Breast cancer pathology and predictive factors

416 POSTER

UK patients are willing to donate biological material for sub-studies in clinical trials

L. Johnson[1], J. Bliss[1], P. Ellis[2], P. Barrett-Lee[3], S. Johnston[4], J. Yarnold[5]. *[1] Institute of Cancer Research, Clinical Trials & Statistics Unit, Surrey, United Kingdom; [2] Guys, Kings and St Thomas' Hospitals, Department of Medical Oncology, London, United Kingdom; [3] Velindre Hospital NHS Trust, Department of Oncology, Cardiff, United Kingdom; [4] Royal Marsden NHS Trust, Department of Medicine, London, United Kingdom; [5] Royal Marsden NHS Trust, Department of Radiotherapy, Sutton, United Kingdom*

With the establishment of a National Translational Cancer Research Network (NTRAC), the UK is now well placed to become an international leader in building a cancer tissue resource to conduct biological research. However, since the Alder Hey scandal in January 2001, there has been much debate about ethical issues and patient consent relating to the collection and use of tissue samples, and a lack of clear guidance on how specific patient consent for future biological research ought to be.

Since 1999, the Clinical Trials and Statistics Unit at the Institute of Cancer Research (ICR-CTSU) has coordinated 2 major national breast cancer clinical trials where biological material was collected. Thirty five UK centres participated in the START (Standardisation of Radiotherapy) Trial, out of which 27 also opted to take part in a sub-study which involved the collection of one blood sample for each patient to be used for DNA testing. 3585 patients from the 27 participating centres agreed to take part in the main START Trial between January 1999 and October 2002, of whom 2849 (79.5%) also donated a blood sample for future research.

Within the TACT (Taxotere as Adjuvant Chemotherapy) Trial, all UK patients were asked to donate breast tumour tissue for future research. Between February 2001 and February 2003, 3507 patients consented to take part in the main TACT Trial, of whom 3445 (98.2%) also agreed to donate tissue.

	No UK centres taking part) (as % of those taking part in main study)	No patients consenting (as % of those from participating centres)
START (blood samples)	27/35 (77%)	2849/3585 (79.5%)
TACT (paraffin blocks)	102/102 (100%)	3445/3507 (98.2%)

Conclusion: Alder Hey has had little impact on the willingness of patients to donate biological material for research purposes, with the overwhelming majority of patients in clinical trials consenting to this type of research. Any impact made by Alder Hey is likely to be due to concerns of pathologists over releasing material, and/or professional anxieties over ethical and legal issues.

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HER2 analysis in breast cancer by two-colour FISH - significance of chromosome 17 polysomy

T. Gaiser[1], M. Hofmann[1], H. Kneitz[1], L.P. Weng[1], C. Schmidtgen[1], G. Maass[2], C. Gross[1], T. Henkel[1], J. Rueschoff[1]. *[1] Klinikum Kassel, Insitute for Pathology, Kassel, Germany; [2] Roche Diagnostics GmbH, Penzberg, Germany*

Background: Knowledge of HER2 status is a prerequisite when considering patient eligibility for treatment with trastuzumab (Herceptin): accurate assessment is essential to ensure that all patients who may benefit are correctly identified. The most common techniques to assess HER2 status in routine clinical practice are IHC and FISH. Two different FISH methods for HER2 determination are available: 1. Colour detecting only HER2 gene specific signals e.g. the Inform test (Ventana). 2. Colour with additional visualisation of chromosome 17 centromere e.g. the PathVysion test (Abbott). Only the latter can detect polysomy 17 (an increase of average chromosome 17 counts per cell): convention dictates that the sample be classified as negative if the ratio of HER2 to chromosome 17 is <2.0, although such cases will be positive according to the one-colour technique. At present, the significance of polysomy 17 concerning therapeutic response is unknown.

Method: Tumour specimens from 289 patients were tested for HER2 status by IHC (HercepTest) and FISH (PathVysion) as part of a study evaluating the efficacy of 3-weekly Herceptin in MBC (WO16229). The degree of polysomy was calculated by dividing the number of centromeric specific signals by the number of counted cells (n=60). Low-, intermediate- and high-level polysomy were defined as ratios of 3–4, 4–5 and >5, respectively. All patients within the study were followed until disease progression, with those showing partial or complete response regarded as responders.

Results: 100 patients demonstrated IHC 3+ or FISH+ tumours and were eligible for Herceptin; 96 were assessable for response. The ORR was 21% (n=20). Of the 96 assessable patients, 22 (23%) showed polysomic tumours (13 low level, 3 intermediate level, and 6 high level, respectively) of which 6 responded to Herceptin. These were distributed as follows: 1. low-level polysomy (IHC 3+, FISH+); 2. intermediate-level polysomy (both IHC 3+, FISH-); 3. high-level polysomy (1 IHC 3+, FISH +; 2 IHC 3+, FISH-).

Conclusions: 1. All responders to Herceptin were IHC 3+ (100% specificity for IHC). 2. Frequency of polysomy 17 within patients assessable for response in this study was 23%. 3. 4 of 6 polysomic responders to Herceptin had a FISH- (FISH ratio <2.0), IHC 3+ phenotype. Based on these findings we suggest that if 2-colour FISH is used as the first-line HER2 test, all polysomic FISH- patients should be retested by IHC; IHC 3+ cases are then eligible for Herceptin treatment.

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Anti-metastatic efficacy of clodronate is associated with a decrease in bone turnover

E.V. McCloskey[1], S. Bal[1], R. Tähtelä[2], T. Powles[3], A.G. Paterson[4], J.A. Kanis[1], S. Ashley[5], A. Tidy[6], S. Atula[2], J. Nevalainen[2]. *[1] University of Sheffield, WHO Collaborating Centre for Metabolic Bone Diseas, Sheffield, United Kingdom; [2] Schering Oy, Clinical Research, Helsinki, Finland; [3] Royal Mardsen Hospital, Institute of Cancer Research, Sutton, United Kingdom; [4] Tom Baker Cancer Centre, Department of Medicine, Calgary, Canada; [5] Royal Mardsen Hospital, Statistics, Sutton, United Kingdom; [6] Royal Mardsen Hospital, Bone Metabolism Unit, Sutton, United Kingdom*

Treatment for 2 years with oral clodronate (Bonefos® 1600mg has been shown to reduce the incidence of skeletal metastases during therapy in a double-blind placebo-controlled study of almost 1100 women with primary operable breast cancer. The mechanisms by which this is mediated are not yet clear and we therefore examined the relationship between clodronate's effects on bone turnover and the incidence of bone metastases in the same study.

BMD and biochemical markers at diagnosis were not predictive for future bone metastatic risk. Furthermore, within the placebo group, changes in BMD or PINP during the first year of treatment were similar in women who subsequently did or did not develop bone metastases (table). On the other hand, women in the clodronate group who remained free of bone metastases had highly significant decreases in bone turnover (PINP) during the first year, whereas women who developed bone metastases during or following clodronate therapy failed to show suppression of bone turnover (table).

	Placebo		Clodronate		P
Incident bone metastases	Yes	No	Yes	No	
Median changes in 1st year in:					
Spine BMD	-1.6%	-1.7%	+0.1%	+0.2%	<0.001
PINP	+13%	+4%	+19%	-29%	<0.001

We conclude that the reduced risk of bone metastases by clodronate therapy is associated with a significant decrease in bone turnover during the first year of therapy. If these biochemical observations are confirmed, it is possible that markers such as PINP will provide useful outcome measures for both clinical trials and routine monitoring of patients.

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Relationship between tumor markers CEA and CA 15-3, TNM staging, estrogen receptor rate and MIB 1 index in patients with pT1-2 breast cancer.

F. Lumachi[1], S.M.M. Basso[1], M. Ermani[2], S. Lonardi[3], A. Tosoni[3], A.A. Brandes[3]. *[1] University of Padua, Endocrine Surgery Unit, Dept of Surg & Gastroenter, 35128 Padova, Italy; [2] University of Padua, Biostatistics Unit, Dept of Neurosciences, 35128 Padova, Italy; [3] Azienda Ospedaliera, Division of Medical Oncology, 35128 Padova, Italy*

Background: Several tumor markers have been proposed to indicate presence and future behavior of breast cancer (BC). However, their sensitivity is usually considered low, especially in patients with early-stage tumors. The aim of this study was to analyze whether a correlation exists between preoperative serum tumor markers CEA and CA 15-3, age of the patients, TNM staging, hormone receptor (ER, PgR) status, and MIB1 proliferation index in patients who underwent curative surgery for primary BC.

Patients and Methods: Data regarding a series of 255 consecutive women (median age 60 years, range 30-85) with pT1-2 (pT1a=9, 3.5%; pT1b=38, 14.9%; pT1c=107, 42.0%, pT2=101, 39.6%) BC were reviewed, while patients with confirmed pT3-4 BC were excluded. The greatest diameter of the tumor measured by the pathologist (size) ranged from 3 and 48 mm (median 19 mm). There were 71 (27.8%) premenopausal and 184 (72.2%) postmenopausal women. Two groups of patients were considered according to the axillary lymph node status: Group A, 70 (27.5%) cases (pN1), and Group B, 185 (72.5%) cases (pN0). All patients underwent pre-operative CEA and CA 15-3 serum levels measurement, and the removed tissue was routinely processed for the detection of ER, PgR, and MIB1 index.

Results: CEA and CA 15-3 serum levels were above the cut-off (10 ng/mL, and 30 U/L, respectively) in 44 (17.2%) and 75 (29.0%) patients (Group A: 22.9% and 47.0%, Group B: 15.1% and 23.0%, respectively; p=NS). Size (23.9±9.0 vs. 18.2±9.3 mm), ER rate (51.3±37.7 vs. 60.4±30.6), MIB1 index (30.1±26.3 vs. 17.8±21.0), CEA (4.3±4.8 vs. 3.4±2.7 ng/mL) and CA 15-3 (26.8±16.3 vs. 18.2±15.1 U/L) serum levels were significantly different ($p<0.05$) in Groups A and B patients. Overall, a significant correlation between size of the tumor and both CEA (R=0.22, p=0.0003) and CA 15-3 (R=0.57, $p<0.0001$) and between ER rate and MIB1 index (R=-0.59, $p<0.0001$) was found. There was no relationship between age of the patients, size (R=0.08, p=0.20), and ER (R=0.13, p=0.71). Among Group A patients, a significant correlation between number of involved nodes and both CEA (R=0.24, p=0.04) and CA 15-3 (R=0.31, p=0.007) serum levels was found, but there was no relationship (p=NS) with age, ER rate and MIB1 index.

Conclusions: In patients with BC, serum markers CEA and CA 15-3 correlate exclusively with the size of the tumor. On account of their low sensitivity and in lack of relationship with others prognostic factors, preoperative CEA and CA 15-3 serum levels measurements are of little value in patients undergoing curative surgery for primary BC.

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Predictive factors for the status of non-sentinel nodes in breast cancer patients with tumor positive sentinel nodes

V. van Iterson[1], M.H.K. Leidenius[1], L.A. Krogerus[2], K.J.A. von Smitten[1]. [1] *Breast Surgery Unit, [2] Department of Pathology, Maria Hospital, Helsinki University Hospital, Helsinki, Finland*

Background: In breast cancer patients with tumor negative sentinel nodes axillary lymph node dissection is omitted and the patient spared from postoperative morbidities. However in patients with tumor positive sentinel nodes, axillary lymph node dissection is routinely performed while a majority of these patients have no tumor involvement in the non-sentinel nodes. The authors tried to identify a subgroup of patients with a tumor positive sentinel node without non-sentinel node tumor involvement.

Methods: In 135 consecutive patients with clinical stage T1-T2 node-negative breast cancer, tumor positive sentinel nodes and axillary lymph node dissection performed, the incidence of non-sentinel node involvement according to tumor and sentinel node related factors was examined.

Results: The size of the sentinel node metastasis, size of primary tumor and number of tumor positive sentinel nodes were the three factors significantly predicting the status of the non-sentinel nodes. The size of the sentinel node metastasis was the strongest predictive factor ($P<0,0001$). In a subgroup of 41 patients with a stage T1 tumor and micrometastatic involvement in the sentinel node only 2 patients (5%) had non-sentinel node involvement.

Conclusion: In patients with small primary tumors is and micrometastatic involvement of the sentinel nodes, the chance of non-sentinel node involvement is small but can not be discarded. Because the clinical relevance of micrometastases in lymph nodes is still unclear it is not advisable to omit axillary lymph node dissection even in these patients.

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Expression of estrogen receptor-β2 and β4 mRNA decreases in breast carcinogenesis

B.-W. Park[1], K. Kim[2], M. Heo[2], W. Yang[3], J. Kim[4]. [1] *Surgery, [2] Brain Korea 21 Project, [3] Pathology, [4] Internal Medicine, Yonsei University College Of Medicine, Seoul, Korea*

Background: Since the discovery of estrogen receptor-* (ER*), more than five variants have been identified. There have been many controversial reports on the role of ER* in breast carcinogenesis and cancer progression, and its prognostic implications. The role of the variant forms has not been yet identified.

Materials and Methods: Using reverse transcription polymerase chain reaction (RT-PCR), we examined the expression levels of ER± and ER* variants in 76 paired normal and cancer tissues, 6 paired normal and benign tumor tissues, and 12 metastatic lymph nodes. We compare the densities of RT-PCR products using Tina version 2.10 (Raytest, Germany). Chi-square test and independent t-test were used for the statistical analysis. Differences were considered significant with a p value of less than 5%.

Results: ER± expression was increased in 51 cancer tissues (67.1%) compared to matched normal tissues and decreased only in 9 (11.8%). On the contrary ER* expression was decreased in 42 cancers (55.3%) compared to matched normal tissues and increased only in 4 cancers (5.3%). Among ER* variants, ER*2 was predominant and expressed 100% in both normal and cancer tissues but the level of expression decreased significantly in cancers compared to paired normal tissues. ER*4 was also expressed in both normal and cancer tissues 77.6% and 73.6%, respectively and it decreased significantly in cancer tissues compared to paired normal tissue, too. ER*5 was expressed more frequently in cancer tissue (57.9%) than in normal tissue (31.7%). ER*1 expression was not significantly different between normal and cancer tissues. There was no ER*3 expression in both normal and cancer tissues.

Conclusions: Among ER* variant forms, ER*2 is predominant in both normal and cancerous mammary tissues and ER*4, ER*5, and ER*1 in descending order but ER*3 is not expressed in the mammary tissue. ER± mRNA expression significantly increases but ER* mRNA decreases in the process of breast cancer development and progression. The decrease of ER*2 and ER*4 expression is a dominant phenomenon during the breast carcinogenesis, which suggests that ER*2 and ER*4 may possess a regulatory function of mammary proliferation. Further investigations to verify the roles of ER* variants are mandatory.

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Soluble adhesion molecules and oxidative stress in patients with breast cancer

P. Tesarova[1], M. Kalousova[2], T. Zima[2], E. Helmichova[1], K. Trskova[1], L. Petruzelka[1]. [1] *Department of Oncology, [2] Department of Clinical Biochemistry, Charles University, Prague, Czech Republic*

Background: Identification of clinically useful prognostic markers and markers of activity could contribute to the improvement of therapy of patients with breast cancer, mainly to the identification of subgroups of patients in higher risk of the formation of metastases and early detection of relapses of the disease. In patients with breast cancer oxidative stress may modify membrane lipids which may then become the target of some autoantibodies. Some receptors (including EGF receptor and apo1/Fas) and adhesion molecules (standard and/or variant CD44 and P-selectin) may detach from the surface of tumor cells and increased levels of their soluble forms may be also identified in sera.

Methods: In our study serum levels of soluble EGF receptor, soluble standard and variant CD44 (CD44s and CD44v6, respectively), soluble P-selectin, soluble apo-1/Fas, advanced oxidation protein products (AOPP), advanced glycation end-products (AGEs), pregnancy associated plasma protein (PAPP-A) and IgG and IgM anticardiolipin antibodies (ACA) were studied in 76 patients (pts) with newly diagnosed, mostly non-metastatic breast cancer (3 pts in stage 0, 37 pts in stage I, 18 pts in stage IIA, 12 pts in stage IIB, 4 pts in stage III and 2 pts in stage IV) and compared with 8 age-matched healthy women.

Results: Patients with breast cancer had significantly higher serum levels of soluble standard form of CD44 (CD44s, 581.5 + 281.1, vs. 406.4 + 48.9 ng/ml, $p < 0.05$), but not soluble variant form, most common on breast cancer cells (CD44v6, 171.4 + 48.4 vs. 160.1 + 48.3 ng/ml, p = n.s.). Serum levels of soluble P-selectin (248.1 + 137.0 vs. 125.5 + 32.0 ng/ml, $p < 0.05$) and serum levels of soluble apo-1/Fas (852.9 + 159.3 vs. 541.5 + 124.5 pg/ml, $p< 0.05$) were also significantly increased in patients with breast cancer. Concerning the markers of oxidative stress patients with breast cancer had higher AOPP (93.6 + 46.8 vs. 68.5 + 23.1 umol/l, $p < 0.05$), but there was no difference in AGEs, PAPP-A and IgM and IgG ACA. We were not able to find any significant difference in serum levels of soluble EGF receptor (3.2 + 3.1 vs. 3.6 + 2.0 ng/ml, p = n.s.). None of measured parameters was able to discriminate the patients with different stages of breast cancer.

Conclusions: Patients with breast cancer (including those in early stages of the disease) may have increased serum levels of some soluble adhesion molecules (sCD44s, sP-selectin), markers of apoptosis (sapo-1/Fas) and oxidative stress (AOPP). Further follow-up should demonstrate the response of these markers to hormonal therapy/chemotherapy and putative prognostic significance of increased levels of these markers in order to improve the current possibilities to monitor the activity of the disease and to predict its