Estrogen, Progesterone and Human epidermal growth factor receptor - 2 in malignant breast lesions: A 5 year study in a tertiary care hospital of Karnataka

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ABSTRACT

Background and Objective: *Breast cancer is the second leading cause of cancer related death in India. Immnuohistochemical study helps to carry out a better management of the disease and to determine the prognosis. This study was conducted to correlate histopathology of the breast tumours, with reference to tumour grade, and common immnuohistochemical markers like Estrogen receptor (ER), Progesterone receptor (PR) and Human epidermal growth factor receptor-2 (HER2) status.*
Methods: *The present study was conducted in the Pathology department of SSIMS&RC, Davangere, from January 2009 to December 2013. Thirty patients with breast carcinoma underwent mastectomy were included in this study. Those specimens were evaluated both for histopathologically and immunohistochemically by Allred scoring for ER, PR, HER-2 markers. All the experimental data were judged for correlations with histopathological features of specimens and immnuohistochemical presentation of the specimens.*
Results: *Majority of the cases founding 41-60 years of age group with Invasive ductal carcinoma Grade 3. ER, PR, Her-2 positivity were found in 56.67%, 50%, and 20% of specimens respectively. Luminal A wasthe most common subtype. Three triple positive IDC cases were seen, suggesting superior response to hormonal therapy.*
Conclusion: *There is geographical and genetic variation among local population groups compared to other Indians. Cost effective immunohistochemistry-based classification on ER, PR, and HER-2 status incorporating in the histopathological evaluation of breast carcinoma assist in better therapeutic management and prognostication.*

Keywords: *Breast cancer, Immunohistochemistry, Estrogen receptor, Progesterone receptor, Human epidermal growth factor receptor.*

INTRODUCTION

Molecular and gene expression pattern studies help us in the prognosis and treatment of breast cancer; important immunohistochemistry (IHC) molecular biomarkers are estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor or, HER2/neu receptor, HER1 receptor, cytokeratin 5/6 and Ki-67.[1,2]In our study we assess the lesions on the basis of ER, PR andHER2 biomarkers. Molecular subtyping of breast cancer specimens by Immunohistochemistry panel can predict the prognosis and also the amplified risk of local and regional relapse.[3] The ER and PR positive lesions have good treatment outcome, while triple-negative or basal-like breast tumours had overall poor prognosis.[4, 5]

In India, breast cancer is the second leading cause of cancer related death. It is the most common cancer amongst women in metropolitan cities of India. In India the average age of detection of breast cancer is 44.2 to 49.6 years.[6, 7]

MATERIALS AND METHODS

A retrospective study was done in the Department of Pathology of SSIMS & R Cover a period of 5 years from January 2009 to December 2013. Relevant information was obtained by request form. Data comprised of 30 patients who had undergone mastectomy and IHC study were included in this study. Patients whose hormone receptor status was not determined were excluded from the study. Information was collected regarding age, sex, site of involvement, histopathological types, estrogen, progesterone and HER2 receptor status and Allred score from cases.

TISSUE PROCESSING

After formalin fixation, 3 μm thick sections were trimmed by LEICA RM 2255 automated microtome and stained by routine Haematoxylin and eosin stain. During histopathology reporting, the

optimum tissue blocks were collected on Super frost glass slides and later was chosen for IHC study.

IMMUNOHISTOCHEMISTRY PROCEDURE:

After brining the tissue sections to water, antigen retrieval was done by microwave oven technique and peroxidase antiperoxidase method of IHC was followed.ER status was decided exercising the BioGenex monoclonal mouse IgG1 (clone ER88from Fremont) and PR status using BioGenex monoclonal mouse IgG1 (clone PR88 from Fremont)upon the tissue sections. HER-2/neu status was revealed using BioGenex monoclonal mouse IgG1 (clone CB11 from Fremont) antibody. Chromogen DAB (BioGenex-3 component DAB pack) imparted the brown tissue colour. Nuclear counterstaining was done with Mayer's Haematoxylin. DPX mounted test slides were examined under light microscopy. [8, 9]

The ER/PR assay measures the intensity and proportion of receptors in cancer cells. Transmembrane HER2 receptor assay measures the amount of HER-2/neu on the surface of cancer cells.

It is seen to overexpresson the surface of 25-30% of metastatic cancers, which usually respond to Trastuzumab.[10] Semiquantative Allred scoring, which also assigns a numerical value based onoverall stain intensity on cell and its patternwas used in this study.[11, 12]

STATISTICAL ANALYSIS

Analysis of data was done on Microsoft Excel sheet 2013. The data was tabulated along with graphical representations.

RESULTS

In our present study of 30 test patients among the females age ranged from 27 to 65years, with a median age of 49 years. Maximum numbers of cases were obtained in the age group of 41-50 years (11 cases/36.67%). In gender distribution 29 (93.33%) cases were females and one was male (6.67%). Left breast pathology was maximum, comprising of 19cases (63.33%) and bilateral involvement was absent.

Table 1: age distribution of patients, IDC cases, ER/PR expression

Age group(years)	No. of patients	IDC cases	ER/PR+	ER/PR-
21-30	2	2	1	1
31-40	4	3	2	2
41-50	11	10	5	5
51-60	8	8	3	5
61-70	5	2	4	0
Total	30	25	15	13

Out of the 30 cases in this study, majority of lesions were infiltrating ductal carcinoma(IDC) comprising of 25cases (83.33%), followed by Ductal carcinoma in situ in 3 cases (10%). Among those IDC cases grade 3 lesions were most commonly encountered in (20/25) 80% cases.

Table 2: Shows numerical and percentage distribution of cases on histopathological lesions

Neoplastic lesions	No(N=30)	Percentage
Ductal carcinoma in situ	3	10
Infiltrating ductal carcinoma	25	83.33
Infiltrating mucinous carcinoma	1	3.33
Infiltrating papillary carcinoma	1	3.33

Table 3: Aistribution of IDC lesions according age group in respect to total patient (n) on that age group. (Modified Richardson Bloom Scoring system)

Age(no of cases)	Grade I	GRADE II	GRADE III
21-30(n=2)	0	0	2
31-40(n=4)	0	0	3
41-50(n=11)	1	2	7
51-60(n=8)	0	2	6
61-70(n=5)	0	0	2

In this study maximum IDC lesions (n=10) were seen in the age group of 41-50 years, out of which 7 cases belonged to grade 3 category.

Table 4: Her-2 status with lesions and distribution in IDC cases

Her-2/Neu status (n=no of cases)	IDC grade(n=no of cases)		
	Grade 1 (n=1)	Grade 2 (n=4)	Grade 3 (n=20)
Positive 6	0	1	5
Negative 21	1	3	13
Equivocal 3	0	0	2

In our study, ER positivity was shown by 17 cases (56.67%), PR positivity was shown by 15 cases (50%) and HER-2/neu positivity was shown by 6 cases (20%).In three cases (10%) HER-2 were equivocal. Only three cases (10%) were positive for all the three markers, on the contrary eight cases (26.67%) were triple negative.
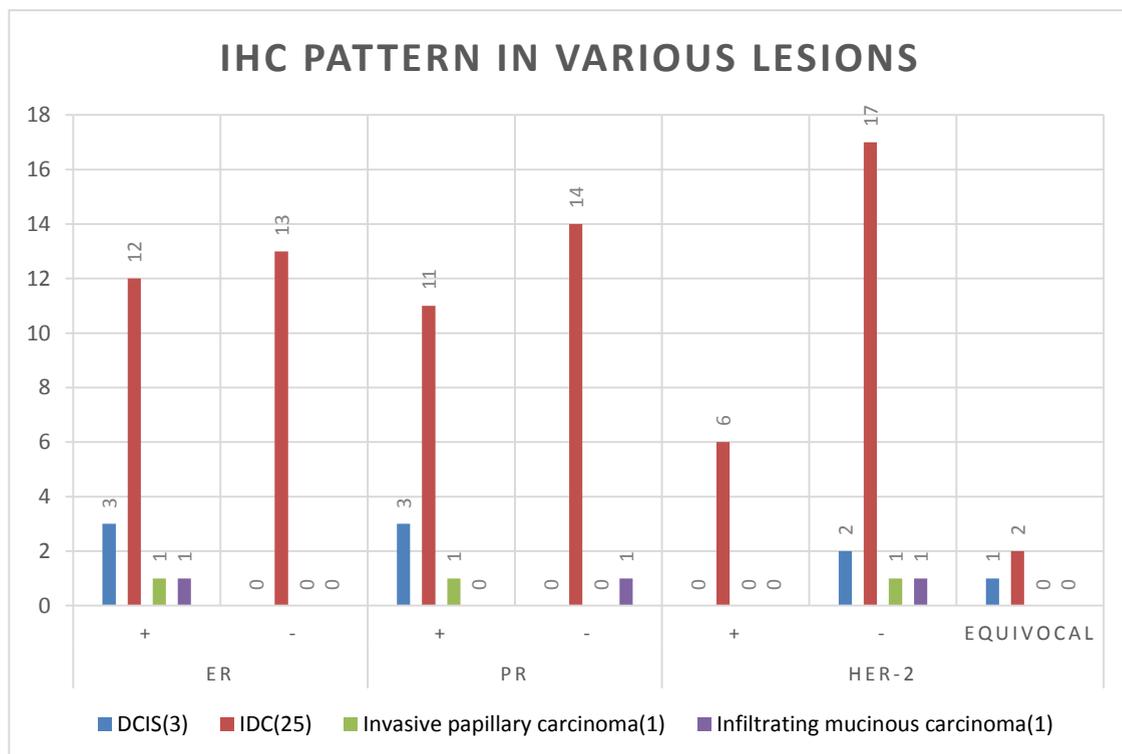
Graph 1: column chart is showing IHC markers response in various histopathological types of breast cancer.

Predominant numbers of triple negative cases (16.7%) (5/30) were seen in 41-60 years of age group, which constitutes 63.33 %(19/30) of total patients. Most common IHC finding was ER$^+$/PR$^+$/Her-2$^-$(Luminal A) were seen in 11 cases (36.67%) and most patients (4 out of 5) were recorded in the eldest age group of our study.

Table 5: Distribution of IDC cases according major histological grade and IHC results

IHC pattern	Grade 1	Grade 2	Grade3
ER/PR+, HER2+	0	1	2
ER/PR+,HER2-	1	3	5
ER/PR-, HER2+	0	0	3
ER/PR-/HER2-	0	0	8

Prevalent IHC pattern in IDC tumors were Luminal A and Triple negative cancers. HER 2 negativity was noticed on 17 (68%) of all IDC breast lesions irrespective of metastasis. All triple negative breast lesions belonged to IDC grade 3.
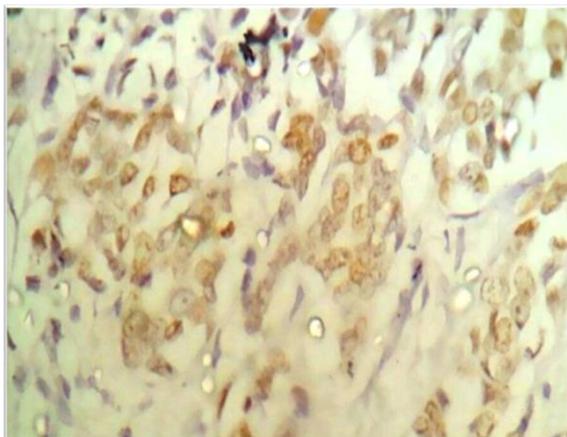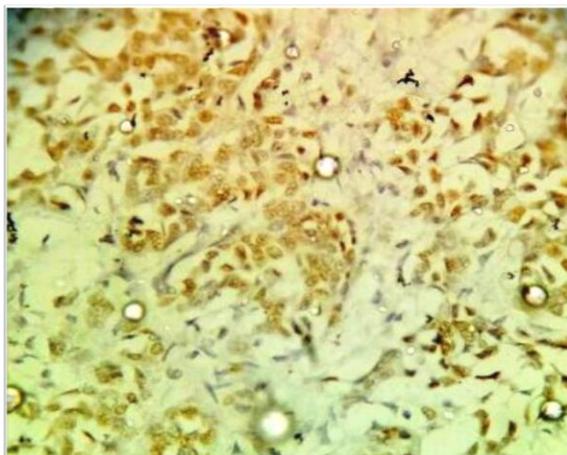

Fig. 1: ER positive in IDC breast

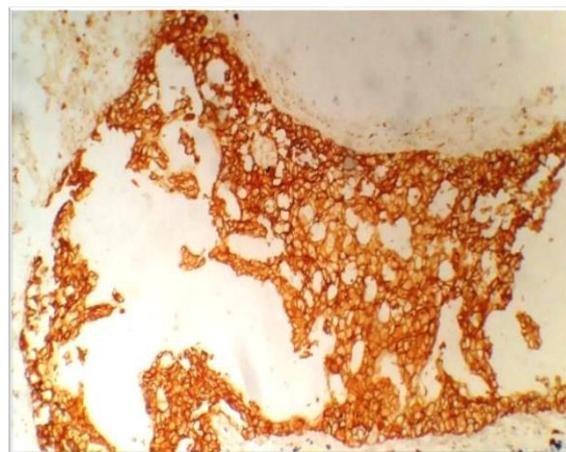**Fig. 2: PR positive in IDC breast**

Fig. 3: HER2 positive in IDC breast

DISCUSSION

Today, IHC is an integral part of breast carcinoma histopathology reporting for prognosis and treatment, in addition to histological diagnosis. In our study maximum cases were noted in the age group of 41- 50 years (36.67%) and 51-60 years (26.67%) with a mean age of 48.6 years similar to other Indian study conducted by Chakrabarti *et al*, Ghosh*et al*.[13,14] In South India majority of breast cancer incidence is observed in women over 40 years of age with late stage of presentation due to lack of awareness about screening, costs, fear and stigma associated with the disease.[15]In the study by Ghosh et al, the age group of patients ranged from 20- 99 years with a mean age of 49 years which is similar to our study.[16]

In present study, the incidence of breast carcinoma was more on the left side in the upper outer quadrant corroborating with the previous reports; the likely explanations are that the left breast being more bulky and comparatively having a larger volume of breast tissue.[17]Out of 27 cases of invasive carcinoma specimens, 25 cases were of IDC (83.33%),and each one case (3.33%)**,** of infiltrating mucinous carcinoma and invasive papillary carcinoma respectively; similar to studies conducted by, Ghosh *et al*, Bhagat *et al* and Adedayo *et al*.[16,18,19] This study as well as reports from India and the

western world show that IDC is the most commonly encountered microscopic diagnosis among breast lesions.[17]

Study carried by Col V Dutta in Army Research Hospital, New Delhi, mentioned majority i.e. 59% of IDC cases were seen among postmenopausal women and the ER/PR status displayed a higher incidence of reactivity with increasing age and receptor negativity was obtained maximum in the menopausal age group. In our study (17/30), 56.67% cases of infiltrating tumours was recorded in postmenopausal female, on the contrary maximum ER/PR positivity and negativity both were observed in the eldest age group.[20] In this study ER positivity was expressed in 56.67% cases and PR positivity was seen in 50% cases, similar to study conducted by Dutta *et al*, Ambroise *et al*.[20,21] In a study from Southern India, the prevalence of ER, PR co-positivity was found to be 32% by Zubeda *et al*.[22] Chariyalertsak *et al* described lower rates of 36.1% ER and 45.8% PR reactivity in breast cancer cases in Thailand.[23]

Study conducted by Patnayak *et al* showed overall 47.6% ER, 48.8% PR, 29.6% HER-2 and 69.2% p53 positivity; there was no significant correlation between HER-2 with age, tumour size, lymph node status, ER and PR status. There was significant correlation between HER-2 and tumour grade.[24] In our institutional study HER-2/neu negativity was observed in 70% of cases.[24] Ambroise *et al* study showed HER-2 positivity in 27.1% of cases.[21] Vaidyanathan *et al* in Bangalore have noted 43.2% HER-2 positivity by IHC.[25] Another study from Varanasi, North India revealed it to be 46.3%.[26] The frequency of HER-2 positivity may change if we take into account cases detected by fluorescence in situ hybridization (FISH) analysis.[22] IHC HER-2 equivocal (2+) cases are a heterogeneous group and FISH is helpful in categorizing these tumours.

Ambroise *et al* in their study showed triple-negative breast cancers constituted 25 % of all cases similar to our study(26.67%).[21] An additional HER2/neu testing in South Indian women with breast cancers demonstrated46% of the breast tumors to be triple negative (Zubeda et al., 2014).[21]

In our study all thirteen ER negative tumours were simultaneously PR negative; other Indian studies by Ambroise *et al*, Patnayak *et al* reported 4.05% and 13.1% for this category.[21,24] Geethamala *et al* study piloted in South India mentioned that among the IDC breast lesions, the most common histologic grade was grade 1 accounting to 54% followed by grade 2 and 3 with 27% and 19% respectively.[27] Ghosh *et al* study showed more of grade 3(75.4%) breast lesions similar to the present study.[14]

Proportionately increasing histological grades of IDC- 25% of grade I, 59% of grade II and 75% of grade III carcinomas displayed over expression of Her- 2/neu by Dutta *et al* study.[20] In the present study Her-2 negativity maximum in IDC grade I (100%) was followed by grade II 75% and grade III 65%. In Nikhra *et al* study HER-2/neu positivity was expressed in 50%, 15.3% and 69.2% in Grade I, II and III IDC respectively.[28] We observed that each case of invasive mucinous (colloid) carcinoma, invasive papillary carcinoma- both of them immunohistochemically had good prognosis. Moreover one 60 years old patient showed IDC with neuroendocrine differentiation that tumour also had good prognosis(ER+PR+HER2-).

CONCLUSION

From this study, it was established that Infiltrating Ductal carcinoma grade 3 is the most common malignant histological subtype. All the DCIS tumours have good prognosis whereas IDC tumours have varying prognosis. In India for better treatment of IDC lesions IHC is avital diagnostic modality. The present study has detected a noticeably different pattern of positivity of various prognostic IHC markers among the local population. As the study revealed a different rate of occurrence of some markers, it can be concluded that this study will act as a guide for appropriate targeted therapy of breast carcinoma occurring among people of this region.

REFERENCES

1. Brien KMO, Cole SR, Tse CK, Perou CM, Carey LA, Foulkes WD, et al. Intrinsic Breast Tumor Subtypes, Race, and Long-Term Survival in the Carolina Breast Cancer Study. Clin Cancer Res 2010;16(24):6101-10.
2. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumors. Nature 2000; 406:747-52.
3. Voduc KD, Cheang MC, Gelmon K, Nielsen TO, Kennecke H. Breast cancer subtypes and the risk of local and regional relapse. J ClinOncol 2010; 28:1684-91.
4. Kumar, Abbas,Fausto, Aster. Pathologic Basis Of Disease, 9th ed. Pennsylvania:Elsevier;2010.p.708-13.
5. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype. Cancer 2007;109(7):1721-8.
6. Preet K. Dhillon.Breast Cancer Factsheet, South Asia Network for Chronic Disease, Public Health Foundation of India. 2013.
7. Advani S. Partner profile: cancer in India. INCTR News 2004;5 .

8. Dabbs D. Diagnostic immunohistochemistry. 2nd ed. Phiadelphia: Elsevier,2006.p.1-41.

9. Miller RT, Wick MR, Swanson PE: Fixation and Epitope Retrieval in Diagnostic Immunohistochemistry: Concise Review of Current Status and Practical Considerations. ApplImmunohistochemMolMorphol 2000;8(3):228-35.

10. Harvey, R.A. Anticancer Drugs. In: et al. (eds.) Lippincott's Illustrated Reviews: Pharmacology. Republic of China: Wolters Kluwer; 2012. p. 505.

11. Qureshi A, Pervez S. Allred scoring for ER reporting and its imapct in clearly distinguishing ER negative from ER positive Breast cancers. J Pak Med Assoc 2010;60(5):350-3.

12. Choudhury KR, Yagle KJ, Swanson PE, Krohn KA, Rajendran JG. A Robust Automated Measure of Average Antibody Staining in Immunohistochemistry Images. J HistochemCytochem 2010;58(2):95-107. doi:10.1369/jhc.2009.953554.

13. Chakrabarti S, Karmakar R, Barui G, Maity PK, Bandyopadhyay A, Roy A. Prevalence of known prognostic factors in female breast carcinoma including oestrogen receptor, progesterone receptor and Her-2/neu status--a study in a tertiary care centre. J Indian Med Assoc. 2012; 110(12):876-9.

14. Ghosh S, Sarkar S, Simhareddy S, Kotne S, Rao PBA, Turlapati SPV. Clinico-morphological profile and receptor status in breast cancer patients in a south indian institution. Asian Pac J Cancer Prev 2014;15(18):7839-42.

15. BabuGR, Lakshmi SB, Thiyagarajan JA. Epidemiological Correlates of Breast Cancer in South India. Asian Pac J Cancer Prev 2013;14 (9):5077-83.

16. Ghosh J, Gupta S, Desai S, Shet T, Radhakrishnan S, Suryavanshi P, *et al*. Estrogen, progesterone and HER2 receptor expression in breast tumors of patients, and their usage of HER2-targeted therapy, in a tertiary care centre in India. Indian J Cancer 2011; 48(4):391-6.

17. Sandhu DS, Sandhu S, Karwasra RK, Marwah S. Profile of breast cancer patients at a tertiary care hospital in north India. Indian J Cancer. 2010;47(1):16-22.

18. BhagatVasudha M, Jha Bharti M, Patel Prashant R. Correlation of Hormonal Receptor and Her- 2/neu Expression in Breast Cancer: A Study at Tertiary Care Hospital in South Gujarat. Natl J Med Res 2012; 2(3): 295-8.

19. Onitilo AA, Engel JM, Greenlee RT, Bickol N. Breast Cancer Subtype Based on ER/PR and Her2Expression: Comparison of Clinicopathologic Features and Survival. *Clinical Medicine & Research* 2009:7(2):4 - 13.

20. Dutta V, Chopra GS, Sahai K, Nema SK. Hormone Receptors, Her-2/Neu and Chromosomal Aberrations in Breast Cancer. MJAFI 2008;64:11-5.

21. Ambroise M, Ghosh M, Mallikarjuna VS, Kurian A. Immunohistochemical profile of breast cancer patients at a tertiary care hospital in South India. Asian Pac J Cancer Prev 2011;12(3):625-9.

22. Zubeda S, Kaipa PR, Shaik NA, Mohiuddin MK, Vaidya S, Pavani B, et al. Her-2/neu status: a neglected marker of prognostication and management of breast cancer patients in India. Asian Pac J Cancer Prev 2013;14(4):2231-5.

23. Chariyalertsak S, Chariyalertsak A, Ruangvejvorachai P. Immunohistochemical detection of Estrogen And Progesterone receptors in primary breast cancer. Asian Pac J of Cancer 1996;16:161-6.

24. Patnayak R, Jena A, Rukmangadha N, Chowhan AK, Sambasivaiah K, Phaneendra BV, *et al*. Hormone receptor status (estrogen receptor, progesterone receptor), human epidermal growth factor-2 and p53 in South Indian breast cancer patients: A tertiary care center experience. Indian J Med PaediatrOncol 2015;36(2):117–22.

25. Vaidyanathan K, Kumar P, Reddy CO, Deshmane V, Somasundaram K, Mukherjee G. ErbB-2 expression and its association with other biological parameters of breast cancer among Indian women. Indian J Cancer 2010;47(1):8-15.

26. Kumar V, Tewari M, Singh U, Shukla HS. Significance of Her-2/neu protein over expression in Indian breast cancer patients. Indian J Surg 2007;69:122-8.

27. Geethamala K, Murthy V S, Vani B R, Sudharao. Hormone receptor expression in breast carcinoma at our hospital: An experience. Clin Cancer Investig J [serial online] 2015 [cited 2015 Sep 21];4:511-5. Available from: http://www.ccij-online.org/ text.asp?2015/4/4/511/157940.

28. Nikhra P, Patel S, Taviad D, Chaudhary S. Study of ER (Estrogen Receptor), PR (Progesterone Receptor) & HER-2/NEU (Human Epidermal Growth Factor Receptor) expression by immunohistochemistry in breast carcinoma. IJBAR 2014;05 (06):275-8.

29. Lal P, Tan LK, Chen B. Correlation of HER-2 status with estrogen and progesterone receptors and histologic features in 3,655 invasive breast carcinomas. Am J ClinPathol. 2005;123(4):541-6.