imprint cytology was performed on all samples, by use of Papanikolaou stain, May Grunwald-Giemsa and Hematolylin –Eosin stain. The cytologic results were compared with those of histopathologic examination of the specimens.

**Results:** Sclerotic adenosis was the most common biopsy finding in patients with benign breast disease, while in women with malignant lesions the incidence of the above mentioned finding was decreased. On the contrary, patients with malignant breast disease had twice as atypical hyperplastic lesions as compared to those with benign breast lesions. Differential diagnosis between benign and malignant lesions by use of imprint cytology had a 99% sensitivity and 96% specificity.

**Conclusions:** Imprint cytology is a rapid diagnostic technique, which might provide the surgeon with further information concerning the cytologic profile of breast lesions.

**154** POSTER

**Breast cancer diagnosis and treatment at the regional center for breast diseases**

M. Ristov. *Medical Center, Surgery, Shtip, Macedonia*

**Aim:** to analyze the three years experience in the diagnosis and treatment of breast cancer at the Regional Center for Breast Disease (RBDC).

**Background:** The Regional Center for Breast Disease in Shtip, Macedonia, has been established in year 2000 as a unit where multidisciplinary team is assessing women with symptoms of breast disease.

**Methods:** The triple test (clinical breast examination, breast imaging and fine needle aspiration biopsy) has been applied to all cases where a breast lump or mammographically suspicious lesion was found.

**Results:** In the three year period (2000–2002) a total of 5080 clinical examinations were performed; for 3288 patients it was their first visit to a breast surgeon. Ultrasound examinations were done on 4432 women; in 1693 was also indicated. Both clinical examination and mammography in 816 patients warranted fine needle aspiration biopsy; the cytological findings confirmed the diagnosis of breast cancer in 155 cases. The final diagnosis was established within 1–7 days after the patient's visit to the RCBD, while the waiting time to surgery in 82 patients, operated on in the Surgical Unit of the Medical Center Shtip, was 3–4 days. Further treatment (radiation therapy, hemotherapy and/or hormonal therapy) continued at the Oncologic Institute in Skopje, the follow-up being organized at the RCBD.

**Conclusion:** The multidisciplinary team approach and the triple test at RBDC have enabled fast and accurate diagnosis in patients with breast cancer and have significantly reduced the waiting time to operation.

## Wednesday, 17 March 2004 16:00–17:15

**PROFFERED PAPERS**

# Molecular biology

**155** ORAL

**High quality gene expression microarray data from a multicentre prospective trial: results of the first microarray analysis in the EORTC 10994/BIG 00-01 study**

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**Introduction:** EORTC 10994/BIG 00–01 study is an intergroup prospective randomized trial of neoadjuvant chemotherapy comparing anthracyclines with taxanes in patients with either large operable or locally advanced/inflammatory breast cancer. A tumour sample (1 incisional or 2 trucut biopsies) must be snap frozen before randomization. 675 patients are already included in this trial (cut-off date 1/11/03) with the objectives of doing p53 assessment with yeast assay and gene expression microarrays. The goal of this first microarray analysis is to test the feasibility of this methodology from the first series of tumours included.

**Methods:** Frozen samples are analysed centrally at the ISREC. Frozen sections are taken for histology and samples are excluded if there is less than 20% tumour. RNA is extracted from 4×25 µm sections of the biopsy. Agilent Bioanalyzer is used to assess the quality and the yield of RNA. T7 amplification is performed with 100 ng RNA. Samples are labelled by Enzo kit and hybridised to U133A Affymetrix arrays.

**Results:** 314 tumours were analysed. 42 tumours were excluded because there were <20% tumour cells. The median RNA yield was 370 ng. 54 tumours were excluded due to bad quality or low yield of RNA (<100 ng). 218 tumours (69%) gave 100 ng or more of acceptable quality RNA. Of these we have tested 49 tumours all of which gave high quality array data, with no evidence of technical bias caused by differences in centre or RNA quality. In two cases, duplicate biopsies were tested on arrays. Hierarchical clustering shows that biopsies from the same patient cluster together. There is a near perfect correlation between ER status assessed by immunohistochemistry in each institution and ER expression level on the chip. The p53 mutant tumours are almost all in the ER negative group. The major split in the tumour dendrogram is between basal and luminal tumours. The first two components in principal component analysis (PCA) identify three groups, which correspond to basal (33%), luminal (55%) and a third group (12%) which may be a subtype of luminal cell tumour. The tumour cells thus dominate the pattern, despite the inclusion of samples with a substantial amount of normal tissue.

**Conclusion:** We have demonstrated that it is possible to obtain high quality microarray data from sections of biopsies collected in a phase III multicentre trial. Ongoing work using an additional 150 samples from patients already randomized in EORTC 10994/BIG 00–01 study will permit the identification of a gene profile which can predict complete pathological response to each treatment arm. This will allow clinicians to select the most appropriate treatment for new patients based on the gene expression profile.

**Acknowledgements:** EORTC 10994/BIG 00–01 study is an intergroup collaboration and we want to thank all the investigators from EORTC Breast Cancer Group (EORTC-BCG), Anglo-Celtic Cooperative Oncology Group (ACCOG), Swiss Group (SAKK) and Swedish Breast Cancer Group (SweBCG).

**156** ORAL

**Breast Cancer ProfileChip: from large scale gene expression profiling to oncodiagnostic device**

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One of the major obstacles to efficient clinical diagnosis and management of breast cancer stems from the significant genetic variability amongst breast cancer patients. In recent studies, microarray technology, allowing gene expression measurement of thousands of genes simultaneously, has contributed towards enhancing the understanding of the diverse molecular mechanisms driving tumorigenesis. However, results from gene expression profiling (GEP) research has yet to be directly translated to the clinical setting. In this study, we present the development of the Breast Cancer Profile*Chip* (BCPC), a device based on GEP for molecular characterization and therapeutic management of breast cancer. The BCPC contains phenotypic (ER, PR, EGFR, VEGFA, HER2/neu, and bcl-2) and prognostic (CD-31, Mib-1) gene expression signatures identified in a large scale study on 220 fully annotated tumor samples (from Institut Paoli-Calmettes, Marseille). Tumors were profiled on Ipsogen's Discovery*Chip* cDNA microarrays (containing 9000 genes), and data processing and analysis were performed using Profile*Software* Corporate (Ipsogen™). Discriminating genes were identified by classical t-test on a learning set (n=160) and leave-one-out method to retain significant genes. For each signature, validity was tested twice independently, first on an independent set (n=60) of tumors from the Institut Paoli-Calmettes (Marseille), and then on another set (n=120) from Centre Léon Bérard (Lyon). Estimated sensitivity and specificity for each identified gene signature was calculated in comparison to standard histopathological, immunohistochemical (IHC), and/or fluorescence *in-situ* hybridization (FISH) techniques. Following robust validation, signatures were transferred to the BCPC. Briefly, the BCPC is a glass-format biochip containing 1200 cDNAs. Tumor profiling from as little as 500 ng total RNA is based on a conservative linear amplification by single primer amplification, colorimetric detection, and image acquisition with a flatbed scanner. Results obtained are quantitative, sensitive, and highly reproducible (mean CV = 5%). This study presents the first example for the use of GEP in a diagnostic device that may contribute to more efficient tumor characterization and patient treatment.