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Prediction of brain relapse by gene expression analysis in HER2-positive metastatic breast cancer patients

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Background: Brain relapse is a common occurrence in HER2 positive breast cancer patients. We earlier demonstrated that clinical and pathological factors may select HER2 positive metastatic breast cancer patient categories with particularly high risk of brain relapse (*EJC Suppl* 2006;4,165−6). In the present study we explored whether early occurrence of brain relapse in HER2 positive metastatic breast cancer patients may be predicted by gene expression analysis.

Materials and Methods: Study group included 90 HER2 positive metastatic breast cancer patients, 43 of whom developed brain relapse after a median of 3 years from diagnosis: 22 and 21 patients with brain relapse at <3 vs >3 years from diagnosis, respectively. Expression of 502 known cancer genes was investigated with cDNA-mediated annealing, selection, extension and ligation (DASL) assay (Illumina Corp). RNA (200 ng) was extracted using HighPure RNA Paraffin Kit (Roche Applied Bioscience) from archived formalin-fixed, paraffin-embedded tumor tissues. RNA was pre-qualified using iScript (Bio-Rad) to reverse transcribe. Quantitative PCR was performed using SYBR Green Master Mix (Applied Biosystems). T-test with unequal variances was applied after sample median normalization. Differentially expressed genes were analyzed using Ingenuity Pathway Analysis.

Results: Among the analyzed genes, 95 were differentially expressed with a p-value <0.05. (false discovery rate = 0.25) and 48 with a p value <0.01 (false discovery rate = 0.1) in patients who developed brain relapse at <3 vs >3 years from diagnosis.

Conclusions: Occurrence of early brain relapse in HER2-positive metastatic breast cancer patients is associated with expression of numerous genes in tumor tissue. Analyses are ongoing to generate a gene-expression signature allowing prediction of brain relapse.

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The Genomic Grade Index (GGI) – a potential predictor of relapse for endocrine-treated breast cancer patients in the BIG 1-98 trial

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Background: We have previously shown that the GGI was able to identify two subtypes of ER-positive tumors that were associated with statistically distinct clinical outcomes in tamoxifen-treated patients. Here, we aim to investigate the ability of the GGI to predict relapses in postmenopausal women with hormone receptor-positive breast cancer who were treated with tamoxifen (T) or letrozole (L) within the BIG 1-98 trial.

Methods: We generated gene expression profiles using Affymetrix and computed the GGI for a matched, case-control sample of frozen tissue specimens of patients participating in the BIG 1-98 trial from the two Belgian hospitals where frozen samples were available. All relapses (cases) were identified from patients randomized to receive either monotherapy or from the switching treatment arms for whom relapse occurred before the switch. Each case was randomly matched with four controls based upon nodal status and treatment (T or L). The prognostic value of GGI upon relapse was assessed using Cox models with GGI as a continuous predictor and divided at the median. Predictive accuracy of GGI was estimated using time-dependent area under the curve (AUC) of the ROC curves (100% = perfect classification, 50% = no discrimination).

Results: Frozen samples were analyzable for 48 patients (10 cases and 38 controls). Seven of the 10 cases had been assigned to receive L at the time of trial enrollment. Cases and controls were comparable with respect to menopausal and nodal status, local therapy, HER2 over-expression, and adjuvant or neoadjuvant chemotherapy. Cases were slightly older than controls and had a larger proportion of large, poorly differentiated ER-positive/PgR-negative tumors. Higher values of GGI were associated with a significant increase in the hazard of relapse, with each 10-unit increase resulting in an 11% increase in the hazard rate (HR = 1.11, 95% CI 1.03 to 1.21). Within the subgroups of patients with node-positive disease or who were randomized to treatment with L, the hazard of relapse was significantly greater for patients with GGI at or above the median. AUC reached a maximum of 78% at 27 months.

Conclusions: This analysis supports the GGI as a good predictor of relapse, even among patients who receive L. Validation of these results, in a larger series from BIG 1-98, is planned using the simplified GGI represented by only four genes and tested by RT-PCR on paraffin-embedded formalin-fixed tissues.

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Is it possible to identify parameters that predict nipple–areola complex involvement in breast cancer patients

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Introduction: A clinically normal-appearing nipple in patients with breast cancer may contain unsuspected neoplastic cells. Preservation of a nipple containing occult malignancy could potentially increase local recurrence rates and affect disease-free survival. Consequently, patients considered for breast conservation operations with nipple preservation must be carefully selected.

Materials and Methods: Information available on 382 patients diagnosed and treated with breast cancer at the Clinical Center Nis from 2000 to 2003 were retrospectively reviewed. Multivariate hazard analyses was used to assess the association between potential risk factors of cancerous nipple involvement.

Results: The frequency of nipple involvement was 12.04%. Nearly half of the patients had disease stage III and IV. Most patients, 29 (63.04%), had a tumor to nipple distance of less then 2 cm. Twenty-five patients (54.34%) had more than four positive axillary nodes. A central/overlap tumor location was present in 28 (60.87%) patients. Cox multivariate analysis of prognostic factors showed that Stage III, central/overlap tumor location and nuclear grade III or greater had a statistically significant effect on malign nipple involvement.

Conclusions: The multivariable model used in this study showed a significant association between stage, centrally located tumors and nuclear grade III or greater in predicting the risk of cancerous nipple involvement. Preoperative magnetic resonance imaging or computed tomography scan examinations are recommended for treatment planning of breast cancer, in particular nipple preserving surgery in patients with central/overlap tumor localization and with a tumor to nipple distance of less than 2 cm.

Friday, 18 April 2008 12:30–14:30

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Response and outcome prediction

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The influence of Lympho Vascular Invasion (LVI) on local (LR) and regional recurrences (RR) and survival: an ONCOPOOL study

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Introduction: This report is of the effect of Lympho Vascular Invasion (LVI) on Survival and on Local and Regional recurrences in 5997. LN neg and 1−3 pos cases from ONCOPOOL in which LVI data had been recorded.

Patients and Methods: ONCOPOOL is a dataset (n = 17,653) of primary breast cancers diagnoses in the 1990's from 12 European units. LVI measured on Histology has been recorded in 5997 of these and compared

with survival; rates of local recurrence (LR) and regional recurrence (RR) have also been investigated in relation to LVI.

Results: See Table 1.

Table 1

LN	LVI	n	% in group	% 10 yr Survival
neg	Neg	3156	52	88.3
	Pos	550	9	76.9
1−3 pos	Neg	1540	26	75.2
	Pos	751	13	43.1
		5997		

Rate of LR was 3% at 10 years for LVI+ and 1% for LVI− (NS). The rate of RR in LN−/LVI+ patients was 3% at 10 years.

Conclusions: These figures verify in a multicentre International large data set that LVI+/LN− have the same survival as LN 1−3 positive cases; In LN+ cases LVI+ has no additional effect on prognosis.

LVI has a clear effect on prognosis of LN− cases whereas the effect of sentinel node micro metastases on survival is unconfirmed and LVI is more commonly found. Furthermore LVI+ does not give significantly higher rates of LR nor RR.

These findings suggest LVI could replace SLNB, being more accurate, easier and cheaper.

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Presence of bone marrow micrometastasis predicts metastatic pattern and disease-free interval in breast cancer patients – results from the Collaborative Group Bone Marrow Micrometastasis

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Background: To establish the prognosis and metastatic pattern in breast cancer patients in relation to bone marrow micrometastasis (BMM), that are readily detectable at the time of first diagnosis of cancer, a large series of patients was analyzed.

Methods: Individual patient data of 9 studies, involving 4,686 patients with breast cancer, were combined to analyze 10-year survival, specifically distant disease-free survival and the site of distant metastasis. We constructed Kaplan–Meier curves and computed incidence rate ratios with 95% confidence intervals. Survival estimates were adjusted for study center. The difference in median disease-free survival interval between BMM positive and BMM negative patients was tested with the Wilcoxon rank sum test.

Results: BMM were detected in 1,432 (30.6%) patients. Median follow-up was 62 months. Overall, distant metastasis occurred in 952 (20.3%) patients. Compared to patients without BMM, patients with BMM experienced twice as often distant metastasis (32.3% vs. 15.1%, $P < 0.001$) and had significantly shorter distant disease-free survival (log rank: $P < 0.001$; IRR 2.36, CI: 2.07−2.69, P(Wald) < 0.001). Among patients with distant metastasis during follow-up, the localization of distant metastasis was visceral (48.4%), bone (30.5%) and multiple sites (21.1%), the latter being defined as simultaneous occurrence of bone and visceral metastasis. The proportion of metastasis at multiple sites was significantly higher in patients with BMM than in patients without BMM (25.8% vs. 16.7%, respectively; $P = 0.003$). For each localization of distant metastasis, the disease-free interval was significantly shorter in patients with BMM than in patients without BMM: the respective medians of distant disease-free intervals were 23 vs. 29 months for visceral metastasis ($P = 0.030$), 24 vs. 33 months for bone metastasis ($P = 0.001$), and 14 vs. 22 months for metastasis at multiple sites ($P = 0.004$). Post-relapse survival was not different between patients with BMM and patients without BMM.

Conclusions: The results provide conclusive evidence that presence of BMM predicts a poor-prognosis pattern of distant metastasis, characterized by earlier distant relapse and first distant metastasis at multiple sites. BMM at the time of first diagnosis of primary breast cancer may be used as surrogate marker of distant metastasis and implemented in treatment strategies.

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Impact of histological grade on prognosis in very young breast cancer patients: pooled analysis of four EORTC trials

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Background: Young age at time of diagnosis of breast cancer is associated with unfavorable prognosis. Current guidelines recommend the administration of adjuvant chemotherapy to patients aged 35 years or less regardless of any tumor characteristics. However, since breast cancer at a very young age is a relative rare event, evidence concerning prognostic factors within this subgroup is lacking. Therefore, the individual patient data of four early stage breast cancer EORTC trials were pooled to study prognostic factors on long term outcome in young breast cancer patients.

Material and Methods: The total dataset consisted of 9938 early breast cancer patients of which 12% was younger than 41 years. Tumor material from 549 patients aged less than 41 years at time of diagnosis was available for renewed pathological analysis. The median follow-up was 7 years.

Results: In multivariate analyses, histological grade remained the only independent prognostic factor for overall survival (Grade III versus I: hazard ratio (HR) 3.92; 95% confidence interval (CI) 1.38 to 11.16). In the subgroup of young node-negative patients who did not receive adjuvant chemotherapy, histological grade was even stronger related to a favourable prognosis (Grade III versus I: HR 8.92; 95% CI 1.17 to 68.20). This association was independent of tumor size, type of surgery and hormone receptor status. Survival rates were excellent for young node negative patients with grade I tumors: 97% at 7 year follow-up compared to 72% for grade III tumors.

Conclusion: Histological grade is a strong independent prognostic factor in young breast cancer patients. These findings support the fact that histological grade is an excellent diagnostic tool to assess disease outcome and to plan systemic treatment strategy in young breast cancer patients.

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Long-term prognostic impact of risk classifications in node-negative breast cancer – comparison between Adjuvant!, St. Gallen, and a novel risk algorithm used in the prospectively randomized Node-Negative-Breast-Cancer-3 trial (NNBC-3)

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Background: defining risk categories in node-negative breast cancer is of great importance. We developed a novel risk classification which is currently evaluated prospectively in the Node-Negative-Breast-Cancer-3 trial (NNBC-3) trial using well-established clinico-pathological criteria. We compared its prognostic utility with the web-based tool Adjuvant! and the St. Gallen risk classification 2007.

Methods: we retrospectively analyzed 410 node-negative breast cancer patients with a median follow-up of 10 years which did not receive adjuvant systemic therapy. Patients with either (I) age <35 years, (II) G III, (III) HER-2 positivity, (IV) vascular invasion, (V) progesterone receptor negativity, (VI) G II tumors >2 cm, or (VII) G I tumors >5 cm were defined as high-risk. All patients were also characterized using Adjuvant! and the established St. Gallen 2007 risk category. We analysed disease-free survival (DFS) and overall survival (OS) for each of these risk classifications.

Results: Adjuvant! and the St. Gallen guideline classified 17% and 18%, respectively, of the patients as low-risk. Use of the novel NNBC-3 algorithm enlarged the low-risk group to 37%. Only the NNBC-3 algorithm retained its prognostic significance for DFS in multivariate analysis ($p = 0.006$; HR 2.02; 95% CI 1.22−3.35). Both Adjuvant! ($p = 0.027$; HR 3.81; 95% CI 1.16−12.47) and the NNBC-3 risk classification ($p = 0.049$; HR 1.95; 95% CI 1.00−3.81) predicted OS in multivariate analysis independently.