

sis-related genes *c-myc*, *p53* and *bcl2* in breast cancers (BC) in association with the main prognostic markers after a long-term follow-up.

Methods: Total RNAs and paraffin-embedded tissue sections were obtained from 175 primary tumors and analysed for *c-myc* transcripts by Northern blot and *p53* and *bcl-2* proteins by immunohistochemistry. All patients were treated by surgery and by adjuvant therapy according to their clinicobiological characteristics. The risk of death associated with the expression of these genes was evaluated by multivariate analysis taking into account the main prognostic factors, namely, tumor size, histoprognostic grade (HG), hormonal receptors (HR) and nodes, after a mean follow-up of 9.5 ± 2 yrs.

Results: *c-myc* overexpression was observed in 35% of BC and *p53* and *bcl2* overexpression was found in 23 and 63% of BC, respectively. A strong association between *c-myc* overexpression and positive nodes ($p = 0.0005$), *p53* expression and both high HG ($p = 0.0001$) and HR-negative tumors ($p = 0.0003$) was shown. In contrast, *bcl-2* expression was found to be associated with favorable prognostic factors including HR-positive tumors ($p = 0.0001$). Multivariate analysis showed that only positive nodes ($p = 0.0001$) and *bcl-2* expression ($p = 0.008$) were independent factors correlated to a higher or lower risk of death, respectively.

Conclusion: Both *c-myc* and *p53* genes favor the development of more aggressive BC. In contrast, the anti-apoptotic function of *bcl2* seems to be most hypothetical in BC. Expression of *bcl2* identifies a particular phenotype of BC with a favorable long-term prognosis and thus may be a useful marker.

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POSTER

Prognostic significance of p53 mutations in the zinc-binding domains (L2/L3) in lymph node-positive breast cancer patients

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Purpose: Mutations of the p53 tumor suppressor gene have been shown to be of prognostic significance in the node-negative breast cancer population. We investigated the prognostic value of p53 mutations in the functionally important L2/L3 zinc-binding domains in lymph node-positive breast cancer patients.

Methods: For detection of p53 mutations we used in vitro amplification by polymerase chain reaction and consecutively performed temperature gradient gel electrophoresis (PCR-TGGE). We evaluated if mutations in the L2/L3 domains provide prognostic information for node-positive breast cancer patients.

Results: Out of 213 tumor samples we found p53 mutations in 48 cases (23%). About a third of the p53 mutations ($n = 17$) were located in the L2/L3 domains. Univariate analysis revealed that patients with p53 mutated tumors had a statistically significant shorter relapse free survival (RFS) ($p = 0.034$). However, in multivariate analysis no significant correlation between p53 mutated tumors and reduced RFS could be detected. We could not detect any significant difference regarding RFS and overall survival (OS) for patients with p53 mutations in the L2/L3 domains.

Conclusion: p53 mutations in the L2/L3 domains in node-positive breast cancer patients do not provide information regarding the clinical outcome of these patients.

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POSTER

mRNA determination of estrogen receptor, progesterin receptor, pS2, PAI-1 by competitive reverse transcription-PCR in human breast cancer

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Purpose: Estrogen receptor (ER) and progesterin receptor (PR) are important regulators of growth and differentiation of the mammary gland as well as in the development of malignant tumors. pS2 is an estrogen inducible protein, which is supposed to be involved in tissue differentiation. Urokinase inhibitor-1 (PAI-1) inhibits the activity of urokinase-type plasminogen activator (uPA) which contributes to the degradation of the extracellular matrix in tumor invasion and metastasis. ER, PR, pS2 and PAI-1 have been reported to be prognostic parameters in primary breast cancer. We developed a competitive RT-PCR system to allow simultaneous quantitative determinations of ER, PR, pS2 and PAI-1 mRNA in tissue samples. We

evaluated 100 breast cancer specimen for mRNA expression. Results were compared with protein status and with clinical data.

Results: We found a statistically significant correlation between mRNA and protein levels of ER ($p < 0.00001$), PR ($p < 0.00001$), pS2 ($p < 0.00001$) and PAI-1 ($p < 0.0147$), respectively. ER, PR, pS2, and PAI-1 showed a statistically significant correlation to each other except to the mRNA expression of PAI-1. Furthermore the mRNA data of ER and PR showed an inverse correlation to tumor size and histological grade.

Conclusion: Our data are in agreement with reports about ER, PR concerning tumor size and grade. We did not find any correlation to lymph node involvement. We did not find any association of pS2 mRNA to the clinical data. PAI-1 was found to be independent of tumor size, grade or lymph node involvement. The present study shows, cRT-PCR is an appropriate method for the simultaneous determination of prognostic factors in breast cancer specimen, requiring small amounts of total RNA.

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POSTER

Medullary breast carcinoma. Ten-year results in 108 patients treated in a single center

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Purpose: To analyse clinical presentation and outcome of patients with medullary breast carcinoma treated at the Institut Curie between 1981 and 1996.

Methods: 108 cases of medullary carcinoma (0.7%) were retrospectively identified in the breast cancer database. All charts were reviewed. All 108 patients were female. Median age was 50 years (24–82 yrs.). 64 pts. (59%) were premenopausal. Median clinical tumor size was 3 cm (0–15). There were 35 T1 (32%), 51 T2 (47%), and 22 T3T4 (20%); 57 were NO (53%), 47 N1 (43%) and 4 N2 (4%). Treatments varied according to tumor sizes. 62 pts. (58%) had a wide tumor excision and radiotherapy (mean tumor size: 2.4 cm), 24 pts. (22%) received irradiation alone (mean size: 6.4 cm) and 22 pts. (20%) had a mastectomy (mean size: 3.8 cm). Overall, 86/108 pts. (80%) had a breast-conserving treatment. 32 pts. (30%) received chemotherapy, either before (13 pts.) or after local treatment (19 pts). Treatment outcome was determined using Kaplan-Meier estimates.

Results: Median followup was 116 months (8–205). Ten-year survival and metastasis-free interval rates were 81% and 79%, respectively. 13 breast recurrences occurred and 8 contralateral breast cancer. 10-year breast recurrence rate was 17%. The 10-year overall breast conservation rate was 66%. It was 83% in patients who had an initial breast-conserving procedure. Survival and metastasis-free interval were similar in patients treated with mastectomy, or breast conservation.

Conclusion: This retrospective study of 108 patients with 10 year follow-up confirms that medullary breast cancer seems to carry a better prognosis than invasive ductal carcinoma. Medullary breast cancer seems very sensitive to chemotherapy and radiotherapy, which allow breast preservation in patients with large tumors. Biological characterization of this particular form of breast cancer is ongoing.

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POSTER

Anti-apoptotic phenotype is associated with decreased loco-regional recurrence rate in breast cancer

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Purpose: Tumour and nodal status are the most important factors predicting loco-regional recurrence in breast cancer. We wanted to investigate the predictive value of some new molecular genetic markers, for the occurrence of a loco-regional recurrence in order to improve the selection of patients for loco-regional adjuvant therapy.

Methods: Bcl-2, p53, MIB-1, pS2, PCNA and CD44v6 were determined immunohistochemically on formalin-fixed and paraffin embedded tumour tissues of 163 patients treated by modified radical mastectomy between 1982 and 1987. Postoperative irradiation was given to only few patients. Node-positive patients had been given CAF adjuvant chemotherapy. A multivariate analysis was performed on a number of potential prognostic factors. The risk for loco-regional recurrence was estimated using the competing risk approach.

Results: After a median period of 7.5 years 28 patients developed a loco-regional recurrence. The cumulative incidence of loco-regional recurrence at 10 years was 17%. Bcl-2 and p53 were found to be independent

factors predicting loco-regional recurrence, whereas a trend was found for MIB-1. Increased Bcl-2 as well as p53 expression were associated with a decreased risk, whereas the presence of MIB-1 was associated with an increased risk.

Conclusion: Results indicate that molecular markers of apoptosis as well as proliferation provide additional information for the risk of loco-regional recurrence after modified radical mastectomy. If confirmed, these markers may play a role in the selection of appropriate loco-regional adjuvant treatment after primary surgery.

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POSTER

Can assessment of angiogenesis, proliferation and apoptosis predict the need for adjuvant therapy in breast cancer?

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Purpose: Currently decision making on the need for adjuvant chemotherapy in patients with breast cancer is mainly based on the axillary lymph node status. In the present study markers of proliferative activity (PCNA, mitotic activity index), apoptosis (bcl-2, bax, p53) and angiogenesis (factor VIII) were studied in an attempt to predict the risk for recurrence.

Methods: Representative tissue specimens of 131 patients with invasive ductal carcinoma (median follow-up 83 months, range 2–165) were immunohistochemically stained. The evaluation of oncoprotein or PCNA immunoreactivity and vessel density was made on a magnification of 400 in at least ten fields.

Results: No relationship could be observed between bcl-2, bax, p53 status or factor VIII expression and tumour grade, pTNM staging or menopausal status. Kaplan-Meier overall survival analysis combining the bcl-2 status and vessel density showed they have a strong prognostic value ($p < 0.001$). Cox proportional hazards analysis demonstrated that bcl-2 status ($p < 0.001$), vessel density ($p < 0.001$) and tumour grade ($p < 0.02$) were as accurate to predict overall survival as a model containing lymph node status, tumour size and tumour differentiation.

Conclusions: If these data are independently confirmed one should consider to design prospective randomised trials to assess the concept of omitting the axillary lymphadenectomy in breast cancer patients with no palpable abnormalities in the axilla.

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POSTER

CA-15.3: Prognostic factor in breast cancer

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Purpose: CA15.3 is the most useful tumor marker in breast cancer. The low sensitivity of CA15.3 explain that this tumor marker is never used for detection of breast cancer. However, The CA15.3 level at time diagnosis may be a prognostic factor.

Methods: During 7 years from 1986 to 1993, 673 women were treated in our institution for not metastatic breast carcinoma. CA15.3 analysis was performed by radioimmunologic assay (CIS-Bio®) at primary clinical diagnosis time, after initial treatment and during the follow-up. The level of CA15.3 was considered normal if ≤ 30 kU/l. Survival time was calculated from the time of primary treatment to the time of death or was censored to the last follow-up date if a patient had not died. The follow-up was updated in December 1997. Survival probabilities were estimated according to the Kaplan-Meier product limit method. The prognostic role of CA15.3 was evaluated by a Cox regression model.

Results: In these population of 673 consecutive not metastatic breast cancer patients, 507 (75%) had normal CA15.3 level (group A) and 166 (25%) had pathological initial level of CA15.3 (group B). The median survival time was 71 months in group A patients and 54 months in group B patients ($p < 0.00001$). The disease free median time survival (without metastasis) was 65 months in group A patients and 40 months in group B patients ($p < 0.00001$). After initial treatment 110 patients in group B normalized the level of CA15.3, the median survival time of these patients was higher than those who did not normalized the CA15.3 level after treatment ($p < 0.001$). However, in metastatic patients there is no statistically difference in median survival time between patients with normal or high levels of CA15.3 at time of metastatic disease diagnosis.

Conclusion: These results suggest that initial level of CA15.3 at initial diagnosis time is an important prognostic factor for recurrence and overall survival in not metastatic breast cancer patient. This inexpensive laboratory test may be an important parameter in initial treatment decision and particularly if the level didn't normalize after initial local and regional treatment.

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POSTER

Prognostic significance of keratins in breast cancer

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Purpose: The normal breast gland contains luminal and basal cells that stains with different keratins. Keratin 8 stains the luminal cells and keratin 14 and 17 mainly stain the basal cells. The prognostic value of keratin 8, 14, and 17 is evaluated in 836 breast cancer patients.

Methods: Sections are cut at 5 μ m. Antigen retrieval is performed using microwave oven boiling at 600W for 25 min in TEG buffer. Sections are stained in a TechMate 1000 immunostainer using the DAKO ChemMate Kit BPR/DAB.

Patients admitted for surgery during the period from 1 January 1980 to 31 December 1990 with primary, operable unilateral breast cancer are included. All patients are followed for at least 5 years.

Results: In 662 cases all cells are stained with keratin 8. Total or clonal staining with cytokeratin 14 and 17 occurs in 90 and 115 cases, respectively. The combined staining pattern reveals negative or clonal keratin 8 staining or total or clonal keratin 14 or keratin 17 staining in 274 cases.

In a multivariate Cox regression analysis including tumour size, lymph node status, and histological malignancy grading, the combined staining pattern of cytokeratin 8, 14, and 17 is significant with a worse outcome related to the basal cell markers.

Conclusion: The combined staining pattern of keratin 8, 14 and 17 is a prognostic factor in breast cancer.

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POSTER

Neural networks improve the prediction of survival in breast cancer

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Traditional prognostic indices are only able to predict outcome for patients with early or advanced breast cancer but are unreliable for the majority of patients who have a moderate prognosis. Neural networks are able to learn the relationships in non-linear complex data sets and thus may provide a more accurate prediction of behaviour in this group. We evaluated the ability of neural networks to prognosticate for breast cancer patients.

Data from 473 patients from the standard local BASO database was utilised. Neural networks were designed with input parameters corresponding to standard NPI parameters, 0–7 perceptrons and a yes/no output neurone. These networks were then trained to predict survival at 36, 48 and 60 months and validated on data not previously seen by the networks. The NPI was also calculated for all patients. Data were censored where appropriate.

Eighty eight percent of patients were in the moderate/good group according to the traditional NPI analysis ($0.2 \times \text{size} + 1 \times \text{grade} + 1 \times \text{no. nodes}$ ($0 = 1, 1-3 = 2, > 3 = 3$)). Results for the neural network prediction of overall survival are summarised below:

Time Period Accuracy (95% C.I.) PPV NPV 36 months 0.84 (0.7–0.99) 0.84 1.0 48 months 0.82 (0.64–0.97) 0.81 0.86 60 months 0.86 (0.7–0.98) 0.88 0.87 In order to detect patients in the moderate group with a poor prognosis the artificial neural network significantly altered the weights used for the NPI parameters with size being considered far less significant (-0.01 – -0.016). In addition, the relative importance of the parameters changed for each time interval predicted.

Neural networks provide a more powerful, robust and flexible tool for the prediction of outcome in breast cancer. The greater accuracy provided by neural network analysis will facilitate the planning of adjuvant treatment and follow up for patients with breast cancer.

NPI = Nottingham Prognostic Index. N/PPV = Negative/Positive Predictive Value, C.I. = Confidence Interval