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Hereditary Predisposition to Breast and Ovarian Cancer Based on BRCA1 and BRCA2 Screening in Women in Croatia

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Epidemiological data indicates 5−15% of all breast cancer cases are hereditary, and germline mutations in Breast Cancer Gene 1 (BRCA1) and Breast Cancer Gene 2 (BRCA2) account for the majority of hereditary breast and ovarian cancers.
The contribution of BRCA1 and BRCA2 mutations to hereditary breast and ovarian cancer in Croatia is unknown. The aim of our study was to estimate the incidence and spectrum of pathogenic mutations in BRCA1 and BRCA2 genes in high risk women in Croatia.
The screening was performed by high resolution melting approach, direct sequencing and semi-quantitative multiplex PCR method (Cvok et al 2008, Clin Chem Lab Med). Protocols were certified by EMQN (European Molecular Genetics Quality Network).
The complete coding sequences and exon-intron boundaries analyses of both genes were carried out on 142 women with hereditary predisposition to breast and ovarian cancer.
Overall, 11 pathogenic mutations were detected, two novel in BRCA1, and three novel in BRCA2. Nineteen BRCA1 and 33 BRCA2 unclassified variants and polymorphisms were also identified, of which two BRCA1 and seven BRCA2 were not previously published.
This is the first molecular investigation of the hereditary predisposition to breast and ovarian cancer in Croatia based on BRCA1 and BRCA2 genes. Samples were collected from different regions of the country and the level of pathogenic mutations and distribution of polymorphic variants will contribute to population statistics.
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Bcl2 Expression Predicts Clinical Outcome of Combined Targeted Therapies of HER2+/ER+ and the Potential Benefit of Anthracycline-based Chemotherapy of HER2+ Breast Cancer (BC)

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Background: Trastuzumab and endocrine targeted therapies are effective in treatment of HER2+ and ER+ breast cancer respectively. However, recent studies showed that HER2+/ER+ BC patients relapse early on the combined Trastuzumab/Tamoxifen treatment. There is an urgent need to determine a biological marker that could help in selecting patients who delivered the most clinical benefit and achieved cost effectiveness from such treatment. In this study we explored if Bcl2 protein, which is a cell cycle/apoptosis regulator and ER downstream response gene, could predict clinical outcome of HER2+/ER+ patients after such agents.
Material and Methods: HER2 expression was assessed according to ASCO/CAP guidelines by using IHC and fluorescence in- situ hybridisation (FISH). Bcl2 expression was immunohistochemically evaluated in high risk (Nottingham Prognostic Index >3.4) HER2+ breast cancer; (a) 140 HER2+/ER+ BC patients treated with surgery (S) + Radiotherapy (RT) followed by endocrine therapy (ET) only; (b) 136 HER2+/ER+ BC patients treated with S + RT followed by sequential Adjuvant anthracycline combination chemotherapy FEC + Trastuzumab and ET; (c) 102 HER2+/ER− BC patients treated with S + RT followed by CMF chemotherapy only; (d) 106 HER2+/ER− BC treated with S + RT followed by sequential FEC + Trastuzumab; and (e) 63 locally advanced HER2+/ER− BC patients treated with neo-adjuvant FEC followed by surgery and adjuvant trastuzumab.
Results: For HER2+/ER+ patients on combined FEC + trastuzumab and ET, the 5-year progression free survival (PFS) of high level of Bcl2 expression was 96% vs. 43% of those with low level of Bcl2 (HR; 0.06, $p = 0.0003$). For HER2+/ER− patients treated with neo-adjuvant FEC followed by S and trastuzumab, the 5-year PFS of low level of Bcl2 expression was 20% vs. 90% of those with high level of Bcl2 (HR; 12.9, $p = 0.002$). Bcl2 expression had no effect on clinical outcome of HER2+/ER+ BC patients treated with S + RT followed by ET or HER2+/ER− BC patients treated with either S + RT followed by CMF chemotherapy, or S+ RT followed by FEC + Trastuzumab.
Conclusions: Immunohistochemical assessment of Bcl2 provided an effective simple and cheap test to predict response/resistance to the combination of endocrine therapy and HER-2 targeted therapies for this subgroup of high risk HER2+/ER+ BC patients. Therefore, it may help to identify the group of patients who may not benefit from the current chemotherapy and targeted therapy, in whom novel therapy would be appropriate.

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Topoisomerase IIα (TOPO2A) Protein Overexpression Predicts Response to Anthracycline-based Chemotherapy Irrespective of HER2 Status

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Background: Recent studies have suggested a link between levels of TOPO2A expression, carcinogenesis and response to anthracycline based chemotherapy. It has been postulated that this relationship may be due the co-amplification of the HER2 and TOPO2A genes. Some investigators have suggested that evaluation of TOPO2A protein level may be more useful than gene alterations.
Methods: In this study; the gene copy number changes using array CGH and chromogenic in-situ hybridization (CISH), the mRNA level using array gene expression and the protein expression using immunohistochemistry (IHC) for both TOPO2A and HER2 genes were evaluated in 171 unselected series of primary breast cancer. The results were validated by using CISH and IHC in four independent series of breast cancers (BC): (a) 240 locally advanced primary BC treated with anthracycline-based combination with or without Taxane followed by surgery + radiotherapy; pathological complete response (pCR) and progression free survival were used as the primary and secondry end points respectively, (b) 245 BC in which all patients were primarily treated with surgery + radiotherapy followed by anthracycline-based chemotherapy, (c) 145 primary BC overexpressing HER-2 treated with surgery + radiotherapy followed by sequential Adjuvant anthracycline combination chemotherapy FEC + trastuzumab and (d) 2000 consecutive cases of primary BC who were treated with surgery + radiotherapy and received adjuvant CMF and/or endocrine therapies according to Nottingham prognostic index and ER status. The association between gene and protein alterations of TOPO2A, HER2 and clinicopathological outcomes was determined. HER2 expression was assessed according to ASCO/ CAP guidelines by using IHC and fluorescence in- situ hybridisation (FISH).
Results: TOPO2A protein overexpression was associated with HER2 amplification/overexpression ($p = 0.001$), p53 mutation ($p = 0.001$), BRCA1 mutation ($p = 0.001$), basal CK5/6 ($p = 0.001$), mitotic index ($p = 0.01$) and high proliferation index ($p = 0.03$). In patients who received anthracycline based treatment, TOPO2A gene amplification predicted a better progression free survival ($p = 0.026$). In the anthracycline-based neoadjuvant chemotherapy series, the pCR rate was 31/132 (24%) in tumours expressing high levels of TOPO2A, compared to 3/49 (6%) in tumours expressing low levels of TOPO2A ($p = 0.008$). In multivariate analysis, TOPO2A expression was an independent predictor for pCR ($p < 0.01$).
Conclusions: Alteration in TOPO2A protein is an independent predictor of response to anthracycline based treatment in both adjuvant and neoadjuvant settings. TOPO2A gene amplification was exclusively asssociated with HER2 amplification/overexpression while TOPO2A protien overexpression was a marker of high proliferative tumours.

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Bcl2 Expression Predicts Clinical Outcome to Adjuvant Hormone Therapy and Response to Anthracycline-based Chemotherapy in ER+HER2- Breast Cancer

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Background: Not all breast cancer (BC) patients benefit from their treatment and there is an urgent need to identify biological markers

which could be used to tailor treatment to individual patients. Most chemotherapeutic agents preferentially target proliferating and cycling cells, which results in mitotic catastrophe and apoptosis. We therefore hypothesized that the level of Bcl2 protein, which is a cell cycle/apoptosis regulator, could predict response and clinical outcome to these agents.
Material and Methods: Bcl2 expression was immunohistochemically evaluated in high risk (Nottingham prognostic index >3.4) luminal "ER+/HER2−" breast cancer from four independent series; (a) 135 BC patients treated with surgery + radiotherapy at Nottingham City hospital before 1986 who did not receive any endocrine therapy, (b) 430 BC patients treated with surgery + radiotherapy followed by Tamoxifen therapies, (c) 179 BC patients treated with surgery + radiotherapy followed by Tamoxifen and anthracycline-based chemotherapy and (d) 70 locally advanced primary BC patients treated with an anthracycline-based combination (FEC) followed by surgery + radiotherapy and Tamoxifen.
Results: Luminal BC patients with low Bcl2 expression had 2 to 4 fold increase risk of death and recurrence compared to those with high Bcl2 irrespective of Tamoxifen treatment (Table 1). After anthracycline-based neo-adjuvant chemotherapy, 33% of low Bcl2 expression luminal BC achieved pCR vs. 7% of high Bcl2 expression luminal BC (p = 0.02). Luminal BC patients with low or high Bcl2 expression who had received anthracycline based combined therapy in addition to Tamoxifen in either neo-adjuvant or adjuvant sittings had similar BC specific survival and progression free survival (p = NS).

Table 1

Variable	Breast cancer specific survival		Progression free survival	
	HR (95% CI)	P	HR (95% CI)	P
High risk luminal breast cancer patients who did not received Tamoxifen (n = 135)				
Bcl2+	1	0.00006	1	0.003
Bcl2−	3.7 (1.9−6.9)		2.3 (1.3−4.0)	
High risk luminal breast cancer patients who received Tamoxifen (n = 430)				
Bcl2+	1	0.00000001	1	0.0000003
Bcl2−	2.6 (1.8−3.7)		2.1 (1.5−2.9)	

Conclusions: Low Bcl2 expression was associated with poor prognosis of high risk luminal BC irrespective of hormone therapy. Bcl2 status could predict the potential benefit of anthracycline based chemotherapy of luminal BC which is resistance to Tamoxifen. Clinical trials based on Bcl2 expression in luminal breast cancer are warranted.

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Gene Expression Profiles Predict Pathological Complete Response to Standard Neoadjuvant Fluorouracil, Doxorubicin, and Cyclophosphamide and Paclitaxel With or Without Trastuzumab in Early Breast Cancer

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Background: To examine the feasibility of gene expression signature as a predictor of pathological complete response (pCR) to sequential fluorouracil, doxorubicin, and cyclophosphamide (FEC) and weekly paclitaxel (P) with or without trastuzumab (T) neoadjuvant chemotherapy.
Materials and Methods: We have conducted consecutive two prospective phase II, establishing training and validation sets, with similar eligible criteria include, stage IIA-IIIC, chemotherapy-naïve, measurable disease, age ⩾20, PS 0/1, and adequate organ function. Patients were treated preoperatively with 4 cycles of FEC (500/100/500 mg/m^2) followed by 12 cycles of weekly P (80 mg/m^2) with or without T (2 mg/kg). Patients underwent pretreatment fine-needle biopsy for cDNA microarray using Affimetrix Gene Chip U133 plus 2.0 arrays with 30,000 differential expressions of various genes. We ranked gene probes from training sets according to a predictive power concerning pCR by Wilcoxon, and validated them using validation sets by SVM.
Results: Between July 2007 and December 2010, 122 patients were enrolled in the two consecutive prospective studies (training: 89 pts, validation: 33 pts). Median age was 51. PS 0/1: 115/7; Stage IIA/IIB/IIIA/IIIB/IIIC: 30/57/20/14/1; Histological subtype: ER+HER2- (LA)/ER+HER2+ (LB)/ER-HER2- (TN)/ER-HER2+ (enrich-HER): 51/18/24/29. All patients have received curable operations. pCR rate was 31.1% (LA; 2.0%, LB; 44.4%, TN; 37.5%, enrich-HER; 69.0%). 104 (85.2%) sufficient mRNA for cDNA microarray from individual primary breast cancer tissues fine-needle biopsy are available. As reported previously, the breast cancers were classified into a Luminal A/B, Basal-like, HER2-enriched, Claudin-low intrinsic subtypes, indicating a high quality of the representative method. In HER2 positive breast cancer, HER2-enriched subtype was a reproductive predictive marker. In contrast, In HER2 negative breast cancer, three genes (N-myc and STAT interacter, Tryptophanyl-tRNA synthetase, and IQCE) and basal-like subtype were validated as the predictors of pCR. The three genes were also identified as predictors of pCR in the triple negative population.
Conclusions: Specific gene expression profiles predict pCR to standard neoadjuvant regimen, especially in triple negative breast cancer.

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Correlation Between PARP-1 Expression and In-vitro Chemotherapy Sensitivity in Patients With Breast Cancer

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Background: Expression of Poly-A-Ribose-Polymerase-1 (PARP-1) has come into scientific focus based on its potential exploitation as a therapeutic target through PARP inhibitors. Furthermore, it could recently be demonstrated that cytoplasmic expression of PARP-1 varies depending on molecular breast cancer subtypes and is correlated with an increased response to neoadjuvant taxane-anthrazykline containing chemotherapy (von Minckwitz et al., J Clin Oncol (in press)). *In-vitro*-chemotherapy sensitivity and resistance assays (CSRAs) allow for the direct measurement of chemotherapy sensitivity in a given tumour independent of host factors.
Methods: We conducted an immunohistochemical tissue-microarray (TMA) analysis of 550 samples of invasive breast cancers with regard to expression of a set of molecular markers including estrogen receptor (ER), progesterone receptor (PR) and HER2 as well as PARP-1. Triple negative breast cancers (TNBC) were identified through lack of expression of ER, PR and HER2. All cancers were analyzed in an *in vitro* CSRA analysis for epirubicin/docetaxel (ED) and epirubicin/cyclophosphamide (EC). *In-vitro*-chemotherapy sensitivity was analyzed using an adenosine triphosphate (ATP) bioluminescence assay.
Results: A moderate/high PARP-1 expression was found in 48 and 33% of cases with TNBC and non-TNBC, respectively (p = 0.015). A correlation between TNBC phenotype and cytoplasmic expression was not observed. Instead, an increased both cytoplasmic and nuclear expression of PARP-1 was correlated with an increased *in-vitro* sensitivity against ED (p = 0.012 and 0.025, respectively) but not EC (p = 0.27 and 0.62, respectively).
Conclusion: Our results support previous observations in that expression of PARP-1 is correlated with an increased sensitivity against taxane-anthracycline chemotherapy independent of tumour phenotype.

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Response of Immunohistochemically (IHC) Defined Breast Cancer Sub-types to Dose-dense Sequential Adjuvant Chemotherapy. Pooled Analysis of Two Randomized Hellenic Cooperative Oncology Group (HeCOG) Phase III Trials

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Background: To investigate the efficacy of adjuvant dose-dense sequential chemotherapy with epirubicin, paclitaxel and CMF in sub-groups of patients with high-risk operable breast cancer, according to immunohistochemically (IHC) defined tumour sub-types.
Materials and Methods: Formalin-fixed paraffin-embedded (FFPE) tumour tissue blocks from 1030 patients (72% of the eligible patients) participating in two adjuvant dose-dense sequential chemotherapy phase III trials (HE 10/97 and HE 10/00) were centrally assessed in TMAs by IHC for 6 biological markers, i.e. estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), Ki67, cytokeratin 5 (CK5), and epidermal growth factor receptor 1 (EGFR). Cases with HER2 IHC 2+ were further evaluated by CISH or FISH. Patients were classified as Luminal A (ER-positive and/or PgR-positive, HER2-negative); Luminal B (ER-positive and/or PgR-positive, Ki67⩾14); Luminal-HER2 (ER-positive