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