Tissue microarrays (TMAs) were prepared from 1000 lesions and immunophenotyped for the expression of luminal (CK7/8,Ck18,CK19) and basal markers (CK5/6,CK14,Vimentin,SMA), ER-α and -β, Her2-neu, MIB-1, Cyclin D1, P53, Bcl-2, E-Cadherin and FHIT.

Results: *TC-pure*: 96% association with CCLs, the majority showing columnar cell hyperplasia with atypia. DCIS was present in 91% cases. Co-localization of CCL, DCIS and TC occurred in 83% patients, all displaying the same cytoplasm-nuclear morphology. LN was seen in 15%.

TC-mixed: Co-existence of CCL, DCIS and TC was seen in 80%. LN occurred in 60% patients.

ILC: 91% cases showed LN. CCL and DCIS were seen in 52% and 41% cases, respectively.

Immunohistochemistry: All TC, ILC and luminal cells of TDLUs, DCIS and LN expressed luminal markers with absence of basal markers. The majority of TC, ILC, TDLUs, CCLs, DCIS and LN were positive for estrogen receptor. TDLUs, CCLs, DCIS, LN, TC and ILC were negative for P53; however P53 was detected in DCIS and invasive tumour. HER-2 was over-expressed only in CCLs, LN and DCIS. TDLUs, CCLs, and low grade DCIS were positive for E-cadherin. In contrast, E-cadherin staining was reduced in TC but absent in LN and ILC. MIB-1 was expressed in >10% of cells comprising DCIS and invasive tumours. Bcl-2 and FHIT were positive in TDLUs, CCLs, DCIS and LN, but were reduced in TC and ILC. The proportion of Cyclin $D1^+$ cells increased progressively from CCLs to DCIS to invasive lesions.

Conclusion: Our findings support the hypothesis that CCLs are associated with pure and mixed forms of tubular carcinoma, and that LN is involved in ILC development. Immunoprofiling suggests that TC, ILC, low grade DCIS, LN, and CCLs belong to a family of luminal low grade breast tumours. Invasive lesions could be distinguished from precursor lesions by decreased Bcl-2 and FHIT staining and their increased expression of Cyclin D1.

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Distinguishing blood and lymph vessel invasion in breast cancer: a prospective study in 95 patients

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Introduction: Blood (BVI) and lymph vessel invasion (LVI) are the histological correlates of the first steps of haematogenous and lymphatic metastasis in solid tumors. New lymphatic endothelium specific markers such as D2−40, make it possible to distinguish blood and lymph vessels. Therefore, the aim of this prospective study was to quantify and compare BVI and LVI in a consecutive series of breast cancer patients.

Materials and Methods: Three consecutive 5 µm sections of all FFPE tissue blocks of 95 consecutive breast cancer resection specimens were (immuno)histochemically stained in a fixed order: HE, anti-CD34 (pan-endothelium) and anti-D2−40 (lymphatic endothelium) antibodies. On every slide, all vessels with vascular invasion were marked and relocated on the corresponding slides. Based on the IHC staining pattern, vascular invasion was assessed as being LVI (CD34?or?/D2−40?) or BVI (CD34+/D2−40?).

LVI and BVI were assessed as intra- (in contact with tumor cells or desmoplastic stroma) or peritumoral. The number of intra- and peritumoral vessels with LVI and BVI per patient was counted as well as the number of tumor cells in every vessel. Results were correlated with clinico-pathological variables, the growth pattern and the presence of a fibrotic focus.

Results: In total 3297 (661 intra, 2636 peri) vessels with LVI and 135 (80 intra, 63 peri) vessels with BVI were seen. The median number of FFPE blocks per patient was 4. 66 (69.5%) patients had LVI (8 intratumoral, 35 peritumoral, 23 intra- and peritumoral) compared to 36 (37.9%) patients with BVI (12 intra-, 8 peri- and 16 intra- and peritumoral). Although LVI and BVI were associated intratumorally ($p = 0.02$), only LVI, not BVI correlated with the presence of LN metastases (p intra = 0.07, p peri = 0.002). Both BVI and LVI were associated with the presence of a fibrotic focus and with an expansive growth pattern. Furthermore, LVI was more extensive ($p = 0.001$) then BVI, and lymphatic emboli were bigger then blood vessel emboli ($p = 0.004$).

Conclusion: Our data demonstrate that it is possible to reliably distinguish BVI and LVI in breast cancer resection specimens using recently characterized specific lymphatic endothelium markers. This is important to study the contribution of both processes to the metastatic process in breast cancer. Furthermore, our data sustain the hypothesis that haematogenous and lymphatic metastasis are specific and biologically different pathways.

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Comparative study of histo-pathological characteristics of breast cancer in women who underwent in vitro fertilization and age matched controls

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Introduction: There has been concern that elevated levels of sex hormones during in vitro fertilization (IVF) may influence future development of breast cancer. Several studies have found an increased risk of breast cancer after IVF, at least in some sub groups. In this study we examine histopathological characteristics of breast cancer in women who underwent IVF, compared with age-matched unexposed cases.

Description: We identified 7162 women who underwent IVF at our institution between 1984 and 2002. These were linked with the National Cancer Registry, and 38 women who developed breast cancer after IVF were identified. Four age-matched unexposed cases for each case were obtained from the institutional oncology database.

Summary of Results: The average age at time of breast cancer diagnosis for women who underwent IVF was 44 years. Patients developing breast cancer after IVF were more likely to have node negative disease: 61 vs. 49%. They were also more likely to have grade 3 tumors: 65 vs. 47%. Despite of the high percentage of high grade tumors, these tumors were more likely to be ER positive (88% vs. 67%) and PgR positive (75 vs. 40%). There was no difference in tumor size distribution: 42% of cases and 43% of controls bearing tumors smaller than 2 cm, 48% and 44% with tumors 2−5 cm, and 10% and 13% larger than 5 cm. The stage distribution was similar (28% and 29% stage 1, 55% and 58% stage 2, 14% and 12% stage 3, 3% and 1% stage 4). The rate of Her2 positive tumors was equal (32 and 33%). The histological types in both groups were similar, with 11% and 7% presenting with DCIS, 79% and 81% with invasive duct carcinoma, and 11% and 9% invasive lobular carcinoma. The rate of breast conserving surgery was similar: 48% and 45%.

Conclusions: breast cancer after IVF was diagnosed at an age significantly younger than the average age for breast cancer diagnosis, perhaps suggesting a promoter effect. The tumors which develop in these patients are more likely to be of high histological grade, but are also more likely to be ER and PgR positive, and node negative. Further study is needed to determine the influence on prognosis.

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Human epidermal growth factor receptor 1 (EGFR) expression was not associated with gene amplification but intimately associated with HER2 gene amplification and protein expression in tissue microarray of clinical breast cancers

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Background: Introduction of anti-epidermal growth factor receptor 1 (EGFR) biologic therapeutics for numerous human malignant diseases mandates the appropriate understanding on the biologic properties of EGFR. We performed the current study to investigate the frequency and clinical implication of EGFR gene amplification and protein expression in breast cancer.

Methods and Results: EGFR gene amplification was assayed by fluorescence in situ hybridization (FISH) and protein expression was assayed by immunohistochemistry (IHC) on tissue microarray (TMA) of 165 non-selected invasive breast cancer. The EGFR was expressed in 34 (20.6%) of 165 studied invasive breast cancers, whereas EGFR gene was amplified in 13 (7.9%). The EGFR protein was expressed in 5 (38.5%) of 13 EGFR amplified tumors, whereas it was expressed in 29 (19.1%) of 152 EGFR non-amplified tumors. The EGFR protein expression was increased in EGFR amplified tumors but the difference was not statistically significant. EGFR protein was expressed in 33.3% of HER2 amplified tumors whereas it was expressed in only 16.3% of HER2 non-amplified tumors. EGFR expression was significantly increased in HER2 amplified breast cancer. The finding was similar when EGFR expression was analyzed according to HER2 protein expression. During the median follow-up period of 56 months

(range: 13–80 months), 41 patients (24.8%) had recurrent disease and 26 patients (15.7%) died due to recurrent breast cancer. EGFR expression was a significant prognostic factor for the disease free and overall survival of the patients together with lymph node metastasis and Ki67 labeling index in univariate survival analysis but lymph node metastasis was an only significant prognostic factor in multivariate analysis.

Conclusions: EGFR expression was independent of EGFR gene amplification and was intimately associated with HER2 amplification and overexpression. Low frequency of EGFR gene amplification hampers its clinical utility as a tool to identify proper patient population for the specific treatment. In contrast, EGFR protein expression seems to have a role as a useful predictive factor if it is rationally integrated with other biologic predictive factors.

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Prevalence of breast cancer-susceptible mutations in women < 36 years with invasive breast cancer and correlation with histopathology features of the primary cancer

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The presence of breast cancer-susceptible genetic mutations BRCA1 and 2 is associated with an increased incidence of early-onset breast cancer. Certain histopathological features (higher grade, hormone receptor negativity, medullary or tubulo-lobular type, 'pushing edge' margins and lymphocytic infiltration) are more commonly seen in BRCA-associated cancers. This prospective study assessed whether the presence of these histopathological features was predictive of a BRCA mutation, irrespective of family history of breast or ovarian cancer.

Method: Consecutive patients with breast cancer diagnosed <36y in Perth, Australia were included. Demographic data including validated family history were obtained. Primary breast pathology was reviewed by a single pathologist. Assessment for BRCA mutations were performed by protein truncation test (PTT), denaturing high pressure liquid chromatography (D-HPLC) and multiplex amplifiable probe hybridisation (MAPH). Patients were interviewed by a clinical psychologist before and after receipt of genetic testing results to assess for anxiety and depression.

Results: From November 2002 to August 2004, 47 women aged <36y at breast cancer diagnosis consented to enter the study. Risk factors for breast cancer included Nulliparity 6(12.8%), First full term pregnancy over age 30y 6 (12.8%), 1 relative with breast cancer 20 (42.6%), 2 or more relatives with breast cancer 7 (14.9%). Three patients had bilateral breast cancer. Complete histopathological review is available in 38 patients thus far. Breast cancer pathology was invasive ductal in 35 (92.1%), mixed ductal-lobular 2(5.3%) and medullary 1 (2.6%). The grade was 1, 2 and 3 in 10.8%, 27% and 62.2% respectively. Pushing margins seen in 15 (44.1%). Peritumoral lymphocytic infiltration in 24 (70.6%). ER negative 48.6%, PR negative 50% and Her2 neu 3+ 10.3%. Genetic testing result is currently available for 43 pts. A breast cancer-susceptible genetic mutation was identified in 6 pts (13.7%); 5 (11.4%) BRCA1 and 1 (2.3%) germ-line p53.

Conclusion: The presence of a breast cancer-susceptible genetic mutation in this cohort of early-onset breast cancer was much greater than the anticipated 5% rate in the general population of breast cancer patients. Correlation of the presence of a mutation with the histopathological features of the primary breast cancer for all patients will be presented. Impact of genetic testing on levels of anxiety and depression will be reported.

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Comparative study of immunohistochemical phenotype in primary breast cancer tissues and lymph node metastases

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Background: Nowadays, in breast cancer patients the immunohistochemical profile of primary cancer tissue is important to determine tumor prognosis and particular treatment strategies. However, potential changes in heterogeneity of tumor cells leads us to consider a hypothesis that immunohistochemical expression of these proteins is permanently modifying during cancer development and spread.

Methods: Immunohistochemical examination with monoclonal antibodies against Ki-67, p53, ER and PgR was performed in 98 lymph node-positive invasive breast carcinomas. Both primary tumor tissues and positive lymph nodes were studied.

Results: 14 primary tumors were ER+PgR+, 38 were ER-PgR-, 17 were ER+PgR-, and 29 were ER-PgR+. Lymph node metastases were ER+PgR+ in 9 cases out of 14 ER+PgR+ patients, ER-PgR- in 31 cases out of 38 ER-PgR- patients, ER+PgR- in 11 cases out of 17 ER-PgR- patients, and ER-PgR+ in 22 cases out of 29 ER-PgR- patients. In primary tumor tissues, p53 was positive in 2 ER+PgR+ cases, 35 ER-PgR- cases, 13 ER+PgR- cases, and 27 ER-PgR+ cases. In lymph node metastases, p53 was positive in 1 out of 2 p53+ and ER+PgR+ cases, 28 out of 35 ER-PgR- cases, 9 out of 13 ER+PgR- cases, and 24 out of 27 ER-PgR+ cases. The proliferation index measured by Ki-67 expression in tumor cells was significantly higher in positive lymph nodes than in the primary tumor (32.6% vs 20.5%).

Conclusion: These results suggest the modification of immunohistochemical expression of Ki-67, p53, ER and PgR between primary tumor tissues and lymph node metastases. It seems that metastatic tumor cells show a higher proliferation activity and perhaps aggressiveness in comparison with the primary cancer cells. These differences in proliferation activity might be taken into account when considering the choice of the adjuvant therapy.

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Atypical Ductular Hyperplasia (ADH); review of 174 cases diagnosed in a series of 1295 macrobiopsies in a single institution

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The diagnosis of ADH on a biopsy is often difficult; the final diagnosis may be different from the one obtained after biopsy. The aim of this study was 1- to compare the diagnosis on the biopsy and after surgery, 2- to evaluate the inter individual reproducibility of the pathological diagnosis.

Patients and Methods: between February 2000 and October 2003, 1295 macrobiopsies have been performed in the centre Oscar Lambret. A diagnosis of ADH according to Page and Tavassoli criteria has been done in 174 patients (13.4%). An evaluable tumorectomy has been performed in 68 cases; the reason for no available surgical specimen in 106 cases was either tumorectomy outside of our center or patient's refusal. A total inclusion of the surgical specimen was performed in 59 cases; in the 9 other cases it was not specified in the pathological reports.

Results: The macrobiopsy scar was found in 55 cases. In 19 patients (26%), the final diagnosis was worse: ductal carcinoma in situ (DCIS) (18), (low grade: 8, intermediate: 7, high grade: 3), and one invasive carcinoma. In 30 patients (44%), preneoplasic lesions were found: ADH (24), lobular neoplasia (LN) (4), flat epithelial atypia (2). In 19 patients (28%), there was only either an usual ductal hyperplasia (UDH) (6), or a fibrocystic dystrophy (13). All the macrobiopsies have been reviewed by two pathologists: 13 out of the 19 cases for whom the final diagnosis was worse have been considered as DCSI (low grade: 6, intermediate: 7); the 6 other cases were considered as initially as ADH. Out of 19 biopsies in which either UDH or fibrocystic dysplasia had been diagnosed on the surgical specimen, after review 6 were considered as UDH. In 4 cases in which the final diagnosis was LN, the retrospective review was ADH. Overall, the diagnosis was truely underevaluated in 7 pts among 72 (10%).

Conclusions: The pathological diagnosis at biopsy is limited by the heterogeneity of the lesions and of the specimens; the interindividual reproducibility has to be further improved; the diagnosis on biopsy specimens should be performed only by trained pathologists.

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Prognostic value of Mib1 in a tissue microarray of 855 invasive breast carcinomas

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Introduction: Grade and mitotic count are important prognostic factors in breast cancer but may be difficult to appreciate in microbiopsy samples. Mib1 may be an alternative to measure proliferation in this setting.

Design: A tissue Microarray (TMA) comprising four 0.6 mm diameter tissue cores of 855 consecutive invasive ductal carcinomas operated on between 01/01/1989 and 12/31/1992 was constructed. Immunohistochemistry for Estrogen Receptor (ER)(1D5), Progesterone Receptor (PR)(PGR636), Her2neu (DA485) and Mib1 was performed. A cut-off of 10% positive tumour cells was chosen for ER and PR. The Herceptest scoring system was used for Her2neu. For Mib1 a cut-off of 20% positive tumour cells corresponding to the 75th percentile in the series was chosen. The prognostic value (probability of metastasis) of these factors as well as patient's age, tumour size, axillary lymph node status (N status), modified Scarff Bloom and Richardson (SBR) grade and peritumoral vascular emboli