5110 POSTER

Research on Microfluidic Chip Detection Method for Breast Cancer Cell Micrometastasis

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Background: To realize micrometastasis training, lysis and fluorescence detection in breast cancer cells with microfluidic chip as platform; and research on the feasibility of early detection and accurate determination of tumour micrometastasis.
Materials and Methods: The cell concentration in breast cancer cell culture RPMI-1640 solution was controlled at ~5×10^6 cells/mL or so. Cell suspension 10uL was adopted and poured into the PDMS chip, the chip was placed into the incubator for culturing, and cells were cultured in micro-channel for 24h. Then cytoperm was added for 10min, CK19 was added in turn then, the chip was placed into the incubator and incubated for 1h, FITC labeled goat anti-mouse IgG was added at dark, the liquid removal gun was used for sucking out the excess FITC labeled goat anti-mouse IgG 30min later, and PBS was repeatedly replaced for multiple times in the process for cleaning, and the chip was placed under fluorescent microscopy for detection finally.
Results: Compared with culture flask culturing cells, the adherent time for culturing MCF-7 cell chips should be short (12 hours vs 6 hours), which can be interpreted as the follows: the chip cell culturing pool is conductive to cell adherence due to larger surface-to-volume ratio. The chip was observed under fluorescence microscope, normal cells were stained for the strong green fluorescence, dead cells showed red fluorescence, and forms of living cells and dead cells were vastly different and easy to distinguish. By calculation, the proportion of living cells can be more than 95%, and core mitotic figures can be observed, which indicates that cells can maintain normal life and division under the condition of chip perfusion culture.
Conclusions: A unique rapid culture, lysis and cell inclusion CK19 fluorescence detection method of breast cancer cell micrometastasis based on PDMS chips is established, and this method meets trace, rapid and real-time requirements demanded for micrometastasis detection. The feasibility of detecting breast cancer cell micrometastases with microfluidic control chip is proved, thereby providing important accumulation for clinical application of early discovering and accurately judge tumour micrometastases.
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Infiltrating Lobular Carcinoma of the Breast − a Hospital General Experience

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Background: Invasive lobular carcinoma (ILC) is the second most common type of invasive breast cancer (BC), that comprise approximately 10% of BC and appears to have a distinct biologic and epidemiologic characteristics.
Patients and Methods: We analyzed data of 205 BC patients diagnosed of ILC who were diagnosed between January 1994 and December 2007. The objective was to determine the clinicopathological features, treatment and patterns of recurrence of ILC.
Results: Median age was 58.5 (range: 29.6–87.3). One hundred thirty six pts (66.3%) were postmenopausal, 131 pts (63.9%) underwent mastectomy and 74 (36.1%) a conservative surgical procedure.
Pathological features were: T1: 79 pts (38.5%); T2: 84 pts (41%); T3: 19 pts (9.3%); T4: 7 pts (3.4%); multifocal: 16 pts (7.8%). Nodal status N0: 131 pts (63.9%); N1: 41 pts (20%); N2: 16 pts (7.8%); N3: 17 pts (8.3%). Regarding phenotype, 90 patients (43.9%) were Luminal A; 82 pts (40%) Luminal B; 14 pts (6.8%) HER2+/RE+; 2 pts (1%) HER2+/RE-, and 7 pts (3.4%) were triple negative.
67 pts (32, 7%) didn't receive adjuvant chemotherapy (QT). Most frequent adjuvant QT received was anthracycline-based (61 pts, 29.8%) followed by CMF (42 pts, 20.5%) and anthracycline+taxane based QT (35 pts, 17.1%). 185 patients (90%) received adjuvant hormonal treatment, being the most commonly used tamoxifen (111 pts, 60%) followed by up-front aromatase inhibitors (AI) (36 pts, 19.5%), 'switch' treatment (19 pts, 10.3%), and extended AI (11 pts, 6%).
With a median follow-up of 97.3 months, 47 pts (22.9%) had a relapse, with a median disease-free survival (DFS) of 184 months. 5-year and 10-year DFS rates were 81.8% and 69.1%, respectively. T1, N0 tumours that received QT/HT/RT had a significantly lower recurrence rate ($p < 0.05$).
Most frequent metastatic site at recurrence was bone (18 pts, 38%), followed by pleuropulmonar (7 pts, 15%), liver (5 pts, 11%) and ganglionar (5 pts, 11%).

Site of relapse	N (47)
Brain	2
Bone marrow	2
Gastrointestinal	3
Contralateral breast	3
Regional recurrence	4
Pelvic	4
Ganglionar	5
Hepatic	5
Pleuropulmonar	7
Bone	18

Median overall survival (OS) was not achieved; 5-year and 10-year OS rates were 94.4% and 81%, respectively. OS was significantly better ($p < 0.05$) for T1, N0 tumours.
Conclusions: In this review of ILC patients, the most common phenotype was luminal A. Recurrence and death rates were low, being the bone the most common site of relapse.

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Influence of Adjuvant Bisphosphonates in the Treatment of Early Breast Cancer on Disease-Free Survival − Results of a Retrospective Analysis of an Unselected Single-Centre Cohort

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Background: Bisphosphonates (BIS) are a standard of care in treating patients with bone metastases for preventing skeletal-related events (SREs) and have demonstrated utility for preventing cancer treatment-induced bone loss (CTIBL). Recently, several trials have demonstrated that BIS may exert anticancer effects in adjuvant and advanced cancer settings. The aim of this retrospective analysis of an unselected single-centre cohort of women with early breast cancer was to evaluate the influence of adjuvant BIS on disease-free survival (DFS).
Material and Methods: Altogether, 1653 women (959 who received adjuvant BIS and 694 without adjuvant BIS) were investigated. All women who started a BIS treatment within the first 12 months of diagnosis and with at least 90 days of treatment were included in the BIS group. There were no significant differences in the 2 patient groups regarding tumour size, hormone receptor (HR) expression, HER-2 expression, and adjuvant treatment modalities except for nodal status. The analysis included women receiving BIS treatment, with the majority receiving zoledronic acid; some also received clodronate, ibandronate, or alendronate. DFS was defined as the time from completion of first-line therapy until the time of disease recurrence or death, whichever occurred first.
Results: DFS was significantly better in patients treated with BIS ($P = 0.01$). This was also apparent in the subgroup of patients with oestrogen receptor (ER)/progesterone receptor (PR)-positive disease ($P = 0.006$), in patients with node-positive disease ($P = 0.00002$), as well as in patients with >3 positive lymph nodes and ER/PR-positive disease ($P = 0.00001$). In this final subset, BIS-treated patients (n = 121) had 30% higher DFS at the 5-year timepoint compared with patients who did not receive adjuvant BIS (n = 56). There was no significant difference in DFS in patients with hormone-receptor-negative disease.
Conclusions: This large-scale retrospective analysis indicates a significant improvement of DFS in patients with early stage breast cancer treated with adjuvant BIS. The benefit was more pronounced in the subgroup of node-positive and ER/PR-positive disease.

5113 POSTER

Getting Deep in the Luminal B Breast Cancer Subtype and Its Ki67 Cutoff Value

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Background: Inside luminal breast cancer (BC) group, B subclass carries a worse prognosis and is less responsive to hormonal treatment. Identification of Luminal B group, by Sorlie et al, has been less consistent

than other subclasses; and gene signatures based in estrogen-related genes or proliferation are better to identify this BC subclass. Cheang et al genetically evaluated 144 luminal ER-positive HER2-negative tumours by IHC; they found a ki67 cutoff value of 13.25% to differentiate B from A subclasses. No differentiation for PR status was done.
Luminal B subgroup is usually defined as ki67 >13 if ER positive, as well as HER2+ or PR negative. The target of this abstract is to evaluate behavior of different Luminal B subsets.
Materials and Methods: We reviewed early BC cases evaluated at Hospital 12 de Octubre between 1995 and 2007 and selected 710 initially operated Luminal B BC. We divided this group in 4 subsets as shown in table 1 and analyzed their clinical- pathologic features and outcomes. Additionally, we evaluated the prognostic behavior of lowering the ki67 cutoff in the ER+PR+HER2− group (820 pts).
Results: Median Ki67 value for the ER+PR− group was 17%. ki67 cutoff at 14% discerns two groups of different prognosis inside the Luminal group (extracting HER2+ and RP−); and comparison of ki67 cutoff between 14 vs 11% found overlapped CI (Median: 6.31 (5.99–6.62) vs 6.49 (6.21–6.78). The table presents different characteristics and prognosis based on molecular features (statistical comparisons exclude ER−PR+ subgroup).

Variables	HER2+ER+	HER2−		
		ER+PR−	ER−PR+	ER+PR+ ki67 > 13
Cases	189	126	10	385
Ductal (p = 0.002)	173 (91.5%)	98 (77.8%)	10 (100%)	314 (81.5%)
III (p = 0.03)	41%	47%	40%	34.1%
Lobular	5.3%	17.5%	0	14%
Median age (p = 0.0021)	53.49	60.29	49.68	57.7
Median ER	85%	83%	0%	90%
Median PR	60%	0%	45%	80%
Median ki67	20%	17%	12.5%	20%
DFS (p = 0.001)	8.21	6.55	9.40	5.67
OS	8.7	7.22	10.53	6.4
Recurrences total (%)	52 (27.5%)	32 (25.4%)	2 (20%)	81 (21%)
LocoRegional	9 (17.3%)	3 (9.4%)	0	15 (18.5%)
Bone	6 (11.5%)	14 (43.8%)	0	25 (30.9%)
Visceral (p = 0.04)	34 (65.4%)	11 (34.4%)	0	36 (44.4%)

Conclusions: Exclusion of ER+/PR−/HER2− subgroup from the Cheang study could have led to a reduction in mean Ki 67 level as the recommended cutoff value. Subsets inside Luminal B subclass according to HER2, ER, PR and ki67 have different features and behaviors. Luminal Ki67 cutoff should be evaluated excluding RP− group.

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Clinical and Histopathological Tumour Characteristics in Patients With Invasive Breast Carcinoma Receiving Metformin

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Background and Aims: Epidemiological studies show that metformin treatment is associated with a reduction in cancer risk. Metformin may exhibit inhibitory effects on cancer cells by inhibiting mTOR signaling pathway. Therefore, it is possible that metformin has also an impact on tumour extension and progression in breast carcinoma (BC) patients. The aim of our retrospective study was to examine if the patients with BC and diabetes mellitus (DM) receiving metformin have lower tumour stage in comparison to patients not receiving metformin.
Patients and Methods: A chart review of 171 patients (mean age 67.4; range 38−93 years) with invasive BC and DM was performed. They were surgically treated at our institute from 2006–2010. Data on clinical and histopathology factors (age, BMI, tumour diameter, TNM tumour stage, number of metastatic lymph nodes, presence of estrogen and progesterone receptors, HER-2 status) were collected. Statistical analysis of these factors (i.e. comparison of metformin group vs. no metformin group) was performed by contingence tables and non-parametric tests.
Results: DM type 1 and DM type 2 was present in 38 and 133 cases, respectively. Altogether 91 patients (mean age 66.3; range 51−88 years) were on metformin, while 80 (mean age 68.6; range 38−93 years) were not receiving metformin. Patients on metformin were younger than patients not receiving metformin ($p < 0.05$). No statistical difference between the study groups (metformin vs. no metformin) were found in TNM stage (T1: 47% vs. 42.5%; T2: 38% vs. 27.5%; T3: 4% vs. 6%; T4: 10% vs. 24%, p = 0.071; N0: 58% vs. 49%, N1 42% vs. 51%, p = 0.21; M0: 97% vs. 97.5%, M1 3% vs. 2.5%, respectively). However, patients on metformin had lower proportion of T3 or T4 tumours than patients who were not receiving metformin (14% vs. 30%; p = 0.013). Axillary lymphadenectomy was performed in patients on metformin and in patients not receiving metformin in 46% and 62% (p = 0.039), respectively. Tumour size (2.5 cm vs. 2.8 cm; p = 0.36), tumour histology, tumour grade, mean number of metastatic lymph nodes (2.4 vs. 2.7; p = 0.23), hormone receptor status or HER-2 status did not show any statistical difference between both study groups.
Conclusion: Our patients with BC and DM on metformin have lower proportion of T3 or T4 tumours in comparison to patients not receiving metformin.

5115 POSTER

Neo-adjuvant Chemotherapy in Breast Cancer; the Possibility of Response Evaluation and Prediction of Response Treatment Using the Internal Mammary Vessels on MR Mammography

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Background: From a previous study in our institute is known that the vascular surfaces of internal mammary artery (IMA) and internal mammary veins (IMV) relate to the the breast with cancer compared to the contralateral side. This difference was not observed in healthy controls. This study investigates the possibility whether the surface of the internal mammary vessels on MR mammography performed on a 1.5T MRI allows evaluation of the effects of neo-adjuvant chemotherapy on the tumour mass and predictions response in breast cancer patients.
Materials and Methods: Eight patients whom received neo-adjuvant chemotherapy underwent a MR mammography before, after 3 and after 6 chemotherapy cycles. Measurements were made on a transverse T2w sequence (scanning parameters: slice thickness 1 mm, field-of-view $280 \times 338 \times 190$ mm, matrix 352). Surface of both the IMA and IMV was determined on the side of the tumour and contralateral, particularly on the second and third intercostal space. The reader was blinded for all clinical data. Differences in vessel surface between the three MR mammography were analyzed using a linear mixed model.
Results: Mean tumour size was 5.6 cm (2.1−9.3) before starting neo-adjuvant chemotherapy. After 6 chemotherapy cycles mean tumour size was 2.1 cm (0.0−4.5). The surface of the IMA and IMV decreases in the present of tumours responding to neo-adjuvant chemotherapy. Probably because of the size of the study population a trend but no significant relation exists (p = 0.245). Furthermore the data suggest a delay in vessel surface decrease compared to the decrease of tumour size.
Conclusion: These data suggests a relation between decreasing tumour and decreasing vascular surface as response on chemotherapy. Future research is warranted to proof whether the vascular surface could be a supplementary parameter in the assessment of the response evaluation and prediction of response treatment on MR mammography.

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Evidence of No Benefit for Extensive Axillary Dissection in Lymph Node-positive Early Breast Cancer Treated With Adjuvant Radiation

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Background: Axillary dissection (AD) in breast cancer (BC) has extensively demonstrated no survival benefit over sentinel node dissection when no ganglionar involvement is found. Recently, a randomized phase III trial (The American College of Surgeons Oncology Group Z0011) found similar results when sentinel node was positive in a sample of 891 T1-T2 BC patients (pN1-N2 in most of the cases) that complemented treatment with adjuvant radiation and standard systemic drugs. Some critics were that the sample size was not the initially planned and that results of this trial changes both oncologists mental paradigms and treatment of a large group of BC patients.
The target of this abstract is to test in a retrospective way the usefulness of extensive AD in a population similar to Z0011 trial.
Materials and Methods: We reviewed BC cases diagnosed at Hospital 12 de Octubre between 1995 and 2007 and selected 337 initially operated T 1−2 N1−2 patients that received adjuvant radiation and standard systemic treatment. We evaluated if number extracted lymph nodes (over or under the median; or in the upper or lower tertile) had a prognostic value.
Results: Median follow up was 7.85 y. Median extracted lymph nodes was 14 and the mean disease free survival (DFS) under or over it were 7.02