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Local recurrence (LR) after breast-conserving therapy (BCT) in node-negative premenopausal patients: Influence of perioperative chemotherapy (PeCT)

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Purpose: PeCT decreases the LR rate after BCT. This study was intended to identify premenopausal patients who benefit most of this adjuvant therapy.

Methods: 361 T1-3 NO premenopausal patients treated with BCT were drawn from a prospectively randomized adjuvant trial (EORTC 10854; POP trial) comparing surgery followed by one course of chemotherapy (fluorouracil, doxorubicin and cyclophosphamide) versus surgery alone. Histology slides were reviewed. Immunohistochemical staining was performed for the following proteins: bcl-2, CD31, cyclin D1, E-cadherin, EGF receptor, ER, PR, Ki-67, c-erbB2/neu, and p53.

Results: In the multivariate analysis young age (<43 years), negative ER and positive p53 are risk factors for developing a LR. All subgroups showed a benefit from PeCT; patients with high vascular count and high mitotic count benefit most from this effect.

Conclusion: Age is an important independent risk factor for LR. PeCT decreases the LR risk in all patients, however no clinical useful subgroups can be identified.

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The occurrence of deletions and point mutations in the estrogen receptor gene in breast cancer specimens

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Purpose: Oestrogen receptor status has been recognised as a useful guide to the likely therapeutic response to Tamoxifen. However it is recognised that not all patients who are ER positive respond well and equally some ER negative patients do respond. One explanation for this would be alterations in the ER gene.

Methods: In order to address this question we have screened 118 breast cancer specimens for large deletions in the estrogen receptor gene by carrying out RT-PCR reactions using standard sets of primers and looking for PCR products of reduced size by agarose gel electrophoresis. Only two out of the 118 specimens yielded smaller than normal PCR products. One of these was a homogeneous deletion of exons 2-5 in the mRNA by alternative splicing. The other specimen was very heterogeneous containing many variants of estrogen receptor mRNA. The individual variants from this specimen were cloned and the deletions characterised.

Conclusion: Automated DNA sequencing of RT-PCR products of estrogen receptor mRNA from breast cancer specimens showed that in this group of specimens there was only a low incidence of point mutations suggesting that simple mutation and deletion is not a major cause of Tamoxifen resistance.

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Tenascin-C expression in invasion border of early breast cancer – A predictor of local and distant recurrence

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Purpose: Tenascin-C (Tn-C) is an extracellular matrix glycoprotein expressed transiently in areas of epithelial-mesenchymal interaction during embryogenesis and neoplasia. Our previous work showed an association between metastasis and Tn-C expressed in invasion border of axillary node-negative breast carcinomas. Our aim was to relate this Tn-C expression with several histopathological and biological variables and to compare their usefulness to predict recurrences.

Methods: The original patient group consisted of 143 women with axillary node-negative breast cancer (one bilateral) treated with breast conserving surgery and postoperative radiotherapy and followed for a median of 8 years. Due to the small number of recurrences an additional group of 15 similarly treated women with a recurrent breast cancer was also studied. The size of the tumour, its histology including a possible intraductal component, and grade were re-evaluated. The expression of erbB-2, p53, Ki-67, and Tn-C was evaluated by immunohistochemistry. Ploidy and S-phase fraction (SPF) were assessed by flow cytometry.

Results: The only statistically significant prognostic factor for local recurrence was Tn-C expression in the invasion border. For metastasis Ki-67 positivity, tumour size, and Tn-C expression in the invasion border were statistically significant, but Ki-67 was the only independent prognostic factor.

Conclusions: Tn-C expression in the invasion border was associated with a higher proliferation rate measured by Ki-67 and SPF, which is consistent with the suggested growth promoting activity of Tn-C. Tn-C may be a useful marker in selecting patients for adjuvant therapies to reduce the rate of both local and distant cancer recurrences.

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Age the limiting factor for breast conservative treatment in T1 breast cancer?

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Between January 1984 and December 1995, 1071 patients with breast cancer were treated with breast conservative treatment, limited surgery and radiation therapy. From the 1071 patients 860 (69%) were pT1 and 203 (28.5%) pT2.

This is an analysis of the 860 pT1 patients. The follow-up ranged from 3-159 months with a mean of 60 and a median of 55 months. The age ranged from 27-89 years with a median of 56 years.

The familiar history was recorded as no, 1 or > 2 first degree relatives (F.D.R.) and was for the 860 pT1 patients 78, 15 and 3% respectively. The pN was 642 (74.5%) pN0 and 207 (24%) pN1. Of the 207 pN1 patients 165 had 1-3 and 42 >3 positive nodes.

The histology was 74% ductal, 10% lobular and 4.5% tubular carcinoma. With 71 (8%) we had histologically positive margins of the lumpectomy and 25% showed also DCIS in the lumpectomy. From the 205 pN1 patients 82% (168) had adjuvant chemo or hormonal therapy depending on the menopausal status.

Results: The local recurrence rate was 3.4% (29/860). Looking at the influence of the familiar history in F.D.R., the pN status, number of positive nodes, positive margins and DCIS on the local recurrence rate, we show for no, 1 or > 2 F.D.R. respectively 25 (3.6%), 3 (2.4%) and 0 local recurrences. The pN0 21 (3.3%) and pN1 8 (4%) local recurrences. Looking to the number of positive nodes we had 3.6% with 1-3 and 4.7% with >3 positive nodes. Positive margins had 3 (4.2%) local recurrences and DCIS 9 (4.2%). These factors were not significant.

Patients were subdivided according to age in three groups, = <40 (67/860), 41-50 (216/860) and >50 (577/860) years. The local recurrence rate was 12 (8/67), 5 (11/216) and 1.7% (10/577) respectively (p = 0.000).

The distant metastasis rate was 11.8% (101/860) and for the three age groups 29.8%, 13% and 9.2% respectively (p = 0.000). In the age group = <40 4/8 (50%) patients had local recurrences and metastasis, of which only 2 patients had local recurrence and metastasis at the same time

Conclusion: Looking to the local recurrence rate age seems to be a limiting factor for breast conservative treatment in T1 breast cancer.

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Platelet-derived endothelial cell growth factor/thymidine phosphorylase expression in macrophages correlates with tumor angiogenesis and prognosis in breast cancer

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Purpose: We evaluated the correlation of platelet-derived endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/TP) expression with tumor angiogenesis and prognosis in patients with invasive breast carcinoma (IBC).

Method: The PD-ECGF/TP expression was evaluated by immunohistochemistry using a monoclonal antibody to PD-ECGF/TP in 117 patients with IBC.

Results: PD-ECGF/TP immunoreactivity was observed in both cancer cells and stromal cells (mainly in macrophages) in the invasive margin. Therefore, we evaluated the PD-ECGF/TP expression separately in cancer cells and stromal cells. Sixty-one (52.1%) cases were defined as PD-ECGF/TP-positive in cancer cells and 44 (37.6%) were positive in stromal cells. The PD-ECGF/TP expression in cancer cells did not correlate with any prognostic factors. However, the expression in stromal cells positively correlated with tumor size and microvessel count, and inversely correlated with estrogen receptor status. Significantly decreased relapse-free survival (RFS) and overall survival (OS) were found in patients with the positive expression of PD-ECGF/TP in stromal cells. A multivariate analysis using the Cox proportional hazards model showed that the PD-ECGF/TP positivity in stromal cells independently correlated with OS as well as nodal status and tumor size.

Conclusion: The PD-ECGF/TP expression in stromal cells correlates with tumor angiogenesis and may predict the prognosis of patients with IBC.

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Tumour angiogenesis by vascular grading is of prognostic significance in breast cancer

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Purpose: The aim of the study was to evaluate the prognostic value of angiogenesis by vascular grading of primary tumours from breast cancer patients.

Material and Methods: The investigation included 841 patients. The median follow-up time was 12.5 years. Recurrence free survival (RFS) and breast cancer specific overall survival (OS) were observed. Adjuvant systemic treatment was given to 40% and radiation therapy to 42% of the patients. The microvessel endothelium was immunohistochemically stained by antibodies against CD34. Angiogenesis was determined by semiquantitative vascular grading, by subjective scoring into three groups according to the number of stained microvessels in the tumour section.

Results: The reproducibility of vascular grading between-observers was estimated to 0.59 (0.41; 0.83), (95% CI). Vascular grading was significantly associated with histologic type, histologic grade, tumour size, vessel invasion, and axillary nodal status. Vascular grading significantly predicted RFS and OS in both the node-negative and node-positive patients. The 5-years RFS probability \pm SE for all patients was $78 \pm 2\%$ in vascular group one, $56 \pm 3\%$ in vascular group two, and $44 \pm 3\%$ in vascular group three. The Cox multivariate regression analysis showed that vascular grading contributed with independent prognostic value in all patients.

Conclusion: The angiogenesis determined by vascular grading has independent prognostic value both for patients with node-negative and node-positive breast carcinomas.

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Multivariate analysis of prognostic factors in lymph node negative breast cancer

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A series of prognostic factors were tested for the prediction of early, late (LRec) recurrence and disease related death (DRD) in lymph node negative (LN-) breast cancer (BRCA) patients.

Design: Formalin fixed paraffin embedded primary LN-BRCA specimens from 220 women (median age 63 years) followed for a minimum of 120 months were evaluated for primary tumor size, histologic grade, estrogen receptor status (competitive binding assay), and HER-2/neu gene amplification by fluorescence in-situ hybridization (FISH). FISH was performed using the Oncor unique sequence HER-2/neu probe (Oncor, Inc. Gaithersburg, MD). No patients received adjuvant treatment prior to the first episode of disease recurrence.

Results: On univariate analysis HER-2/neu gene amplification by FISH independently predicted ERec; LRec ($p < 0.0005$); and DRD ($p < 0.0001$). When stratified into HER-2/neu non-amplified, borderline amplified and highly amplified groups, patients with highly amplified tumors had a relative risk (adjusted relative hazard) of ERec of 8.3 (range 2.1–32.4); LRec of 4.3

(range 1.7–11.0) and DRD of 11.0 (range 3.0–40.7). Tumor size, histologic grade and estrogen receptor status did not predict ERec, LRec or DRD. On multivariate analysis HER-2/neu gene amplification by FISH predicted ERec, LRec and independent of tumor size, grade and hormone receptor status.

Conclusions: In this series of LN BRCA patients, HER-2/neu gene amplification by FISH significantly predicted early and late disease recurrence and disease related death independent of tumor size, grade and estrogen receptor status.

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Independent role of p53 expression and reduction in kinetic cell activity in predicting clinical complete response (CR) to primary chemotherapy in breast cancer (BC) patients

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Mutant p53 and reduction in proliferative activity have been evaluated in order to assess whether they may play a role in modulating response of BC to primary chemotherapy. 145 patients bearing T2–4, N0–1, M0 primary BC were submitted to 2 different chemotherapeutic regimens before surgery. The first 64 received the CMF regimen (days 1.8 every 28) associated with tamoxifen (30 mg daily) in those with estrogen receptor positive (ER+) BC, the remaining 81 were submitted to single agent epirubicin (120 mg/m² every 21 days). The expression of p53, bcl-2, Ki67, ER, progesterone receptor and c-erbB2 was evaluated in BC specimens obtained at diagnosis by incision biopsy and at post-chemotherapy surgery. 35 patients (24.1%) attained a clinical CR, 72 a partial response (PR) (49.7%), and 37 a stable disease (SD) (25.5%). p53 expression was significantly associated with a lower CR, the maximum difference being observed for a cut off of $>20\%$ positive cells (7.1% vs 28.2%, respectively; $p < 0.02$). p53 was a stable phenotype, only 4 cases with p53 negative BC before chemotherapy became positive afterwards. In multivariate regression analysis, p53 expression was an independent variable inversely associated with clinical CR, while reduction in Ki67 expression ($>50\%$) was an independent variable directly associated. Menopausal status, T, N, histology grade, ER, PgR, c-erbB2, Ki67 and bcl-2 did not enter the model. The clinical CR mainly confined to p53 negative BC confirms in vivo the finding that the responsiveness of BC to chemotherapy in part derives from the capability of tumor cells to undergo apoptosis. The chemotherapy induced antiproliferative effect concurs independently in obtaining the tumor responsiveness.

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Menstrual phase and timing of breast cancer surgery: Statistical aspects

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Purpose: The impact of menstrual cycle dependent timing of surgery on long-term outcome of breast cancer patients is still discussed controversially. It is our experience that many colleagues are dissatisfied with the uncertainty related to this potentially simple and beneficial therapeutic tool. New arguments would be helpful to explain the contradictory data reported in the literature. In accordance with McGuire (1991) and Jager and Sauerbrei (1995), we feel that the statistical side of the problem is of the utmost interest, since looking for the optimal splitting of the menstrual cycle is equivalent to cut-point searching involving a cyclic covariate. Referring to this problem Altman et al. (1994) described "optimal" cut-point searching in connection with a simple continuous prognostic factor. They reported an approximative formula by Lausen and Schumacher to be a useful tool for correction of the obtained minimum p-value.

Methods: Since the mathematical theory for an analogous correction in case of a cyclic covariate such as the menstrual cycle is not yet available, we designed a simulation study using randomly generated exponentially distributed survival data. We randomly assigned a menstrual cycle value between 1 and 28 days to every survival time. We varied the sample size ($n = 140$, $n = 280$, $n = 1400$), the amount of censoring (33% and 67%) and the minimum selection interval (between 7 days and 14 days). When using minimum lengths of the selection interval ranging from 7 days to 14 days, a total of 210 different partitions were possible. We generated 2000 simulated samples for each of these 210 scenarios.

Results: Neither the sample size nor the amount of censoring had any remarkable influence on the inflation of type I error rate (Fig. 1a + 1b).