ER, PR, HER2 was previously determined. IHC molecular subtypes were defined based on expression of these markers: Luminal A: ER+ and/or PR+, HER2− and Ki67−; Luminal B: ER+ and/or PR + and HER2+ and/or ki67+; ERBB2: ER−, PR− and HER2+; Basal-like: ER−, PR−, HER2− and EGFR+ and/or CK5/6+; Unclassified: ER−, PR−, HER2−, EGFR− and CK5/6−. IHC molecular subtypes were validated against gene expression defined molecular subtypes. Assessment of distribution and prognostic effect of molecular subtypes was stratified to age (<65 versus ⩾65 years).

Results: Validation of molecular subtypes determined by IHC against gene expression revealed a substantial agreement in classification (Cohen's kappa coefficient 0.77). A statistical trend to an association ($p = 0.056$) was found between molecular subtypes and age, where Luminal tumors were more often found in elderly patients, while ERBB2, basal-like and unclassified subtypes were more often found in young patients. Molecular subtypes showed a prognostic association with outcome in young patients concerning relapse free period (RFP) ($p = 0.03$) and relative survival (RS) ($p < 0.001$). No statistically significant prognostic effect was found for molecular subtypes in elderly patients (RFP $p = 0.7$; RS $p = 0.3$). Additional analyses showed that no molecular subtypes showed a statistically significant difference in outcome for elderly compare to young patients, apart from Luminal A tumor where elderly patients had a worse RS.

Conclusion: We have shown that molecular subtypes have a different distribution and prognostic effect in elderly compared to young breast cancer patients, emphasizing the fact that biomarkers may have different distributions and prognostic effects and therefore different implications in elderly compared to their younger counterparts. Our results support the premise that breast cancer clinical behavior is significantly affected by patient age, but we suggest that competing risks of death in elderly patients and ER-driven differences in biology are underlying these age-dependent variations in patient prognosis, rather than the general belief that elderly breast cancers are of a more indolent biological character.

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Survival in Early Breast Cancer Patients is Influenced by Circulating Tumor Cells

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Background: There is good evidence for Circulating tumor cells (CTCs) in the peripheral blood to be a predictor of shortened progression-free and overall survival in metastatic breast cancer patients. Now we evaluated whether the presence of CTCs in patients with early breast cancer before the initiation of systemic adjuvant chemotherapy increases the likelihood of subsequent relapse and death.

Methods: In 2,026 patients with early breast cancer, CTCs were analyzed using the CellSearch System (Veridex, USA) right after complete resection of the primary tumor and prior to the initiation of systemic adjuvant treatment. All patients were randomized in the SUCCESS A trial, which compared FEC-Docetaxel vs. FEC-Docetaxel-Gemcitabine and 5 vs. 2 years of treatment with zoledronic acid in primary breast cancer patients and node positive or high-risk node negative disease. Patients were followed for a median of 35 months (range 0 to 54 months). The prognostic significance of CTCs for disease-free and overall survival was assessed using the Cox regression models.

Results: CTCs were detected in 21.5% of patients (435 out of 2026; median 1.3, range 1–827). Axillary lymph node involvement was more prevalent in patients with CTCs ($p < 0.001$), but no association was found with tumor size, histopathological grading or hormone receptor status.

There were 114 events of recurrence and 66 patients died of their disease. The presence of CTCs before systemic treatment was an independent predictor of poor disease-free survival (DFS) ($p < 0.0001$), distant disease-free survival (DDFS) ($p < 0.001$) and overall survival (OAS) ($p = 0.0002$). Patients with at least 5 CTCs had the worst prognosis with a four-fold increased risk of recurrence and a three-fold increased risk of death (hazard ratio (HR) 4.0 for DFS and 3.1 for OAS).

Conclusions: This is the first study to prospectively evaluate in a large patient cohort with early breast cancer the relevance of CTCs observed in the peripheral blood prior to the initiation of systemic treatment to the prognosis of early disease recurrence. CTC detection may be a clinically useful tool for monitoring treatment and should be tested as an indicator for secondary adjuvant treatment interventions in clinical trials.

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Using the 21-gene Breast Cancer Assay in Adjuvant Decision-making in ER-positive (ER+) Early Breast Cancer (EBC) is Cost-effective: Results of a Large Prospective German Multicenter Study

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Background: The Onco*type* DX® Recurrence Score (RS) is accepted as a predictive marker of adjuvant chemotherapy benefit in patients (pts) with ER+ EBC. We performed a clinical study to evaluate the impact on treatment decisions when using the RS including a pharmacoeconomic assessment of cost-effectiveness of using the assay in Germany.

Materials and Methods: Pts with ER+, HER2-negative N0 and N+ (1−3 positive lymph nodes) EBC and no contraindication for chemotherapy were included. Treatment recommendations before and after knowledge of the RS and actual treatment data were recorded. A Markov model was developed to estimate the long term costs and life expectancy associated with chemotherapy decisions in ER+, N0 and N+ EBC including 3 health states (recurrence, no recurrence and dead). Transition from one state to another was based on published recurrence risk data. The model compared costs and life expectancy associated with treatment decisions either based on criteria currently used in German clinical practice or on the RS. The study was conducted in the perspective of German sick funds' and over a 30 year time frame. Costs and outcomes were discounted at 3% per year. One-way sensitivity analyses were conducted on key variables.

Results: Of the 366 evaluable pts 244 were N0 and 122 N+, 54.1% had low, 38.0% intermediate and 7.9% high RS values. Initial recommendation changed in 33.1% of all cases.

Prior to the RS 50.5% of low, 62.6% of intermediate and 75.9% of high RS pts were recommended chemotherapy. Net changes in chemotherapy use from the study were −18.9% for all pts, −36.9%, 1.4%, and +20.7% for pts with low, intermediate and high RSs. Using Onco*type* DX to guide chemotherapy decisions was associated with an increase in survival (4.83 life years) due to the high number of pts reclassified by the RS as likely to benefit from chemotherapy and an incremental cost of €757 per patient. Thus, using the test in Germany is expected to be cost-neutral to the sick funds (i.e. incremental cost-effectiveness ratio of €206/life year). Considering the societal perspective, the incremental cost-effectiveness ratio associated with the use of the test is €6/life year. One-way sensitivity analyses confirmed the robustness of the main results.

Conclusions: Onco*type* DX guided chemotherapy decision-making for ER+ EBC resulted in a significant reduction of adjuvant chemotherapy usage and was cost-neutral versus current clinical practice.

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Could the Axilla Be Managed Less Aggressively in Selected Node-positive Breast Cancer Patients?

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Background: With advances in staging, and increasingly effective systemic treatments, management of the axilla is becoming less aggressive. Until recently, when sentinel lymph node biopsy became the standard axillary staging technique, our institutional policy has entailed four-node sampling, with node-positive (<4) patients receiving axillary radiotherapy (ART) without axillary clearance. This has been retrospectively evaluated for outcomes of regional recurrence, in a consecutive cohort with over 10 years of follow-up. Our hypothesis is that this management protocol is efficient and associated with low risk of regional recurrence (RR), and that there is a difference based on the number of positive nodes.

Methods: The study population was selected from 2607 consecutive patients with operable cT1-T2 breast cancer at our institution between the years 1990 and 2000. Surgery and radiotherapy to breast or chest wall, and systemic therapy, were given according to standard local guidelines.

Number of positive nodes did not affect management decision. Regional radiotherapy was given to the axilla from an anterior field, 50 Gy D_{max} in 25 daily fractions. Outcomes of recurrence and 'breast cancer specific survival' (BCSS) were assessed.

Results: 387 patients with 1−3 positive nodes who received ART were identified. The number with 1, 2 or 3 nodes involved was 222, 106 and 59 respectively. Median follow up was 129 months (5–247) There were 28 (7%) RR, with median time to recurrence of 50 months (12–175). Estimated RR free survival (SE) was 95.3% (0.011) at 5 years, 93.1% (0.014) at 10 years.

Univariate survival analysis demonstrated that 1 node positive status predicted a lower risk of RR compared to those with 2/3 nodes positive (HR 0.376, 95% CI 0.173–0.815, p = 0.013.) This remained significant on multivariate analysis, adjusting for tumour grade and tumour size (p = 0.02).

Estimated BCSS (SE) was 85.8% (0.018) at 5 years, 72.4% (0.024) at 10 years, with appearances of improved survival in the 1 node positive group; however, this was of borderline significance, p = 0.086.

Conclusion: RR is uncommon in 1−3 node positive patients treated with ART, but the risk is significantly lower in the 1 node positive sub-group. Less aggressive strategies of regional management should be considered and investigated for these patients.

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Clinical Significance of Stem Cell Phenotype (CD44+/CD24−) Relating to Molecular Subtype of Breast Cancer − a Multi-institutional Retrospective Study

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Background: The breast is the first solid organ that has been discovered its cancer stem cells. $CD44^{+}CD24^{-/low}Lineage^{-}$ tumorigenic cells showed stem cell properties. However, the clinicopathologic significance of stem cell phenotype has not been clarified yet. To investigate the prognostic significance of stem cell phenotype ($CD44^{+}/CD24^{-}$) in breast cancer cells and its relation to molecular subtype, we performed a multi-institutional retrospective study.

Materials and Methods: From 8 institutions of South Korea, 1401 consecutive breast cancer cases were collected which were resected from 1997 to 2003. Median follow-up period is 73.1 month. Immunohistochemical (IHC) stainings of ER, PR, EGFR, CK5/6, CK14, c-kit, CD44 and CD24 were done on slides of 64 tissue microarray blocks. To identify the stem cell phenotype ($CD44^{+}/CD24^{-}$), double IHC staining for CD44 and CD24 was done. The status of HER2/neu amplification was investigated by dual-color silver-enhanced *in situ* hybridization (SISH). Clustering of CD44/CD24 expression pattern was done using k-means clustering (stem cell phenotype). In addition, clustering of stem cell phenotype and molecular subtype markers was done using k-modes clustering. Univariate and multivariate survival analyses were done using Kaplan-Meier method and cox regression test.

Results: Seven hundred forty five cases were analyzed which had all available IHC stain, SISH results and follow up data. CD44/CD24 expression pattern was classified into stem cell rich/poor phenotype using k-means clustering. The pattern of stem cell phenotype and molecular subtype markers was classified into five subtypes using k-modes clustering. Those were hormone receptor positive and stem cell rich subtype (19.5%), hormone receptor positive and stem cell poor subtype (41.3%), basal-like subtype (4.7%), null subtype (23.2%) and *HER2* positive subtype (11.3%). The hormone receptor positive and stem cell rich subtype shows best prognosis, followed by basal-like, hormone receptor positive and stem cell poor, null, and *HER2* positive subtypes (p < 0.001, log-lank test) and the significance remained after adjustment with TNM stage.

Conclusions: By clustering analysis, hormone receptor positive breast cancer is divided stem cell rich/poor phenotypes. The stem cell rich phenotype shows better prognosis than stem cell poor phenotype. The stem cell phenotype could be a prognostic marker in hormone receptor positive type. In addition, the stem cell rich phenotype is associated with basal-like and null subtypes and stem cell poor phenotype is associated with *HER2* positive subtype.

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A Validated Web-based Nomogram for Predicting Positive Surgical Margins in Breast-conserving Therapy

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Background: Breast-conserving therapy (BCT) is considered standard treatment for early-stage breast cancer. One of the most important risk factors for local recurrence following BCT is the presence of positive surgical margins. We aimed to develop and validate a user-friendly prediction model (nomogram) based on preoperatively obtainable variables to predict for positive surgical margins in BCT.

Patients and Methods: Breast cancer patients who underwent BCT throughout the North-East region of the Netherlands between June 2008 and July 2009 were selected from the Netherlands Cancer Registry (n = 1185). Results from multivariate logistic regression analyses served as the basis for development of the nomogram. Nomogram calibration and discrimination were assessed graphically and by calculation of a concordance index, respectively. Performance of the nomogram was validated on an external independent dataset (n = 331) from the University Medical Center Groningen.

Results: The following clinicopathological variables were associated with positive surgical margin status in BCT and were included in the final model: microcalcifications on mammogram (OR: 1.37), absence of preoperative MRI (OR: 1.80), suspicion of multifocality (OR: 2.81), non-palpable tumor (OR: 1.51), positive preoperative N-stage (OR: 1.73), large tumor size (OR: 1.33), high density of the breast (OR: 1.22), lobular histological type (OR: 2.90), high histological grade (OR: 1.44), positive ER status (OR: 1.80), and presence of DCIS (OR: 3.11). Concordance indices were calculated of 0.70 (95% CI: 0.66–0.74) and 0.69 (95% CI: 0.63–0.76) for the modeling and the validation group, respectively. Calibration of the model was considered good in both groups. A nomogram was developed as a graphical representation of the model.

Conclusions: We developed and validated a nomogram to predict the probability of positive surgical margins in BCT using preoperatively obtainable clinicopathological variables. Moreover, a web-based version (*Breast Conservation!*) of the nomogram is accessible at http://www.breastconservation.com (login name: review; password: ArrBaw5X). Our nomogram could support clinicians in counseling patients regarding the likelihood of requiring further surgery, identify high risk patients who could benefit from more extensive surgery, and allow for the stratification of patients in clinical trials.

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The Influence of Education Level On the Survival of Breast Cancer

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Background: The prognostic role of education level (EL) in breast cancer has been not consistent but still controversial. We tried to investigate the role of EL as a prognostic factor of breast cancer.

Materials and Methods: Data of 36,299 primary breast cancer patients diagnosed between 1987 and 2008 from the Korean Breast Cancer Registry was analyzed. EL was classified into the low EL group (<12 years; n = 13,178) and the high EL group (⩾12 years; n = 23,121) according to time spent in education.

Results: The high EL group had younger age, earlier tumor stage, more estrogen receptor positivity, less HER2 positivity, lower histologic grade, less lymphovascular invasion, and less body mass index compared to the low EL group. The high EL group received more lumpectomy and/or more adjuvant therapy than the low EL group regardless of tumor size, node positivity, tumor stage, and operation methods. Both overall survival rate and breast cancer specific survival rate of the high EL group were higher than those of the low EL group (log-rank test, both $P < 0.001$). The differences in survival rates between the high and low EL groups were more evident under clinicopathologically more favorable conditions.