

thus mimicking hormone receptor distribution in normal breast cells. Whether this differential effect of age and probably more important, of menopausal status on combined ER/PR expression has a prognostic value is the object of currently ongoing studies.

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POSTER

Correlation of serum HER-2/neu extracellular domain levels in metastatic breast cancer with the expression of HER-2/neu in corresponding primary tumors

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Background: A positive HER-2/neu status is a requirement for antibody treatment with Herceptin in metastatic breast cancer (MBC). As a consequence, reliable and clinical relevant assessment of HER-2/neu status is a matter of interest. The aim of our study was a) to determine if HER-2/neu extracellular domain (ECD) concentrations determined at the onset of MBC reflect the HER-2/neu status of the primary tumor, b) to determine the influence of other tumorbiological factors and c) of HER-2/neu expression and ECD levels on disease-free survival and overall survival.

Methods: HER-2/neu ECD serum concentrations were determined at the first diagnosis of metastatic disease in 82 patients. Serum HER-2/neu ECD was quantified by a commercially available ELISA (Oncogene Science, part of Bayer Diagnostics, Cambridge, USA). ECD levels above 15 ng/ml were regarded as elevated. For HER-2/neu immunohistochemistry from paraffin-embedded tissue sections of primary tumors the monoclonal antibody CB 11 (Novocastra Laboratories, Newcastle upon Tyne, UK) was used. Staining was evaluated according to the DAKO scoring system (0, 1+, 2+ and 3+).

Results: a) HER-2/neu ECD levels at the onset of MBC are correlated with the HER-2/neu expression of the corresponding primary tumor ($p=0.006$). b+c) In patients with non-MBC at primary diagnosis only nuclear grading and nodal status had a significant impact on disease-free survival and overall survival in the multivariate analysis ($p<0.05$). In the univariate analysis patients with HER-2/neu positive tumors (DAKO-Score 2+ and 3+) tended to have a shorter disease-free-survival than patients with HER-2/neu negative tumors ($p=0.05$). In patients with MBC visceral metastases correlated with shorter OS compared with bone or locoregional metastases ($p=0.007$). In our group of patients, HER-2/neu ECD levels had no impact on overall survival.

Conclusion: HER-2/neu ECD levels correlate with the HER-2/neu expression of the primary tumor. Our results indicate that the ELISA method could be an option to obtain a real-time status of HER-2/neu in MBC. We were able to observe an impact of HER-2/neu expression in the primary tumor on disease-free survival, but not on overall survival. In our cohort of patients a prognostic relevance of HER-2/neu ECD levels in MBC was not demonstrated.

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POSTER

Menopausal status and breast cancer (BC) characteristics: analysis of 3143 consecutive patients

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Background: The physiological characteristics of a woman's life (age at menarche and menopause, etc.) are major factors affecting the risk of developing BC, but there are few data concerning the impact of menopausal status on its characteristics, particularly the significance of new prognostic factors such as p53, p21, BCL2 levels, vascular invasion and multi-focality.

Materials and methods: The aim of this study of 3143 patients was to verify the differences between the 966 diagnosed before and the 2177 diagnosed after menopause in terms of T (DCIS, T1, T2, T3, T4); nodal involvement (positive vs negative); ER and PgR (positive vs negative); Ki-67 proliferative index (low 0–15%, intermediate 16–25%, high 26–100%); grading (G1, G2, G3); c-erbB2 (positive vs negative); type of diagnosis (asymptomatic vs symptomatic); p53, p21, BCL2 levels; vascular invasion and multi-focality. Among the patients diagnosed in post-menopause the impact of age at menopause was also considered. The data were analysed using the χ^2 test.

Results: The BC of the patients with a post-menopausal diagnosis, compared with BC of patients with a pre-menopausal diagnosis, was less frequently DCIS (10.5% vs 14.9%; $p<0.001$), G3 (29.4% vs 35.2%;

$p=0.008$) and c-erbB2+ (38.1% vs 48%; $p<0.001$), and more frequently without nodal involvement (64.5% vs 59.37%; $p=0.01$) and ER+ (83.3% vs 78.8%; $p=0.006$).

There were no differences in terms of PgR, Ki-67, type of diagnosis, p53, p21, BCL2, vascular invasion or multi-focality.

Among the patients diagnosed in post-menopause, only nodal involvement correlated with age at menopause: N0=70.4% in those aged <45 years at menopause, 66% in those aged 46–50 years, 60.1% in those aged 51–55 years, and 56.6% in those aged >55 years; $p=0.003$.

Conclusions: Menopause seems to have a considerable effect on the prognostic characteristics of BC: a post-menopausal status correlates with good prognostic factors, such as no nodal involvement (particularly in patients entering menopause at a young age), ER positivity, a low grading and c-erbB2 negativity.

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POSTER

Correlation between Her-2 status in primary tumour and response to anastrozole in patients with metastatic breast cancer

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Background: The aim of our study was to determine the predictive value of Her-2 in primary tumour for the response to anastrozole treatment in postmenopausal patients with metastatic breast cancer.

Patients and methods: In a retrospective study, 60 postmenopausal females with metastatic breast cancer treated with anastrozole were included. Most of the patients (90%) had been previously treated with tamoxifen. All patients had estrogen receptor (ER) positive and/or progesterone (PR) positive primary tumours. For the study, tissue array was constructed from formalin fixed paraffin-embedded primary tumours of all patients. On tissue array sections, ER and PR were determined by immunohistochemistry (IHC) only, whereas HER-2 was analysed by IHC and FISH. Chi-square test was used for statistical analysis.

Results: In the group of 9 FISH positive tumours there were two immunohistochemically negative (one 0 case, one 1+ case) and 7 positive (two 2+ cases, five 3+ cases). Relatively high response rate to anastrozole (73%) were observed in both Her-2 + and Her-2 – group. If determined by IHC, response rates were similar in Her-2 + and Her-2 – cases (70% vs. 74%; NS). If determined by FISH, response to anastrozole was even better in Her-2 + than in Her-2 – patients (100% vs. 69%; $P=0.067$).

Conclusion: According to our results, Her-2 positive tumours respond to anastrozole treatment equally good as HER-2 negative tumours. In addition, the response to anastrozole was found to be even higher in HER-2 positive tumours when determined by FISH.

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POSTER

Improved prognosis for breast cancer across the prognostic spectrum from improved therapeutic management

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The Nottingham Prognostic Index (NPI) was originally derived from multivariate analysis of prognostic factors, it recognised three groups with significantly differing survivals and was prospectively validated, intra and inter centre and internationally in series totalling over 20,000 cases. Later analysis divided patients into 5 groups. Mortality from breast cancer in the UK has fallen since the 1980s with operable 10 year survival rising in Nottingham from 55% to 77%. Earlier detection is partly responsible for this (by raising the percentages lying in the best prognostic groups and by detection of DCIS).

Group	Breast Cancer Specific 10 year % survival				
	NPI ≤	1980–86	1990–96	Reduction in deaths	
				Absolute %	Relative
Excellent	2.4	88	95	7	0.58
Good	3.4	72	90	18	0.64
Moderate I	4.4	61	79	18	0.46
Moderate II	5.4	42	71	29	0.50
Poor	6.4	14	44	30	0.34
V. Poor	7.0	12	33	22	0.24
All cases		55	77	22	0.49

Prognosis has greatly improved within each prognostic group which is largely explained by better therapeutic management (eg) adjuvant

therapies. The relative reductions are smaller in the poorer prognostic groups; this may reflect failure to respond to adjuvant therapies.

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POSTER

Expression of ErbB family members and levels of phosphorylated ErbB2 in breast tumours

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Background: ErbB2 (Her2/Neu) receptor tyrosine kinase is overexpressed in 10–30% of human primary breast tumors depending on patient's age. The overexpression of ErbB2 in breast cancer correlates with a more aggressive phenotype and increased risk of metastases resulting in reduced survival. Recently, we have shown prognostic and predictive significance of ErbB2 overexpression as well as increased levels of phosphorylated (Y1248-P) ErbB2 in breast tumors. ErbB2 is known to be a preferred heterodimerisation partner for other ErbB family members, therefore the purpose of our study was to correlate ErbB2 phosphorylation with the expression patterns of ErbB1 (EGFR), ErbB2, ErbB3 and ErbB4.

Material and Methods: Quantitative expression levels of ErbB1, 2, 3 and 4 was measured by means of TaqMan Q-RT-PCR and quantitative levels of phosphorylated (Y1248-P) ErbB2 by means of a sandwich ELISA on membrane extracts from 70 well-characterized primary breast cancers. For ErbB1 quantification EGF binding assay was also performed. ER concentrations were assessed from tumor cytosolic extracts by commercial quantitative ER EIA kit.

Results: Increased Y1248-P ErbB2 content was found in ErbB2-overexpressing tumors (median 2.6 U/mg) as compared to low-ErbB2-expressing tumors (median 0.65 U/mg). ErbB2 phosphorylation significantly correlated with poor survival ($p=0.005$) and inversely correlated with ER status ($p=0$, $rs = -0.65$). Comparison of ErbB family expression on mRNA level with ErbB2 phosphorylation revealed significant correlation with ErbB2 ($p=0.0002$, $rs = 0.49$) and ErbB1 ($p=0.05$, $rs = 0.26$) and inverse correlation with ErbB4 ($p=0.0005$, $rs = -0.47$). Even better correlation was found between ErbB1 levels, detected by EGF binding assay, and ErbB2 ($p=0.08$, $rs = 0.43$). No correlation between Y1248-P ErbB2 content and ErbB3 mRNA levels was found.

Conclusions: Phosphorylation of ErbB2 is associated with poor prognosis and reduced ER content. Significant correlation of phosphorylated ErbB2 with ErbB2 and ErbB1 expression suggests the role of ErbB1/ErbB2 heterodimers in malignancy of tumors. Inverse correlation between phosphorylated ErbB2 and ErbB4 is in agreement with findings describing association of ErbB4 with good prognosis.

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POSTER

Bilateral breast cancer – prognostic significance of synchronous and metachronous bilateral breast cancer (BBC)

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Purpose: to evaluate the impact of the type of bilateral breast cancer and mutations of BRCA 1 and 2 genes on survival.

Material and methods: 131 patients treated at Institute of Oncology MCS Memorial in Gliwice between 1969–2002 developed bilateral breast cancer. There were 39 (29.7%) synchronous BBC, defined as tumor arising in both breast simultaneously or within a maximum 12 months interval and 92 (70.3%) metachronous BBC, defined as tumors of second breast diagnosed more than 12 months following ipsilateral breast cancer, in absence of distant metastases. The time interval between metachronous tumors ranged from 1.26 to 29.6 years (mean 7.3). BRCA 1 and 2 gene mutations analysis were performed in 92 patients with ASA-PCR technique. Survival distributions from the date of primary and opposite side tumors were compared in group of patients in synchronous and metachronous BBC and in patients with and without BRCA 1 and 2 genes mutations by the log-rank test.

Results: Mean age of diagnosis of cancer in SBBC and MBBC group was 52 and 46 ($p=0.002$). The proportion of early stage tumors T0–1 was significantly higher in opposite side tumors compared to the first primary tumor in both groups ($p<0.0001$). The mean tumor diameter of first primary and contra lateral breast tumors were 3.6 and 2.4 cm ($p<0.00001$). BRCA 1 and 2 mutations were recognized in 25 patients (27.6%). All but two mutations were detected in MBBC ($p=0.006$). Median follow-up from the diagnosis of primary and BBC was 9.34 and 3.78 years. Five year DFS for SBBC and MBBC were 67 and 93% respectively (log-rank $p=0.005$).

MFS were 76 and 97% (log-rank $p=0.07$), RFS were 77 and 95% (log-rank $p=0.03$). If the same analysis was performed since the date of the second breast tumor DFS, MFS and RFS in both groups did not reach statistical significance. Differences in 5-year survival-DFS, MFS, RFS in BBC patients with and without BRCA 1 and 2 mutations did not reach statistical significance.

Conclusion:

1. A close follow-up policy based on clinical examination and annual mammography enables detection the second breast cancer at an earlier stage than the primary one.
2. Patients with metachronous bilateral breast cancer (MBBC) have better survival characteristics in comparison with SBBC patients.
3. BRCA 1 and 2 gene mutations do not have the adverse impact on survival in bilateral breast cancer.

BBC – bilateral breast cancer; MBBC – metachronous bilateral breast cancer; SBBC – synchronous bilateral breast cancer; DFS – disease free survival; MFS – meta free survival; RFS – Recidiva free survival.

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POSTER

Women under age 50 with an ER-positive breast cancer are more likely to have positive lymph nodes if PR is not expressed

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Background: Women with an ER-positive breast cancer have a better prognosis if PR is also expressed and this prognostic significance is independent of treatment. This study examines qualitative and quantitative PR-expression in women with a primary invasive ER-positive breast cancer, in predicting axillary lymph node involvement.

Patients and Methods: 1008 consecutive women with a complete axillary clearance for a primary operable invasive breast cancer were evaluated retrospectively between Jan 2000 and Jun 2003 in the Multidisciplinary Breast Centre, UZ-KULeuven. We excluded those with a lobular type breast cancer, those with ER-negative breast cancer and those who received neoadjuvant therapy or the sentinel lymph node procedure only for axillary staging. The following variables were tested in an uni- and multivariate analysis: tumour diameter (less or more than 20 mm) and grade, histologic type, quantitative steroid hormone receptor status, hormone replacement therapy, menopausal status and family history of breast cancer. Age at diagnosis is stratified as less or more than 50 years. Immunohistochemical stains for ER (6F11/2) and PR (312) were categorised using the H-score; ≤ 50 was defined as ER/PR negative.

Results: Univariate analysis shows that tumor grade, size and PR status are significantly related with lymph node status in patients less than 50 years of age. Selected PR<200 versus PR>200 and size > 20 mm versus size ≤ 20 mm were determined by ROC curve. Multivariate analysis also demonstrates grade, tumor size and PR status as independent predictors for lymph node positivity in women under age 50.

Univariate Analysis: (correlation between predicting variables and LN metastasis)

Variables	P-value		
category	All patients	50 years	≥ 50 years
No of patients (%)	1008	282 (28%)	726 (72%)
PR (≤ 200 vs > 200)	0.035*	0.010*	0.170
ER (≤ 200 vs > 200)	0.225	0.118	0.776
Histology	0.428	0.523	0.679
Grade (I/II vs III)	0.000*	0.001*	0.000*
Size (≤ 20 vs > 20 mm)	0.000*	0.000*	0.000*
HRT (– vs +)	0.761	0.807	0.603
Family History (– vs +)	0.907	0.730	0.543
BMI	0.761	0.393	0.241

Multivariate analysis in patents under age 50:

	Odds ratio	95% Confidence Interval
PR (<200/300 vs >200/300)	1.9	1.15–3.30
Grade (3 vs 1.2)	5.3	1.86–15.30
Size (>20mm vs <20mm)	4.4	2.55–7.66

Conclusion: PR expression and its quantitative value, tumour grade and size are all independent predictors for axillary lymph node involvement in women under age 50 with an ER-positive breast cancer.