Significance of immunohistochemical markers in Breast cancer

Nilima S. Desai
Gujarat Cancer and Research Institute, India

Background: The incidence of breast cancer at The Gujarat Cancer & Research Institute, Ahmedabad, India is approx. 23.76% according to hospital base registry 1997 and it is rising and a major health problem affecting a large number of women. Among women with early-stage breast cancer treated with lumpectomy and local radiotherapy, 10% to 20% will experience local recurrence and 30% to 40% will develop distant metastatic disease, which is often fatal. So, a need exists for identifying prognostic markers that accurately predict long term outcome in those patients, thus permitting rational choices among therapeutic options such as adjuvant chemotherapy, surgical resection, radiation therapy, hormonal therapy and application of newly discovered experimental therapeutics. Predictive biomarkers are greatly needed that can help guide clinicians and patients in treatment related decisions about the necessity (or lack thereof) for adjuvant chemotherapy, hormonal therapy and new treatments as they become available.

Aim: The aim of the present study was to investigate the prognostic significance of ER, PR, Her2 Neu, Bcl2, BAG1, P53, Ki67, Topoisomerase II alpha, FOXA1 and GATA-3 binding protein, MDM2 protein, Cyclin E and basal subtype marker Cytokeratin 5/6 in primary breast carcinomas and further compared with Clinicopathological parameters.

Methods and Materials: Paraffin blocks were obtained from the Pathology Department of our Institute. Study conducted during the period 1997-2016. Routinely morphological diagnosis done by our histopathologist on H&E sections was noted. The clinical information regarding age, menopausal status, tumor size, lymph node status, stage, histological type and grade and treatment offered were obtained from the case files maintained at medical record department. Disease staging was done according to TNM Classification of malignant tumors, T (tumor), N (nodal) & M (distant metastasis) Staging system of (AJCC) American Joint Committee on Cancer, Staging Manual 6th edition, New York, 2002, Springer). The treatment of these patients was decided by the clinicians. The patients were treated with surgery followed by (CT) chemotherapy (CMF) cyclophosphamide, methotraxate, 5-florouracil, or (FAC) 5 fluorouracil, doxorubicin, & cyclophosphamide, & (AC) doxorubicin, cyclophosphamide and/or radiotherapy (RT) and/or hormonal therapy (HT) (Tamoxifen). Majority of the (93%) patients treated with surgery followed by CT and RT.

Immunohistochemical Localisation: In brief 3 Xm thin paraffin sections were cut and after treating with milk for 5 mins heated at 60XC overnight. Slides were then stained in automatic Immunostainer Ventana Bench Mark XT Tm Machine where deparaffinization, the endogenous peroxidase activity, Bluing and DAB are done automatically according to the protocol already feed. Sections were then washed and mounted in DPX outside. Known positive controls for each of these antibodies as well as negative controls (ie. sections in which the primary antibody were substituted by non-immune mouse serum were also stained in each run. Using

Ventana BenchMark XT automated Immunostainer Estrogen receptor (ER), Progesterone receptor (PR), Her2 neu, CK5/6 were evaluated. Bcl2 Novocastra , UK NCL-bcl-2 1:40, P53 Dakocytomation CA USA DO-7 1:25, BAG-1 Dakocytomation, CA, USA KS-6C8 1:100,Topoisomerase IIalpha Dakocytomation, CA, USA SWT3D1 1:50, Ki67 Antigen DAKO, Glostrup, Denmark MIB-1 1:25, CerbB2 Novocastra CB11 1:30, MDM2, Cycline E were evaluated. ER procured from (Dako, 1:100), PR (Dako, 1:50), Her2Neu, Mdm2 from Lab Vision Corporation, Neomarkers, USA used in 1:50 giving Nuclear staining, Cycline E from Thermo Scientific Lab Vision in dilution 1:10 showing Cytoplasmic staining. Biogenex, 1:30) and CK5/6 (1: 10) (Ab 2 Clone B4) Thermo scientific Neomarker, UK.

Scoring interpretation: The semi quantitative scoring was done as follows: tumors with marker staining in <10% cells considered negative, staining in 10-20 % cells considered 1+ and staining in 20-50% cells considered 2+ and staining in >50% cells considered 3+.

Statistical Analysis: The data were statistically analyzed using SPSS statistical software version 13. The two - tailed chi-square test was used to assess the association between two parameters. Correlation between two parameters was calculated using Spearman's correlation coefficient (r) method. Overall Survival (OS) were evaluated using the Kaplan-Meier method. The log rank test was used to assess the prognostic significance of OS. P values less than 0.05 were considered significant.

Results: Our earlier study reveals Patients who subsequently developed metastases had significantly high MV counts than patients without metastatic disease ($p < 0.001$). Patients who subsequently died of the disease had significantly high mean microvessels counts than patients who remained alive at the end of 5 years ($p < 0.001$). As density of factor VIII antigen staining increased the survival decreased ($p < 0.001$). All the patients having > 25 MV per 200x field had tumour recurrence faster as compared with patients having < 25 MV ($p < 0.02$). MV count correlates with the prediction for metastasis and poor survival. Such an indicator would be useful in selection of a subgroup of patients with breast cancer who are at high risk for having occult metastasis at presentation and subsequently would benefit from aggressive therapy.

Protein expression of Bcl2, BAG-1 and P53 were studied by immunohistochemical localisation on paraffin embedded tumour tissue sections as well as by molecular methods of breast cancer patients. Protein expression of Bcl2, BAG-1 and P53 was noted in 57%, 68% and 61% in tumours of breast tumours (carcinoma patients), respectively. Further, a significant decrease in Bcl2 expression was noted with increase in tumour size, disease stage and tumour grade in younger patients. In univariate survival analysis, Bcl2 overexpression significantly associated with better overall survival in lymph node negative patients. Moreover, in trivariate analysis patients with three marker positivity had reduced overall survival as compared to patients with one or two marker positivity followed by negative subgroup.

Molecular study reveals When markers were inter correlated a trend of positive correlation was observed between p53 exon 5 &7 (P=0.069) while no such correlation was observed between Bcl2 gene with Exon 5&7.

Expression of exon 5 was found to be higher in patients expressing p53 (92%) protein also a trend of Inverse correlation was observed between Exon 5 and PR (P = 0.06). Within LN status subgroup patients, a significant positive correlation was noted between LN positive patients and p53 exon 5 and 7expression (P=0.027).A trend of positive correlation was also observed with Bcl2 gene and protein within Older age group patients (>48 years; P=0.068). No such correlation was observed with in LN status subgroup patients.

In other study, sixty-two patients of primary breast carcinoma were enrolled. Out of total patients, ninety two percent tumors were invasive ductal carcinoma, 2% Medullary and Metaplastic carcinoma respectively and 5% tumors had Lobular subtype. Nodal involvement was observed in 35% patients and 31% having histological grade III. ER and PR positivity was observed in 68% and 81% of the tumors respectively.37% Her2/neu positivity was noted.

We studied the expression of FOX1 and GATA-3 binding protein FOXA1expression was documented as follows; positive 1+ (16%), 2+ (26%) and 3+ (55%). While GATA-3 was detected in 21% and CK5/6 expression was observed in 29% of the total sections. We observed that FOXA1 expression was inversely associated with Age, LN stage and grade while GATA-3 expression showed inverse association with lymph node and Her2 over expression. Significant correlation was observed between basal marker CK5/6 and grade (P<0.02). FOXA1 was positively correlated with Her2 overexpression (P<0.001). FOXA1 was found to be associated with reduced overall survival (P<0.032).

Protein expression of MDM2, Cyclin E and Basal sub type marker Cytokeratin5/6 was observed in 79%, 18%, and 29% respectively. Fifty three percent patients were in early stage, 45% patients had age >51years, 92% patients had IDC and 31% patients had HG III tumors and 35% patients had LN status negative. Among them, 68% patients had ER positive tumors, 81% patients had PR positive tumors and 37% patients had 3+ Her2Neu positivity. A significant inverse correlation was noted between LN status (p=0.055) and MDM2. A trend of inverse correlation was also noted with established clinicopathological markers ER, PR and Her2 Neu. A significant positive correlation was noted between Ck5/6 and grade (P=0.020). A trend of reduced overall survival was observed with in breast cancer patients with Mdm2, Ck5/6 and Cyclin E markers.

Conclusion: The fact that breast cancer is not a uniform cancer entity but consists of several different subtypes with different molecular profiles, biological behaviour, and risk profiles poses a challenge for the clinical management. Prognostic and predictive factors constitute important tools for the individualization of breast cancer therapy to provide efficient treatment and to spare patients with excellent low-risk profiles from unwanted side effects of overtreatment. The established clinicopathologic markers, in particular ER and HER2, have clearly defined clinical applicability, but deficiencies in the methodologies of assessment may still affect their use. Additional tools are required to facilitate clinical decision-making processes especially for the optimal treatment of early hormone receptor-positive breast cancer.

MV count correlates with the prediction for metastasis and poor survival. Such an indicator would be useful in selection of a subgroup of patients with breast cancer who are at high risk for having occult metastasis at presentation and subsequently would benefit from aggressive therapyThus, Bcl2 negativity and BAG-1 and P53 positivity indicated aggressive phenotypes in breast cancer patients. p53 exon 5 & 7 expression was found to be associated with hormonal receptors. Down regulation of p53 protein noted as compared to incidence of p53 gene expression. p53 protein expression was associated with hormone receptors negativity. FOXA1 was found to be associated with reduced overall survival. A trend of reduced overall survival was observed with in breast cancer patients with Mdm2, Ck5/6 and Cyclin E markers. A change from the traditional approach of using only established markers appears to be an inevitable next step in newly diagnosed patients with breast cancer however more patients to be analyzed.

Biography

Nilima S. Desai joined Division of Immunohistochemistry in 1992 as a Research fellow. She is involved in clinical research aspects of Immunohistochemistry and molecular pathology. She has presented her data in 20 National and International meetings, notable among them being 5th International symposium on Impact of Biotechnology on Cancer Diagnostic and Prognostic indicators by International Society for Preventive Oncology at Geneva, Switzerland in 2000. "Seeking Excellence in Breast Cancer Care" American Cancer Society sponsored and organized by John Hopkins Institute Nursing, Baltimore, USA in 1999; and reach to Recovery International 2nd Asia Pacific UICC breast cancer support conference in 2004, Singapore. She has undergone observer-ship in Immunohistochemistry laboratory at Memorial Sloan Kettering Cancer Centre (MSKCC), New York, USA for one week in October 1999.

Received: June 01, 2022; **Accepted**: June 03, 2022; **Published**: August 01, 2022