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

13%), tumor size (T1 59% T2 39% T3 1.6%) and grade (G1 32% G2 39% G3 15%).
Results: Median follow-up was 47 months. Actuarial (OS and DFS) at 4 years were luminal A (99.3% and 95.8%), luminal B (95.6% and 80.4%), Her2 (89.8% and 80.9%) and triple negative (74.6% and 58.7%), p = 0.0001; for N0 (98.8% and 93.9%) N1−3 (90.8% and 83.6%) and N > 4 (87.1% and 71.8%), p = 0.0001. Significant independent prognostic factor for OS were BC subtypes (with relative risk (RR) luminal B 4.7, Her2 3.6 and triple negative 12.08 referent to luminal A, p = 0.0001), and the number of positive nodes (N1−3 RR = 6.6 and N > 4 8.3 referent to N0 category, p = 0.004), respectively. Significant independent prognostic factor for DFS were BC subtypes (with relative risk (RR) luminal B 4.8, Her2 3.9 and triple negative 7.7 referent to luminal A, p = 0.0001), the number of positive nodes >4 (RR 2.01 referent to N0 category, p = 0.049) and tumor size (T2 RR 2.28 referent to T1 category, p = 0.004).
Conclusions: A simple immunopanel can divide breast cancers into biologic subtypes with independent prognostic effects and provides additional information to nodal status. Triple negative status emerged as a strong adverse prognostic factor.

5163 POSTER

Methylation in breast cancer and correlate ER with tumor phenotypes and prognostic factors

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Background: To investigate the association between ESR1 gene hypermethylation and tumor phenotype including diagnosis and treatment response are the objetive of this studies. Other gene as 14−3-3σ were also analized.
Materials and Methods: Since January 2002 to June 2005, 107 women with breast cancer and 108 control subjects were recruited. Real Time QMS-PCR SYBR green (methylation-specific PCR) was used to analyze the methylation of *ESR1* and *14−3-3σ* gene promoter regions as breast cancer biomarkers. Tumours were classified as phenotype basal, luminal A, Luminal B and phenotype HER2+.
Results: Ours analyses revealed low or absent methylation *ESR1*and *14−3-3σ* in healthy controls and significant differences between breast cancer patients (pts) and healthy controls in relative serum levels of methylated gene promoters *ESR1 (p* = 0.0112) and *14−3-3*s (p = 0.0047). Presence of methylated *ESR1* in serum of breast cancer patients was associated with *ER-negative* phenotype (*p* = 0.0179). Of the available cases, 60 pts (56%) were Luminal A, 10 pts (9.3%) Luminal B, 13 pts (12%) Basal like and 9 pts (8.4%) HER2+. We observed that methylated *ERS1* was preferably associated with phenotype Basal Like and worse interval progression free and survival global though p > 0.05. We observed that hypermethylation of *ERS1* and *14−3-3σ* combined differentiated between breast cancer patients and healthy controls (p = 0.0001) with a sensitivity of 81% (95% CI: 72−88%) and specificity of 88% (95% CI: 78−94%). In addition observed lower methylated *ERS1* and *14−3-3σ* value after surgery, respect pretreatment levels, but without an overall statistically significant difference. With a median follow up of 6 years, we found that patients with a significant decrease of sera methylated levels of both genes after surgery had better time to progression an overall survival respect patients without this observation.
Conclusions: This study identifies the presence of variations in global levels of methylation promoters genes in healthy controls and breast cancer with different phenotype classes and shows that these differences have clinical significance. In the future this panel of genes detected could be useful as markers for early detection of breast carcinoma. These findings cast some doubts on the utility for early cancer diagnosis of highly sensitive techniques to identify hypermethylation of specific gene promoters in DNA extracted from serum. Although numerous issues remain to be resolved, the quantitative measurement of circulating methylated DNA is a promising tool for cancer risk assessment.

5164 POSTER

Impaired glucose tolerance in non-diabetic women during adjuvant chemotherapy for breast cancer

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Background: Up to 16% of patients with breast cancer have diabetes and diabetic individuals tend to have poorer outcomes following treatment for breast cancer. During chemotherapy, dexamethasone is widely used to prevent side effects. However, glucocorticoid administration is associated with impairment of insulin sensitivity, elevations in peripheral glucose levels as well as suppression of the hypothalamic-pituitary-adrenal axis for up to 3 weeks. We measured blood glucose levels in a group of non-diabetic women receiving adjuvant chemotherapy for breast cancer.
Materials and Methods: 39 women (age 58.6±12.8 years, BMI 27.2 +/-4.9 kg/m^2) participated in this study which was approved by the local ethics committee and all patients gave informed consent. Patients received either 6 cycles of fluorouracil, epirubicin, cyclophosphamide (FEC) (18) or 3 cycles of FEC followed by 3 cycles of docetaxel (21). Before each cycle of FEC, patients received 8 mg of dexamethasone (po). The patients who received docetaxel had 8 mg of dexamethasone (po) 24 hours, 12 hours and immediately before docetaxel. For each cycle of chemotherapy non-fasting glucose was measured before the treatment cycle began, immediately after the pre-chemotherapy Dexamethasone was administered but before chemotherapy and, immediately after chemotherapy and 10 days after each cycle.
Results: There was an increase in blood glucose levels with later cycles among women who received the higher dose of Dexamethasone in combination with docetaxel (cycle 5: P < 0.001; cycle 6: P = 0.002 [paired *t* tests]) (Table). Before the first cycle of chemotherapy, none had blood glucose levels in either the impaired glucose tolerance range (ie, 7.8−11.1 mmol/L) or the diabetic range (ie, >11.1 mmol/L). Increasing number of patients developed glucose intolerance as cycles progressed; 6 had blood glucose levels in the impaired tolerance range and 8 had levels within the diabetic range following the 5th cycle.

Cycle	Before treatment cycle	Immediately after Dexamethasone but before chemotherapy	After Dexamethasone and chemotherapy	10 d after chemotherapy
1	5.8 [1.1]	4.8 [0.7]	5.7 [2.0]	5.7 [0.7]
2	5.5 [0.9]	5.6 [1.0]	5.5 [0.8]	5.5 [1.0]
3	5.3 [0.9]	5.5 [1.3]	5.9 [1.5]	5.8 [1.4]
4	5.6 [0.9]	6.0 [1.6]	6.4 [1.8]	5.6 [0.8]
5	5.3 [1.0]	7.7 [3.0]	7.8 [2.7]	6.0 [2.0]
6	5.5 [0.8]	8.0 [2.7]	8.1 [3.1]	6.0 [1.2]

Conclusions: The implications of transient hyperglycaemia on the efficacy of chemotherapy in this setting is uncertain and further investigation is indicated.

5165 POSTER

Microarray based determination of ER, PR and HER2 receptor status compared to local IHC assessment in 11 hospitals

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Background: The level of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression is predictive for prognosis and/or treatment response in breast cancer patients. However, differences in immunohistochemistry (IHC) methods and interpretation can substantially affect the accuracy and reproducibility of the results. The recently developed TargetPrint test measures the mRNA expression level of ER, PR and HER2 and provides an objective alternative to IHC. This study describes a