group. Control group consisted of patients with more than 10 years follow-up and without disease progression. For both groups demographic, clinical, pathological and molecular markers were analyzed. Tissue micro-arrays were constructed to study P-cadherin expression from 86 tumors with available paraffin embedded blocks.

Results: Mean time to recurrence was 41 months and mean survival time after recurrence was 33 months, with a 5-year survival rate of 55%. Tumour size, nodal status and histological grade were identified as independent markers of prognosis. P-cadherin was associated with higher histological grades and hormone negative tumours. P-cadherin was identified as an independent prognostic marker for disease free survival, but not for overall survival.

Conclusion: P-cadherin was related to other known factors of worse prognosis and had an independent relation to disease-free survival. P-cadherin might constitute a novel therapeutic target, but its real biological value is yet to be determined. Doubt persists whether it is an independent marker of tumour behaviour or only a surrogate marker of a set of clinical and molecular features related with worse prognosis.

RESUMO

Introdução: O cancro da mama é o tumor maligno mais frequente e a principal causa de morte nas mulheres em Portugal. Devido à sua relação com a metastização à distância e morte subsequente, a recidiva loco-regional é uma das maiores preocupações no seguimento destas doentes. São conhecidos diversos factores clássicos de prognóstico para recidiva local, tais como o tamanho do tumor, o estádio tumoral, grau histológico, positividade HER2 e a expressão de receptores hormonais. Contudo, existe heterogeneidade no prognóstico e no comportamento do tumor em doentes com estadiamento semelhante e com a mesma expressão de marcadores moleculares de prognóstico. Daí advém a necessidade de descobrir novos factores prognósticos. Uma das possibilidades é a P-caderina, previamente descrita como um possível marcador independente de prognóstico no cancro da mama. O objective deste trabalho foi estudar a correlação da expressão de P-caderina com a recorrência loco-regional do cancro da mama.

Material e métodos: Analisámos os registos clínicos de 1432 doentes consecutivos com cancro da mama e tratados na nossa instituição durante um período de 10 anos. Os doentes com recorrência loco-regional (n=101) sem evidência ou história de metastização à distância foram selecionados como casos. O grupo de controlo consistiu em doentes com mais de 10 anos de seguimento, sem progressão da doença oncológica. Em ambos os grupos foram analisadas variáveis demográficas, clínicas, patológicas e moleculares. Para estudo da expressão da P-caderina, foram construídos Tissue Micro-Arrays a partir de 86 tumores com blocos de parafina disponíveis.

Resultados: O tempo médio livre de doença foi de 41 meses e a sobrevida media após a recorrência foi de 33 meses. A taxa de sobrevivência aos 5 anos foi de 55%. O tamanho do tumor, estadiamento ganglionar e grau histológico foram identificados como marcadores independentes de prognóstico. A P-caderina associou-se com graus histológicos mais altos e tumores sem expressão de receptores hormonais. A P-caderina foi identificada como uma marcador independente de prognóstico para a recidiva livre de doença, mas não para a sobrevivência global.

Conclusão: A P-caderina surgiu associada a outros factores já conhecidos de pior prognóstico e a uma relação independente com a sobrevivência livre de doença. A P-caderina pode vir a constituir um alvo terapêutic a explorar, mas o seu real valor biológico ainda não está determinado. Subsiste a dúvida sobre se a P-caderina é um marcador independente de prognósico ou apenas um marcador de um conjunto de características clínico-patológicas relacionadas com pior prognóstico.

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INTRODUCTION

Breast cancer is the most frequent malignant tumour and the leading cause of cancer related death in woman, with one million cases and half a million deaths each year worldwide.[1] Cumulative individual risk of breast cancer is estimated in 12% (approximately 1 in each 8 women) and the risk of death might be up to 5% (approximately 1 in each 20 women).[2]

Loco-regional relapse of breast cancer is a frequent concern in the treatment of this disease as it has been established as an independent prognostic factor for distant metastasis and subsequent death.[3-6] However, whether it constitutes a cause for distant metastasis or only a marker of an existing risk remains a matter of debate.[5,7,8]

Several clinical and pathological parameters have been used to determine not only prognosis but also the need of adjuvant systemic therapies. The most common of these are: age, size, nodal staging, histological grade, hormonal receptors and HER2 positive.[9-12]

With the development of new microarrays techniques, it became possible to simultaneously analyze thousands of genes and classify tumours according to their profile of genetic expression. As such, a new classification of breast cancer was developed, based on profiles of genetic expression. Five different groups with prognostic differences were identified: luminal A, luminal B, basal, normal-like and HER2.[13]

Although this new classification was based on the hierarchical cluster analysis of genetic expression, some currently available imunohistochemistry markers allow the translation of this classification to the routine pathology practice. Specifically, based on 3 markers (ER, PR and HER2), groups can be divided into luminal type (positive for ER or PR), HER2 positive or triple negative (ER, PR and HER2 negative).[14] The prognostic evaluation of patients in the triple-negative group revealed at least 2 groups of tumors: one of them expressing markers of basal differentiation (CK5, EGFR, P-cadherin) and another without expression of these markers considered unclassified.[13]

Loco-regional relapse is an early and important marker of disease progression. However, regarding patients with identical staging and a similar pattern of expression of molecular markers there is a significant discrepancy in disease progression and prognosis, hence the need to further discover new prognostic factors and stratify risk for disease progression.[12]

One of the molecules used to classify the tumour as basal-like, is P-cadherin, which is associated with increased proliferation and undifferentiated phenotype.[15]

Unlike epithelial cadherin (E-cadherin), P-cadherin expression is usually related to tumorogenic properties, allowing for cellular invasiveness and tumoral aggressive-

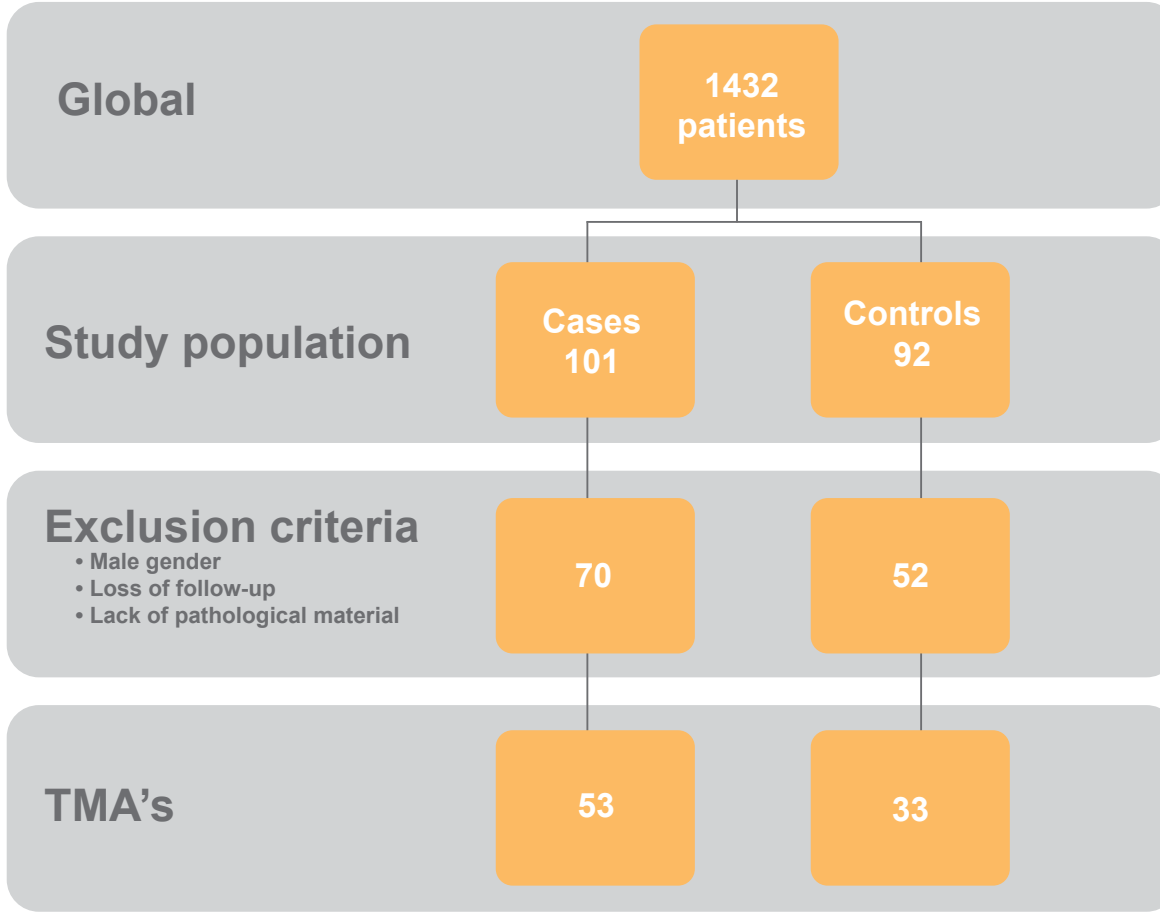

Fig. 1 - Schematic representation of the cohort.

ness, translating into a worst prognosis in breast cancer patients.[16] Its expression is usually associated with other known factors of worse prognosis (high histological grade, high proliferative rate and lack of estrogen receptors).[17,18]

Our objective, in this retrospective series of patients, was to evaluate the correlation of P-cadherin expression in breast cancer loco-regional relapse, disease-free and overall survival.

MATERIAL AND METHODS

We performed a nested case-control study and analyzed the clinical records of 1432 consecutive patients treated and followed at Hospital de São João (University Hospital of Porto Faculty of Medicine) during a 10-year period (January 1st 1997 to December 31st 2006). The case group consisted of all the patients (n=101; 7%) with loco-regional relapse without previous or concurrent systemic progression.

Loco-regional relapse after breast cancer surgery was defined as the onset of histologically confirmed carcinoma at least in one of the following locations: remaining breast tissue; skin, subcutaneous tissue or muscle of ipsilateral thoracic wall; axillary, supraclavicular or internal mammary lymphnodes.[11]

As the majority of loco-regional relapses occurs before 10 years after the initial diagnosis,[19] for control group we selected patients with more than 10 years of follow-up without disease progression: 92 patients surgically treated between January 1st 1997 and June 30th 1998.

Male patients, patients lost to follow-up and those whithout material available for pathological re-evaluation were excluded from the study. Final case group consisted of 70 patients (69.3% of the initial sample) with loco-regional relapse (cases) and 52 patients (56.5% of the initial sample) without disease progression (control group).

Classical clinical and pathological parameters were evaluated in all patients (age, size, type of surgical treatment, TNM staging, histological type, histological grade, presence of associated DCIS, size, lymphatic and venous invasion, Nottingham Prognostic Index [NPI](20) and estrogen receptors. Molecular classification (Luminal, HER2 [HER2(+)/RE(-)/RP(-)], triple negative) and P-cadherin expression were studied using immunohistochemistry in Tissue MicroArrays *(*TMA's*)*.

In our series, only 53 cases and 33 controls had paraffin-embedded blocks available for the construction of TMA's (Fig.1). New sections of the tumour stained with hematoxylin-eosin were undertaken in those blocks. Representative areas were selected and marked for TMA construction.[11]

Representative areas of invasive breast carcinoma were carefully selected on haematoxylin and eosin stained sections and marked on the correspondent individual paraffin blocks. Two tissue cores (2mm in diameter) were obtained from each selected specimen (donor block) and deposited into a paraffin block (receptor block) using a TMA workstation (TMA builder ab1802, Abcam, Cambridge, UK). Twenty-two TMA blocks were constructed, each containing 24 tissue cores (4x6). In each TMA block, non-neoplastic breast and liver tissue cores were also included as controls and TMA guide, respectively. After construction, two 2µm tissue sections were cut and adhered to Superfrost Plus glass slides. An HE stained section from each block was reviewed to confirm the presence of morphological representative areas of the original lesions. Sections were immunostained with primary monoclonal antibodies against ER, PR, HER-2, and P-cad. Immunostaining for ER and HER-2 were performed using the streptavidin-biotin-peroxidase technique (LabVision, Fremont, CA, USA), whereas PR and P-cad immunostaining used the HRP labeled polymer (DakoCytomation, Carpinteria, CA, USA).

Antigen unmasking for ER, PR and HER-2 was carried out using a dilution of 1:100 from a commercially available solution of citrate buffer, pH=6.0 (Vector Laboratories, Burlingame, CA, USA) at 98ºC, whereas a dilution of 1:10 from tris-ethylenediaminetetraacetic (EDTA) solution with pH=9.0 (DakoCytomation) was used for P-cad.

Antigen retrieval time, antibodies, dilutions and suppliers are listed in Table 1. After antigen retrieval procedure, slides were washed in a phosphate buffer solution (PBS), and submitted to blockage of the endogenous peroxidase activity by incubation of the slides in a 3% hydrogen peroxide (Panreac, Spain) in methanol (Sigma-Aldrich). Slides were further incubated with a blocking serum (LabVision Corporation kit) for 15 min and then incubated with the primary antibodies. After washing, slides were incubated with biotinylated secondary antibody, followed by streptavidin-conjugated peroxidase (LabVision). Diaminobenzidine (DAB) was used as a chromogen (DakoCytomation). For PR and P-cad staining, secondary antibody was associated with HRP labelled polymer (DakoCytomation) and immediately revealed with DAB. Tissues were then counterstaining with Mayer`s haematoxylin, dehydrated and coverslipped

Table 1 - Sources and dilutions of primary antibodies used in this immnohistochemistry

Antibody	Clone	Manufacturer	Incubation time (m)	Dilution	Antigen retrieval (m)
ER	SP1	Neomarkers	30	1:150	30
PR	1A6	Neomarkers	30	1:40	30
HER-2	SP3	Neomarkers	30	1:80	30
P-cad	56	Transduction	60	1:50	30
CK5	XM26	Neomarkers	60	1:50	30

Table 2 - Characterization of the clinical and pathological pattern according to group.

	Cases (n=70)	Controls (n=52)	*p*
Age	N(%)	N(%)	0.84
Age	53.9 (± 16.3)	53,4 (± 11.5)	
Type of Surgery			< 0.001
Lumpectomy	13 (18.6%)	27 (51.9%)	
Mastectomy	57 (81.4%)	25 (48.1%)	OR – 1.69 (1.25 – 2.29)
Surgical Margin			0.751
Distance to margin (mm)	7.95 (± 12.11)	8.69 (± 10.47)	
Chemotherapy			0.059
No	25 (39.1%)	17 (34.0%)	
Adjuvant	22 (34.4%)	27 (54.0%)	
Pre-operative	17 (26.6%)	6 (12.0%)	
Adjuvant Radiotherapy			0.103
No	44 (62.9%)	25 (48.1%)	
Yes	26 (37.1%)	27 (51.9%)	
Hormonal treatment			0.003
No	31 (44.3%)	8 (17.4%)	
Yes	39 (55.7%)	38 (82.6%)	OR = 0.67
TNM Staging			< 0.001
I	6 (8.6%)	23 (45.1%)	
IIa	19 (27.1%)	20 (39.2%)	
IIb	18 (25.7%)	7 (13.7%)	
IIIa	11 (15.7%)	1 (1,9%)	
IIIb	16 (22.9%)	0 (0.0%)	
Histological grade			< 0.001
1	1 (1.5%)	13 (26.5%)	
2	36 (54.5%)	26 (53.1%)	OR = 1.46 (1.16 - 1.83)
3	29 (43.9%)	10 (20.4%)	OR = 2.22 (1.39 – 3.56)
T			< 0.001
1	15 (21.4%)	30 (58.8%)	
2	31 (44.3%)	18 (35.3%)	OR – 1.80 (1.18 – 2.72)
3	8 (11.4%)	3 (5.9%)	OR – 3.82 (1.13 – 12.9)
4	16 (22.8%)	0 (0.0%)	OR – 2.08 (1.43 – 2.97)
N			< 0.001
0	15 (23.9%)	37 (72.5%)	
1	38 (60.3%)	14 (27.5%)	OR = 2.6
2	9 (14.2%)	0 (0.0%)	
3	1 (1.6%)	0 (0.0%)	
Lymphatic invasion			< 0.001
No	21 (33.3%)	38 (77.6%)	
Yes	42 (66.7%)	11 (22.4%)	OR = 2.97
Venous invasion			< 0.001
No	42 (57.7%)	48 (98.0%)	
Yes	20 (32.3%)	1 (2.0%)	OR = 15.8
Nottingham Prognostic Index			< 0.001
Average NPI	5.56 (± 1.42)	3.80 (± 0.98)	
ER			0.004
Neg	25 (42.4%)	6 (15.0%)	
Pos	34 (57.6%)	34 (85.0%)	OR = 0.68
Molecular Classification			0.167
Luminal	46 (70.7%)	41 (85.4%)	
HER2	6 (9.2%)	3 (6.3%)	
Triple Negative	13 (20.0%)	4 (8.3%)	
P-cadherin			0.17
Negative	29 (54.7%)	23 (69.7%)	
Positive	24 (45.3%)	10 (30.3%)	OR = 1.49

Table 3 - Logistic regression for loco-regional relapse

	p	OR
Type of surgery	0.241	0.307
Histological grade	**0.049**	**3.802**
T	**0.034**	**4.672**
N	**0.014**	**8.849**
Lymphatic invasion	0.251	3.215
Venous invasion	0.495	2.857
ER	0.530	0.464

Table 4 - P-cadherin expression according to other markers of prognosis

P-cadherin	Positive (n=34)	Negative (n=52)	*p*
Molecular sub-type			**0.002**
Luminal	19 (55.9%)	46 (88.5%)	
HER2	7 (20.6%)	2 (3.8%)	
Triple negative	8 (23.5%)	4 (7.7%)	
MIB-1			**0.003**
Positive	16 (52.9%)	9 (82.7%)	
Negative	18 (47.1%)	43 (17.3%)	
TNM stage			**0.807**
I	7 (20.6%)	8 (15.4%)	
IIA	11 (36.4%)	15 (28.8%)	
IIB	5 (14.7%)	13 (25.0%)	
IIIA	4 (11.8%)	7 (13.5%)	
IIIB	7 (20.6%)	9 (17.3%)	
Histological grade			**0.008**
1	4 (12.1%)	5 (10.0%)	
2	10 (30.3%)	32 (64.0%)	
3	19 (57.6%)	13 (26.0%)	
T			**0.325**
1	12 (35.3%)	15 (28.8%)	
2	14 (41.2%)	20 (38.5%)	
3	1 (2.9%)	8 (15.4%)	
4	7 (20.6%)	9 (17.3%)	
N			**0.779**
0	13 (39.4%)	17 (36.2%)	
1	16 (48.5%)	25 (53.2%)	
2	4 (12.1%)	4 (8.5%)	
3	0 (0.0%)	1 (2.1%)	

using a permanent mounting solution (Zymed, San Francisco, CA, USA).

Positive controls were included in each run, to guarantee assay reliability. All cases showing an unequivocal nuclear staining for ER and PR in at least 10% of the neoplastic cells were considered positive. We also considered positive cases with membranous staining for P-cad and in at least 10% of the neoplastic cells. HER2 expression was evaluated according to the DakoCytomation HercepTest scoring system. Cases were considered positive (overexpression) for HER2 when immunostaining was classified as 3+. All the samples were blinded and reviewed by the same experienced pathologist.

Statistical analysis was done using SPSS 15.0 (SPSS Inc. Chicago, Illinois, USA). The chi-square contingency test was used for categorical variables and the t-student was used for continuous variables. A p value of less than 0.05 was considered to reflect a significant association. The multivariate analysis was performed with a model of binary logistic regression. The time-dependent variables were analyzed with the Cox regression model and the Kaplan-Meier curves were based on life tables. For the multivariate regression models, we selected the variables with significant association with the outcome on univariate analysis and in the Cox regression model, we also included the type of systemic treatment to check for potential confounding on the effect of P-cadherin.

RESULTS

Mean age at diagnosis was 53.7 years. Mastectomy was the type of surgery performed on the majority of patients (67.2%) and 77% of the patients were classified as stage I or II, according to TNM classification. Predominant histological type was invasive ductal carcinoma (87%) and half the patients (50.8%) had grade II carcinomas (Nottingham grading system). Forty-seven percent of patients had lymphatic invasion and only 19% had venous invasion. Axillary staging was negative (N0) in 45.6%. ER expression was positive in 68.7% of the patients and PR in 52.3% of the patients. The majority of local relapses occurred in the remaining breast tissue or in the thoracic wall (73%) and 56% of the cases were re-excised. After 93 months of mean follow-up, 69% of the patients are alive.

The expression of classical prognostic factors is listed in Table 2. Mastectomy was associated with higher rates of loco-regional relapse but also with the expression of several markers of worse prognosis (larger tumours [70% T1/T2 vs 92.5% for breast conserving surgery; *p*=.02]; nodal metastasis [67.5% vs 27%; *p*<.001], lymphatic [60.5% vs 19.4%; *p*<.001] and venous [24.0% vs 8.3%;*p*=0.05] invasion and higher TNM stages [29.6% stage III/IV vs 10.0%; *p*=.001]).

The specimen's surgical margins were not different between the groups and post-operative radiotherapy was not associated with a decrease in local relapse risk.

Staging was directly associated with relapse risk, as well as histological grade and NPI index. The presence of lymphatic and venous invasion was also strongly associated with loco-regional relapse. Expression of ER was identified as a marker of better prognosis.

Molecular classification was achieved by the use of routine immunohistochemistry and tumours were divided into 3 categories (ER or PR positive, HER2 overexpressing or triple-negative). The majority of patients expressed luminal type markers in both groups (70.7% of the cases vs. 85.4% of the controls). Triple-negative tumours were more frequent in patients with loco-regional relapse (20% vs. 8%) although this value did not reach statistical significance (Table 2).

The logistic regression model (Table 3) identified histological grade, size and nodal invasion as independent markers of prognosis for loco-regional relapse. Once corrected for other prognostic factors, the type of surgery was no longer related with loco-regional relapse.

P-cadherin was positive in 45.3% of cases and 30.3% of controls (*p*=0.17), OR=1.49. There was a positive relation of P-cadherin expression with the non-luminal molecular types and with higher proliferative index (*p*=0.003) as measured by MIB-1. There were no significant relations between P-cadherin expression and other prognostic markers, with the exception of higher histological grade. (Table 4)

P-cadherin expression was related to a significant decrease (*p*=0.017) in disease-free survival, from 90.5 months to 55.2 months (Fig.2). However, these earlier recurrences were not related with a decrease in overall survival (135.5 months vs 136.2 months – Fig.3), despite the differences observed in the 5-year survival rate (82.7% vs 58.3%).

Multivariate analysis of prognostic factors for disease-free survival (Table 5) identified P-cadherin expression as an independent factor of prognosis, (HR=2.1) together with the known classical factors of prognosis: tumor size, nodal staging and histological grade. For overall survival the only identified independent factors were tumor size and histological grade.

DISCUSSION

The research around new molecular markers has rised tremendously not only because they have the capacity to add some information and enhance discriminant power to scores already available[12] with classical markers but also because they can bring some new understanding over the oncological biology or arise as new putative therapeutic targets.[21]

The major limitation of this study is the shortness of the sample, as we could only retrieve 86 tumors for TMA construction. Additionally, this is a retrospective study with a 10-year span and during this period the treatment of breast cancer suffered significant variations.

Table 5 - Cox regression – Overall survival and disease free survival

	Disease-free survival		Overall survival	
	p	HR [95%CI]	p	HR [95%CI]
P-cadherin	**0.047**	**2.108** [1.009; 4.402]	0.129	2.087 [0.807; 5.395]
T	**0.004**	**1.822** [1.217; 2.729]	**0.003**	**2.317** [1.325; 4.053]
N	**< 0.001**	**2.780** [1.609; 4.802]	0.061	1.957 [0.969; 3.954]
Grade	**0.001**	**3.326** [1.666; 6.643]	**< 0.001**	**8.541** [3.188; 22.883]
Molecular class	0.336	0.870 [0.643; 1.178]	0.093	0.688 [0.445; 1.064]
Chemotherapy	0.632	0.891 [0.556; 1.427]	0.270	0.702 [0.374; 1.316]
Hormone therapy	0.234	0.668 [0.344; 1.298]	0.831	0.909 [0.378; 2.188]
Anti-HER2 therapy	0.903	1.057 [0.431; 2.591]	0.980	1.017 [0.275; 3.764]

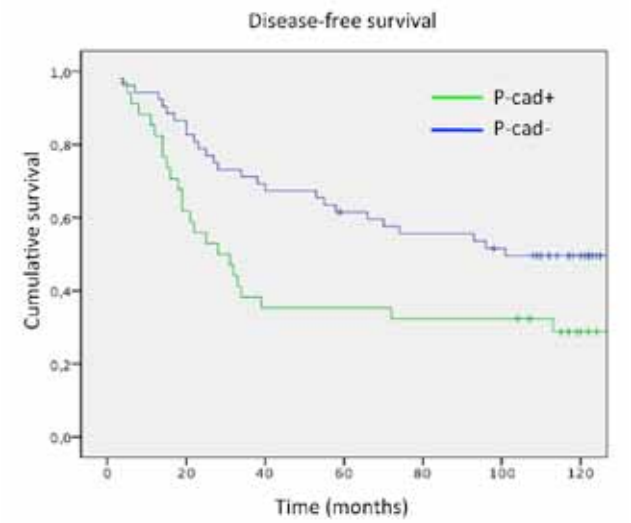

Fig. 2 - Kaplan-Meier curves for disease free survival according to P-cadherin expression.

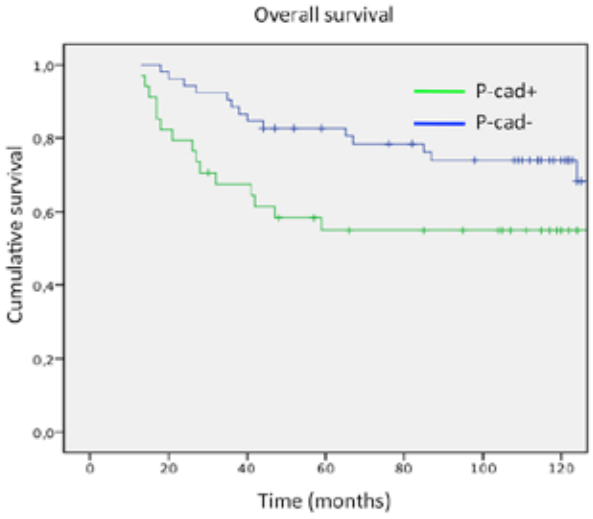

Fig. 3 - Kaplan-Meier curves for overall survival according to P-cadherin expression.

Several studies have reported the risk of local recurrence after breast cancer treatment as being 5-40%.[11,22] Despite therapeutic improvements in the last decade, 40% of the women with local recurrence will have disease progression and eventually death. In our series, local recurrence rate was 7% (101/432) and 54% of these women died from breast cancer, as in most clinical reported studies.[22,23]

Several studies have reported either a similar[24] or increased survival[25] with breast conserving surgery when compared to mastectomy. In our series, breast conserving surgery has a longer median survival (132 vs. 64.5 months for mastectomy). Mastectomy is also related to an increased risk of local relapse (OR = 1.69). However, these results maybe the consequence of a a selection bias, as tumors of patients who had mastectomy, in our series, presented with features of worse prognosis (size, nodal metastasis, histological grade, TNM staging and NPI). Once corrected for these factors, the benefit of conservative surgery is no longer detectable.

Tumor size (p=0.002) and nodal staging (p<0.001) were two important factors of prognosis for local recurrence, which confirms the data of several other studies,[22,26,27] and patients with tumors larger than 5cm had a 4-fold increase in local recurrence as compared with tumors smaller than 2cm (OR=3.82).*4* Also as described in the literature,[28,29] patients with axillary invasion had an almost 3-fold inrease in local recurrence as compared to patients with node-free disease (OR=2.6). According to some authors, axillary invasion might be not just an event related to tumor progression, but a biological marker of tumor aggressiveness[27] independently of tumor size, recurrence type or time-to-recurrence. Also according to several studies,[5,11] there was a significant relation between high histological grade and local recurrence (OR=1.46 for Grade 2 and OR=2.22 for Grade 3; p<0.001). Regarding all well-known factors our results were identical to others of similar series.

In one of the first studies about P-cadherin expression in breast cancer, the molecule was only identified in 4% of invasive breast cancers. In the following studies, its expression was observed in approximately 20% of tumors and inversely related to E-cadherin expression and directly to higher histological grades.[17] With the development of anti-P-cadherin monoclonal antibodies its expression was registered in 30% to 50% of all the invasive ductal cancers.[15,30-32] In our series, P-cadherin was expressed in 39.5% of all cases. P-cadherin was more often positive in patients with local recurrence (OR=1.49; p=0.1), although without a statistically significant difference.

Several studies reported that the P-cadherin expression in cancer cells was directly related to other known factors of worse prognosis, such as: tumor size;[30] histological grade;[17,30,32] ER negativity[17,30,32] and nodal metastization.[30] In multivariate analysis only relation with nodal metastization and histological grade has kept significance.[30] Other reports found no association between P-cadherin expression and tumor size or axillary invasion.[32] These conflicting reports and differing association with known prognostic factors, suggests that P-cadherin might be related to oncological progression of breast cancer, but its real biological behavior is not yet determined.[30] In our series, P-cadherin expression was directly related only with histological grade and ER status.

Several other reports have shown a direct relation of P-cadherin with other known factors of worse prognosis, such as triple-negative type[33-35] and proliferative index.[32,36] In this study, we also confirm these findings of a direct relation between P-cadherin expression and triple-negative tumors (p<0.001) and higher proliferative index (p=0.003), as measured by MIB1.

Several reports observed an inverse relation between P-cadherin expression and hormonal receptors. Most of the P-cadherin expressing tumors lack hormonal receptors expression[17,31,32,37,38] and are positive for HER2, EGFR, higher histological grades and proliferative index, which are asso-

ciated with worse prognosis.[17,30-32] These authors suggest that the hormonal negative state is a requirement to the expression of P-cadherin, probably through the differentiation of luminal type cells into myoepithelial cells where P-cadherin is usually expressed.[32] It has been suggested by some authors that P-cadherin expression in breast cancer cells might represent the differentiation in an embrionary phenotype, similar to the ductal-extremity cells, which are highly proliferative, negative for ER and positive for P-cadherin.[17] Our results, as other before,[30] support this hypothesis as P-cadherin expression was found more often in high histological grade and ER negative cancers.

Although some studies described impairment in survival for patients with P-cadherin expression, in multivariate analysis,[30,31,37] our results only confirm a reduction in disease-free survival (Cox regression; p=0.047), without differences for overall survival (p=0.129). Nevertheless, the Kaplan-Meyer survival curves suggest that there is an effect of P-cadherin on survival, visible at 5-years follow-up and fading progressively, nearly unnoticed at 10 years. Similar data were reported in other studies,[30-32] suggesting this fade-out of effect in long-term follow-up, which explains the lack of association with overall survival but the significant differences of survival at 5 years (82.7% vs 58.3%). More studies directed to the underlying pathophysiology of P-cadherin will be necessary, in order to unravel this effect and to understand the molecular mechanisms and signaling involved in this process.

CONCLUSION

Breast cancer is one of the most prevalent diseases worldwide, being the leading cause of death for cancer in women.[1] In the last few years, the mortality due to breast cancer has been following a downward trend, due to better screening programs and most effective medical care.[39] Local recurrence has been described as a marker of disease progression and an important risk factor for death.[3] As a consequence, several studies have tried to identify risk factors for local recurrence.[11]

One of the most promising markers for loco-regional disease progression seems to be P-cadherin and in the future, it might even constitute a novel therapeutic target.

P-cadherin, in our study was related to other known factors of worse prognosis, was more frequent in non-luminal type tumors and had an independent relation to disease-free survival. Although it did not affect overall survival or relapse rate, it seemed to be associated with earlier relapse and mortality.

The real biological value of P-cadherin is still undetermined raising the question to whether it has an independent relation to tumor behavior or if it constitutes just an indirect marker of a group of clinical and molecular characteristics related to worse prognosis.

CONFLICTS OF INTEREST

The authors declare there are no conflicts of interest.

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