Results: In our database 7.0% of patients were younger than 40 year of age, 41.0% and 38.4% were respectively grade 3 and LN positive; 15.4% of breast cancers in our institution were ER− and 11.6% were HER-2+. HER-2 over-expression correlated with tumour grade only in ER+ tumours ($p < 0.0001$, Mantel-Haenszel χ^2). HER-2 was also related with LNI involvement (10.1% in LN− and 13.8% in LN+ cases; $p = 0.0038$, χ^2). Although more women in the ER− '4 or more lymph node' group were HER-2+, this figure was not significant ($p = 0.2894$, χ^2). However, in ER+ cases, HER-2 was related to the degree of LN involvement ($p = 0.0022$, χ^2). Also, tumours with an ER+HER-2+ phenotype were more likely LN+ than tumours of any other ER-HER-2 status.

Conclusion: The risk of LN involvement among operable HER-2+ breast cancers is associated with the ER status.

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High preoperative plasma TIMP-1 is prognostic for early relapse in primary breast carcinoma

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TIMP-1 is a natural inhibitor of extracellular matrix degrading enzymes called matrix metalloproteinases (MMPs). In addition to its capacity to inhibit matrix degradation, TIMP-1 has been shown to promote cell growth and inhibit apoptosis. The expression of TIMP-1 in tumour tissue, as well as in circulating blood, has therefore been shown to associate with worsened survival in several malignancies.

In this study, a prospective series of 213 patients with primary breast carcinoma was assessed. Circulating pre- and postoperative TIMP-1 levels were assayed using ELISA analysis.

It was shown that high preoperative plasma TIMP-1 was a powerful predictor of systemic early relapse in breast carcinoma, with HR 8.1 (95% CI 1.8−37.6) ($P = 0.007$) as a log-transformed continuous variable in Cox regression univariate analysis. It was shown to be independent of, and superior to, nodal status as a prognostic variable in multivariate analysis, and not associated with any known prognostic clinicopathological parameters. Kaplan–Meier analysis showed that the patients belonging to the highest quartile of circulating TIMP-1 levels had a worsened recurrence-free survival of 79% compared to 94% RFS among patients in the lower quartiles ($P = 0.016$).

The postoperative levels of circulating plasma TIMP-1 were not found to be prognostic for relapse.

In conclusion, preoperative plasma TIMP-1 was found to be a powerful prognostic factor for early systemic relapse in primary breast carcinoma.

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EGFR genetic mutations of exons 19 and 21 are rare in male breast carcinoma

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Background: Breast carcinoma is a rare male neoplasm with poor outcome, despite its histological low grade features. We studied 12 cases of male breast carcinoma (MBC) to evaluate over-expression and genetic mutations of EGFR and Her 2/neu comparing clinical characteristics.

Material and Methods: All cases of MBC were collected in the period between 2000–2006. Histological features were reviewed; immunophenotyping analysis was performed for ER, PgR, Her 2/neu EGFR, and ki-67 with avidin-biotin method as previously described. Her 2/neu/CEP17 sonde from PathVysis was used for FISH assay. Genomic DNA was extracted and used to assay EGFR mutation analysis of exons 19 and 21 by PCR and confirmed by automated fragment analysis (PE Applied Byosystems, Foster City, CA) using fluorescent-labeled primers.

Results: Twelve cases were identified with a median follow-up of 17months (1−35mo). The mean age was 68yr (48−87yr). Ten patients (83%) showed advanced clinical stages; 5 patients (41.7%) presented lymph node involvement. The median size of the tumors was 4.5 cm (2−9 cm).Ten cases were ductal invasive carcinoma (83.3%), one case was papillary carcinoma (8.3%), and one case was mixed secretory and ductal carcinoma (8.3%). ER was expressed in 83.3%, PgR in 66.7%, Her 2/neu in 8.3%, EGFR in 25%, and ki-67 in 50% of the cases. None FISH amplification was found. Eleven cases amplified by PCR of EGFR; all amplified cases showed wild type sequences of exons 19 and 21 by fragment analysis.

The outcome showed: 5/12 patients (41.7%) were death with disease, 2/12 (17%) were alive with disease, and 5/12 patients (41.7%) were alive without disease. Large tumor size or advanced clinical stage were associated with poor outcome in 5/12 (41.7%) patients. Over-expression of EGFR was related with bad prognosis in 1/12 patient (8.3%), and ki-67 in 4/12 patients (25%).

Conclusions: Over-expression and genetic mutations of EGFR are two uncommon phenomena in MBC. The prognostic factors involved with poor outcome are related with clinical and histological aspects. In our study, the expression of ki-67 was associated with bad prognosis. More studies are necessary to establish different prognostic factors and new treatments.

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EGFR analysis in metaplastic carcinoma of the breast

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Background: Metaplastic carcinoma (MC) of the breast is considered a triple-negative receptor carcinoma, which over-expresses EGFR in 80%, and gene amplification is observed in 25% of the cases. EGFR is regulated by other proteins like ph-EGFR. We studied different molecular aspects of EGFR in a group of MC, in order to recognize alterations at transcriptional or regulatory protein levels.

Material and Methods: In a group of 22 MC, we assessed IHC studies for ER, PgR, Her 2/neu, EGFR and ph-EGFR with avidin-biotin method as previously described. Genomic DNA was extracted and used to assay EGFR mutation analysis of exons 19 and 21 by PCR and confirmed by automated fragment analysis (PE Applied Byosystems, Foster City, CA) using fluorescent-labeled primers. Sequencing was performed in suspicious mutant cases (PE Applied Byosystems, Foster City, CA).

Results: The assessment of immunophenotyping was negative for both hormonal receptors, and Her2/neu in all cases. The 100% of the cases were positive for EGFR with high intensity in 13/22 cases and moderate intensity in 9/22 cases. The expression of ph-EGFR was positive in 11/22 cases (50%), 9 cases were mixed tumors, and 2 cases were purely epithelial neoplams. Twenty of 22 cases amplified by PCR, and a 316bp band in the electrophoresis gel was observed. The fragment analysis of exon 19 showed 5/20 cases (25%) with two peaks at 192 and 207bp. Exon 21 was wild type in all cases. The sequencing of 1/5 cases (20%) presented a deletion in exon 19 of 15bp, and 4/5 cases (80%) presented wild type sequences. The case with genetic deletion was purely epithelial type, and negative for ph-EGFR; the 4/5 cases with wild type sequences were positive for ph-EGFR, 3 were mixed type and one case was purely epithelial tumor.

Conclusions: MC of the breast over-expresses EGFR and is a triple-negative receptor carcinoma. In our study, we observed the mutations of EGFR are rare (4.5%), but the high expression of ph-EGFR (50%) may explain the signalling of EGFR. EGFR presents different alterations at transcriptional levels, and regulatory proteins also are involved in over-expression in MC of the breast. New treatments may focus on different pathways of EGFR expression.

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Semi-quantitative transcript analysis of Portuguese breast/ovarian cancer families with the BRCA1 founder mutation R71G of Galician origin

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Background: We identified three unrelated Portuguese breast/ovarian cancer families with the c.211A>G (R71G) variant in the BRCA1 gene. In order to evaluate the functional effect of this variant localized at position −2 of the exon 5 donor splice site, we performed semi-quantitative transcript analysis. Furthermore, we have evaluated whether Portuguese and Galician families with this mutation share a common ancestry.

Material and Methods: RNA analysis of three carriers and control individuals was performed by RT-PCR. All amplification fragments were sequenced and semi-quantitative fragment analysis of the RT-PCR products was performed. Furthermore, segregation and loss of heterozigozity (LOH; in a patient with bilateral disease) analyses were performed. Histopathologic data from one proband with bilateral carcinomas and from an affected family member of a second family were obtained from medical records. BRCA1 haplotype analysis was performed using six microsatellite markers in the three Portuguese families and in one Galician family with the same R71G BRCA1 mutation.

Results: From both mutation carriers and controls, three RT-PCR products were obtained: one corresponding to the full length transcript with the expected size (214 base pairs (bp)), another with 192bp corresponding to a deletion of 22bp of exon 5 (BRCA1-Δ22ntex5), and a third with 134bp corresponding to the in frame skip of exon 5 (BRCA1-Δex5). Semi-quantitative fragment analysis showed a relative amount of BRCA1-Δ22ntex5 more than eight-fold higher in patients and only the wild type allele was present in the full length transcript. The haplotype identified in the three Portuguese families and in the Galician family is compatible with a common origin of this mutation. The mutation segregates with the disease in the family with two affected members. Of the three breast cancers, one was an atypical medullary carcinoma and two were invasive ductal carcinomas with medullar features. All breast carcinomas were grade III and two of them were hormone receptor negative (data not available from the third case). No LOH was detected.

Conclusions: We conclude that disruption of alternative transcript ratios is the mechanism causing hereditary breast/ovarian cancer associated with the BRCA1 R71G mutation, and segregation and histopathologic data are consistent with its pathogenicity. Furthermore, our findings indicate a common ancestry of the Portuguese and Galician families sharing this mutation.

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Heat Shock Protein 60kDa in breast cancer tissue and cell lines

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Background: Breast cancer has been reported as the most common cancer of women in U.S.A., Western Europe and Korea. Breast cancer is curable with an early diagnosis, and many researchers have made efforts to find a marker for this malady. Heat shock protein (HSP) consists of 6 groups, it is highly preserved throughout both the prokaryotic and eukaryotic cells and it acts as a molecular chaperone that is involved in protein folding. HSPs have been recently reported to be related with breast cancer. In this study, we investigated the changes of expression of HSP60 in breast cancer tissues and cancer cell lines.

Materials and Methods: We obtained breast cancer tissues and normal tissues from twenty breast cancer patients, and we purchased several cancer cell lines from ATCC. We treated the human breast cancer tissues and cancer cell lines with heat shock protein. Proteins and mRNAs were isolated from the tissues and the cancer cell lines and then we performed Western bloting, RT-PCR and FACS on them.

Results: On Western blot, HSP60 was more overexpressed in the cancer tissue and the cancer cell lines than in the normal breast tissue and in the normal cell lines. The Expression of HSP60 showed 2 types of molecular weight differences in both the breast cancer tissues and the cancer cell lines, and specifically, low HSP60 was over-expressed in the cancer tissues. There was no difference between the expression of HSP60 protein and mRNA according to the treatment with heat shock protein in both the breast cancer tissue and the normal cell lines. Also, there was no relationship between phosphorylation and the structural difference of HSP60 protein according to HSP60 protein molecular weight.

Conclusion: We conclude that HSP60 may be used as a diagnostic marker for breast cancer. Detailed investigation of the usefulness and significance of the HSP60 expression as a prognostic factor is required in further studies.

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Is triple negative a prognostic factor in breast cancer?

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Background: Breast cancer is characterized by hormone dependency and endocrine therapy is a key treatment in breast cancer. Recently, targeted therapies such as Trastuzumab treatment for HER2 positive breast cancer has been important. Triple negative breast cancer is characterized by lack of expression of estrogen receptor (ER) and progesterone receptor (PgR), and the absence of HER2 protein overexpression, and so there is no targeted therapy for this subtype. In this study, we examined the biological and prognostic characteristics in triple negative breast cancer.

Patients and Methods: Between January 1998 and September 2006, 1552 patients with primary breast cancer were investigated retrospectively in this study and ER, PgR and HER2 status were evaluated in all cases. Furthermore, p53 overexpression and Ki67 values were examined immunohistochemically.

Results: Patient distribution according to ER, PgR or HER2 status were as follows; ER and PgR positive: 57.9% and ER and PgR negative: 25.1%. With regards to the HER2 status, HER2 positive was 23.3%, and triple negative (TN) was 14.0%. TN breast cancer has a high proliferation rate, high nuclear grade and frequent p53 overexpression. Patients with TN tumors had a significantly poorer disease-free survival (DFS) than those with non-TN tumors. After recurrence the overall survival (OS) rate in TN cases were significantly lower than that of the non-TN cases. Multivariate analysis revealed that TN was a significant factor for DFS and OS after recurrence.

Conclusion: TN breast cancer is a rare subtype and has a high proliferation rate, a high nuclear grade. p53 overexpression, and lower DFS/OS. To improve the prognosis of TN breast cancer, a new effective strategy needs to be developed.

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Simultaneous analysis of HER-2/neu gene amplification and protein overexpression in single cells of pleural and ascitic effusions from patients with breast and ovarian cancer

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Background: HER2/neu protein overexpression is found in 20−30% of breast cancers and correlates with poor clinical outcome. Patients are selected for anti-HER2/neu-therapy by examination of tumour specimens by IHC (immunohistochemistry) and FISH (fluorescence-in-situ-hybridisation). Good correlation between both methods has been found for score 1+ and 3+ samples, but not for score 2+ samples. Combined approaches using FISH and immunofluorescence on the same tumour specimen have been described. In this study we examined pleural and ascitic effusions with a method allowing simultaneous analysis of protein expression by IHC and gene amplification by FISH. We regarded the following aspects: 1. the frequency of HER2/neu protein expression and gene amplification in effusions. 2. the correlation between protein expression, gene amplification and chromosome 17 polyploidy.

Methods: We examined 35 effusions from patients with breast cancer (n = 31) and ovarian cancer (n = 4). The same cytospins were analysed by IHC using two anti-HER2/neu antibodies and by FISH with HER2/neu/CEP17 probes. Amplification was defined as: 1. HER2/neu gene copy number of >4 and 2. HER2/neu/CEP17 ratio $\geqslant$2.0.

Results: 35 tumour-cell-positive effusion specimens were examined. 25 of them were scored HER2/neu positive (score 2+, 3+). All of them contained cells with heterogeneous protein scores. Single cells were analysed for HER-2/neu gene amplification and chromosome 17 ploidy with regard to their scores. 9 of these 25 samples showed mean HER2/neu copy numbers of >4 in cells with a 2+ and 3+ score, but only 12% (n = 3) of these samples were amplified according to HER2/neu/CEP17-ratio. 32% (n = 8) were polyploid (mean CEP17 >4). In some samples we found tumour cells with gene amplification but without protein overexpression (score 1+) and cells without gene amplification but strong protein expression.

Conclusion: The combination of IHC and FISH allows a differentiated analysis of single cells, which is especially important for effusions that often contain heterogeneous cells. In this study only few samples showed HER2/neu amplified cells. Protein overexpression was not always correlated with gene amplification. For the selection of patients for an anti-HER2/neu-therapy protein overexpression might be more important since it might sometimes be caused by CEP17 polyploidy rather than by gene amplification.

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Clinical features of BRCA1/BRCA2 positive hereditary breast cancers

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Background: BRCA1 and BRCA2 mutations cause hereditary breast cancer (BC). Patients (pts) who carry this type of mutations have a significantly cumulative lifetime risk of developing breast and ovarian cancer. The authors review all cases of BRCA1/BRCA2 positive BC in their Institution.

Material and Methods: Retrospective analysis of consecutive pts with BRCA1/BRCA2 positive BC followed at the Portuguese Institute of Oncology, Porto. Clinical data were obtained from medical records. Data were analyzed using the statistical package SPSS 13.0. Survival curves were calculated by the Kaplan–Meier method.

Results: A total number of 30 pts were evaluated.