radiotherapy – 152 (73%); disease status – AWOD – 164 (82%), DWD – 31 (15%), AWD – 6 (3%).
The only statistically-significant prognostic parameters for OS on CPHM were tumor size ($p < 0.001$), lymph-node-status ($p < 0.001$), location ($p < 0.001$), and grade ($p = 0.007$), and for PFS were tumor size ($p < 0.001$), location ($p = 0.01$), and lymph-node-status ($p = 0.03$). The only statistically-significant prognostic parameters for OS on LGM were menopausal status ($p < 0.001$), grade ($p < 0.001$), and progesterone-receptor status ($p < 0.001$), and for PFS was tumor size ($p < 0.001$).
Conclusions: This retrospective, single-institution experience validates: a) the prognostic importance of classic clinicopathological parameters including the stats of menopause, tumor size and location, lymph-node, grade and progesterone-receptor, and b) The limited prognostic value of various immunohistochemical parameters commonly-used in practice.

5153 POSTER

The influence of polymorphism in TYMS, MTHFR and GSTP1 genes on toxicity and response in breast cancer patients treated with adjuvant chemotherapy

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Background: Genetic polymorphism in drug-metabolising enzymes and drug targets are known to be responsible for inter-individual differences in cancer treatment response and toxicity of various types of chemotherapeutics. *TYMS* and *MTHFR* variants may affect 5-fluorouracil (5-FU), capecitabine and methotrexate metabolism, while *GSTP1* influences detoxification of cyclophosphamide, doxorubicin, etoposide and platinum compounds. In the present study, 28-bp tandem repeat in *TYMS* promoter (TSER), *MTHFR* Ala222Val and *GSTP1* Ile105Val polymorphisms were investigated in relation to adverse effects in 135 breast cancer patients receiving neoadjuvant (4−9 cycles) and adjuvant (4−6 cycles) chemotherapy based on 5-FU, i.e. CAF, CMF, (n = 100) or AC, AT, TAC (n = 35) regimens.
Material and Methods: There were 135 women (mean age: 54 years, range 27−78) diagnosed with breast cancer stage I-IV included in the study. Ductal carcinoma accounted for the most frequent type of cancer (65%). Physical examination and full blood count were performed before each chemotherapy cycle. Toxicity was recorded according to the WHO criteria, after each cycle. The assessment of response concerned patients receiving neoadjuvant chemotherapy. It was based on physical examination, magnetic resonance and histopatological examination of tumor and lymph nodes of axilla. DNA was isolated from peripheral blood and the genotypes were identified using standard PCR-RFLP assay.
Results: Overall toxicity grade 3−4 was observed in 20% of patients. There were more *TYMS* 3R/3R carriers among cases with overall toxicity grade higher than 2 in all group ($P = 0.016$) and in patients receiving 5-FU-based therapy ($P = 0.047$). Also hematotoxicity grade 2−4 was noticed more often among 3R/3R homozygotes ($P = 0.076$). When gastrointestinal toxicity has been considered, grade > 2 was observed exclusively in *GSTP1* Ile/Ile or Ile/Val carriers in 5-FU treated subgroup ($P = 0.020$). Nausea and vomiting grade 2−3 were also associated with *GSTP1* Ile allele ($P = 0.058$). Response rate (complete or partial) in the group was 42%. There was slight preponderance of *TYMS* 3R/3R homozygotes and heterozygotes among responders *versus* nonresponders ($P = 0.077$). In treated with 5-FU in turn, 2R allele was found to be prevalent among good responders ($P = 0.051$).
Conclusions: Our preliminary results show that polymorphisms in *TYMS* and *GSTP1* may serve as useful predictors of toxicity and efficacy of chemotherapy in breast cancer patients, however large-scale, prospective studies are warranted.

5154 POSTER

Triple-negative high grade invasive ductal breast carcinomas are biologically heterogeneous: differences between the basal and non-basal subtypes

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Background: Triple-negative breast carcinomas (TNBC) are characterized by their unique molecular profile, aggressive behavior, distinct patterns of metastasis, and lack of targeted therapies. Although not synonymous, the majority of triple-negative breast cancers carry the “basal-like” molecular profile on gene expression arrays. Our objective was to explore the clinicopathological characteristics of TNBC.
Material and Methods: We selected 31 consecutive cases of invasive ductal carcinomas, histological grade 3, negative for estrogen and progesterone receptors (ER/PR) and HER2 protein. We examined differences between the basal subtype determined by expression of basal cytokeratins and/or epidermal growth factor receptor, and the non-basal phenotype in relation to age, tumor size, axilla involvement, presence of lymphvascular embolization and immunoexpression of p-cadherin and p53.
Results: The results are summarized in table 1.

Table 1: Differences between the basal and non-basal subtypes of triple negative breast carcinomas of histological grade 3

subtype	n	Age range (median)	Tumor size range (median)	LVE positive (%)	Axilla positive (%)	p-cadherin (%)	p53
basal	23	30−94 y (46)	2−16 cm (4.0 cm)	8 (34.8%)	12/18 (66.7%)	4 (17.4%)	16 (69.5%)
Non-basal	8	43−66 y (51)	2−10 cm (5.3 cm)	1 (12.5%)	4/7 (57.1%)	2 (25%)	5 (62.5%)

LVE = lymphvascular embolization.

Conclusions: TNBC of the basal subtype occur in younger patients and show more aggressive pathological characteristics compared to non-basal subtype, confirming the heterogeneity of the group.

5155 POSTER

Time-dependence of hazard ratios for prognostic factors in patients with early breast cancer

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Background: After surgical treatment of early breast cancer a life long risk for relapse persists. Hazard ratios for relapse can change during the follow-up period. The aim of our study was to study time-dependence for classical prognostic factors for relapse (age, tumor stage and grade, histological type, estrogen receptors, lymphovascular invasion (LVI) and of axillary lymph nodes involvement).
Materials and Methods: In 1035 patients with early breast cancer treated between 1983−87 at the Institute of Oncology Ljubljana (median follow-up was 17 years) we retrospectively analysed prognostic factors for relapse with Cox proportional hazard model and with test for violation of proportional hazard assumption (method of Schoenfeld’s residuals).
Results: Tumor size, grade and estrogen receptors (ER) were time-dependent prognostic factors for breast cancer relapse. Tumor size >2 vs. $\leqslant$2 cm (HR 1.59; 95% CI 1.21−2.10) and grade G2/3 vs. G1 (HR 2.08; 95% CI 1.39−3.13) were unfavorable prognostic factors in the first five years after surgery and lost their prognostic role thereafter. Positive ER (ER+ vs. ER) were a favorable prognostic factor only in the first year after surgery, from 1−5 years they had no prognostic role, after 5 years they became borderline unfavorable prognostic factor (HR 1.50 95% CI 0.98−2.30). Positive axillary lymph nodes (4−9 lymph nodes: HR 2.55; 95% CI 1.91−3.40; $\geqslant$10 lymph nodes: HR 4.65; 95% CI 3.29−6.58) and LVI (HR 1.58; 95% CI 1.21−2.06) were time-independent risk factors throughout the follow-up.
Conclusions: Tumor stage and grade are time-dependent unfavorable prognostic factors, being significant only in the first 5 years after surgery. Positive ER changes their prognostic role with time, from being shortly favorable to being unfavorable. More then 3 involved axillary lymph nodes and LVI are time-independent unfavorable prognostic factors throughout the follow-up period.

5156 POSTER

A tool to improve personalized cancer care: measuring the response of circulating epithelial tumour cells (CETC) and tumour stem cell subpopulations to therapy in the individual patient

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Background: Cells released from the primary tumour persisting and recirculating in the host can lead to the formation of distant metastases. We can show that CETC are detectable and can be quantified in the peripheral blood of almost all cancer patients including early-stage solid malignancies, although it was claimed that such cells are detectable only in a minor fraction of early-stage cancer patients.
Material and Methods: Using anticoagulated peripheral blood and red blood cell lysis as the only enrichment step, one centrifugation step, staining live cells with fluorochrome labelled anti-epithelial antigen as a search antibody, automated image analysis for detection of positive events

and evaluation of exclusively surface located epithelial antigen on vital unfixed cells, CETC were detected in most patients with early stage cancer. Subsequently cells could be stained with anti-ALDH-antibody and in situ hybridized for HER2/neu amplification and quantified repeatedly during neo/adjuvant chemotherapy and during maintenance therapy with hormones or trastuzumab.
Results: 497 breast cancer patients were analyzed more than three times during the course of disease, 248 during neoadjuvant/adjuvant chemotherapy, 249 during trastuzumab and or hormone therapy. Different pattern of therapy response were obtained with rapidly responding CETC changes over several logs in response to chemotherapy and slow and long-lasting changes extending over several years in response to hormone therapy and trastuzumab. Stem cell like staining was seen in a minor fraction of cells (1%) in about 10% of patients. An increase in cell numbers and in the fraction of HER2/neu amplified cells was under all treatment conditions unequivocally significantly correlated to highly increased risk of relapse.
Conclusions: CETC and subpopulation monitoring provides an invaluable tool for prompt gauging of systemic therapy in early stage solid tumours as a tool for therapy guidance and optimal personalized therapies to improve therapy results and spare unnecessary treatments.

5157 POSTER

Survival and prognostic factors in patients with early-stage breast cancer after conservation therapy

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Background: To improve the access of patients with early breast cancer after conserving surgery to radiotherapy, in 1999 standard 5 weeks of whole breast irradiation was shortened to 3.5−4 weeks regimen and since then, according to international recommendations more often adjuvant chemotherapy regimens were used.
Purpose: Comparing disease-free and overall survival and prognostic factors in patients treated in two periods of time: between 1995–1998 and 1999–2002.
Material and Methods: The retrospective analysis included 552 early breast cancer patients consecutively treated between 1995–2002. During that time patients were entered to conservative treatment according to the same protocol. There were not differences in clinical and pathological characteristics between the two compared groups. Systemic treatment has been given in older and recent group in 50% and 82% of patients respectively. Cox regression survival analysis was used to study the effects of clinical, histological and some biological factors on disease-free and overall survival.
Results: 7-years disease free survival (DFS) and overall survival (OS) for all patients were 0.86% (0.82−0.89) and 0.92% (0.90−0.95) respectively. The Cox regression model by stepwise selection showed some parameters such as ductal carcinoma (HR 2.3; CI 1.3−4.4), G3 (HR 2.0; CI 1.2−3.4) negative steroid receptors (HR 10.1; CI 4.5−22.4), amplification HER2 (HR 7.4; CI 3.1−17.6) as independent significant predictors for DFS. Nodal index (HR 1.5; CI 1.0−2.1) and palpation of the tumor (HR 2.0; CI 0.95−4.1) appeared to have effect on DFS either but on the limit statistical significance ($p > 0.05$). The significant independent predictors of OS on multivariate analysis were ductal carcinoma (HR 4.1; CI 1.6−10.6), G3 (HR 3.2; CI 1.6−6.4), negative steroid receptors (HR 9.4; CI 3.1−28.5) and amplification HER2 (HR 4.1; CI 1.2−15.2). Neither regimen of irradiation ($p = 0.5$) nor period of treatment ($p = 0.4$) were significant predictors of DFS. However the risk of death for patients treated between 1999–2002 was nearly three times more then for patients treated between 1995–1998 (HR 2.9; CI 1.1−7.4).
Conclusion: The prognosis of patients treated between 1999–2002 compared with 1995–1998 appeared worse in spite of more aggressive systemic management. Adjuvant treatment in early breast cancer should be based mostly on histological and biological features of malignancy because TNM classification is less useful in this group of patients.

5158 POSTER

Predictive value of circulating angiogenic factors in the neoadjuvant treatment of breast cancer

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Background: Neoadjuvant chemotherapy (NCT) is used in non-metastatic breast cancer to treat systemic disease earlier, try to achieve a complete pathological response (pCR), and increase the rate of conservative surgery. A useful strategy to improve knowledge about such treatments is the early identification of features associated with response or resistance. Over the last decade, research on angiogenic factors has greatly evolved. However, their exact prognostic and predictive value remain unclear, mainly due to the lack simultaneous evaluations. In order to identify the predictive value of a panel of circulating angiogenic factors, we evaluated the efficacy of a homogeneous NCT treatment in 79 patients treated in our centre. The association of a pCR with a serial determination of theses different factors was evaluated in order to determine their predictive value.
Material and Methods: To study the predictive value of the serum level of a panel of angiogenic factors (VEGF, PlGF, VEGFR2, bFGF, TGFa, PDGF, Ang1, Ang2, Tie2) during NCT, a serial measurement of their value using ELISA or Luminex techniques was performed in a population of breast cancer patients treated by the association of 3 cycles of FEC100 then 4 taxane cycles. Serum samples were withdrawn before the first CT cycle, after 3 cycles of CT and before surgery. A correlation between classical clinicopathological factors, the initial levels of theses factors, their kinetic variation and the achievement of a pCR was evaluated.
Results: 79 patients were evaluated. The clinicopathological characteristics of the population were classical of a neoadjuvant setting. 23% of the patients achieved a pCR. The following factors were significatively associated with a pCR in univariate analysis: ER positivity, PR positivity, low pretherapeutic TGFa level and the increase of VEGF levels between the first and the second sample. In multivariate analysis, ER positivity (OR = 0.233, $p = 0.028$) and the increase of VEGF levels between the first and the second sample (OR = 5.13, $p = 0.027$) were the only 2 factors significatively associated with a pCR, allowing us to develop an index able to predict the probability of pCR.
Conclusions: In such a NCT population, the initial kinetic variation of serum VEGF levels appears to be the most discriminating predictive angiogenic factor. Its association with ER status allows the development of a predictive index. The validation of theses results on an independent population is ongoing.

5159 POSTER

The effects of E-Cadherin and bcl-2 on prognosis in patients with breast cancer

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Background: Breast cancer is the most common malignancy in women. Axillary lymph node involvement and tumor size are the most significant prognostic factors in breast cancer. However, more factors are needed for prognosis evaluation and individualization of treatment in these patients. Intracellular adhesion molecule E-cadherin, an antiapopitotic protein bcl-2, and p53 might have predictive and prognostic properties in breast cancer.
Materials-Methods: We have investigated the effects of E-cadherin, bcl-2, and p53 on disease free survival and overall survival in patients with breast cancer. Positivity of aforementioned genes was detected with immunohistochemistry staining. Seventy-six women patients with invasive ductal and lobular breast cancer who had received adjuvant therapy were included in the study. Chi-square test for the comparison of qualitative data, log-rank test for the comparison of variables that were used, Kaplan-Meier method for the evaluation of the relationship of these variables with disease free survival (DFS) and overall survival (OS), Cox-Regression test for multivariate analysis were used.
Results: Bcl-2, E-cadherin, and p53 expression in tumor tissue specimens were found 26.31%, 35.52%, and 9.21%. Mean duration of follow-up was 93.58 ± 3.40 months. Sixty-six patients (86.8%) were diagnosed as invasive ductal carcinoma, 10 patients (13.2%) were invasive lobular carcinoma. Median DFS (25%) and OS (16%) could not reach. In univariate analyses, we couldn't find any statistical significant between DFS and all parameters. Only there was statistical significant between OS and both of lymph node