EL was a statistically significant independent factor with adjustment for various clinicopathologic parameters.

Conclusions: EL was a strong independent prognostic factor of breast cancer and these results were more evident under clinicopathologically more favorable conditions. Earlier diagnosis and active treatments are suggested as the main causes of superior survival in the women with higher EL.

Table: Univariate and multivariate analyses of overall survival according to clinicopathologic characteristics

	Univariate analysis			Multivariate analysis								
Characteristic	Cox's proportional hazard model			Biological model[a]			Treatment model[b]			Combined model[c]		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Education level, high vs low	0.759	0.709-0.812	<0.001	0.776	0.684-0.880	<0.001	0.804	0.743-0.870	<0.001	0.837	0.727-0.964	0.014
Age, >35 vs ⩽35	0.652	0.593-0.718	<0.001	0.628	0.527-0.748	<0.001				0.578	0.479-0.698	<0.001
Tumor size, >2 cm vs ⩽2 cm	3.064	2.829-3.320	<0.001	1.895	1.648-2.178	<0.001				1.613	1.369-1.900	<0.001
Node positivity, yes vs no	3.762	3.484-4.061	<0.001	2.648	2.292-3.058	<0.001				2.247	1.892-2.668	<0.001
Metastasis, yes vs no	12.024	10.720-13.487	<0.001	5.221	3.994-6.825	<0.001				4.413	3.156-6.170	<0.001
Hormone receptor, positive vs negative	0.501	0.466-0.540	<0.001	0.477	0.421-0.540	<0.001				0.546	0.444-0.671	<0.001
HER2, positive vs negative	1.345	1.234-1.465	<0.001	1.046	0.931-1.176	0.446				1.014	0.892-1.153	0.833
Histologic grade, 3 vs 1, 2	2.257	2.082-2.445	<0.001	1.665	1.463-1.894	<0.001				1.608	1.394-1.855	<0.001
Lympho-vascular invasion, yes vs no	2.537	2.280-2.821	<0.001	1.492	1.307-1.704	<0.001				1.475	1.268-1.716	<0.001
Body mass index, >25 vs ⩽25	1.109	1.031-1.193	0.005	1.021	0.902-1.157	0.740				1.104	0.962-1.266	0.159
Operation, mastectomy vs lumpectomy	2.799	2.558-3.062	<0.001				4.608	4.104-5.173	<0.001	2.816	2.346-3.380	<0.001
Radiation therapy, yes vs no	0.926	0.861-0.997	0.040				2.178	1.990-2.384	<0.001	1.498	1.289-1.739	<0.001
Chemo-therapy, yes vs no	1.629	1.473-1.802	<0.001				1.276	1.138-1.431	<0.001	0.779	0.599-1.015	0.064
Hormonal therapy, yes vs no	0.593	0.551-0.639	<0.001				0.613	0.566-0.663	<0.001	0.865	0.707-1.058	0.157

HR, hazard ratio; CI, confidence interval; HER2, human epidermal growth factor receptor 2. [a]Cox's proportional hazard model adjusted with nine factors including age, tumor size, node positivity, metastasis, hormonal receptor, HER2, histologic grade, lymphovascular invasion, and BMI. [b]Cox's proportional hazard model adjusted with four factors including operation, radiation therapy, chemotherapy and hormonal therapy. [c]Cox's proportional hazard model adjusted with all thirteen factors described above.

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Ki-67 as Predictive Biomarker for Systemic Chemotherapy in Breast Cancer

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Background: Biological markers that reliably predict clinical or pathological response to primary systemic therapy early during the course of chemotherapy may have considerable clinical potential. Aim of the study is to evaluate changes in Ki-67 (MIB-1) labeling index and apoptotic index (AI) before, during, and after neoadjuvant anthracycline chemotherapy in breast cancer.

Materials and Methods: Breast cancer tissue were collected from Grant Medical College and Sir J.J. Groups of Hospitals, Mumbai, India. Twenty-seven patients receiving neoadjuvant FEC (5-fluorouracil, epirubicin, and cyclophosphamide) chemotherapy for operable breast cancer underwent repeat core biopsy after 21 days of treatment.

Results: The objective clinical response rate was 56%. Eighty patients (31%) achieved pathological response by histopathological criteria; two patients had a near-complete pathological response. Increased day-21 AI was statistically significant predictor of pathological response ($p = 0.049$). A strong trend for predicting pathological response was seen with higher Ki-67 indices at day 21 and AI at surgery ($p = 0.06$ and 0.06 respectively).

Conclusion: The clinical utility of early changes in biological marker expression during chemotherapy remains unclear. Until further prospectively validated evidence confirming the reliability of predictive biomarkers is available, clinical decision-making should not be based upon individual biological biomarker profiles.

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Triple-negative Breast Cancer − Which Classical Prognostic Factors Can Help in Identifying Patients with Early Relapse?

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Background: Triple-negative breast cancer (TNBC) belongs to a poor prognostic group with the highest risk of relapse during the first few years after radical surgery. We investigated the influence of classical prognostic factors and adjuvant CHT (A-CHT) on 2-year prognosis in early TNBC pts treated at the Institute for Oncology and Radiology of Serbia (IORS).

Patients and Methods: We identified a group of 165 stage 1/2 TNBC pts diagnosed during 2006–2008 treated with radical surgery ± postoperative radiotherapy and A-CHT as per protocol. TN status was defined as IHC ER0−3/PR0−3/HER2:0−1 or IHC HER2:2+/CISH−. We analyzed the following prognostic factors: patients' age, menopausal status, medullar histology, tumor size, tumor grade, nodal status, HR/HER2 phenotype (ER0/PR0/HER2:0 vs. non-ER0/PR0/HER2:0) and A-CHT (anthracyclines vs. non-anthracyclines). Disease free survival (DFS) and overall survival (OS) were the main end points. Fisher Exact test, Pearson Chi-squared test and Log-rank test were used for statistical analysis.

Results: Median age of analyzed group was 58 years (range 26−84) and median follow-up was 24 months (range 3−56). Disease relapse experienced 31/165 (18.8%) pts, and 21/165 (12.7%) pts died, all from BC. Women ⩽50 years more frequently undergone subcutaneous mastectomy with immediate reconstruction ($p < 0.0001$) and received more frequently anthracycline − containing CHT ($p < 0.0001$) compared to women >50 years. Medullar BCs were more frequently associated with grade 3 tumors than non-medullar BCs ($p < 0.0001$). Breast conserving surgery was more frequently performed in pts with tumors ⩽2 cm compared to pts with tumors >2 cm ($p < 0.0001$) and in N0/N1−3 pts compared to N⩾4 pts ($p = 0.0003$). Grade 3 BCs were more frequently associated with ER0/PR0/HER2:0 phenotypes than grade 2 BCs ($p = 0.03$). Pts with N⩾4 more frequently experienced disease relapse than pts with N0/N1−3 ($p = 0$), especially bone and liver metastases ($p < 0.0001$ and $p = 0.0002$, respectively). There was no difference in DFS and OS in subgroups divided according to age, menopausal status, tumor histology, size and grade, HR/HER2 phenotype, and type of adjuvant CHT. N0/N1−3 pts subgroups had significantly better DFS (Log-Rank test; $p = 0$) and OS (Log-Rank test; $p = 0$) than N ⩾4 subgroup.

Conclusion: Nodal status was the only prognostic discriminator for 2-year outcome in pts with stage 1/2 TNBC.

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PR Negative Tumors ñ Prognosis and Results of Oncotype Dx

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Several studies demonstrated that breast cancers that are ER positive/PR negative carry a worse prognosis than ER/PR positive tumors if treated only by adjuvant hormonal therapy. In these studies PR was assessed by methods that measure protein content. Oncotype Dx measures mRNA expression. In clinical trials the discrepancy between IHC and RT PCR for the expression of PR was about 20%, but this was not assessed in a community setting. The meaning of high levels of PR mRNA without detectable PR protein is unclear. This discrepancy might result from technical issues or might be explained by a biological mechanism such as translational inhibition of PR by miRNA's.

We sought to verify the prognostic value of the expression of the progesterone receptor by IHC in women with early stage HER 2 negative, ER positive BC treated with systemic hormonal therapy only at our institution. Next, we aimed to characterize the results of Oncotype Dx in ER positive, PR negative HER 2 negative tumors.

Methods: 1st cohort: Files of consecutive patients with ER positive HER 2 negative tumors that were treated by adjuvant hormonal treatment during 2000–2006 were reviewed.

2nd cohort: The characteristics of PR negative tumors tested by Oncotype Dx during 2007–2011 were analyzed.

Results: 1st cohort: 286 patients' files were reviewed. 82% of tumors were node negative. 80 were PR negative and 209 were PR positive. No significant differences existed between the groups with respect to nodal involvement or grade. After a median follow up of 7.24 years (0.78 to 9.5) 8 women with PR negative and 9 women with PR positive tumors experienced disease recurrence (10% vs 4.3%, RR 2.32, p 0.014 by univariant analyses). 33% of patients (5 out of 15) with grade 3 PR negative tumors experienced recurrence.

2nd cohort: 81 PR negative tumors were included. The rate of discordance between IHC and Oncotype Dx for PR expression was 47.3%. Recurrence scores were: 50% intermediate risk, 32.5% low risk and 17.5% high risk. Grade 3 tumors were more likely to be high risk (37.5%) and PR negative by RT PCR (84.6%).

Conclusion: In our hands PR negative tumors carry a worse prognosis when treated by hormonal adjuvant treatment compared with ER/PR positive tumors. We found a high rate of discrepancy between IHC and RT PCR concerning PR expression.

Most PR negative tumors are labeled intermediate risk. The value of the test in this setting is unclear.

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Breast Density Change as a Predictive Surrogate of Adjuvant Anti-estrogen Therapy Response in Estrogen Receptor Positive Breast Cancer

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Background: Mammographic breast density is an established risk factor of breast cancer. Previous studies showed that adjuvant anti-estrogen therapy lowers breast density. We hypothesized that the change of breast density can be a surrogate marker predicting response to anti-estrogen therapy.

Materials and Methods: We analyzed data of 1,542 estrogen receptor positive breast cancer patients who underwent surgery in Seoul National University Hospital between Oct 2003 and Dec 2006. Of them, patient who accomplished at least 2 year of adjuvant hormone therapy (mmg available) were included and total 1065 cases were evaluated. Percent mammographic density (PMD) was evaluated by comparing mammography taken preoperatively and after 8−18months of adjuvant hormone therapy. PMD was measured with Cumulus software 4.0. Factors associated with the change of PMD (dPMD = postPMD − prePMD) were analyzed and recurrence-free survivals were compared with respect to dPMD.

Results: After median follow up of 67.69 months, overall recurrence rate was 7.5% (80/1065). Mean dPMD was 5.92% (−17.22 to 36.87). In a univariate analysis, younger age, tamoxifen use (vs aromatase inhibitor), high prePMD, high histologic grade, positive lymph node, and adjuvant chemotherapy were associated with higher dPMD (p value <0.05). In a multivariate analysis, age <50, high prePMD and adjuvant chemotherapy were significantly associated with higher dPMD (p value <0.05). In a survival analysis, duration of anti-estrogen therapy, size, LN status, high Ki-67, and dPMD were independent factors associated with recurrence-free survival.

Conclusions: PMD change after about 1 year of adjuvant hormone therapy was a significant predictive factor for long term recurrence-free survival in patients with ER-positive breast cancer. Mammographic density change might be used clinically for the prediction of prognosis in patients taking anti-estrogen therapy.

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Vitamin D Receptor and Prognosis in Breast Cancer

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Background: The Vitamin D receptor (VDR) belongs to the nuclear class II receptor family. It is involved in cell growth and differentiation in healthy and malignant breast tissue through its binding capacity for vitamin D and shows anti-proliferative effects.

Material and Methods: In this study we analyzed VDR (1,25(OH)2D3 receptor) expression and survival in a breast cancer patient cohort of 82 patients. 75/82 (91.5%) patients showed immunohistochemical results after detection of the VDR expression with monoclonal antibodies and the ABC method. Staining results were classified as Immunoreactive Scores (IRS), which were assigned according to Remmele and Stegner. The IRS score was calculated by multiplication of the staining intensity and percentage of cells stained positive. IRS 0−1 was classified as negative/very low, IRS 2−4 as moderate-high and IRS 6−12 as high. Statistical analysis was performed by Spearman's correlation test ($p < 0.05$ significant). Overall survival was analyzed using Kaplan-Meier estimations.

Results: Only 6 patients (8%) had a negative or very low IRS. Moderate IRS values (2−4) were present in 20 patients (26.7%). Most of the patients had a high IRS (49 patients, 65.3%). For survival analysis, data were dichotomized (IRS 0−4: negative to moderate and IRS 6−12: high VDR expression) because of the small number of patients in the IRS 0−1 group. In univariate analysis, VDR expression showed significant differences in progression-free survival (PFS), $p = 0.046$; HR 0.83, 95% CI 0.73–0.94) and overall survival (OS), $p = 0.014$; HR 0.35; 95% CI 0.15–0.81). Patients with high IRS scores showed a significantly better PFS (log rank: $p = 0.037$) and OS ($p = 0.008$) than patients with moderate or negative IRS scores for VDR expression. VDR expression showed a trend towards a correlation with OS ($p = 0.06$; HR 0.39; 95% CI 0.14–1.04), and clearly non-significant correlation to PFS ($p = 0.436$; HR 0.67; 95% CI 0.24−1.9). When analyzed separately, the 3 different IRS groups (IRS 0−1, 2−4 and 6−12) showed significant differences in VDR expression (multivariate analysis for OS: $p = 0.034$, HR 0.45, 95% CI 0.21–0.94).

Discussion: Our data suggest that VDR expression in breast cancer tissue may be of clinical significance, and the results provide evidence that VDR may be a factor with prognostic relevance.

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TROP-2 Expression and Mutation in Familiar Breast Cancer

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Background: TROP-2 is a transmembrane calcium signal transducer, involved in the regulation of cell-cell adhesion. It is a stimulator of human cancer growth and a marker of metastatic tumor, so it could be a target of diagnostic and therapeutic procedures.

The aim of this study was to analyze TROP-2 expression in two different subset of familiar tumors (patients were selected to have BRCA mutation), to identify patients with a worst outcome disease.

Materials and Methods: We analyzed 32 Ductal Invasive Breast Cancer (CDI), 16 were defined Triple Negative (TN), whereas 16 were Non-Triple Negative (NTN) CDI. All cases were tested for BRCA mutations, TROP-2 mutations and expression, hormonal receptor status (ER, PgR), Ki-67, ck5/6 and EGF-R expression.

BRCA 1/2 and TROP-2 mutations were screened by direct sequencing.

Results: All 16 TN cases had a high proliferation index with an average of 50% (from 25% to 90%), 12 out of 16 samples were negative to ck 5/6 expression, whereas 10/16 were negative to EGF-R reaction.

Moreover, TROP-2 immunoreactivity high or moderate was detected in 6 (37.5%) out of 16 TN breast samples, whereas 10 (62.5%) case had low TROP-2 IR. 5 out of 6 cases with high/moderate TROP-2 score resulted also ck5/6 negative and 4 were EGF-R+. The follow-up data (mean FU: 50.4 months; range: 24–132 months) of these 6 patients revealed that in 5 cases there was disease relapse.

Only 8 out of 16 NTN breast cancer showed a high ki-67 proliferation index with an average of 46% (from 2% to 80%), 12 out of 16 samples were negative to ck 5/6 expression and 6 were EGF-R +. A high and moderate TROP-2 immunoreactivity was detected in 6 (37.5%) out of 16 NTN breast samples, whereas 10 had low IR. 5 out of 6 cases with high/moderate TROP-2 score resulted also ck5/6− and EGF-R−. The follow-up data (mean FU: 40 months; range: 12−72 months) of these 6 patients revealed that in 3 cases there was disease relapse.

Our results show that 8 (67%) out of 12 tumor with TROP-2+ had disease relapse.

As expected, cases with TROP-2 protein overexpression did not presented gene mutations with the exception of 2 cases.

Conclusions: Our data suggest that: (1) there is not difference in TROP-2 expression between TN (37.5%) and NTN (37.5%) familiar breast cancer and (2) TROP-2 positive tumor do not express ck 5/6. TROP-2 overexpression could be predictive for poor disease-free survival and useful as an immunohistochemical prognostic factor related to tumor progression.