

ADJUVANT THERAPY IN NODE NEGATIVE BREAST CANCER

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ABSTRACT:

This is a retrospective and prospective study carried out in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from October 2000 to October 2002. The objective was to assess the role of adjuvant therapy in node-negative breast carcinomas. A total of fifty-breast carcinoma specimens were investigated comprising of forty invasive ductal carcinomas and ten invasive lobular carcinomas. Ki-67 antigen was immunostained on formalin-fixed paraffin embedded tissue and the positivity index was determined. Thirty-three cases showed positive nuclear staining. In ILC, 07 lymph node positive cases had a mean Ki-67 index of $13.1 \pm 5.7\%$ while 01 lymph node negative case had a Ki-67 index of 14.3%. In IDC, 19 lymph node positive cases had a mean Ki-67 index of $16.5 \pm 11.0\%$ and 06 lymph node negative cases had a mean Ki-67 index of $14.3 \pm 4.5\%$. No significant correlation was found between Ki-67 labeling rates and overall extent of lymph node metastases. So it is concluded that the benefits of adjuvant cytotoxic therapy are independent of lymph node status.

INTRODUCTION

There have been many attempts to identify prognostic factors in breast cancer, but few have stood the test of time and the rigors of detailed analysis (McGuire, 1991). The past decade has seen major advances in the basic sciences, with the development of many new investigational techniques and the discovery of a large number of macromolecules that are important in biological processes. Many investigators have applied these advances to the problem of identifying prognostic and predictive variables in breast cancer (Ravdin, 1997).

We still lack knowledge of prognostic and predictive factors. So the therapeutic guidelines in patients with node-negative breast cancer (NNBC) are more variable than in those with node-positive breast cancer (Jean, 2003)

Node-negative patients treated with tamoxifen had a 4-year disease free survival that was statistically greater to those treated with placebo (83% versus 77%) (Fisher *et al.*, 1989). Combination of ovarian ablation (GnRH analogs) plus or minus tamoxifen is an alternative to chemotherapy-plus-tamoxifen in premenopausal, endocrine-responsive node-negative breast cancer patients of average/high risk (Arnesson *et al.*, 1994). In patients with NNBC considered at high risk for relapse, various studies assessed different protocols of adjuvant chemotherapy compared to observation only. CMF-like regimens were compared to doxorubicin-containing combinations and chemo/hormonal therapy was tested against tamoxifen alone (Jean, 2003). The results of chemo/hormonal combinations are better than tamoxifen alone in all age groups (Fisher *et al.*, 1997). Despite the best current chemotherapy or chemo/hormonal therapy, at least 15% to 20% of women with average/high-risk

NNBC will continue to present with recurrent disease (Jean, 2003). This highlights the continuing need to investigate potentially more active regimens.

Growth Rates:

Carcinogenesis can be a multistep process in which an initiating genetic change may remain occult until subsequent genetic change or changes result in growth of a tumor (John S. Spratt and John A. Spratt., 2002). Carcinogenesis is linked to the development of proliferative abnormalities. Cell kinetics of breast carcinoma is a prognostic parameter (Keshgegian, 1995; Elias, 1996 and Goodson, 2000). Growth is a multiplicative or geometric increase in proliferating cells. Random errors in such a system are to be expected. The increasing documentation of the rates of growth of human breast cancers provide insight into the many problems of breast cancer control. In cases of node-negative breast cancers the ability to identify only those cancers with a high probability of recurrence would permit selection of candidates for controlled clinical trials for adjuvant therapy (John S. Spratt and John A. Spratt., 2002). It has been documented that the women with node-negative breast cancer who receive adjuvant therapy have a longer disease-free interval and a longer overall survival (Press *et al.*, 1993).

Ki-67 Antigen:

Many markers of proliferation have been studied in breast cancer, including thymidine labeling, bromodeoxyuridine incorporation, flow cytometry, and immunostaining with the Ki-67 antibody (Hall and Levison, 1990; Hall and Woods, 1990 and Silvestrini, 1991). This antibody recognizes a nuclear antigen encoded by a gene located on chromosome 10q25 (Fonatsch *et al.*, 1991; Gerdes *et al.*, 1991). The antigen Ki-67 is regarded as a marker for proliferating cells. It was identified as a protein(s) (pki-67) which exists free or associated with DNA as evidenced by DNA digestion of cells before or after immuno-labelling with Ki-67 (Lopez *et al.*, 1994). As

Ki-67 identifies the proliferating cells in a tumor, it reflects the percentage of dividing cells (Isola *et al.*, 1990). The positivity of the cells correlates with the degree of differentiation, vascular invasion, and lymph node metastases, and it relates inversely to the presence of steroid hormone receptors (Isola *et al.*, 1990).

MATERIALS AND METHODS

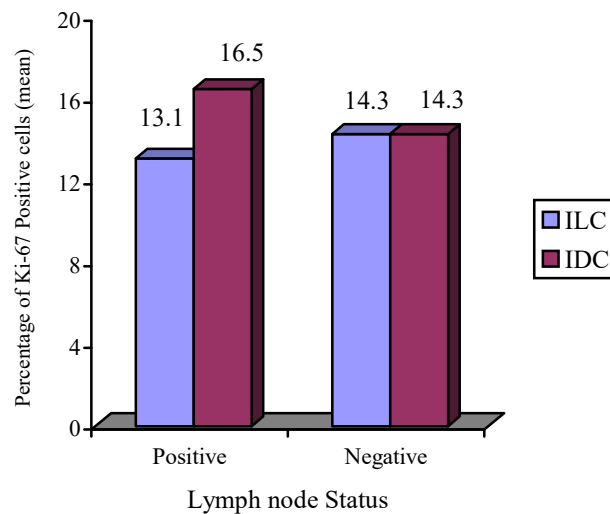
This study was performed on formalin fixed paraffin embedded blocks of cases diagnosed as invasive ductal carcinoma and invasive lobular carcinoma of breast with and without lymph node involvement, in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan from October 2000 to October 2002.

Fifty cases were selected comprising of 10 cases of invasive lobular carcinoma (ILC) and 40 cases of invasive ductal carcinoma (IDC) and were subjected to immuno-staining for Ki-67 positivity. Five-micron thick sections were retrieved for H&E staining. Extra slides were prepared for immuno-staining by cutting 4µm thick sections from representative paraffin embedded blocks and were applied to already positively charged slides. Antigen retrieval was done by trypsin digestion (Zymed Cat No. 00-3003) followed by heat induced antigen recovery. Specific staining is accomplished by localizing the Ki-67 antigen with Ki-67 polyclonal antibody. The antigen/antibody complex is then identified using the LAB-SA biotinylated secondary antibody detection method. A streptavidin enzyme is then added which binds to the biotinylated secondary antibody. A substrate solution is then added that forms a coloured deposit in the presence of the enzyme that is complexed to the antigen. The location of the antigen is then revealed by the presence of the colored deposit that forms around it. Any nuclear staining was regarded as positive. Positivity index of Ki-67 was determined by counting the number of positively stained nuclei in 1000 tumour cells

Table
Correlation of KI-67 positivity with lymph nodes positivity in invasive lobular carcinoma and invasive ductal carcinoma of breast

S. No.	Lymph Node Status	ILC		IDC	
		No. of Cases	Ki-67 Mean $\pm$ SD	No. of Cases	Ki-67 Mean $\pm$ SD
1.	Positive	07	13.1 $\pm$ 5.7	19	16.5 $\pm$ 11.0
2.	Negative	01	14.3%	06	14.3 $\pm$ 4.5
3.	Total	08		25	
4.	P Value		N.A.		P>0.05

Key: ILC = Invasive Lobular Carcinoma
IDC = Invasive Ductal Carcinoma
N.A. = Not applicable



Key: ILC = Invasive lobular carcinoma
IDC = Invasive ductal carcinoma

Fig. Correlation of Ki-67 positivity with lymph nodes positivity in invasive lobular carcinoma and invasive ductal carcinoma of breast.

in at least five representative high power fields across the slide.

STATISTICAL ANALYSIS

The computer package “Microsoft Excel” was used for data feeding and “EPI-INFOR”

was used for statistical analysis. The results were given in the text as number and percentage for quantitative variables and mean and standard deviation for quantitative data. To compare the difference between two means, Student t-test was employed. For the comparison of more than two means Analysis

of Variance (F-test) was performed. In all statistical analysis, only 'P' values less than '0.05' were considered significant.

OBSERVATIONS AND RESULTS

In this study, 50 diagnosed cases of human breast carcinoma including invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC) were subjected to immunohistochemical staining for Ki-67 antigen. Out of 50 cases, 10 were invasive lobular carcinoma and 40 were invasive ductal carcinomas. In ILC, 09 cases were lymph node positive and only 01 case was lymph node negative. In IDC, 31 cases were lymph node positive and 09 cases were lymph node negative.

Out of a total of 50 cases of invasive breast carcinomas, 33(66%) cases showed positive nuclear staining for Ki-67 antigen while remaining 17(34%) cases showed absence of any nuclear staining.

Table and Fig. show correlation of Ki-67 positivity with lymph node status in invasive lobular and invasive ductal carcinoma of breast. In ILC, 07 lymph node positive cases had a mean Ki-67 index of $13.1 \pm 5.7\%$ while 01 lymph node negative case had a Ki-67 index of 14.3%. In IDC, 19 lymph node positive cases had a mean Ki-67 index of $16.5 \pm 11.0\%$ and 06 lymph node negative cases had a mean Ki-67 index of $14.3 \pm 4.5\%$. The results of ILC versus IDC were statistically significant ($P < 0.05$), while the results of lymph node positive versus lymph node negative groups in cases of IDC were statistically insignificant.

DISCUSSION

Measurements of cell kinetics have been found to correlate with clinical course. Biological factors are dependent on cell kinetics and bear important relationship in predicting clinical outcome of the disease and response to the treatment (McGurrin *et al.*,

1987; Barzanti *et al.*, 2000). The benefits of adjuvant cytotoxic therapy are independent of nodal status. The proportional reduction in recurrence and death are similar for patients who are node-negative or have multiple nodal involvement (John S. Spratt and John A. Spratt., 2002).

Previous studies have been quite divided on an association between Ki-67 positivity index and extent of lymph node metastases, with several studies suggesting that no correlation between cell cycle kinetics and overall extent of lymph node metastases has been found (Meyer *et al.*, 1978; Gentili *et al.*, 1981; Kute *et al.*, 1981; Moran *et al.*, 1984; Tubiana *et al.*, 1984). Other studies showed a suggestively positive but weak relationship to the number of positive axillary nodes (Lelle *et al.*, 1987; Lelle, 1989; Viehl *et al.*, 1990).

In the current study, we do not find any significant correlation between Ki-67 labeling rates and overall extent of lymph node metastases in cases of IDC ($P > 0.05$).

CONCLUSION

The benefits of adjuvant cytotoxic therapy are independent of the fact that whether the patient is node-negative, have a small number of nodes involved, or have large number of involved nodes. Adjuvant therapy reduces the odds of recurrence and death for patients with early stage breast cancer.

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