screening, reporting symptom-GP periods of 2.5 and 4 years. The median period between the first GP- and breast clinic visit was 7.0 days (95% CI 5.9−8.1) in symptomatic screened patients and 6.0 days (95% CI 4.0−8.0) in control patients.

Conclusion: Our results show that false reassurance played, at most, only a minor role in breast cancer screening.

Patient group	Symptomatic screened group	Control group	P value
Total number included	N = 32	N = 42	
Time in days between discovery of the (first) symptom and the first GP visit			
(Median, 95% CI)	7.0 (0.0−15.3)	13.5 (7.3−19.7)	0.9 [a]
(⩾30 days: n, %)	10 (31.2)	13 (31.0)	0.9 [b]
(⩾90 days: n, %)	4 (12.5)	8 (19.0)	0.4 [b]
Time in days between first GP visit and first breast clinic visit			
(Median, 95% CI)	7.0 (5.9−8.1)	6.0 (4.0−8.0)	0.9 [a]
(⩾10 days: n, %)	7 (21.9)	11 (26.2)	0.6 [b]

[a]Kaplan–Meier, [b]Chi square test.

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Development of blood based gene expression test to detect early stage breast cancer in an Indian population

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Background: We have previously reported in 3 separate studies [1−3] the potential use of gene expression profiling in peripheral blood cells for early detection of breast cancer. Recently, we presented results from a study using Scandinavian/American women and a 96 transcript-set for the classification of breast cancer with an accuracy of 82%, sensitivity of 87% and specificity of 76% [4]. ROC analysis showed an area under the curve for these studies to range from 0.80 to 0.89. The current study investigates the efficacy of the blood based test with an Indian cohort.

Methods: We have initiated a large clinical trial tot test the efficacy of a 96 transcript set for detecting breast cancer in an Indian population. The patient population includes approximately 720 subjects with or without breast cancer from various geographical locations within India, including the North, South, East and West of India. The healthy population includes women with benign lesions, and women with no mammographic findings. Recruitment for breast cancer patients includes early and late stage cancers. The standard of truth for benign and cancerous findings was histopathology or cytology. Recruitment for all cohorts is age balanced to include women below and above the age of 50 in order to obtain both pre- and post-menopausal women. All laboratory handling of blood and gene expression testing was performed in India outside of the DiaGenic laboratory. The study population will be divided into a training set and a test set for validation of diagnostic efficacy. Recruitment for this study is planned to continue until early 2008 and the latest interim data is presented.

Results: An interim analysis has been performed with 113 subjects from multiple centres. The results obtained indicate that the informative transcripts identified from Scandinavian/American women efficiently discriminate breast cancer from non-breast cancer in Indian women. The sensitivity and specificity of the test lies in the same range as that presented above for previous studies, with an area under the curve (AUC) from receiver operator curve (ROC) analysis of 0.83.

Conclusion: The interim data from 113 Indian subjects suggests that transcripts identified from a Scandinavian/American cohort are informative for discirminating breast cancer from non-breast cancer. The AUC from ROC analysis of 0.83 suggests a potential role of this test as an additional tool in the breast cancer diagnostic work-up in India.

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Internal mammary lymph drainage and sentinel node biopsy in breast cancer – a study on 1008 patients

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Background: Nowadays, axillary sentinel node (SN) biopsy is a standard procedure in the staging of breast cancer. Although the internal mammary (IM) lymph node status is a major independent prognostic factor in breast cancer patients, sampling of IM sentinel nodes (IMSNs) is not performed routinely. The aim of this study was to evaluate the relevance of IMSN biopsy as a method to improve staging and determine the likelihood of finding IM lymph node metastases in case of IM hotspots on lymphoscintigraphy.

Material and Methods: Between April 1997 and May 2006, a total of 1008 consecutive patients with clinically node-negative operable primary breast cancer were enrolled in a prospective study on SN biopsy. Both axillary and IMSNs were sampled, based on lymphoscintigraphy, intraoperative gamma probe detection and blue dye mapping, using 10 mCi (370 MBq) 99mTc-nanocolloid injected peritumorally, and 0.5–1.0 ml Patent Blue V injected intradermally.

Results: Lymphoscintigraphy showed axillary sentinel nodes in 98% (989/1008) and IMSNs in 20% of the patients (196/1008). Sampling the IM basin, as based on the results of lymphoscintigraphy, was successful in 71% of the patients (139/196) and revealed metastases in 22% (31/139). In 29% percent of the patients with positive IMSN's (9/31) no axillary metastases were found.

Conclusions: Evaluation of IMSNs improves nodal staging in breast cancer. Patients with IM hotspots on lymphoscintigraphy have a substantial risk (22%) of metastatic involvement of the IM chain. In addition, true IM node-negative patients can be spared the morbidity associated with adjuvant radiotherapy.

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The sensitivity of breast tomosynthesis compared to digital mammography in the detection of breast cancer in patients referred to an outpatient breast clinic, a prospective analysis

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Background: Mammography is the first radiological method of investigation in symptomatic patients with breast abnormalities, despite its well-known false-negative rate. Tomosynthesis is a new method to detect breast cancer. We conducted a prospective study in which we investigated the value of Tomosynthesis in a group of patients, referred to our outpatient breast cancer clinic.We compared the sensitivity of tomosynthesis alone with digital mammography alone.

Material and Methods: From 1−6–2006 until 1−6–2007, 1028 women visited our outpatient clinic. 513 participated in the study. In these patients, digital mammography and tomosynthesis were performed. The sensitivity to detect breast cancer was compared.

Results: Malignancy was diagnosed in 193 patients. In 85 of these cases, the Birads-classification was 6. The Birads score of the other 108 breasts with carcinoma is presented in the table.

Birads	Mammography	Tomosynthesis
0	0	1
1	5	6
2	2	0
3	19	11
4	40	37
5	42	53

Without further workup (ultrasound and biopsy), 6 of 108 carcinomas would have been missed using tomosynthesis alone (classified: Birads 1), 7 carcinomas using Mammography alone (Birads 1 or 2).

Two carcinomas would have been missed using both techniques combined. The addition of tomosynthesis to standard digital mammography detected five more carcinomas.

Conclusion: The addition of tomosynthesis to mammography detected five more carcinomas, but four of them (in our group of symptomatic

patients) were also found with other techniques (2 with ultrasound used in the standard clinical workup in symptomatic patient). Tomosynthesis is promising for lesion detection and lesion characterisation because of reduced superposition. In symptomatic patients the role of tomosynthesis is not yet fully established, but this technique might be useful, especially when used in combination with mammography.

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Examination of sentinel lymph node in breast cancer by the combination of computed tomography lymphography, blue dye method and fluorescence navigation

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Background: The sentinel lymph node (SLN) biopsy technique is established in the treatment of breast cancer. It is considered that the combination of scintigraphic and blue dye method is needed for SLN biopsy. Recently, computed tomography lymphography (CTLG) with iopamidol and fluorescence navigation with indocyanine green (ICG) was developed for detecting SLN in breast cancer.

We examined efficacy of CTLG and fluorescence navigation.

Method: An iopamidol was injected subareolarly in 51 patients with operable breast cancer. Within two minutes after the 15–30 sec gentle massage of the injection sites, 1.25 mm-thick cross-sectional CT images of the breast and axilla were taken. These images were reconstructed into 3D images to identify the location, size and number of the SLN. All the subjects underwent blue dye method SLNB.

SLNB using the fluorescence navigation was performed in the last 15 patients.

Fluorescence images were obtained using an ICG fluorescence high sensitivity near-infrared video camera system (PDE: Hamamatsu Photonics, Hamamatsu, Japan). When ICG was injected subareolarly, subcutaneous lymphatic vessels draining from the areola to the axilla were visible by fluorescence within a few minutes. The SLN was then dissected by CTLG and fluorescence navigation.

A backup axillary dissection was performed on 31 patients. The accuracy of the procedure was evaluated histologically.

Results: A backup axillary dissection was performed on 31 patients, the identification rate was 93.5% and the false-negative rate was 12.5% (1/8 patients). The identification rate in CTLG was 100% afterwards. Subcutaneous lymphatics and SLN were detectable by fluorescence in all patients. The presumptive region of the skin incision was the same as CTLG by the fluorescence navigation for all cases. There was the case that SLN was not stained with by blue dye method.

Conclusion: SLN navigation by ICG fluorescence imaging and CTLG are a promising technique for further clinical exploration.

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Significance of preoperative Fluorodeoxyglucose-PET for detection of axillary lymph node metastasis

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Introduction: The axillary lymph node status is still considered the single most important prognostic indicator in patients who have breast cancer. Clinical examination is generally unreliable for staging the axilla. Lack of conventional imaging techniques to determine the axillary lymph node involvement with acceptable accuracy has been the main reason for axillary lymph node dissection; however, up to 70% of patients who have stage T1 and T2 tumors have negative axillary lymph nodes. The extent, morbidity, and cost of the staging procedure of axillary lymph node dissection are often greater than those of the surgical treatment of the primary tumor. In anatomical based imaging modalities, such as computed tomography, ultrasound, and MRI, the size of a particular lymph node is of crucial importance to determine the tumor involvement. Generally, lymph node enlargement over 1 cm in diameter is the decisive criterion. In contrast, metabolic imaging with FDG-PET is suggested to provide more specific information, based on detecting increased glucose consumption of cancer tissue. This study was undertaken to evaluate the diagnostic accuracy of preoperative positron emission tomography.

Methods: A retrospective review from January of 2007 to December of 2007 was performed in all patients (n = 109) undergoing a preoperative FDG-PET. PET imaging with the radiolabeled glucose analogue (F-18 FDG) was used to visualized the primary breast tumor and metastatic lesions.

Results: In 109 patients, the sensitivity and specificity of PET for detection of axillary lymph node metastasis were 74% and 80%, respectively. Overall accuracy was 79%. In patients (n = 42) who had primary breast tumors larger than 2 cm (>stage pT1), the sensitivity increased of 100%, with corresponding specificity of 82.3%.

Conclusion: FDG-PET cannot replace the axillary dissection, not only because of the limited sensitivity, but also because the number of involved lymph nodes and extranodal extension cannot be determined. But, among patients who have larger tumors, sentinel biopsy can be avoided in those who have positive FDG-PET, in whom complete axillary lymph node dissection should be the primary procedure.

Wednesday, 16 April 2008 12:30–14:30

POSTER SESSION

Tumour biology and immunology

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Breast cancer-derived factors stimulate ERK-mediated survival of osteoclasts and limit the effectiveness of bisphosphonates in treatment of bone metastases

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Introduction: Bone metastasis is a common complication of advanced breast cancer, which causes distressing symptoms, including pathological fractures and bone pain. Osteoclasts are critical mediators of bone osteolysis induced by breast cancer. Moreover, osteoclasts further drive tumor growth by releasing IGF and TGF-β stored in the bone matrix. Osteoclast-directed therapy is thus an ideal strategy to treat breast cancer metastases to bone. Bisphosphonates are the only available anti-osteoclastic agents used in metastases but their effect is limited in the metastatic setting relative to other osteolytic conditions. We sought the explanation of osteoclast resistance to bisphosphonates and the potential avenues to inhibit osteoclasts in breast cancer metastatic bones.

Materials and Methods: Osteoclasts formed from RAW 264.7 cells under effect of the physiological osteoclastogenic mediator RANKL (50ng/ml) were treated with Alendronate for 48 hours in the presence or absence of conditioned media from the MDA-MB-231 breast cancer cell line culture. The numbers of multinucleated osteoclasts stained positive for tartrate-resistant acid phosphatase were assessed in parallel samples, and the levels of ERK1/2 phosphorylation were determined by immunoblotting. Some cultures were treated with the ERK inhibitor PD98059.

Results: Raw 264 cells develop mature osteoclasts after 5 days of treatment with RANKL. Exposure of mature osteoclasts to 10% MDA-MB-231 conditioned media significantly increased the cell count at day 7 compared to cultures that were continuously treated with RANKL treatment, indicating that soluble mediators released from breast cancer cells support osteoclast survival in vitro. ERK pathway has been previously shown to play a critical role ion osteoclast survival. We have found that exposure to soluble factors from breast cancer cells strongly induced phosphorylation of ERK1/2 in mature osteoclasts. Alendronate treatment of RANKL-treated mature osteoclasts resulted in significant and dose-dependent decrease in the osteoclasts count. In contrast, in the presence of breast cancer-derived factors, mature osteoclasts failed to respond to alendronate in the concentration range from 10^{-8} to 10^{-4} M, demonstrating that osteoclast exposed to breast cancer soluble factors are resistant to the apoptotic effect of alendronate. Inhibition of ERK pathway using PD98059 (10 μM) before treatment with Alendronate (10^{-4} M) partially restored the responsiveness of mature osteoclast to Alendronate.

Conclusion: Osteoclasts exposed to cancer-derived factor demonstrate improved survival likely counteracting pro-apoptotic effects of alendronate. Identification of the mechanism of alendronate resistance in breast metastases may pave the way to more effective breast cancer bone metastases.

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CD44+/CD24−/low cells derived from long-term cultured human breast carcinosarcoma

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The CD44+/CD24−/low cells have been recently identified as breast cancer-initiating cells. They retain tumorigenicity in vivo and display stem cell-like properties. We have obtained CD44+/CD24−/low (>90%) cells from carcinosarcoma using mammosphere culture method which was previously