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

and C-erb-B2 (p = 0.029 and p = 0.031). In multivariate analyses axillary lenf node presence and C-erb-B2 overexpression were a strong negative prognostic factor on disease free survival and overall survival (p = 0.04, p = 0.03 for DFS and p = 0.03, p = 0.007 for OS). E-cadherin and bcl-2 failed to have an effect on disease free survival and overall survival in our study. In addition, p53 mutation positivity was observed in seven patients (9.2%), there was not any effect on prognostic parameters (p = 0.419 for DFS and p = 0.218 for OS).
Conclusions: The results of this study showed that E-cadherin, bcl-2, and p53 did not have any significant prognostic value for our patients. We need studies which include more patients and long follow-up periods to get a decision.

5160 POSTER

Long-term prognostic effects of fasting insulin in early stage breast cancer (BC) patients

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Background: Hyperinsulinemia, a likely mediator of adverse prognostic effects of obesity in early BC, has been associated with increased risk of distant recurrence and/or death during the first 3−4 years after diagnosis; long-term effects are unknown.
Materials and Methods: An inception cohort of 512 women with T1−3, N0−1, M0 BC diagnosed at University of Toronto hospitals between 1989–1996 was followed prospectively to 2007. Tumor and treatment variables were obtained from clinical records, and women were followed for recurrence and death. Insulin was measured on fasting blood obtained postoperatively, prior to systemic therapy, using a 2-epitope immunometric chemiluminescent method (Beckman-Coulter). Distant disease-free and overall survival were analysed using Cox multivariate models adjusted for age, T stage, N stage, hormone receptors, grade, adjuvant chemotherapy and hormone therapy.
Results: Mean age was 50.5±9.7 years. Tumor characteristics were as follows: T1 = 287, T2 = 158, T3/TX = 59; N0 = 352, N1 = 152; ER positive = 337; PgR positive = 285; grade 1 = 73, grade 2 = 199, grade 3 = 170. Median follow-up was 11.9 years. Mean insulin level was 44.6±31.1 pmol/L. 193 (37.7%) received adjuvant chemotherapy and 197 (38.5%) received adjuvant hormone therapy. Short and long-term prognostic effects of insulin are provided in the table below. Adverse effects were present in short-term, but not long-term, analyses. Short-term effects were present in both hormone receptor positive and negative BC but were greater in hormone receptor negative BC [HR death Quartile 4 vs. Quartile 1 = 6.35, 95% CI 1.1 vs. 36.8] than in hormone receptor positive BC [HR death Quartile 4 vs. Quartile 1 = 3.03 (1.18−7.75)]. Smoothed HR curves over time show an increased risk of distant recurrence and death for the first 5 years after diagnosis, with no excess risk after 5 years.

	Short-Term [1] (median 4.2 years)	Long-Term (median 11.8 years)	4+ Years only
Distant recurrence HR, 95% CI Q4 vs. Q1	2.1, 1.2−3.6	1.2, 0.8−1.9	0.8, 0.5−1.4
Death HR, 95% CI Q4 vs. Q1	3.3, 1.5−7.0	1.1, 0.7−1.7	0.9, 0.6−1.5

* Insulin Quartile 1 <27 pmol/L; Quartile 4 >51.9 pmol/L.

Conclusions: Adverse prognostic effects of hyperinsulinemia are seen in hormone receptor positive and negative BC in the first 4 years after diagnosis but are not present beyond 4−5 years post-diagnosis. Interventions targeting insulin should focus on the first 4−5 years post-diagnosis.

References

[1] Short-term effects were previously reported in Goodwin PJ et al J Clin Oncol 2002;20:42−51.

5161 POSTER

Clinical and pathological aspects of 90 infra-centimetric HER2+ invasive breast cancers: a 3-centres joint AERIO/REMAGUS series

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Background: HER2+ and invasive infracentimetric breast carcinomas (BC) have been extensively described separately. Few fata have been published regarding the combining of both features in the same cases (InfraHER2). According to adjuvant trastuzumab (TZM) trials, InfraHER2 stand for less than 10% of HER2+ tumours. Our purpose was to describe this particular subset.
Material and Methods: We performed a retrospective study of patients (pts) with InfraHER2 tumours treated at 3 major French Comprehensive Cancer Centres between 2002 and 2008. Data were extracted from databases. Tumours with >80% of *in situ* component or multifocal were excluded.
Results: Of 90 cases listed, median age was 56 years (range 24−84). Median tumour size was 8 mm (range 2−10), 18 being ⩽5 mm (T1a). There was no significant difference in characteristics between T1a and T1b (table). Invasive ductal carcinoma (IDC) was the main histological subtype (89%). In 86 cases (96%), HER2 was overexpressed by immunohistochemistry. For equivocal cases, HER2 amplification was confirmed by FISH (range 8−20 HER2 copies). Estrogen (ER) and progesterone receptors (PgR) were expressed in respectively 44/90 cases (49%) and 22/79 cases (28%), and 19 pts (21%) had a pN+, pN0i+ or pNmi+ status. Elston-Ellis grade was III in 34% (30/89 cases) without any significant difference between T1a and T1b; 29% of tumours showed lymphovascular invasion (LVI). In one case of pN0 IDC, initial work up revealed a single bone metastatic deposit while hepatic metastases were discovered 2 years later. All patients were treated by surgery; radiotherapy, chemotherapy and TZM were delivered in 75%, 54% and 45% of pts respectively. With a 27 months median follow-up, 2 invasive recurrences have occurred. Those 2 pts had initial IDC classified as pN0, ER−, PgR− and LVI−. In one pure micropapillary case, *in situ* local recurrence occured 5 years later.
Conclusions: InfraHER2 tumours may present with aggressive features including node invasion, high grade or LVI, irrespective of T1a or T1b subclassification. These finding should stimulate further prospective research to assess the value of adjuvant treatment for these tumours.

Main characteristics, N (%)	T1a (n = 18)	T1b (n = 72)	Total (n = 90)
Discovered by screening	15 (83)	44 (61)	59 (66)
Lymphovascular invasion	3 (17)	23 (32)	26 (29)
Elston-Ellis grade II/III	15 (83)	67 (93)	82 (91)
Mitotic Index 2/3	6 (33)	41 (57)	47 (52)
ER±PgR positive	9 (50)	37 (51)	46 (51)
pN1 (including pN0i+/mi+)	1 (6)	18 (25)	19 (21)
Recurrence	0 (0)	2 (3)	2 (2)

5162 POSTER

Prognostic significance of breast cancer subtypes and nodal status

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Background: To investigate the prognostic and predictive significance of subtyping breast cancer (BC) by immunohistochemistry. We analyzed and correlated breast cancer subtypes with overall survival (OS) and disease-free survival (DFS) in nodal +/− patients treated with adyuvant therapy.
Methods: A case series of 567 breast cancer patients treated at Granada University Clinical Hospital between 1998 and 2004 were identified retrospectively. Patients were classified by tumor characteristics as (14.5%) triple negative (estrogen receptor ER-negative, progesterone receptor PR-negative, HER2/neu HER2-negative), (8.5%) HER2 (HER2-positive, ER-negative, PR-negative), (68%) luminal A (ER-positive and/or PR-positive and not HER2-positive) and (4%) luminal B (ER-positive and/or PR-positive and HER2-positive). For mutivariante analysis, stratified cox models were built to determine de hazard ratios of breast cancer subtypes adjusting for age (median 54±11 yrs), nodal involvement (N0 62%, N1−3 24%, N > 4