Original Article

Diagnostic Value of Cytokeratin 5 and Cytokeratin 6 in Benign and Malignant Lesions of Breast

Kafil Akhtar[1]*, Sanjay Bharduaj[1], Mohammed Naim1, Tariq Mansoor[2], Rana K Sherwani[1]

1Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India
[2]Department of General Surgery, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India

Keywords: *Breast Carcinoma, Histopathology, Immunohistochemistry, Cytokeratin*

ABSTRACT

Background: Correlation of Cytokeratin 5 and Cytokeratin 6 expression in benign and malignant lesions of the female breast-ducts and to find out the utility of Cytokeratin 5/6 in the prognosis of carcinoma breast.

Methods: The present study was carried out on 78 benign and malignant lesions of breast. Tissues were fixed in formal saline, processed for paraffin sections and stained with Haematoxylin and Eosin (H&E) stain. Immunohistochemical staining was performed using mouse antihuman polyclonal D5/16B4 antibody for cytokeratin 5/6 and visualization obtained with DAB and the slides were examined for staining pattern (cytoplasmic or membrane), proportion and intensity of staining of tumour cells.

Results: Amongst the 78 cases of breast lesions, 38 (48.7%) cases were benign breast disease, 18 (23.1%) were ductal carcinoma in situ and 22 (28.2%) cases were of malignant breast carcinoma. Out of 22 cases of malignant breast disease, 16 (72.7%) cases showed negative cytokeratin reaction with staining score of <2 and 6 (27.3%) cases of triple negative breast carcinoma (TNBC) showed positive cytokeratin expression with staining score of 5-8.

Conclusions: Immunohistochemistry is efficiently used for differentiating the UDH (usual-ductal-hyperplasia) from the DCIS (ductal carcinoma in situ), ruling out micro-invasion, distinguishing invasive carcinoma from pseudo-invasive lesions, identifying breast cancer histological sub-type and molecular phenotype.

***Corresponding author:**
Dr. Kafil Akhtar, Associate Professor, Department of Pathology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh. (U.P) India.
E-mail: drkafilakhtar@gmail.com

Introduction

The rising prevalence of breast cancer and molecular diagnosis of the breast cancer variants continue to concern the medical community. Molecular diagnosis of the breast cancer types depends on highlighting with tumor-marker stains, the molecules considered as signals, symbols or representatives of tumor cells and which are increased in the cancerous conditions. [1] Normal cells also express most of the tumor markers, but quantum of marker molecules distinguishes the tumor cells from the normal cells.

Immunohistochemistry (IHC) had already become very important tool in the molecular diagnosis of breast diseases. IHC is efficiently used for differentiating the UDH (usual-ductal-hyperplasia) from the DCIS (ductal carcinoma in situ), ruling out micro-invasion, distinguishing invasive carcinoma from pseudo-invasive lesions, identifying breast cancer histological sub-type and molecular phenotype, and confirming the breast as the primary site in a metastatic carcinoma. In addition, IHC markers are useful for estimating prognosis and predicting the therapeutic response. The best approach to the use of IHC markers is to couple them with standard haematoxylin-eosin histology and to use panels of the markers. [2,3]

The present study was undertaken to correlate Cytokeratin 5 and Cytokeratin 6 expression in the benign and malignant lesions of the female breast-ducts and to find out the utility of Cytokeratin 5/6 in the diagnosis and prognosis of carcinoma breast.

Material and Methods

The present study was carried out on 78 benign and malignant lesions of breast in the Department of Pathology, JNMC, Aligarh, from Nov 2012 – Dec 2014. The patient's clinical history and examination findings with relevant investigations were obtained from the medical records department at our department and hospital.

Tissues were fixed in formal saline, processed for paraffin sections and stained with Haematoxylin and Eosin (H&E) stain. IHC marker staining was performed on the sections mounted on lysin coated clean glass slides. IHC staining was performed using mouse antihuman polyclonal D5/16B4 antibody for cytokeratin 5/6 and visualization was obtained with DAB (3, 3'- diaminobenzidine, Dako). For the assessment of cytokeratin 5/6, the immunohistochemically stained slides were examined for staining pattern (cytoplasmic or membrane) and proportion and intensity of staining of tumour cells. IHC staining intensity, score and staining proportion score were calculated as below:

Final immuno- staining score (FIS) was obtained by adding scores from the two categories with a maximum score not > 8. The breast lesion with score < 2 were termed cytokeratin 5/6 negative while those with score >2 were termed cytokeratin 5/6 positive.[1,4] The statistical analysis was done using basic descriptive statistics, mean and chi-square tests. Differences at p value <0.05 were considered significant. The analyses employed SPSS 10 software.

Intensity score	Proportion score
0= No staining. 1= Weak staining. 2= Moderate staining. 3= Strong staining.	1= <10% positive staining 2= 10-25% positive staining 3= 26-33% positive staining 4= 34-66% positive staining 5= 67-100% positive staining

Results

Amongst the 78 cases of breast diseases presently studied, 38 (48.7%) cases were benign lesions of breast, 18 (23.1%) were premalignant and 22(28.2%) cases were malignant breast carcinoma. All the 38 benign lesions of breast showed positive cytokeratin 5/6 expression with variable staining score (Table I). Fibrocystic disease showed cystically dilated ducts lined by cytoplasmic and membranous cytokeratin 5/6 positive flattened epithelium with mild pericystic fibrosis and inflammation (Figure 1).

Cases of duct ectasia showed dilated ducts lined by cuboidal epithelium and granular amorphous debris in the lumen with sparse peri-ductal chronic inflammatory cell infiltration. Fibroadenoma showed proliferating breast ducts with predominantly fibrous stroma around dilated and compressed ducts with cytoplasmic and membranous cytokeratin 5/6 positivity in the outer myoepithelial cell layer only (Figure 2).

All the 18 (23.1%) cases of ductal carcinoma in situ and intraductal carcinoma showed proliferative monomorphic tumor cells with low to high N:C ratio, evenly distributed in duct spaces lined externally by a basement membrane, while all the twenty two (28.2%) cases of infiltrating ductal carcinoma showed clusters of malignant cells with high N:C ratio, pleomorphism with surrounding stromal invasion and infiltration into adjacent fatty tissues and vascular spaces. ($p<0.05$)

Out of 22 cases of malignant breast diseases including heterogenous breast duct carcinoma, 16 (72.7%) cases showed negative cytokeratin 5/6 immunoreaction in luminal cells and strong membranous and cytoplasmic cytokeratin 5/6 positivity in the basal cells with staining score of <2 (Figure 3 and Table 2). Six (27.3%) cases of triple negative breast carcinoma (TNBC) showed positive cytokeratin expression with staining score of 5-8 (Figure 4 and Table 3).

Table 1: Correlation between benign breast disease and Cytokeratin positivity

S No	Histopathological Diagnosis	No. of cases	Age (year)	CK positive cases	CK 5/6 staining Score -Range	Mean of Score ± SD
1	Fibrocystic disease	06	18-52	06	5-6	5.7±0.10
2	Usual ductal hyperplasia	04	23-53	04	5-7	6.6±0.11
3	Duct ectasia	08	35-50	08	5-6	5.4±0.13
4	Fibroadenoma	20	16-40	20	5-8	7.2±0.14
	Total	**38**		**38**		

P value: 1:2 = 0.106 (insignificant); 1:3=0.105 (insignificant); 1:4=0.048 (significant), 2:3p=0.003 (insignificant), 2:4= 0.112 (insignificant)

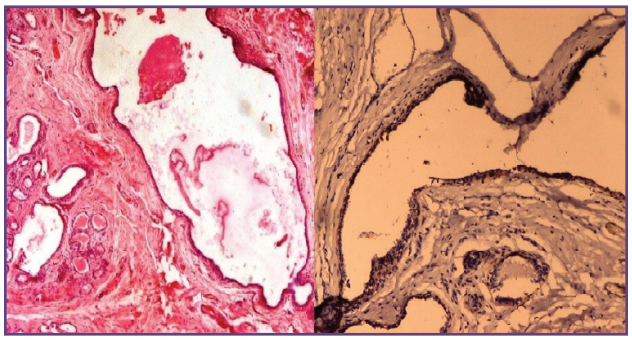

Fig. 1: Fibrocystic disease shows cystically dilated ducts lined by cytoplasmic and membranous cytokeratin 5/6 positive flattened epithelium with mild pericystic fibrosis and inflammation. (H & E and Immunostain Cytokeratin, 125x)

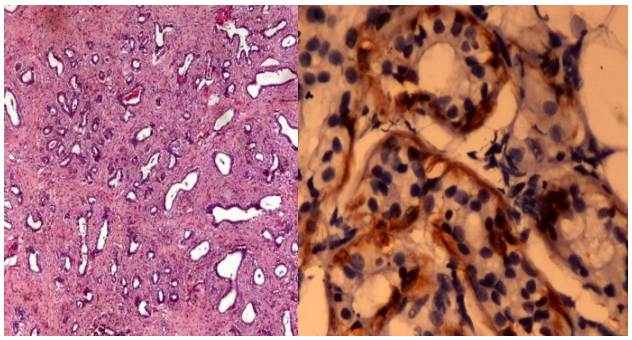

Fig. 2: Fibroadenoma shows proliferating breast ducts with predominantly fibrous stroma around dilated and compressed ducts with cytoplasmic and membranous cytokeratin 5/6 positivity in the outer myoepithelial cell layer only. (H & E and Immunostain Cytokeratin, 125x)

The hererogenous carcinoma specifically showed multiple tumor-cell components with secretory pattern and solid dedifferentiated carcinoma. The solid component showed triple (ER, PR and Her2) negativity and strong cytokeratin 5/6 positivity. The secretory component showed triple positivity and negative cytokeratin 5/6 immunoreaction (Figure 5).

Out of the total 78 cases, 44 (56.4 %) cases showed positive cytokeratin staining with a decline in the number

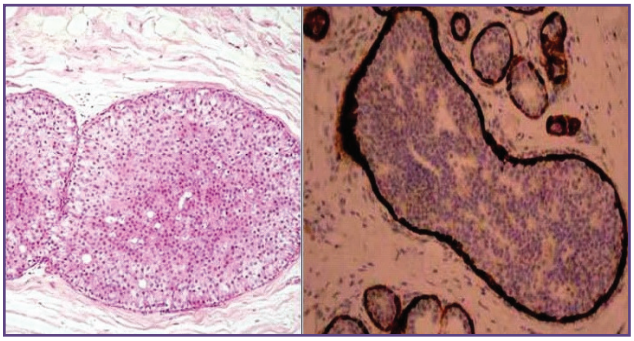

Fig. 3: Cribriform Intraductal carcinoma showing strong membranous and cytoplasmic cytokeratin 5/6 positivity in basal cells and negativity in the luminal cells. (H & E and Immunostain Cytokeratin, 125x)

of cytokeratin positive cases from age group 20-30 year to 50-60 year followed by an increase in number above 60 years of age.

The mean age of presentation in 16 (72.7%) cases of invasive ductal carcinoma and 6 (27.3%) cases of triple negative breast carcinoma was 57.7 years and 53 years respectively. (Table 3) Lymph node metastasis was positive in 4 cases (66.7%) and negative in 2 cases (33.3%), out of the 6 cases of triple negative breast carcinoma. Amongst the 16 cases of invasive ductal carcinomas, 6 cases (37.5%) were lymph node positive and 10 cases (62.5%) were lymph node negative, with a p value < 0.05, which was statistically significant. Vascular invasion was seen in 5 (83.3%) cases of triple negative breast carcinoma and 12 (75.0%) cases of invasive ductal carcinoma. (Table 3), with a p value < 0.05, which was statistically significant. All the 6 cases of triple negative breast disease showed grade III and variable cytokeratin positivity with staining score between 5 to 8, whereas other breast carcinomas showed a variable grade, from I to III with negative cytokeratin expression and a p value < 0.05, which was statistically significant. (Table 3)

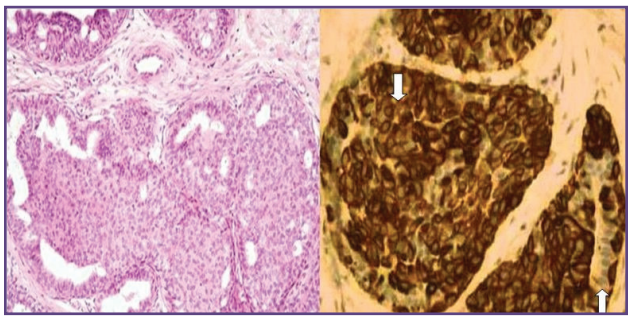

Fig. 5: Heterogenous Breast Duct Carcinoma shows multiple pattern with solid and secretory pattern of growth, with the solid component with triple negativity and strong cytokeratin 5/6 positivity (↓) and the secretory component with triple positivity and negative cytokeratin 5/6 immunoreaction (↑) (H & E and Immunostain Cytokeratin, 500x)

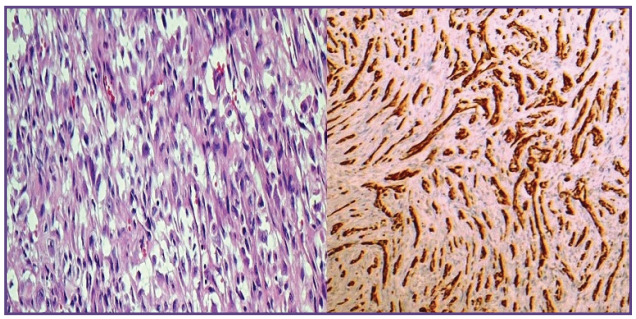

Fig. 4: Triple negative breast carcinoma with metaplasia showing cytoplasmic and membranous cytokeratin 5/6 positivity. (H & E and Immunostain Cytokeratin, 125x)

Table 2: Correlation between Premalignant and Malignant Breast disease with Cytokeratin 5/6 expression

Histopathological diagnosis	No of cases	Lymph node status	Grade (Bloom Richardson)	cytokeratin 5/6 positive cases	Cytokeratin 5/6 staining score	Mean of Score ± SD
Premalignant (Fibro-adenotic neoplasms)	18	-	I-8 II-10	0	<2	1.8±0.12
Malignant Breast disease Luminal A Luminal B Basal like	22 12 06 04	+/_	 III-8 II-6 I-8	6	 2-6 <2 <2	 5.8±0.10 1.8±0.13 1.4±0.12
Total	**40**					

p=0.021(p<0.05); significant

Table 3: Characteristics of triple negative breast cancer versus other breast cancer

Variable	Triple negative breast cancer (Total no=6)	Other Breast Cancer (Total no=16)
Mean age of diagnosis (years)	53	57.7
Lymph node status:		
Positive	4 (66.7%)	06(37.5%)
Negative	2(33.3%)	10(62.5%)
Mean tumor size(cm)	3.0	2.1
Tumor grade		
III	06	02
II	-	06
I	-	08
Lympho-Vascular Invasion		
Present	05 (83.3%)	12 (75.0%)
Absent	01(16.7%)	04 (25.0%)
ER/PR/HER2 status		
Positive	06	-
Negative	-	16
CK 5/6 positivity SC	5-8	-

p value=0.123 (>0.05) insignificant

Discussion

The present study on 78 cases of breast lesions showed a positive immunohistochemical staining for Cytokeratin 5/6 in the 38 benign lesions with immunoscore of 5-8. All the cases (100%) of UDH showed positive immunostaining for cytokeratin 5/6. None of the 18 (100%) cases of DCIS presently showed positive immunostain for cytokeratin 5/6. Thus cytokeratin 5/6 can be useful in distinguishing UDH from the spectrum of ADH/DCIS. Magali Lacroix-Triki at el reported positive immunostaining of cytokeratin 5/6 in 31 cases of UDH, 5 cases of ADH and 54 cases of DCIS on a study on 100 breast lesions.[5] Rabban et al, demonstrated higher immunoscores for cytokeratin in benign breast lesions than DCIS. [6] Presently, the in situ and intra-ductal carcinoma cases were observed to be showing positive CK 5/6 staining of the basal cells and negative CK 5/6 in the lining malignant cells.

Tan et al have reported that DCIS lesions with spindle cells may show neuroendocrine differentiation and negative immunoreactions with Cytokeratin 5/6.[4] Usual ductal hyperplasia with apocrine change, columnar cells in papillary lesions also do not stain with Cytokeratin 5/6. [7] Therefore, a careful evaluation of the H&E stained sections along with immune-histochemical analysis with Cytokeratin 5/6 is useful for diagnosis of these lesions.

Out of the 22 cases of malignant breast lesions in our study, 16 cases (72.7%) showed negative immunoreaction for Cytokeratin 5/6, and 6 cases (27.3%) showed positive immunoreaction. All the 6 cases of breast carcinoma positive for CK 5/6 expression were triple (ER, PR, EGFR) negative. Bocker et al, studied CK 5/6 expression in 23 cases of benign and 25 cases of malignant breast lesions. [8] They found that 100% benign lesions showed positive immunoreaction while 5% of malignant cases showed CK 5/6 expression. None of the cases of DCIS in our study showed a positive immunoreaction whereas 6 cases of advanced malignant lesions (27.3%) showed positive CK 5/6 expression. The slightly higher percentage positivity of CK 5/6 in the present study can be attributed to different patient cohorts or a smaller study sample. No case of medullary carcinoma breast was included in our study. However, Tot reported CK 5/6 positivity in 25% of the typical, 43% of the atypical and 20% of the metastatic medullary carcinomas. [9]

Cytokeratin 5/6 antibody is very frequently applied to help differentiate invasive from non invasive lesions. The common examples include radial scar versus invasive cancer, intraductal papilloma versus papillary intraductal carcinoma and microglandular adenosis versus tubular carcinoma. In radial scar the myoepithelial layer is retained around glandular structures and therefore these can be expected to show positive staining with CK 5/6 and myoepithelial cell markers. In a foci of sclerosing adenosis, the staining is heterogeneous. In low grade invasive carcinomas, the myoepithelial layer is absent and CK 5/6 staining is negative. [10]

Out of the total 78 cases, 44 (56.4%) cases showed positive Cytokeratin staining. Number of Cytokeratin positive cases decreased from third decade to sixth decade, followed by an increase in number beyond the seventh decade of life. No definitive correlation was observed between age of the patient and Cytokeratin 5/6 expression in our study. However, Rehim et al showed an inverse correlation of Cytokeratin 5/6 with the patient's age. [11]

In the present study, all the triple negative malignant cases showed positive immunoreaction for Cytokeratin 5/6 in the polygonal faint PAS positive non-secretory tumor areas, while the secretory tumor components were negative for CK 5/6 expression. These findings suggested that CK 5/6 positive polygonal components in the breast carcinoma represented de-differentiation of secretory tumor into the breast duct stem cell components, which could be a determinant of poor prognosis. CK 5/6 may be of utility in distinguishing well differentiated luminal cell carcinoma. All the cases were grossly necrotic with mean tumor size of 3 cms and were of grade III. Lakhani et al have also reported that necrosis was a common factor in all the CK 5/6 positive tumors. [12] Naim et al have reported strong CK 5/6 positivity in triple negative breast carcinomas. [13]

The mean age of presentation of triple negative breast malignancy was at an younger age (53 years) than other breast cancers. Rehim et al reported a positive correlation of tumor size, local and regional recurrence, distant metastases with CK 5/6 expression and an inverse correlation with patient's age and metastasis.[11] The present study showed no consistent correlation with size and age.

In the present study 4 cases (66.7%) of triple negative breast carcinoma cases showed nodal metastasis whereas only 6 cases (37.5%) of other malignant breast carcinomas were lymph node positive. Vascular invasion was seen in 5 cases (83.3%) of triple negative breast carcinoma and 12 cases (75.0%) of other malignant breast carcinomas. Our findings were consistent with the study conducted by Dent et al who demonstrated lymph node positivity and lymph vascular invasion in 55% and 45 % cases of triple negative carcinoma respectively while among other breast carcinomas, 40% and 30% cases showed lymph node and lympho-vascular invasion respectively. [14]

Van de Rijn et al observed that expression of basal type cytokeratin in node negative breast carcinoma was a prognostic factor independent of tumor size and tumor grade. [15] It was associated with significantly shorter survival, but held no predictive value in patients with known lymph node metastases. On the contrary, Takei et al reported that there was no significant difference in overall survival and relapse free survival in tumors expressing high molecular weight keratin as compared to tumors not expressing it. [16] However further studies with larger sample size are required to established conclusive results.

Conclusions

Immunohistochemistry has an increasing role in the modern pathology of breast disease. IHC markers Cytokeratin 5 and 6 are efficient in differentiating the UDH from the DCIS, ruling out micro-invasion, distinguishing invasive carcinoma from pseudo-invasive lesions, identifying breast cancer histological sub-type, especially triple negative breast carcinoma and heterogenous breast duct carcinomas.

Acknowledgements

We acknowledge the technical staff of the cytopathology lab and the departmental head for the general support.

Funding

None

Competing Interests

None declared

References

1. Bhalla A, Mridu M, Kahlon SK, Parbodh K, Nikita K. Cytokeratin 5/6 expression in benign and malignant breast lesions. Ind J Pathol Microbiol 2010; 53: 676-9.
2. Yeh I-Tien and Carolyn M. Application of Immunohistochemistry to Breast Lesions. Archives of Pathol Lab Med 2008; 132: 349-358.
3. Abdel-Fatah, Tarek MA, Desmond GH, Zsolt RF, Jorge SL, Andrew H, et al. Morphologic and molecular evolutionary pathways of low nuclear grade invasive breast cancers and their putative precursor lesions: further evidence to support the concept of low nuclear grade breast neoplasia family. Am J Surg Pathol 2008; 32:513-523.
4. Tan M, Puayhoon AW, Meng Yuan BS, Bay B, Huat S, Lang H et al. Cytokeratins in Papillary Lesions of the Breast: Is There a Role in Distinguishing Intraductal Papilloma From Papillary Ductal Carcinoma In Situ. Am J Surg Pathol 2005; 29 (5): 625-632.
5. Magali Lacroix-Triki, Eliane M, Jean-Jacques V, Luc I, Philippe R. Value of cytokeratin 5/6 immunostaining using D5/16 B4 antibody in the spectrum of proliferative intraepithelial lesions of the breast. A comparative study with 34beta E12 antibody. Virchows Archiv 2003; 442(6):548-554.
6. Rabban JT, Frederick C, Koerner M, Melinda FL. Solid papillary ductal carcinoma in situ versus usual ductal hyperplasia in the breast: a potentially difficult distinction resolved by cytokeratin 5/6. Hum Pathol 2006; 37(7): 787-793.
7. Raju U, Crissman JD, Zarbo RJ. Epitheliosis of the Breast- An Immunohistochemical Characterization and Comparison to Malignant Intraductal Proliferations of the Breast. Am J Surg Pathol 1990; 14(10): 939-947.
8. Bφcker W, Moll R, Poremba C, Holland R, Van Diest PJ, Dervan P et al. Common adult stem cells in the human breast gives rise to glandular and myoepithelial cell lineages: A new cell biological concept. Lab Invest 2002; 82:737-745.
9. Tot T. The cytokeratin profile of medullary carcinoma of the breast. Histopathol 2006; 37: 175-181.
10. Otterbach F, Bànkfalvi A, Bergner S, Decker T, Krech R, Boecker W. Cytokeratin 5/6 immunohistochemistry assists the differential diagnosis of atypical proliferations of the breast. Histopathol 2000; 37(3):232-240.
11. Rehim DM, Pinder SE, Paish CE, Bell J, Blamey RW, Robertson JF et al. Expression of luminal and basal cytokeratins in human breast carcinoma. J Pathol 2004; 203(2):661-671.
12. Lakhani SR, Reis-Filho JS, Fulford L, Penault-Llorca F, Van der Vijver M, Parry S et al. Breast cancer linkage consortium: Prediction of BRCA 1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clin Cancer Res 2005; 11:5175-5180.
13. Naim M, Soni M, Khan A. Modified Naim Murad classification of female breast cancer in Breast cancer classification, serum adiponectin and SNP rs 2241766. Lap Lambert academic publications, Germany, 2014; pp 19-59.
14. Dent R, Trudeau M, Pritchard KI, Hanna WM. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007; 13(15): 4429-4434.
15. Van de Rijn M, Perou CM, Tibshirani R, Haas P, Kallioniemi O, Kononen J et al. Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. Am J Pathol 2002; 161:1991-1996.
16. Takei H, Iino Y, Horiguchi J, Maemura M, Oyama T, Yokoe et al. Low and high molecular weight cytokeratins in invasive breast carcinoma. Oncol Rep 1997; 4: 33-38.