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ER AND PgR IN BREAST CANCER, 1993, MORE THAN A QUESTION OF POSITIVITY VERSUS NEGATIVITY

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Outcome of breast concer is now widely recognized as being associated with the content of steroid hormone receptors in the primary tumor issue.

The estrogen receptor (ER) is the only receptor that is considered in most settings; moreover, in nearly all cases receptors are considered in a bivariate manner. Meanwhile, biochemical assay methods (EIA and LBA) yield quantitative data demonstrating a wide spectrum of receptor concentrations in tumor tissues. Semi-quantitative data can be harvested using immunohistochemical methods.

Because large numbers of patients are enrolled in the Danish Breast Cancer Cooperative Group (DBCG) trials for treatment of primary breast cancer it is possible to evaluate the potential significance of implementing receptor data in a more nuanced manner.

Data will be presented evaluating clinically optimal cutoff points for receptor concentrations among more than 3,000 patients enrolled in the DBCG project. Some of these patients receive while others do not receive adjuvant treatment following primary surgery. The significance of progesterone receptor (PgR) concentrations is evaluated in relation to ER concentrations. Both receptors are evaluated in multivariate analysis together with the traditional histopathological and morphological prognostic factors (grade of anaplasia, lymph node involvement, tumor size).

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CATHEPSIN D AS A MARKER OF BREAST CANCER METASTASIS

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Molecular markers to predict relapse and metastasis in primary breast cancer are important both to classify tumors to orientate towards adjuvant therapy and to understand mechanisms of tumor progression. At least three types of independant markers available to clinicians are useful to assay primary tumors in order to obtain informations on (i) hormone responsiveness and degree of differentiation, (ii) degree of cell proliferation and (iii) invasive and metastatic potential of tumor cells.

Cathepsin D is one of the first proteases providing prognostic information on breast cancer. Its immunometric assay in the cytosol, routinely used for hormone receptor assays, is available, reproducible and easy to perform. Several retrospective studies have indicated that concentrations higher than the median value are of bad prognostic significance. The validity of semi-quantitative immunohistochemistry in small tumors will be discussed, in addition to our recent data on the role of cathepsin D overexpression in facilitating metastasis.

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PROGNOSTIC VALUE OF UROKINASE-TYPE PLASMINOGEN ACTIVATOR (UPA) AND ITS INHIBITOR PAI-1 IN BREAST CANCER.

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Serine proteases such as the urokinase-type plasminogen activator uPA and its activation product plasmin, metalloproteases, cysteine proteases (cathepsins B and L) and the aspartic protease cathepsin D promote the dissolution of tumor stroma and basement membrane components in solid cancer. By this action, tumor spread and metastasis is facilitated. Proteolysis is modified by the respective inhibitors PAI-1/2, TIMP-1/2, cystatins and stefins. We determined uPA, PAI-1 and cathepsin D by immunoassays in tumor tissue extracts of 316 breast cancer patients (median follow-up: 40 months) and found that patients with either high uPA (>3.5 ng / mg protein) or high PAI-1 (>13.7 ng / mg protein) in their primary tumors experienced an increased risk of relapse and death. Cathepsin D was of no prognostic value. Multivariate analysis revealed that PAI-1 was the strongest independent factor regarding prognosis even surpassing lymph node status. We also investigated by ELISA the metalloproteases MMP-8 and MMP-9, the metalloprotease inhibitor TIMP-1 and the cysteine protease cathepsin B in a group of 149 breast cancer patients. MMP-8 (p=0.0257) and TIMP-1 (p=0.043) were of prognostic relevance (univariate analysis). MPP-9 (p=0.0907) and cathepsin B (p=0.015) were less significant. Multivariate analysis, however, revealed that only uPA and PAI-1 remained statistically significant variables. These results implicate a key role of the proteolytic factors uPA and PAI-1 in breast cancer.

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MUCIN LIKE PROTEIN IN BREAST CANCER

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Polymorphic high molecular weight glycoproteins are abundantly expressed in human breast carcinomas. These proteins designated MUC1 (also referred to as H23Ag, PEM, EMA, episialin, etc.) are heavily glycosylated with O-glycosidic linked carbohydrate side chains and, as such, have mucin-like characteristics. Increased MUC1 expression may reflect a change in the differentiation status of the malignant epithelial cells. High levels of MUC1 expression are also observed in lactating mammary epithelial tissue, where it is localized to the apical surface. Molecular studies, including cDNA and gene cloning, have shown that one of the major MUC1 gene products contains a transmembrane domain that anchors the protein in the cell membrane, and bisects it into a large heavily glycosylated extracellular domain that contains a polymorphic tandem 20 amino acid repeat array and a smaller 69 amino acid cytoplasmic domain. The MUC1 protein can also be detected in the body fluids of breast cancer patients indicating the existence of a secreted MUC1 form. The serum levels of MUC1 correlate with extent of disease, and may be used for evaluation of disease progression. Insights into the function of MUC1/H23 have been obtained from analyzing MUC1 transfectants, that showed reduced cell-cell as well as cell-substrate adhesiveness. Due to the loss of cellular architecture in breast cancer tissue, MUC1/H23 is no longer expressed solely on the apical surface and this, in conjunction with the finding that MUC1/H23 expression reduces cell-cell adhesiveness, and may enhance the invasiveness of the breast cancer cell.

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PREDICTING POTENTIAL OF c-erbB-2 ONCOPROTEIN AND LAMININ RECEPTOR EXPRESSION ON PRIMARY BREAST CANCER

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Two biological markers of breast cancer have been evaluated by immunohistochemistry in paraffin sections by avidin-biotin indirect immunoperoxidase assay and their predicting potential compared to that of lymph node status: the c-erbB-2 (neu) oncogene product and the monomeric laminin receptor (LR). The study included a series of 1117 primary tumors from breast cancer patients, surgically treated in our Institute from January 1968 to December 1971, with 20 years follow up and without any systemic adjuvant therapy. Among them, 679 patients showed no clinical lymph node involvement. Pathologically, 494 of the patients were found to be N-. The univariate analysis showed that lymph node status, neu overexpression and LR expression all significantly affected the patients' survival. The multivariate analysis showed that the 3 parameters were independent prognostic factors. Among the 1117 primary tumors 101 (9%) expressed both neu and LR, 468 (42%) were double negative, 395 (35%) were LR+ and neu- and 153 (14%) LR- and neu+. Patients with double positive tumors had a significantly poorer survival in comparison to patients with double negative ones. The survival of patients according to neu and LR was also evaluated in the 494 N- patients. In these patients, in absence of LR expression, the survivals of neu+ and neu- patients were superimposable, whereas when the tumors were LR+ the overexpression of neu was associated with a poorer prognosis. The predicting potential of the pathological assessment of the lymph node status versus the evaluation of the two markers on primary tumors was compared in the group of 679 patients with clinically uninvolved lymph nodes. The survival of patients with double negative tumors was analogous to the survival of N- patients, whereas the survival of patients with double positive tumors was even worse than that of N+ patients. Furthermore, the evaluation of the survival according to marker expression and lymph node status allowed the identification of subsets of N+ and N- patients with identical prognosis depending on neu and LR expression. In conclusion, by evaluation of the two markers in the series of 1117 breast cancer examined, subgroups with a different disease aggressiveness among each of the two conventional N- and N+ groups were identified, such as N- patients (those positive for both neu and LR) with a prognosis analogous to that of N+ patients (those negative for both markers) as well as N+ patients (those positive for both markers) with a very severe prognosis.

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NEW PROGNOSTIC FACTORS BY IMMUNO-HISTOCHEMISTRY