Thursday, 17 April 2008 12:30–14:30

POSTER SESSION

Pathology

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Prognostic significance of lymphovascular invasion in node-positive patients with primary operable breast cancer

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Background. Lymphovascular invasion (LVI) is a major and significant predictor of distant recurrence for node-negative breast cancers. The aim of this study is to assess its prognostic impact for distant recurrence in node-positive patients with primary operable breast cancer.

Methods. The study group consisted of 374 node-positive breast cancer patients operated on between January 1989 and December 1992 at the Bergonié Institute (median follow-up: 126 months). For each case, LVI was determined on three haematoxylin and eosin-stained (HE) sections. Only unequivocal emboli located at distance from the tumour were considered.

Results. LVI was identified in 46% of tumours and was significantly associated with age $\leqslant$40 ($p = 0.02$), histological mSBR grade III ($p = 0.01$), negative estrogen receptor ($p = 0.032$). No significant correlation was found with pathological tumour size, number of involved axillary lymph node or HER-2 over-expression. After performing multivariate analyses, HE LVI appeared to be a significant predictor of distant recurrence not only in the whole group (HR: 1.70 – 95% CI: 1.21−2.40 – $p = 0.002$), but also in the subgroups of non HER2-overexpressing tumours ($n = 324$) (HR: 1.82 – 95% CI: 1.24−2.67 – $p = 0.002$) and non HER2-overexpressing endocrine sensitive tumours ($n = 282$) (HR: 1.71 – 95% CI: 1.12−2.62 – $p = 0.013$).

Conclusion. In our study, LVI was found to be statistically correlated with age, histological grade and estrogen receptor status, but not with pathological tumour size and number of axillary lymph node involved, suggesting that LVI is an integral part of the tumour genotype rather than an event during evolution of the tumour. In addition, LVI appeared to be an independent significant prognostic factor for distant recurrence in the studied N+ tumours.

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Morphological and molecular analysis of Circulating Tumor Cells (CTCs) in breast cancer: a real possibility

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Background: Detection and characterization of CTCs in peripheral blood may have some clinical utilities to choose targeted therapy and monitor treatment response and disease recurrence. The first main step to reach this aim consists in employing reproducible systems to isolate and typify CTCs.

Materials and Methods: 20 ml blood samples were collected from 84 patients with breast cancer in different stages of desease. CTCs were isolated and counted with CellSearch System® (Immunicon Co.) by means of immunomagnetic separation, using ferrofluid nanoparticles binding anti-epithelial cell adhesion molecules (EpCAM), and fluorescently stained with Epithelial Cell Kit®. CTCs were defined as nucleated epithelial cells, positive for DAPI, CK 8, 18, 19 and negative for CD45. A case was defined as CTCs positive if more than 1 cell was identified. Concomitantly, we also used Tumor Phenotyping Reagent® to investigate HER-2/neu and EGFr expression. Moreover, using Profile Kit® CTCs were isolated to performe morphological and molecular analysis: slides were set up and investigated by Papanicolaou staining, Fluorescence in situ hybridization (FISH) to investigate HER2/neu and Immunocytochemestry (ICC) to investigate CK, ER, PGR, Ki67, C-erbB2.

Results: 54 patients were CTCs positive, with a median value of 12/7.5 ml. HER-2/neu and EGFr expression were investigated in 48 cases (48% positive, 52% negative) and 12 cases (17% positive, 83% negative) respectively. Morphological analysis by Papanicolaou staining showed a clear evidence: CTCs differ from original neoplastic tissue's cells presenting a rounded shape probably modified by the liquid medium, blood, in which they circulate. However, in our experience, two cellular kinds generally appear: the most representative one characterized by small size, round cells with large nucleus (high nucleus/cytoplasmic ratio), both isolated (similar to blood cells) or in clusters; the other group characterized by larger and sometimes elongated cells.

Conclusions: Counting, biocharacterisation and morphological analysis of CTCs are possible using a good, simple, automated and standardized method to isolate and better investigate them in order to give useful informations about patient's care and probably, in the future, individual therapies.

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Decrease of expression of androgen receptor in triple negative breast cancer

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Background: The role of ER, PR and HER2/neu as prognostic and predictive factors in human breast cancer is well established. Triple negative breast cancer is characterized by lack of expression of estrogen receptor (ER), progesterone receptor (PR) and HER-2/neu. Previous studies have showed that breast cancer tumors are expressing androgen receptor (AR) in 40−70% of the cases. However, the functional role and clinical value of AR expression in breast cancer have still not been clearly defined. This study was set up to investigate the expression profile of AR in relation to ER alpha/beta, PR and HER2/neu in tumors of patients with breast cancer.

Methods: We developed a retrospective study in 76 patients with confirmed diagnostic of invasive ductal carcinoma. We assessed by immunoblotting and IHC (using specific monoclonal antibodies) the distinct expression pattern of ER alpha/beta, PR, HER2 and AR. Chi-square and log rank tests were used to determine differences between proportions of each marker and mortality and survival distributions respectively (P value $\leqslant$0.05 was considered significant).

Results: The 76 patients were women. The median follow-up was 58 months (1–346). The median age of the patients was 48 years (22−81 ys). The number of patients and percent with positive tumors for ER alpha/beta, PR, HER2/neu and AR are showed in the table.

	ER		PR	HER2/neu	Triple negative	AR
	alpha	beta				
No. of patients	34	28	27	16	18	32
%	45%	42%	35%	21%	24%	42%

N = 76 patients

Only three patients (4 %) with triple-negative tumors were positive for AR expression. This relationship was significant ($P = 0.01$) in comparison with the 58 (76%) patients with non-triple negative tumors where we found 31 (41%) of the patients were positive for the expression of AR. In triple negative cases, we did not found correlation with the overall survival.

Conclusion: Our findings suggest loss of AR expression may be involved in tumor progression in breast cancer patients. We need further studies to demonstrate clinical significance of AR in these patients.

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Cancer of the stomach in breast cancer patients is often metastatic disease

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Background: In patients with breast cancer, the differential diagnosis between metastatic disease or primary cancer of the stomach can be challenging in a gastric biopsy and these diagnoses have completely different clinical implications. Since the incidence of primary gastric cancer in western countries is low compared to the incidence of breast cancer, we hypothesized that cancer of the stomach in a breast cancer patient is often metastatic disease.

Materials and Methods: Patients with breast cancer and cancer of the stomach in the years 1988 until 2005 were retrieved from our files. In our laboratory approximately 300 new patients with breast cancer are diagnosed each year. Patients with gastric cancer preceding the diagnosis of breast cancer were excluded. Age at the time of the breast cancer diagnosis and interval-time until the diagnosis of cancer of the

stomach were recorded. Slides of the stomach and breast were reviewed to determine the type of tumour and histological similarity. Cancer of the stomach was classified according to Lauren. All cases were stained with antibodies for cytokeratin 7, cytokeratin 20, E-cadherin, estrogen receptor and progesteron receptor and slides were stained in a Benchmark XT automatic stainer (Ventana).

Results: A total of 26 patients were retrieved from our files and in 18 patients slides of the breast and stomach could be reviewed and sufficient material was available for additional staining. Median age was 67 years (37−79 years). In 7 patients (37−71 years) the cancer of the stomach was shown to be metastatic disease. In 6 patients the gastric metastasis had a diffuse growth pattern and in 5 patients the breast cancer was of the invasive lobular type. Median time between diagnosis of breast cancer and gastric metastasis was 32 months (1–167 months). In 2 patients a diagnosis of gastric metastasis was made only after resection of the stomach. All gastric metastases were positive for hormone receptors.

Conclusion: Cancer of the stomach can be metastatic disease in breast cancer patients, even in those with a remote history of breast cancer. Proper clinical information and staining of the gastric biopsy are most helpful in avoiding misclassification.

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Clinicopathological features of inflammatory versus non-inflammatory locally advanced non-metastatic breast cancer

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Background: Inflammatory breast cancer (IBC) is a rare but aggressive form of breast cancer. It is mainly a clinical diagnosis. The aim of this study was to compare IBC to clinically diagnosed non-inflammatory locally advanced non-metastatic breast cancer further (cLABC) with respect to clinicopathoIocial features.

Material and Methods: 108 patients were studied: 49 with IBC and 59 with cLABC. The following features were analysed: age at diagnosis, body mass index (BMI), axillary lymph node status (cN), oestrogen receptor status (ER), progesterone receptor status (PR), HER2 status, histological tumour grade and subtype. Short term disease-free and overall survival (DFS, OS) were also assessed in both groups.

Results: Compared with cLABC, IBC was less often PR positive (41.7% vs 66.1%, $p = 0.01$) and showed a trend to be more often HER2 positive (34.7% vs 19.3%, $p = 0.07$) The 3-year DFS was 63% and 77% respectively for IBC and cLABC ($p = 0.01$); these figures were 83% and 85% for OS ($p = 0.17$). No significant differences in age at diagnosis, ER, cN, BMI, histological tumor grade or subtype were detected.

Conclusion: Differences in PR, HER-2 and DFS confirm the distinctive biological nature of IBC and cLABC. Age at diagnosis, ER, cN, BMI, histological tumour grade and subtype show no difference and therefore these features might be more determined by or related to the locally advanced stage than to the inflammatory component itself.

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HER-2/neu amplification detected by fluorescence in situ hybridization in touch imprint cytology in comparison with tissue sections

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Background: HER-2/neu status has been used in breast cancer as a prognostic and predictive factor to select patients for trastuzumab treatment. The analysis of HER-2 is usually performed on formalin-fixed paraffin tissue sections and testing with fluorescence in situ hybridization (FISH) is preferred. The objective of our study was to evaluate the reliability of HER-2/neu determination by FISH on touch imprints (TI) of breast core needle biopsies (BCNB) from primary breast cancer patients in comparison with the results obtained by FISH on the corresponding tissue sections (TS).

Material and Methods: The sections tissue of the BCNB were touched a lot of times to one slide, it was made another mirror slide and then, one of them was stained whit H&E to detect malignant cells and other was utilized for FISH: The slides of TI of the breast core needle biopsies and corresponding TS from breast cancer patients were eavluated for HER-2 gene amplification by determining the HER-2/CEP17 signal ratio in 20 tumor nuclei. If the ratio was <2.2, the specimen was considered to lack gene amplification; if the ratio was ⩾2.2, the specimen was considered to show HER-2 gene amplification. Chi square test was made.

Results: A total of 55 BCNB were examined and paired results by FISH cytology and FISH histology were available in 48 cases. Concordance was 83.33% (40/48). Eight cases didn't show concordance. It was not stastistically significant ($p > 0.05$) by chi square on both samples

Conclusion: We conclude that HER-2 gene analysis by FISH on TI is easily done and reliable tecnique. TI provided results earlier and quicker, were easier to score and were more accurate. However, the use of TI sacrifice the arquitectural tissue.

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Pathology and biology

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Detection of homologous recombination defects in biopsies of sporadic breast cancers

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Background: It has been suggested that up to 30% of sporadic breast cancers may have defective homologous recombination (HR), the only cellular mechanism that reliably repairs DNA double strand breaks. DNA-damaging drugs including alkylating agents can cause double strand breaks. Tumours with deregulating mutations in the key players of HR may be extremely sensitive to these and to a novel class of agents targeting DNA-repair: the poly (ADP-ribose) polymerase (PARP) inhibitors. The identification of such a subgroup of breast cancers before treatment could be of great clinical utility. This study was initiated to develop a test that can be incorporated in a routine clinical workup.

Materials and Methods: So far, 38 patients with HER2 negative tumours and scheduled for preoperative chemotherapy have been tested prospectively. We investigated the expression levels of BRCA1, FANCC, and FANCF by quantitative RT-PCR and amplification of the EMSY gene first by FISH and additionally by Multiplex Ligation-dependent Probe Amplification (MLPA). Triple-negative patients were additionally checked for BRCA1 germ line mutations by sequencing the BRCA1 gene locus.

Results: EMSY amplification assessment by FISH is technically challenging and is not an optimal choice for clinical routine. MLPA is a reliable alternative that can also detect amplification missed by FISH because of high background staining. Quantitative RT-PCR detected a number of tumours with a considerably lower expression of BRCA1 ($n = 5$) than the other ones ($n = 33$). Of those 5 tumours, four had a triple-negative phenotype, whereas the other one was a luminal tumour with a high expression of the estrogen receptor.

Conclusion: Amplification of the EMSY gene locus is a rather rare event. Detection by FISH may miss samples that can be detected by MLPA. The main changes in the investigated sporadic samples are low expression of the BRCA1 and FANCC protein. Gene expression arrays are available of these samples and an update and comparison of the applied techniques will be presented.

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TP53-mutated breast carcinomas are associated with specific array comparative hybridization (aCGH) patterns involving deletions of 3p, 4p, 4q and 5q

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The TP53 tumor suppressor protein acts as a major defense against cancer. Among its most distinctive features is the ability to elicit both apoptotic death and cell cycle arrest. TP53 plays a key role in mediating cell response to various stresses: one of these is DNA-repair. When *TP53*