fine needle aspiration as part of the triple diagnostic procedure. Non-conclusive or non-representative triple test results were reason to perform a histological core needle biopsy. Malignant lesions, non-conclusive and non-representative histology of the core needle biopsy and suspected lesions, that did not undergo one of the previous diagnostic procedures underwent an excisional biopsy. This excisional biopsy and 1 year follow up was used as standard for reference.

The performance of the diagnostic modalities were described in an inconclusive rate, sensitivity and specificity, and area under the receiver operating characteristic curve.

**Results:** From October 1999 till August 2000, 2020 patients underwent physical examination of the breasts and imaging. In 271 suspicious palpable breast lesions additional diagnostic procedures were indicated. Fine needle aspiration was performed in 241 lesions. Histological core needle biopsy was performed in 70 cases. 191 palpable breast lesions were surgically excised. See Table 1.

**Conclusion:** The diagnostic performance of the histological core needle biopsy as a less invasive diagnostic modality seems better compared to the triple diagnostic procedure, including fine needle aspiration cytology. Whether we should abandon the triple diagnostic procedure all together is uncertain. Probably there is some place for better-defined indications about the previous diagnostic results and some characteristics of the lesion about palpability.

Table 1

| | Inadequate rate | Sensitivity | Specificity | AUC-ROC |
|---|---|---|---|---|
| Fine needle aspiration cytology | 0.12 | 0.81 | 0.74 | 0.95 |
| Triple test | 0.20 | 0.85 | 0.12 | 0.76 |
| Histological core needle biopsy | 0.06 | 0.95 | 0.87 | 0.95 |
| Diagnostic excision | 0.00 | 1.00 | 1.00 | 1.00 |

**150** POSTER

**Impact of introducing a preoperative vacuum assisted biopsy on the surgical outcome of suspicious microcalcifications**

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**Purpose:** To evaluate whether a preoperative vacuum assisted biopsy (VAB) for suspicious microcalcifications reduces open biopsies for benign lesions and reduces the re-excision rate.

**Materials and methods:** Retrospectively the results of surgical procedures after preoperative localisations of 1998 and of 2001 were reviewed and compared. In 2001 VAB was introduced in the evaluation of non-palpable clusters of microcalcifications.

**Results:** In 2001, 213 lesions and in 1998, 146 lesions needed a preoperative localisation. 23% (50 of the 213 lesions) were clusters of microcalcifications in 2001 and 50% (73 of the 146 lesions) in 1998.

Only 22% (11 of the 50 clusters) showed benign histology after excision where this was 49% (36 of the 73 clusters) in 1998. Of these 11 clusters, 5 clusters were proliferative fibrocystic lesions (radial scar and atypical hyperplasia).

Of the malignant (39 of the 50) clusters of microcalcifications, 67% had a VAB prior to surgery. 5 patients needed a re-excision (4 mastectomies, and 1 axillary dissection).

Comparing with 1998, there was an important decrease in the re-excision rate (from 22% to 12%), where re-excision was more frequent when no biopsy was done preoperatively.

**Conclusion:** The introduction of VAB reduces the number of surgical procedures for benign cluster of microcalcifications and facilitates one-step surgery.

**151** POSTER

**Review of lymphomas diagnosed in a Breast Unit**

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**Background:** A pathological result of lymphoma is almost always an unexpected result in a Breast Unit. Our purpose is to study the clinical aspects of lymphomas diagnosed in our Breast Unit.

**Material and methods**: We have included 2651 patients who had a diagnosis of malignancy in our Breast Unit between January 1995 and July 2003 and who were included in the Unit computer database. We have found patients who have had a pathological result of lymphoma and we have performed a retrospective study using their case-histories.

**Results**: Ten patients had a pathological result of lymphoma and represent 0.38% of cases with a malignant diagnosis. Nine patients consulted us because of clinical or radiographic breast abnormalities, with or without axillary adenopathies, and one patient consulted because of axillary nodes without breast pathology. The ages range from 22 to 87.

Three cases were primary breast lymphomas (B-cells) and one more (T-cells), who was diagnosed in terminal stage, probably also was one of former (in total, 0.15% of total malignant breast pathology). Two of them had antibodies against hepatitis C virus and one against HIV. A fifth case with a lymphoma localised in the breast had a mammary recurrence of a B-cell lymphoma treated in another centre. The mammographic findings in these cases varied from benign features to a high suspicion of malignity. The diagnosis was performed by a surgical biopsy in one case, by a core biopsy in three cases and a punch in the last one. There was no need of axillary biopsy.

The other five cases were extramammary lymphomas: four B-cell lymphomas with mammography with benign findings, and one Hodgkin's disease with a clinical presentation and radiographic features compatible with an inflammatory breast cancer, having breast swelling without a dominant mass and axillary and supraclavicular adenopathies. Four patients had a negative breast biopsy and we needed a surgical axillary biopsy in three cases.

**Conclusions:** Primary breast lymphoma is an uncommon lesion and can present diverse mammographic images, from benign features to high-suspicion lesions. We must not forget the possibility of an extramammary lymphoma when the patient has axillary adenopathies without evidence of a breast lesion. We do not always need an open biopsy to make the diagnosis. We must be aware of the possibility of a lymphoma in patients with antibodies against HIV and to perform an accurate control and follow-up.

**152** POSTER

**How often unsuspected cytologically nipple discharge is a symptom of underlying breast cancer**

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**Background:** Nipple discharge is can be caused by benign diseases of the breast, but also may by a symptom of an underlying breast cancer. Fear that nipple discharge might be a symptom of underlying cancer is the main issue motivating patients and their physicians to treat this disorder surgically.

**Objective:** To assess the rate of false negative results of cytologic examination of nipple discharge in patients qualified to surgical treatment.

**Material and methods:** From 1977 to 2002, 414 women were operated on for nipple discharge in our Department. The study group was composed of 234 women, in whom no palpable tumor was identified on palpation, no cancer or suspected cells were identified on cytologic examination of the nipple discharge. In 177 of them discharge was unilateral and in 57 was bilateral. Altogether 291 occurrences were analysed. We evaluated the incidence of cancer diagnosed on pathological examination of the excised breast tissue in these patients.

**Results:** Breast cancer was diagnosed in 4 cases. Therefore the results of cytologic examination of nipple discharge were false-negative in 1.4% of cases (4/291). In all these cases the character of nipple discharge was described as bloody.

**Conclusions:** The rate of false-negative results of cytologic examination of nipple discharge is very low. Therefore there is no necessity to treat surgically all such patients in order to verify the possibility that the discharge is caused by underlying cancer. Further diagnostic work-up should be undertaken in such patients.

**153** POSTER

**The contribution of intraoperative cytology in the diagnosis of hyperplastic lesions of the breast**

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**Background:** To report the incidence of the hyperplastic lesions in benign and malignant breast tumors, as well as to evaluate the contribution of the intraoperative imprint cytology in the diagnosis of hyperplastic breast lesions.

**Material and Methods:** 486 biopsy specimens from breast cancer patients who underwent surgical treatment were evaluated. Intraoperative

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.