act by either suppressing the local estrogen formation or by competitively inhibiting receptor binding. Nevertheless, little is known about the local expression of aromatase and sulfotransferase, which are key modulators of intratumoral estrogen levels.

We have performed immunohistochemostry to investigate the expression of aromatase and sulfotransferase in 42 samples obtained directly from malignant breast tumors, and compared it to biopsies obtained from uninvolved tissue in the vicinity of the front and to distant breast tissue. We found that aromatase was equally detectable in both tumor epithelial and stroma, but was mostly confined to the epithelium in non-malignant tissues ($p = 0.00008$, Fisher's Exact Test). Also, aromatase protein expressin was significantly more common in tumoral stroma when compared to peritumoral and distant breast stroma ($p = 0.00005$, and $p < 0.00001$, respectively). By contrast, sulfotransferase protein was only detectable in epithelial tissues, regardless of the location within the diseased breast. Epithelial sulfotransferase was, however, correlated with epithelial aromatase ($r = 0.35461$, $p = 0.0009$, Spearman's Rho test) and with the epithelial ER status ($r = 0.29313$, $p = 0.005$).

Taken together, we have demonstrated a differential aromatase and sulfotransferase protein expression pattern that is dependent of the spacial relation to a malignant breast tumor. Our results indicate a net increase in intratumoral active estrogen levels through increased stromal aromatization, while physiological local inactivation by sulfotransferase activity remains essentially unchanged.

Thursday, 23 March 2006 16:00–16:45

POSTER SESSION

Predictive and prognostic factors

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Prognostic factors and impact of contralateral cancer on survival of hereditary breast cancer

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Introduction: A high incidence of contralateral breast cancer (CBC) has been reported in BRCA1 mutation carriers and high risk familial patients. Data are scarce on the influence of hereditary CBC on survival, while 10- 25% of new familial patients opt for a risk-reducing contralateral mastectomy. Here we assess differences in CBC incidence, ipsilateral recurrence (ILR) and breast cancer specific survival (BCSS) in 3 risk groups and which factors influence prognosis.

Methods: We assessed tumour characteristics, CBC incidence, ILR and BCSS in 223 BRCA1 mutation carriers with invasive BC and 311 BC patients with $\geqslant 3$ breast and/or ovarian cancers in the family but no BRCA1/2 gene mutation (non-BRCA1/2). They were matched to 759 sporadic controls for year and age at detection.

Results: Median follow-up was 5.4 yrs. Tumours were $\leqslant$ T1 in 50% of the BRCA1, 60% of non-BRCA1/2 and 45% of sporadic patients ($p = 0.02$), node-negative in 66%, 53% and 48% respectively ($p < 0.001$), grade 1 or 2 in 8%, 30% and 26% respectively ($p < 0.001$). Risk-reducing contralateral mastectomy was performed in 23%, 11% and 1% respectively ($p < 0.001$.

After correction for the selection bias of offering DNA-testing with preference to patients with CBC and longer living patients, by exclusion of the patients with a DNA test performed 2 years or more after their diagnosis, 10 years metachronous CBC incidence was 25% in 170 unselected-BRCA1 patients, 6% in 238 unselected-non-BRCA1/2 patients and 5% in the sporadic patients ($p < 0.001$).

After correction for age, stage, grade, estrogen receptor and adjuvant therapy there was no significant difference in BCS survival between the 3 groups (unselected-BRCA1 vs. sporadic HR 1.1; p0.6) (unselected-non-BRCA1/2 vs. sporadic HR 0.9; p0.7), nor did ILR differ (multivariate HR 0.81 for BRCA1 vs. sporadic $p = 0.6$; HR 1.5 for non-BRCA1/2 vs. sporadic $p = 0.2$).

Independent prognostic factors for BCS survival in the total BRCA1, non-BRCA1/2 and sporadic group were tumour size (HR T2 vs. T1: 2.3; $p < 0.001$) nodal status (HR+ vs. HR−: 3.2; $p < 0.001$), age (HR 0.98 per year increase; $p = 0.009$), adjuvant therapy (HR 0.5; $p < 0.001$), and positive estrogen receptor (HR 0.6; $p < 0.001$) Metachronous CBC was associated with favourable BCSS,using follow-up from first diagnosis HR 0.6;p0.01, reflecting longevity before CBC. After CBC, BCSS was comparable (H 1.1; $p = 0.7$)

Conclusion: After correction for selection bias, stage and treatment factors we found no significant difference in BCS survival between both hereditary groups and sporadic breast cancer patients. Stage at detection of the first BC and adjuvant therapy are also in hereditary patients key determinants of prognosis, whereas the occurrence of metachronous contralateral breast cancer is not. We will discuss the impact of CBC and risk-reducing contralateral mastectomy. Decisions on breast-conserving treatment can be made on the same grounds in hereditary and sporadic patients.

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Expression of the HOXB13-to-IL17BR-gene ratio in oestrogen receptor positive primary breast carcinomas: Relation with tumour aggressiveness and response to tamoxifen

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Using a genome-wide screening, Ma et al. [(1)] identified the HOXB13-to-IL17BR expression ratio to predict clinical outcome of breast cancer patients treated with adjuvant tamoxifen. However, in the adjuvant setting this ratio may predict both a tumour's response to tamoxifen and its intrinsic aggressiveness. Therefore, we evaluated the two-gene expression ratio in retrospectively collected frozen specimens from 917 oestrogen receptor (ER) positive primary breast tumours. Using a quantitative RT-PCR assay we have assessed: 1) the relation with tumour aggressiveness and 2) the association with response to first-line tamoxifen monotherapy. Patients who received adjuvant systemic therapy were excluded in this study.

To investigate the relation with tumour aggressiveness, 619 tumours were analysed from 468 lymph node-negative and 151 node-positive patients of whom 332 patients showed a recurrence. The association with therapy response was determined in 193 tumours from patients treated with first-line tamoxifen for advanced disease. Expression levels were compared to housekeeper genes and correlated with clinical outcome. The hazard ratio (HR) and 95% confidence interval (95% CI) were calculated and all statistical tests were two-sided.

As continuous variable, the two-gene ratio had a statistically significant correlation in univariate analysis with disease-free survival (DFS) and progression-free survival (PFS), irrespective of lymph-node status. When dichotomised, high expression levels of HOXB13-to-IL17BR ratio showed a strong association with a shorter DFS for both node-negative (HR = 1.52 [95% CI: 1.16–1.99]; P = 0.002) as well as node-positive patients (HR = 1.66 [95% CI: 1.14–2.44]; P = 0.009). In addition, a shorter PFS for patients treated with first-line tamoxifen (HR = 3.43 [95% CI: 2.18–5.40]; P < 0.0001) was observed.

In conclusion, these results indicate that the HOXB13-to-IL17BR ratio is able to identify 1) patients at risk for earlier recurrence as well as 2) patients who fail to respond to first-line tamoxifen monotherapy for advanced disease. As a consequence, these patients may benefit more from other treatment modalities.

References

[1] Ma XJ, Wang Z, Ryan PD, Isakoff SJ, Barmettler A, Fuller A, et al. A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. Cancer Cell 2004; 5: 607–616.

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Risk of second non breast cancer in relation to BRCA1 and BRCA2 mutation status following breast-conserving treatment

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Purpose: *BRCA1* and *BRCA2* germline mutations are associated with a strong risk of breast and ovarian cancer. The increased risk of other cancers is not clearly established. We investigated whether mutation status influenced the rate of second non breast malignancies (SNBM).

Patients and Methods: *BRCA1* and *BRCA2* genes were screened for germline mutations in 131 patients with a family history of breast and/or ovarian cancer, treated with breast conserving surgery and radiotherapy. The 131 patients with familial history were matched to 261 patients without, according to age at diagnosis and year of treatment. The follow-up of controls was at least equal to the time-interval between diagnosis and genetic testing in familial cases. SNBM free interval was calculated from the

date of the first treatment. SNBM were scored as an event with censoring of the other patients at the time of the last follow-up or of death. Survival curves were derived from Kaplan-Meier estimates and were compared using the log-rank test. Incidence rates of each localisation SNBM found in our groups have been calculated for 100,000 persons – year (PY) for all population of patients and for each of the three groups. The incidence of SNBM was compared between the groups and between each group and the French women population incidence. These comparisons were realised after indirect standardization using the French population as reference, by calculating and testing the Standardized Incidence Ratio (SIR).

Results: *BRCA1/2* mutations were found in 20.6% patients with a family history. Nineteen patients had a *BRCA1* mutation, and 8 had a *BRCA2* mutation. At 10 years of follow up, SNBM free interval was 0.98 [0.97–1.00] in the control group, 0.97 [0.94–1.00] in the *BRCA1/2* non carriers and 0.79 [0.65–0.97] in the *BRCA1/2* carriers ($p < 10–5$). From 8 cancers in "familial group", there were 5 in *BRCA1/2* carriers (4 ovaries and 1 pancreatic carcinoma), and 3 in *BRCA1/2* non carriers (2 ovarian and 1 meningioma). In the group of controls, there were 3 gynaecological tumours (uterine body and cervix) and 1 small cell lung cancer. Of the 5 cases in the group of carriers, there were 4 ovarian and one pancreatic carcinoma in *BRCA1*-carriers and one ovarian cancer in *BRCA2*-carriers. *BRCA1/2* carriers presented more SNBM than the general French women population, SIR = 1099.2 [354.3–2565.4], $p < 10^{-3}$. No differences have been found between non carriers, controls and the French population, SIR = 149.3 [30.0–436.2] and SIR = 80.0 [21.5–204.7]. Familial group of patients (*BRCA1/2+ et BRCA1/2-*) presented higher incidence of ovarian cancers compared to the French population, respectively SIR = 9640.1 [2593.6–24,680.6] with $p < 10^{-3}$ and SIR = 1155.7 [129.8–4172.6] with $p < 0.05$), but no difference was found between controls and the French population, SIR = 0 [0–846.5]. No differences were found between the incidence rates of digestive, gynaecological and lung carcinoma between BRCA1/2 carriers, non carriers and controls and the French population.

Conclusion: At a 10-year median follow-up, the rate of SNBM was higher in *BRCA1/2* mutation carriers than in non-carriers with a family history or sporadic cancers. This difference is related to significantly higher rates of ovarian cancers in this population of patients. There were no differences in the other types of cancers (GI, lung). No difference has been found in the incidence of SNBC in the population of sporadic breast cancer and the general French population.

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Age-specific tumour features, contralateral breast cancer (CBC) risk and survival in BRCA1-associated breast cancer

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Introduction: Breast cancer (BC) in BRCA1-mutation carriers is characterized by specific tumour features, such as a more frequent occurrence of medullary carcinoma, ER-/PR-negativity, grade III tumours and a high incidence of CBC. We investigated whether these characteristics are maintained throughout different age categories considering the age at BC onset. Further, we assessed the prognostic impact of a young age at diagnosis in BRCA-associated and sporadic BC cases.

Methods: In 207 BRCA1-associated and 446 sporadic BC cases we compared tumour characteristics, the CBC rate and BC-specific survival (BCSS) between BRCA1- and sporadic cases separately within three different age groups: first BC diagnosis $\leqslant 35$, 36–49 and $\geqslant 50$ years.

Results: In the age groups < 36, 36–49 and $\geqslant 50$, ER-negativity was 71, 81, and 58%, respectively, in the BRCA1-group, whereas these percentages were 39, 31 and 29%, respectively, in the sporadic group. The difference between BRCA1- and sporadic cases was significant in all three age groups. A higher frequency of the medullary tumour type in the BRCA1-group was only observed in the group affected with BC before the age of 50. In all age groups, grade III tumors were more frequent in BRCA1-cases. However, while the frequency of grade III tumours clearly declined with increasing age at BC diagnosis in sporadic cases (76, 69 and 50%, respectively, p for trend = 0.02), this trend was not found in BRCA1-cases (89, 85 and 89%, respectively, p for trend = 0.83).

The rate of metachronous CBC was significantly increased in the BRCA1-groups as compared to sporadic BC in the age groups $\leqslant 35$ and between 36–49 years (HR 7.1, $p < 0.001$; and 7.6, $p < 0.001$, respectively). After the age of 50, no significantly increased CBC risk was seen for BRCA1-carriers as compared to sporadic cases (HR 1.5 ($p = 0.63$)).

In neither of the three age groups significant differences in BCSS were observed between BRCA1 as compared to sporadic cases. A young age at BC diagnosis ($\leqslant 35$) was an independent unfavourable prognostic factor both for BRCA1- and sporadic BC.

Conclusion: The frequency of the typical tumour characteristics of BRCA1-BC, including the high incidence of a CBC, is age-dependent. Differences between BRCA1 and sporadic cases are most outspoken under the age of 50, with the exception of the frequency of grade III tumours. These findings should be taken into account into the consideration of DNA-testing and prophylactic (contralateral) mastectomy.

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Young patients after BCT are at higher risk of loco-regional recurrence but not for distant metastases

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Background: Breast conserving surgery is associated with a higher risk of loco-regional recurrences, as compared to mastectomy. However, the impact of loco-regional recurrences on overall survival has not been demonstrated in trials which randomized between breast conserving therapy and mastectomy. This means that there is a group of isolated loco-regional recurrences after primary treatment that not lead to distant metastases or death and that are potentially curable. It would be useful if we could identify at the time of primary treatment risk factors associated with an increased risk to develop an isolated loco-regional recurrence followed by distant metastasis. Those risk factors could guide primary treatment choices. We studied whether the effects of risk factors at primary diagnosis associated with distant metastases and primary treatment change after the incidence of isolated loco-regional recurrences. To do this, we re-analysed the data of 3602 patients with early breast stage cancer surgically recruited in three EORTC trials (study 10801, 10854, and 10902).

Method: We modelled breast cancer disease progression as a multi-state model with three states: without any recurrence, with isolated locoregional recurrence, with distant metastasis or death. The following characteristics were considered for each transition: age at diagnosis, tumour size, axillary nodal status, surgical therapy, perioperative chemotherapy, adjuvant chemotherapy, adjuvant radiotherapy, and Tamoxifen®. The predictive ability of all independent variables was measures by adjusted hazard ratios (HR).

Results: Young age ($\leqslant 35$; HR: 2.31, 95%-CI: 1.46–3.67), surgical therapy (breast conserving therapy; HR: 2.14, 95% CI: 1.53–3.01) and having no adjuvant radiotherapy (HR: 1.69, 95%-CI: 1.17–2.45) are significant risk factors for locoregional recurrences.

The incidence of locoregional recurrences is a significant risk factor for distant metastases (HR: 3.95; 95%-CI: 2.00–7.81). This risk remains over time, and will only slowly decrease (HR: 0.95; 95%-CI: 0.87–1.04). Baseline prognostic factors as young age ($\leqslant 35$); breast conserving therapy and having no adjuvant radiotherapy are no significant risk factors for distant metastases after locoregional recurrences.

Discussion/Conclusion: Young patients after BCT are at higher risk of loco-regional recurrence but not for distant metastases.

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Importance of number of examined axillary lymph nodes for assessing the risk of locoregional recurrence (LRR) among breast cancer patients with 1–3 lymph node metastases

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Background: LRR remains a problem in breast cancer, and several studies have shown that postoperative radiotherapy (PRT) may improve survival. PRT is generally accepted when there are more than three involved lymph nodes and the cumulative ten-year LRR risk is at least 20%. In a review of International Breast Cancer Study Group (IBCSG) data, we showed that the subgroup with >20% LRR risk based on peritumoral vascular invasion (VI), tumor grade and tumor size also included some patients with 1–3 involved nodes (Wallgren, et al., Risk factors for locoregional recurrence among breast cancer patients: results from International Breast Cancer Study Group trials I through VII, J Clin Oncol 21:1205–1213, 2003). We have expanded this analysis to include patients from two additional trials. The number of lymph nodes found to be uninvolved on pathological examination was included as a potential risk factor.