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POSTER

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 to 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 secondary 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 associated with HER2 amplification/overexpression while TOPO2A protein 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