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Breast Cancer Patients Resistant to Endocrine Therapy Show Decreased Number of Cytotoxic Supressor Cells and Enhanced Production of Neoangiogenetic and Immunosupressive Factors

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Background: Endocrine therapy is an essential modality in patients with hormone receptor positive breast cancer. Even with high therapeutic efficacy of first-line hormonal treatment, most patients with metastatic breast cancer will develop resistance. It appears that a factor contributing to the resistance may be a transforming factor-beta (TGF-beta). It is highly immunosuppressive factor that inhibits the natural and specific immunity against tumors and stimulates vascular endotelial growth factor (VEGF). The purpose of the study was to monitor immune responses in patients with hormone receptor positive breast cancer, particularly the examination of cellular (CD4, CD8) as well as humoral immunity, TGF beta and VEGF production.

Materials and Methods: Patients included in the research project were implemented routine cancer teratment with hormonal therapy. Basic parameters (histological type and grade, the degree of expression of ER and PR, HER2, and the proliferative marker) were established. Patients were evaluated by a cancer clinical immunologist to exclude immune disorders, allergic or autoimmune origin. TGF beta, VEGF were mesured by ELISA and anti-tumor cellular immunity (CD4, CD8, antigen presenting cells) was measured by flow cytometry.

Results: In patients with resistance to endocrine therapy mainly depression in cellular immunity was found, mainly CD 8, cytotoxic T lymphocytes were significantly [$p < 0.05$] decreased. Immunglobuline plasma level was decreased as well (mainly IgG4 subtype[$p < 0.05$]). Most patients have shown clinical symptoms of immunodeficiency (frequent infections of respiratory or urinary tract, herpetic infections). TGF beta as well as VEGF plasma level were significantly increased.

Conclusions: Correlation of these factors with resistance to endocrine therapy could help in the future with the the prediction of therapy response and contribute to the selection of targeted therapy in breast cancer cancer patients.

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Prediction of Non Sentinel Nodal Metastases After Positive Sentinel Lymph Node Biopsy for Early Breast Cancer − Burney Breast Unit Experience

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Background: Sentinel lymph node biopsy (SLNB) is an established method of assessing axillary node status in women with early breast cancer. The primary aim of SLNB is to understand lymph node status with minimal morbidity. Current practice is to offer completion axillary clearance for those women with metastases in sentinel lymph node (SLN). However the majority of patients, SLN is the only nodal involvement and so axillary dissection serves no therapeutic purpose. The aim of this study was to identify the biological predictors associated with non SLN metastases after positive SLNB.

Materials and Methods: A consecutive series of patients underwent SLNB and further axillary dissection for sentinel nodal metastases between 2008 and 2010. The outcomes of SLN and nodal disease were observed. Logistical regression analysis was performed on the data set to identify the tumour related factors associated with non sentinel nodal metastases.

Results: SLNB was performed on 350 patients (median age, 59 years). The median number of SLNB was 1. Of these, 297 (85%) had invasive ductal carcinoma and 174 (78%) were grade 2 cancer. The median tumour size was 14 mm. Lymphovascular invasion (LVI) was present in 58 patients (17%). 57 patients underwent axillary node dissection (median number of axillary nodes retrieved, 11.5). Of these, 20 (35%) patients had further axillary nodal disease. Multivariate regression analysis showed that lymphovascular invasion ($p < 0.000$), primary tumour size ($p < 0.045$) and tumour grade ($p < 0.039$) were associated with non SLN disease after a positive SLNB.

Conclusions: The results of this study demonstrate that LVI, tumour size and tumour grade are associated with non sentinel node metastases. These biological markers could be implemented as a tool in the selection of patents that would benefit from complete axillary clearance.

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Prognostic Implications of Invasive Lobular Breast Cancer

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Introduction: In our centre, invasive lobular carcinoma (ILC) constitute 13% of all primary operable breast cancer (Vandorpe et al. Breast Cancer Res Treat July 2011). Controversy remains whether ILC has a distinct prognosis compared to other tumor types. The Milano group (Colleoni et al. Ann Oncol Nov 2011) recently reported a 87.4% DFS at 5.8 yrs of follow-up for ILC, significantly worse than other luminal type breast cancers. We examined whether ILC carries a different prognosis over a similar follow-up time in a large consecutive cohort of breast cancer patients.

Patients and Methods: A total of 4251 consecutive women with invasive breast cancer, primary operated at UZ Leuven between 2000 and 2009 were included. Primary metastatic or neo-adjuvant treated patients were excluded. We studied disease free survival (DFS) defined as first event (distant or loco-regional including contra-lateral) comparing pure ILC with non-ILC type breast cancers taking all other established prognostic factors into account. The diagnosis of ILC was confirmed on histology and by lack of E-cadherin expression. Median follow-up was 6 years.

Results: ILC patients (n = 555) were significantly ($P < 0.05$) older at diagnosis with larger tumors size, more likely grade 2, lymph node (LN) positive, multifocal, bilateral, steroid receptor positive and HER-2 negative than those with non-ILC lesions (n = 3696). As compared to non-ILC mastectomy, axillary LN dissection, adjuvant endocrine therapy were more frequently used in ILC; chemotherapy was less frequently given but radiotherapy administration did not differ. Considering recurrences by their first event, the distant metastasis free interval was 93.0% for ILC vs 92.1% for non-ILC; loco-regional or contra-lateral relapse free interval was 97.5% for ILC vs 96.7% for non-ILC. Neither breast cancer specific nor overall survival differed between ILC and non-ILC.

Conclusion: Our findings add to the controversy whether ILC carries a distinct prognosis. Taking all established prognostic markers into account in a multivariate model, we did not find ILC to have a different DFS than non-ILC within this time frame of follow-up.

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Risk Factors of Breast Cancer Relapse. a Case-control Study and Results of Multivariate Analysis in a Cohort of 348 Patients Who Underwent Curative Surgery

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Background: Breast cancer (BC) is the first leading cause of cancer-related deaths in women, and a major public health problem, especially in western countries. All patients with BC may potentially develop disease progression and will thus need an effective lifelong follow-up. The aim of this study was to assess the overall weight of the main demographics, pathological and biochemical parameters considered as risk factors (RFs) for cancer relapse, obtained from a population of BC patients followed up for at least 60 months.

Patients and Methods: We retrospectively reviewed data regarding a series of 348 consecutive women (median age, 60 years, range 28−85 years) who underwent curative surgery for pT1−2, N0−1 (stage I and IIA) invasive ductal breast carcinoma. During five-year follow-up, 54 (15.5%) patients developed cancer relapse (cases), while 294 (84.5%) were cancer-free (controls). In both groups, the analysis was restricted to women who gave complete information. Final pathology defined the size of the tumor (pT), and axillary lymph node involvement (N+). Baseline carcinoembryogenic antigen (CEA, ng/mL) and cancer antigen 15−3 (CA 15−3, U/mL) serum levels were measured by automated testing using a two-site enzyme-linked immunosorbent assay. Both ER and PR were assayed using a quantitative standard immunoenzymatic method and results were expressed as percentage of positivity in the overall cell population. Immunostaining of the Ki-67 antigen was performed using the monoclonal antibody MIB-1 using a microwave antigen retrieval technique, and the MIB-1 labeling index was expressed as a percentage. For each parameter, the number of cases considered as positive were those above the median value. Odds ratio (OR) estimates and the associated 95% CI were obtained, and the significance level was set at $p < 0.01$.

Results: As expected, older (>60 years) age (OR = 0.82, 95% CI 0.46–1.47, $p = 0.51$), and high ER (OR = 0.29, 95% CI 0.24–0.78, $p < 0.0001$) and

PR (OR = 0.43, 95% CI 0.24–0.78, $p = 0.005$) rate were protective (OR < 1) factors, while only axillary node positivity (OR = 8.05, 95% CI 4.29–15.0, $p < 0.0001$) and high MIB-1 rate (OR = 2.28. 95% CI 1.27–4.11, $p = 0.006$) represented the two statistically significant RFs for cancer recurrence. Size (OR = 1.25, 95% CI 0.70–2.25, $p = 0.44$), and CEA (OR=1.05, 95% CI 0.66–2.07, $p = 0.62$) and CA 15−3 (OR = 1.27, 95% CI 0.71–2.27, $p = 0.42$) baseline serum levels, were not significantly related to cancer recurrence.

Conclusions: In this population, axillary node negativity and ER positivity were the most sensitive RFs for cancer recurrence at long-term follow up, while MIB-1 labeling index and PR rates were weak RFs.

References

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Cell-cycle Phase Specific Markers' Expression in Breast Cancer

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Background: Cell-cycle phase specific markers have been identified to be of high prognostic relevance in breast cancer. Ki67 expression is traditionally the most widely justified method for the assessment of mitotic state of cancer cells. Although, its utilization to date lacks standardization.

Methods: A total of 387 breast carcinomas were analyzed for the immunohistochemical expression of Cyclin A (CYCA), Geminin, Histone H3F3 (HH3F3), MCM2, MCM6 and Polo-like kinase 1 (PLK1) based on tissue microarrays. A two dimensional score reflecting intensity and frequency of staining was established and evaluated semi-quantitatively (0−11). The data were tested for their prognostic utility and reflection of mitotic index and DNA content measured by FACS.

Results: CYCA (p = 0.004), Geminin (p = 0.002) and MCM2 (p = 0.020) were able to distinguish prognostic subgroups by themselves. A meta-score of all cycle-specific markers was established bearing robust prognostic power (p = 7.7E-5). All outperformed Ki67 for prediction of prognosis, but the latter reflected mitotic index in a more accurate way (p = 3.8E-29) than CYCA (p = 8.7E-4), HH3F3 (p = 0.039), MCM6 (p = 1.5E-8) or PLK1 (p = 8.0E-7). Among the investigated proteins CYCA reflected DNA index (p = 0.008).

Conclusion: Cell-cycle specific markers may add further prognostic information. Ki67 remains, however, the most promising marker related to mitotic activity, and Cyclin A expression could reflect aneupoidy.

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High Level of Ki67 (⩾10%) is Not a Adverse Prognostic Factor in Younger (<35years) Patients with a Hormone Receptor-positive Breast Cancer

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Background: Young age is regarded as an adverse prognostic factor in patients with breast cancer, especially in those with a hormone receptor (HR)-positive tumor. This study aims to investigate whether the high level of Ki67 is strong prognostic factor in younger (<35years) patients with hormone receptor (HR)-positive breast cancer.

Materials and Methods: Data from 1,023 HR positive breast cancer patients in the Seoul National University Hospital Breast Care Center (SNUHBCC) who underwent surgery between 2000 and 2003 were reviewed. Patients were classified into two groups according to the level of Ki67: low (<10%) and high(⩾10%).

Results: In an analysis of 1,023 patients form a single institution, an age of <35years (hazard ratio 2.62; 95% confidence interval [CI]: 1.58–4.35; P < 0.001) and high level (⩾10%) of Ki67 (hazard ratio 1.64; 95% CI: 1.09–2.47; P < 0.018) were found to be independent predictors of distant metastases.

According to the level of Ki67, distant metastasis free survival was not significantly differ in an age of <35years(Ki67 <10%, hazard ratio 1.66; 95% CI: 0.82–3.37; P = 0.158; Ki67⩾10%, hazard ratio 1.67; 95% CI: 0.71–3.90; P = 0.240, respectively).

Conclusions: Young age (<35 years) and high level (⩾10%) of Ki67 were adverse prognostic factors in patients with HR-positive breast cancer. However, high level of Ki67 was not a strong predictor of distant metastasis in the young age (<35 years) patients.

Table 1. Multivariate analysis for prognostic factor associated with distant metastasis free survival among HR+ breast cancer according to the level of Ki67

	p	HR	95.0% CI of HR	
			LL	UL
Ki67 <10%				
Age of <35 years (vs ⩾35 years)	0.158	1.66	0.82	3.38
Tumor size >2 cm (vs ⩽2 cm)	0.001	2.65	1.47	4.80
Node positive (vs negative)	0.000	4.39	2.33	8.25
Histological Grade3 (vs G1 or G2)	0.262	1.36	0.79	2.32
Ki67⩾10%				
Age of <35years (vs ⩾35years)	0.240	1.67	0.71	3.90
Tumor size >2 cm (vs ⩽2 cm)	0.043	3.52	1.04	11.91
Node positive (vs negative)	0.002	4.32	1.73	10.79
Histological Grade3 (vs G1 or G2)	0.882	1.06	0.49	2.27

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Comparison of Molecular Subtyping with BluePrint and MammaPrint to Local IHC/FISH Based Subtype Classification According to St Gallen 2011 in 133 Breast Cancer Patients

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Background: Molecular subtypes in breast cancer are increasingly important in guiding adjuvant treatment decisions. We compared (locally assessed) molecular subtyping as defined by St Gallen treatment recommendations 2011 with three microarray based assays: BluePrint which classifies samples into Luminal, Basal and HER2 types, MammaPrint which classifies Luminal patients into High Risk Luminal B and Low Risk Luminal A, and TargetPrint which measures mRNA levels of Estrogen Receptor (ER), Progesterone Receptor (PR) and Her2neu (Her2).

Material and Methods: BluePrint, MammaPrint and TargetPrint were performed on fresh tumor samples from 133 breast cancer patients (T1−4, N0−2) between Dec 2008 and Jul 2011 at 11 institutions in US and Europe.

ER, PR, Her2 (n = 130) and Ki-67 (n = 79) IHC/FISH assessments were performed according to local practice at each institution. FISH was performed on 11 Her2neu samples if Her2 2+.

For 86 patients St Gallen subtype classification is possible.

Results: Concordance of TargetPrint with IHC/FISH is 97% for ER, 78% for PR and 95% for Her2.

Concordance of BluePrint with IHC/FISH subtyping is 88% for Luminal type and 93% for both Her2 and Basal type.

BluePrint	Subtyping according to St Gallen with local PA assessments					
	Luminal A (HR+, Her2−, Ki67 <14%)	Luminal B/Her2− (HR+, Her2−, Ki67 high)	Luminal B/Her2+ (HR+, Her2+)	Her2+ (HR−, Her2+)	Basal (HR−, Her2−)	Total
Luminal A-type	26	9	0	0	0	35
Luminal B-type	12	18	1	1	1	33
HER2-type	0	0	4	2	0	6
Basal-type	0	3	1	1	7	12
Total	38	30	6	4	8	86

Of 35 MammaPrint Low Risk (BluePrint Luminal A) 26 are Luminal A according to St Gallen definition (HR+, Her2−, Ki67 <14%) and 9 are Luminal B according to St Gallen definition (HR+, Ki67 high or Her2+).

Of 31 MammaPrint High Risk (BluePrint Luminal B) 12 are Luminal A according to St Gallen definition (HR+, Her2−, Ki67 <14%) and 19 are Luminal B according to St Gallen definition (HR+, Ki67 high or Her2+).

Resulting in a concordance of Ki67 and MammaPrint of 68%.

Conclusions: There is high concordance between IHC/FISH and TargetPrint. There is fair concordance with subtyping according to St Gallen and BluePrint. Concordance between MammaPrint and Ki67 is poor.

Implementation of TargetPrint, BluePrint and MammaPrint may improve the clinical management of breast cancer patients.