A). No correlation (p = NS) was found between hormone receptors and CEA, CA 15−3, and MIB-1 in both groups.

Conclusions: Our results suggests that (1) baseline STM CEA and CA 15−3 are not useful for prognostic purposes, (2) age, size, and ER are week isolated prognostic factors, and (3) the cumulative risk of relapse increases in the presence of multiple factors such younger age associated with highest levels of STMs and MIB-1, and low ER rate, together.

References

Lumachi *et al*. Anticancer Res 2010; 30: 2331–2334.

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Expression of MMTV-homologous Sequences in the Patients with Breast Hyperplasia and Ductal Carcinoma in Situ

E.G. Zenit-Zhuravleva[1], A.A. Lushnikova[1], L.N. Lyubchenko[2], E.B. Polevaya[2]. [1]*N.N.Blokhin Cancer Research Center, Carcinogenesis Institute, Moscow, Russian Federation;* [2]*N.N.Blokhin Cancer Research Center, Clinical Oncology Institute, Moscow, Russian Federation*

Background: Earlier, mouse mammary tumor virus (MMTV)-related sequences were revealed in significant proportion of human breast cancer (BC) tissues and blood sera samples. Mammary hyperplasia (MH) and ductal carcinoma in situ (DCS) are considered as a first steps for BC progression. A relationship between expression of MMTV-related provirus (hMTV) genome sequences and BC initiation/ progression is mostly unclear.

Material and Methods: 25 DCS and 10MH fresh or fixed archival tissues and blood samples from 35 primary patients (age is 21−56 years old) were studied by PCR and one-tube RT PCR after DNAase treatment to avoid pseudo positive results. Tumor histology was verified by routine staining of BC or normal tissue sections. Specific PCR products were sequenced and compared by BLAST and/or CLUSTAL programs.

Results: DNA sequences with 92%-95% homology to the *env* MMTV gene and 89−97% homology to 3′LTR MMTV were revealed in 11/25 (44%) DCS and 3/10 (30%) MH tissue samples, as well as in 17/25 (68%), 5/10 (50%) blood samples, correspondingly, *vs* 0/7 normal mammary tissue control samples. All hMTV-positive patients, except five ones, had pathological immune system status: rheumatoid arthritis, chronic lung infections, dental or nosofaringeal diseases. The hMTV sequence expression was found in 9/25 DCS and 3/10 H tissue samples. The sequences integration sites were studied by RACE method and were sporadic, without any predominant genome localization. An analysis of more number of malignant mammary tissue samples would clear provirus integration pattern and its possible localization near cell oncogenes.

Conclusions: a preliminary data suppose a role of hMTV sequences expression as a risk factor for genome instability and MH or DCS development. Cloning and sequencing of *env, gag*, Sag and HRE-coding hMTV sequences revealed in DCS and MH samples and its prognostic evaluation is in process.

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Leptin and VEGFR Expression in Sporadic Breast Cancer Patients

D.N. Kravchenko[1], E.V. Peredereeva[2], A.A. Lushnikova[2]. [1]*N.N.Blokhin Cancer Research Center, Clinical Oncology Institute, Moscow, Russian Federation;* [2]*N.N.Blokhin Cancer Research Center, Carcinogenesis Institute, Moscow, Russian Federation*

Background: Hormone leptin (Lep) is produced by adipose tissue and regulates cell metabolism and growth through signal transduction by interaction with leptin receptor (LepR). It is found that LepR-dependent signal pathways have common points with VEGF-dependent ones. Lep signaling regulates VEGF activity mainly through HIF-1α and NFkappaB. The effects of Lep/LepR expression on mammary cell proliferation and proteins associated with Lep /LepR signaling are important for understanding of breast cancer (BC) induction and progression, especially in BC patients suffered with obesity and other metabolic diseases.

Material and Methods: Expression of Lep/LepR and VEGFR-2 was studied in tumor tissues samples obtained from 15 sporadic BC patients (4 − obesity, 3 − high body mass, 8 − normal body mass, 24−45 yo, mean age 32$\pm$6.4 yo) using immunohistochemistry and RT PCR. Tumor and normal mammary tissue images (100–150 per sample) obtained by using monoclonal antibodies anti-LepR, anti-VEGFR-2 (Dako) and immunohistochemical staining (EnVision system, Dako) were analysed by MatLab 7.0 program. The data were compared with RT PCR results obtained by using total tumor RNA as a matrix and specific primers for *Lep, LepR* long isoform and *VEGFR-2* gene regions.

Results: Mean density of stained anti-LepR granules was significantly higher in tumor mammary tissues than in normal tissue: LepR − 37.4$\pm$3.6 *vs* 8.2$\pm$2.3, $p < 0.002$; mean density of anti-VEGFR-2 granules per length unit of vascular endothelium was 1.78$\pm$0.62 *vs* 0.82$\pm$0.34, $p < 0.05$. RT PCR results confirms that Lep, LepR and VEGFR-2 expression levels in BC tumors under study were significantly higher in tumor mammary tissue than in normal mammary tissue of sporadic BC patients. Moreover, LepR expression correlated with Lep and VEGFR-2 expression. There were no any relationships between body mass index and Lep/LepR/VEGFR-2 expression levels, probably, because of small patient number.

Conclusions: The results confirm high expression level of Lep/LepR/VEGFR-2 in mammary tumor cells in sporadic BC patients. A concordance between high LepR − Lep and LepR/Lep − VEGFR-2 expression levels in BC tissues in spite of patient body mass was also revealed. The data supports an involvement of Lep/LepR signalling into mammary tumor carcinogenesis; it is indicative of Lep regulation of VEGF/VEGFR expression. Continued study of prognostic value for Lep/LepR expression using results of immunohistochemistry, ELISA and RT PCR is in process.

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What is the Influence of Early Loco-regional Recurrence in Triple-negative Breast Cancer Patients on Disease Outcome?

V. Posarac[1], S. Susnjar[2], M. Milovic-Kovacevic[2], A. Karaferic[2], J. Aleksic[3], M. Jevric[1]. [1]*Institute for Oncology and Radiology of Serbia, Department of Surgical Oncology, Belgrade, Serbia;* [2]*Institute for Oncology and Radiology of Serbia, Department of Medical Oncology, Belgrade, Serbia;* [3]*Health Center Dr Ristic, Belgrade, Serbia*

Introduction: Triple negative breast cancer (TNBC) is a biologically heterogeneous group of breast tumors with generally poor prognosis. The aim of this analysis was to investigate how early occurrence of loco-regional relapse (LRR) influenced the outcome of these patients (pts).

Patients and Methods: From June 2006 to the end of 2009 a total of 243 stage I-IIIB TNBC pts were diagnosed at the Institute for Oncology and Radiology of Serbia. Since 21 pts were lost to follow up, 222 pts were analyzed. TN status was defined as IHC ER0−3/PR0−3/HER2:0−1 or IHC HER2:2+/CISH−. The majority of them had radical surgery +/− postoperative radiotherapy (RT) and adjuvant chemotherapy (CHT) as per protocol. The main end points were disease-free survival (DFS) defined as the time between surgery and BC relapse or death without BC relapse, and overall survival (OS) defined as the time from BC diagnosis to death from any reason. Statistics included Pearson Chi-squared test and Log-rank test.

Results: During the median follow up period of 25 months (range 3−58) LRR occurred in 15/222 (7%) pts, 5 of whom developed synchronous distant metastases. At the same time 28/222 (13%) pts were diagnosed with distant relapse only. Significantly higher proportion of LRR pts are older than 65 years (60%) and had positive nodal status (67%) compared to pts without LRR (27% and 36%, respectively) (c_1^2 test, p = 0.019 and c_1^2 test, p = 0.006, respectively). We looked separately at a group of pts with LRR only (10/222) and compared their disease outcome with pts who developed distant metastases. There was no significant difference in DFS [15 ms (95% CI 4.2–25.8 vs. 13 ms (95% CI 7.8–18.2)] and OS [39 ms (95% CI 10.0–67.9) vs. 40 ms (95% CI 33.6–46.4)] between LRR only and distant metastasis groups of pts (Log rank test, p > 0.05 for both).

Conclusion: Our results seem to point out that early relapse in TNBC pts means decreased survival irrespective of the first relapse site (loco-regional only vs. distant metastases).

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Evaluation of Molecular Parameters and Risk Factors of Breast Cancer in Therapeutic Decisions

R. Soares[1], Y. Shvets[1], N. Afonso[1], H. Rodrigues[1]. [1]*Instituto Portugues de Oncologia, oncology, Oporto, Portugal*

Introduction: Biological characteristics of breast cancer (BC) are of increased importance in treatment decision. Molecular parameters (MP) considered of prognostic importance can be positively correlated with more traditional risk factors (RF) or might change an initial prognosis based on these RF. We purposed to review our clinical practice using data of patients admitted in our institution with BC from 1.Jan.2007 until 31.Dec.2007 and identify the association between MP and RF and how this affected treatment decision and survival.

Methods: Demographic and clinical characteristics of patients were reviewed using clinical records. As RF considered in treatment options we considered: age (<35 years old and >35 years old) and TNM stage. The MP investigated were: grade (G), hormonal receptors (HR), HER2. An indication for chemotherapy was used as surrogate of clinical consideration of worse prognosis. Statistical package SPSSv.17 was used for statistic analysis and categorical variables were compared using x^2 test and continuous variables using Mann-Whitney U test. A p value <0.005 was considered of statistically significance. A multivariate analysis using

linear regression was used to identify independent factors associate with chemotherapy indication.

Results: A total of 509 patients were included for analysis. Thirty patients (6%) were younger than 35 years old. No statistical significant difference was found between age (<35 and >35) according to HR, HER2 and G. Fifty percent of younger patients presented in TNM stage III comparing to 29% of older patients, without statistically significant difference. Distribution by stage was not associated with HR, but 30% of HER2 positive patients were in stage III/IV comparing to 18% of HER2 negative patients ($p = 0.008$). Among stage III /IV patients 40% were Grade 3 comparing to 26% of patients stage I/II ($p = 0.002$). A significant difference was found between G3 and HR negative patients ($p < 0.0001$), G3 and HER2 positive patients ($p < 0.0001$) and HR negative and HER2 positive ($p < 0.0001$). All RF and MP were independent factors for chemotherapy proposing.

Discussion: No clear association is found between all RF and MP, although MP correlate between them. All these factors are considered for treatment decision and are independently associated with chemotherapy indication by oncologists.

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Risk Factors for Postoperative Complications After Breast Conserving Therapy in 255 Patients with Breast Cancer

P. Panhofer[1], V. Ferenc[1], R. Exner[1], A. Gleiss[2], P. Dubsky[1], R. Jakesz[1], M. Gnant[1], F. Fitzal[1]. *[1]AKH Wien, General Surgery, Wien, Austria; [2]AKH Wien, Center for Medical Statistics Informatics and Intelligent Systems, Wien, Austria*

Background: Postoperative complications impair quality of life and cosmetic results. Aim of the study was to detect risk factors for in breast complications and reoperations.

Material and Methods: 255 patients (mean age 59.6±13.5 years) underwent breast conserving therapy due to breast cancer between 2008 und 2010 at a single center (Medical University of Vienna). Patients with positive sentinel macrometastases undergoing axillary dissection were excluded. The Clavien Dindo Classification (CDC: Grade 1−5) evaluates the severity of postoperative complications under the aspect of the therapy management. In this respect, Clavien Dindo classification 3 and 4 stands for surgical re-operation due to in breast morbidity.

Results: Mean follow up was 10.2±4.1 months. Ductal carcinomas in situ were found in 7.8%. Invasive carcinomas included pT1 (71.8%), pT2 (19.6%) and pT3 (0.8%) tumors. Neoadjuvant chemotherapy was performed in 3.5% and oncoplastic sugery in 9% of patients. The majority of patients (94.8%) had no or minor postoperative complications (CDC 1+2). 13 reoperations (5.1%) were performed due to postoperative complications (CDC 3+4). There was no hospital mortality (CDC 5). No prognostic factor could be detected for reoperation necessity. Univariate analysis showed that the BMI >30 was an predictor for wound infections ($p < 0.05$, odds ratio: 2.94). In the multivariate analysis, oncoplastic operations were independent risk factors for necrosis ($p < 0.01$, odds ratio: 10.7) and a positive Her2 status for bleeding ($p < 0.006$, odds ratio: 13.4).

Conclusions: Oncoplastic operations and positive Her2 status but not neoadjuvant therapy were independent predictors for postoperative morbidity. However, none of the above mentioned factors were predictive for morbidity related re-operations (CDC 3+4).

Univariate and multivariate* analyses of predictors for postoperative complications

Predictor	No. (%)	p-value (top) and odds ratio (bottom)						
	Total	>65 a	BMI >30	DM II	Oncoplast. Operation	Neoadjuvant Chemother.	Triple negative	Her2 positiv
	255	119 (46.7%)	49 (19.2%)	18 (7.1%)	23 (9.0%)	9 (3.5%)	29 (11.4%)	19 (7.5%)
Abscess	8 (3.1%)	0.14	0.68	0.31	0.70	0.09	0.58	0.81
		3.08	1.37	2.64	0.56	5.61	0.44	0.69
Wound infection	15 (5.9%)	0.30	**0.05**	0.76	0.39	0.87	**0.05**	0.81
		1.73	**2.94**	1.32	1.89	0.78	**3.31**	1.24
Bleeding	4 (1.6%)	0.46	0.28	0.09	0.96	0.51	0.91	**0.006***
		0.48	3.60	5.74	1.08	2.83	0.84	**13.40**
Hematoma	7 (2.7%)	0.87	**0.05**	0.25	0.77	0.74	0.64	0.27
		0.89	**5.22**	3.05	0.64	1.67	0.50	2.88
Necrosis	4 (1.6%)	0.46	0.53	0.09	**0.01***	0.52	0.91	0.86
		0.48	0.39	5.74	**10.7**	2.83	0.84	1.33
CDC 3+4	13 (5.1%)	0.06	0.96	0.57	0.51	0.19	0.40	0.14
		3.28	1.04	1.69	0.38	3.60	0.29	3.08

oncoplast.: oncoplastic; chemother.: chemotherapy.

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Prognostic Factors in Triple Negative Breast Cancer Patients Treated with Neoadjuvant Chemotherapy

N. Wada[1], K. Yoneyama[1], C. Yamauchi[1], S. Fujii[2]. *[1]National Cancer Center Hospital East, Breast Surgery Division, Kashiwa Chiba, Japan; [2]National Cancer Center Hospital East, Pathology Division, Kashiwa Chiba, Japan*

Background: Triple negative breast cancers (TNBC) are aggressive neoplasms and associated with a poor prognosis, despite showing a good response to neoadjuvant chemotherapy (NAC). The purpose of this study was to investigate a prognostic factor for TNBC patients after NAC.

Patients and Methods: In our prospectively collected database, we identified 350 patients with Stage II-III invasive breast cancer treated with anthracycline and/or taxane based regimens for NAC between Feb 2002 and Mar 2011. Outcomes for 83 patients with TNBC were analyzed in this study. TNBC is defined as the lack of ER, PR, and HER2 expression in both pre-therapy core-needle biopsy and post-therapy surgical excision specimens. The expression of Ki-67 was also assessed using immunohistochemistry (MIB-1) in the both specimens. Levels of Ki-67 index (percentage of Ki-67 positive cancer nuclei) were dichotomized as high (over 10%) and low (less or equal 10%). Statistical analysis performed included Kaplan-Meier survival analysis, log-rank test, and Cox proportional hazard model.

Results: Median age was 53 years (28−68 years). 21 cases (28%) of all achieved a pathological complete response (pCR: no residual invasive disease in the breast). Ki-67 index in pre-therapy was not correlated with the pathological response and the prognosis after NAC. At a median follow-up time of 38 months (1–107month), no patients with pCR had an unfavorable event. The patients with non-pCR, however, developed 27 recurrences and 26 deaths. 5-year overall survival estimates were 100% for pCR versus 51% for non-pCR. There was a significantly difference between them in overall and relapse free survival curve (both $p < 0.01$). In 62 non-pCR patients, cox multivariate analyses showed that mastectomy [$p < 0.05$, HR 3.5, 95% CI 1.3−9.2], presence of lymphovascular invasion [$p < 0.05$, HR 2.7, 95% CI 1.2−5.9], evidence of lymph node metastasis [$p < 0.05$, HR 2.6, 95% CI 1.1−5.7] and high Ki-67 index of post-therapy [$p < 0.01$, HR 11.0, 95% CI 4.5–26.6] were independently associated with worse overall survival.

Conclusions: In patients with TNBC, attainment of pCR after NAC yields promising survival results. Once there is evidence of residual invasive disease in the breast, Ki-67 index in post-therapy may be a useful predictor on clinical outcomes in addition to the known pathological factors.

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FOXP3 Expression in Tumor Cells Associated with the Prognosis in Breast Cancer Patients

M. Takenaka[1], U. Toh[1], N. Seki[2], A. Kawahara[3], S. Hattori[4], N. Iwakuma[1], R. Yamaguchi[3], H. Yano[3], K. Shirouzu[1], M. Kage[3]. *[1]Kurume University School of Medicine, Surgery, Kurume, Japan; [2]Research Center for Innovative Cancer Therapy Kurume University, Surgery, Kurume, Japan; [3]Kurume University School of Medicine, Pathology, Kurume, Japan; [4]Biostatistical Center Kurume University, Statistics, Kurume, Japan*

Purpose: The transcription factor forkhead box protein3 (FOXP3) is highly expressed not only in regulatory T cells, but also in tumor cells. We investigated the prognostic significance of FOXP3 expression in cancer cells and infiltrating lymphocytes.

Patients and Methods: The expression patterns of FOXP3 were characterized by immunohistochemistry in 100 patients with primary invasive breast carcinoma. Kaplan-Meier analysis and Cox regression model were used to assess overall survival and relapse-free survival, according to the presence of FOXP3 expression in cytoplasm or nuclear of tumor cells.

Results: Of 100 tumor specimens, FOXP3 expression was found in cytoplasm in 37% of the cases, in the nucleus in 32%, and in infiltrating lymphocytes in 57%. FOXP3 expression in cytoplasm and infiltrating lymphocytes was associated with worse overall survival (OS) and relapse-free survival (RFS) of patients. Moreover, FOXP3 expression in both cytoplasm and lymphocytes was associated with significantly worse OS ($p < 0.001$) and RFS ($p < 0.001$). Inversely, FOXP3 expression in nucleus was associated with better OS ($p = 0.016$).

Conclusion: These data indicate that concomitant FOXP3 expression in both lymphocytes and cytoplasm could be a prognostic marker for breast cancer, which might help identify high-risk patients for appropriate therapy.