Section: Pathology

Original Research Article

EXPRESSION OF CYTOKERATIN 5/6 IN BENIGN AND MALIGNANT BREAST LESIONS

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Abstract

Background: The incidence of breast cancer is rising in India and is now the second most common cancer diagnosed in women after cervical cancer. Breast carcinoma is a group of very diverse diseases that can be detected at a clinical, histopathological and molecular level. A panel of antibodies, improved antigen retrieval techniques have all contributed to the use of immunohistochemistry (IHC) in solving diagnostic problems in breast pathology. The aim of the present study was to study the frequency and diagnostic utility of CK 5/6 expression in benign and malignant breast disease along with its use as a surrogate marker. **Materials and Methods:** The study was done at a tertiary centre in the Department of Pathology, for a period of 18months which is a cross-sectional, descriptive and analytical study. Clinical data was retrieved from HPE records. **Result:** Total specimens collected were 60, of which benign lesions accounted for 20 and malignant lesions were 40. All benign breast lesions (fibroadenomas, fibrocystic disease, benign papillomas, UDH) showed strong positivity with a staining index of 6+. Atypical papillary lesions (2 cases), atypical ductal hyperplasias (2 cases) were negative for CK 5/6 immunostaining in the luminal proliferation with a retained myoepithelial layer in the periphery positive for ck5/6. In case of malignancies, 6 cases (15%) were positive for CK 5/6 immunostaining, indicating a basal like phenotype. Out of six cases,4 cases were that of IDC NOS, one case of medullary carcinoma, one case of metaplastic carcinoma. All these cases were triple negative. **Conclusion:** CK 5/6 positivity is used as a component of panels to differentiate benign and malignant breast lesions.

INTRODUCTION

Cancer is now the second leading cause of death in Indians after cardiovascular disease. Amongst women, cervical cancer is still the most frequently diagnosed cancer but breast cancer is now the most commonly diagnosed cancer in urban Indian women.[1] The reasons for the recent observed increase in incidence of breast cancer in the Indian population are not clearly understood but thought to be largely explained by westernization of lifestyles and changes in reproductive behaviour. Breast cancer accounts for 5-8% of all cancers in India and the incidence is on the rise. It is the most common cancer of urban Indian women, and the second most common in rural women. Locally advanced breast cancer accounts for 50% of all breast cancers. Several molecular components related to development of breast carcinomas and associated with therapeutic and prognostic value, viz. p53, RB, ER, HER-2; have been studied in great detail.[2]

Perou and colleagues classified breast cancers into distinct molecular subtypes based on gene expression into luminal A, B, Her 2 neu type, triple negative and normal breast like.[3] Molecular subtypes are potentially predictive of patterns of response to specific therapeutic agents. For instance, luminal A tumors are expected to be sensitive to endocrine therapy, HER-2– overexpressing tumors can be targeted with monoclonal antibodies against HER-2 or HER-2 tyrosine kinase inhibitors, and basal-like cancers may respond to specific therapeutic regimens and inhibitors of the poly (ADP-ribose) polymerase (PARP)enzyme….. Basal like breast cancer (BLBC), despite its low frequency, has been the focus of extensive research during the last years, principally due to its more aggressive behaviour, non-responsiveness to routine endocrine therapy, poor

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prognosis and early relapse, and similarities with tumors arising in BRCA 1 carriers.[4] Epithelial lesions of the breast are not only the most frequent lesions encountered by the surgical pathologist but also are the greatest source of concern in the differential diagnosis of benign versus malignant lesions. The lesion categories that typically need to be differentiated include florid epithelial hyperplasia's of usual type (UDH) in ducts and papillomas from atypical ductal hyperplasias (ADH) in ducts and papillomas and ductal carcinoma in situ (DCIS). These atypical ductal epithelial hyperplasia (ADEH), papillary lesions and microinvasive tumors lend themselves to IHC clarification in many instances. In all of these diagnostic situations, the presence of myoepithelial cells (MECs) that envelop ductal-lobular epithelium, situated on the epithelial basal lamina, has always been considered to be the important criterion that separates benign from malignant lesions. CK5/6 routinely intensely decorate florid ductal hyperplasia of breast which may be useful in separating florid UDH in ducts or papillomas from atypical epithelial hyperplasis / DCIS. CK5/6 antibody immunostaining of the DCIS is rare to negative. In conjunction with histomorphology, CK5/6 may help distinguish DCIS from florid hyperplasia and further aid in distinguishing intraductal papilloma and intraductal papillary carcinomas. Cytokeratin5/6 is a type 2 basic/neutral high molecular weight keratin, encoded by type 2 keratin gene cluster on chromosome 12q and is expressed by myoepithelial cells.[5] It is used as a surrogate marker for identification of basal like breast cancer.

Aims & Objectives

1. To study the frequency of CK 5/6 expression in benign and malignant breast disease.
2. To study diagnostic utility of CK 5/6 to differentiate florid epithelial hyperplasia in ducts and papillomas from atypical epithelial hyperplasia (ADH) / and ductal carcinoma in situ (DCIS).
3. To study expression of CK 5/6 in breast carcinomas where it is used as a surrogate marker to identify basal like breast cancer (BLBC).

MATERIALS AND METHODS

The study was done in the Department of Pathology, at a Tertiary Centre, Hyderabad for a period of 18 months from January 2012 to August 2013. Clinical data was retrieved from HPE records. The specimens were fixed in 10% buffered formalin, grossed and sections were taken from representative sites. The sections were then processed in automated tissue processor and embedded in paraffin wax.

Inclusion Criterion for Selection of Cases

- Benign epithelial lesions
  - Non proliferative breast changes (fibrocystic change)
  - proliferative breast diseases without atypia
  - proliferative breast diseases with atypia.
- Fibroadenomas
- Diagnosis of invasive breast carcinoma
- No prior treatment history.
- Adequate tumor tissue for analysis.
- Complete Clinico-pathological data (age, sex, histopathological diagnosis)

Exclusion Criterion

- Congenital disorders
- Inflammatory lesions.

Methods

Two micro sections of 4-5 micron thickness were prepared from the corresponding paraffin blocks, one on albumin coated slide for H&E staining and the other on poly-L-lysine coated slide for immuno-histochemical staining.

RESULTS

Total specimens collected were 60, which were reported histopathologically as follows-

Fibroadenomas – 5, fibrocystic diseases – 5, papillomas – 4, usual ductal hyperplasia – 4, atypical ductal hyperplasia – 2, IDC NOS – 33, metaplastic carcinoma – 2, papillary carcinoma – 2, medullary carcinoma – 1, mucinous carcinoma – 1, cribriform carcinoma – 1.

From the above [Table 1]: The benign lesions consisted of 5 cases of fibroadenoma, 5 cases of fibrocystic disease, all of which were positive for CK 5/6 with a staining index of 6+. Out of four cases of papilloma, 2 cases were positive with 6+ staining index, 2 cases were negative with 1+ staining index. 2 cases of UDH were positive with 6+ staining index and 2 cases of ADH were negative with 1+ staining index.

From the above [Table 2]: 33 cases of IDC NOS were included of which 4 were positive with a staining index of 5-6+. One case of metaplastic carcinoma, 1 case of medullary carcinoma were also positive for CK 5/6. 1 case of metaplastic carcinoma, mucinous carcinoma, cribriform carcinoma, 2 cases of papillary carcinoma were negative for CK 5/6.

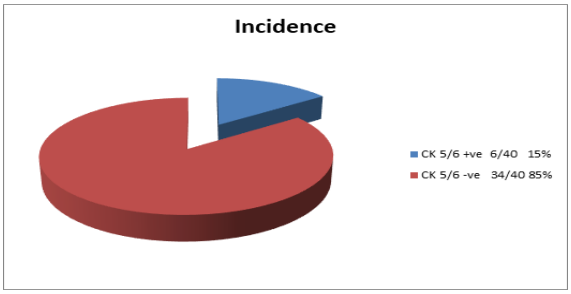

Figure 1: Incidence of CK 5/6 positive cases.

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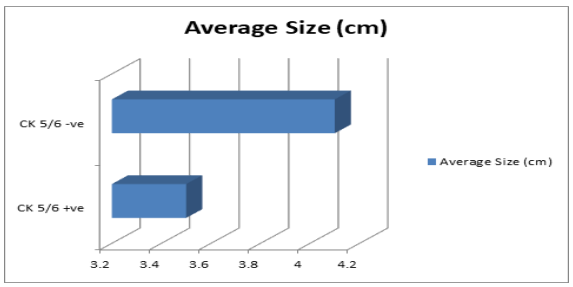

Figure 2: Tumor size of CK 5/6 positive and negative cases

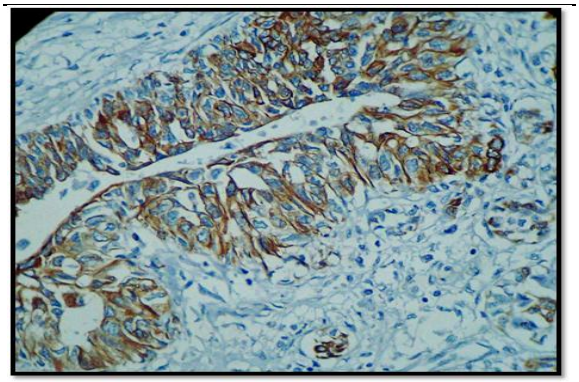

Figure 3: Fibroadenoma with UDH – IHC – CK5/6 – 40x

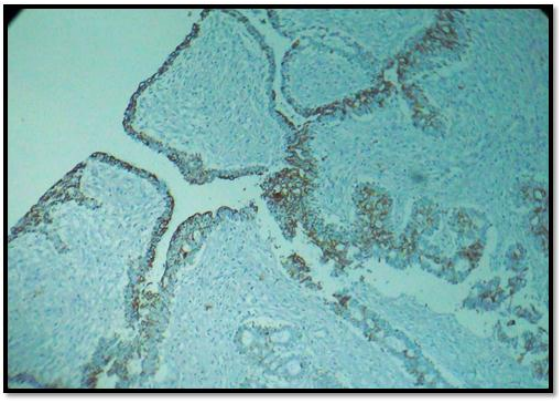

Figure 4: Fibroadenoma – IHC 5/6 10x.

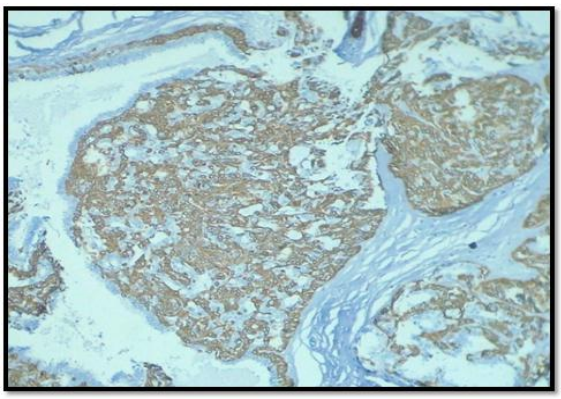

Figure 5: Fibrocystic disease with UDH – IHC-5/6-10x.

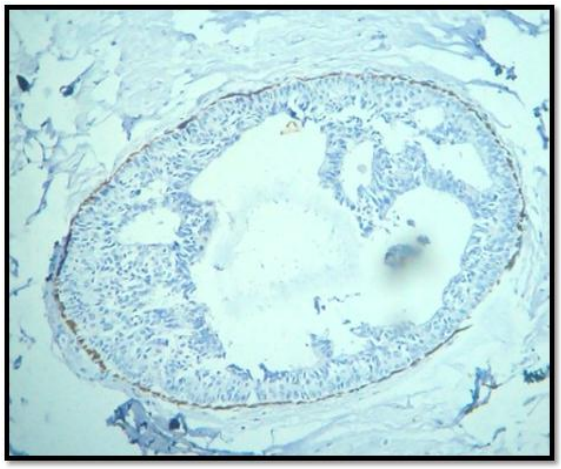

Figure 6: Atypical ductal hyperplasia IHC 5/6 – 40x.

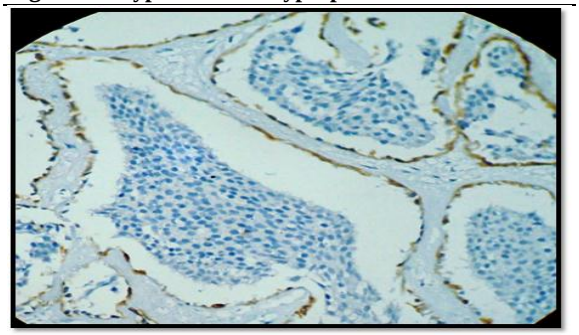

Figure 7: Ductal carcinoma in situ IHC 5/6 – 40x.

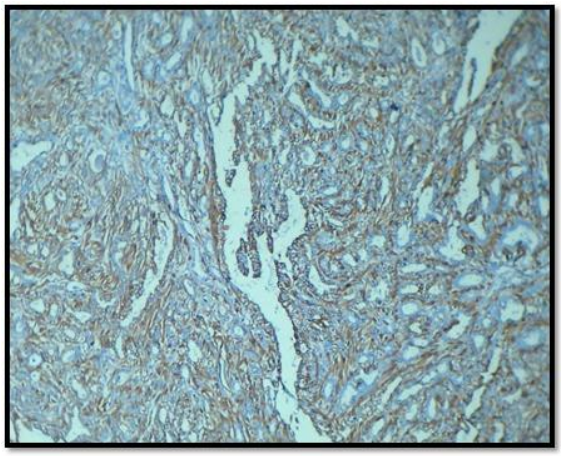

Figure 8: Adenosis with papillomatosis – IHC 5/6 10x.

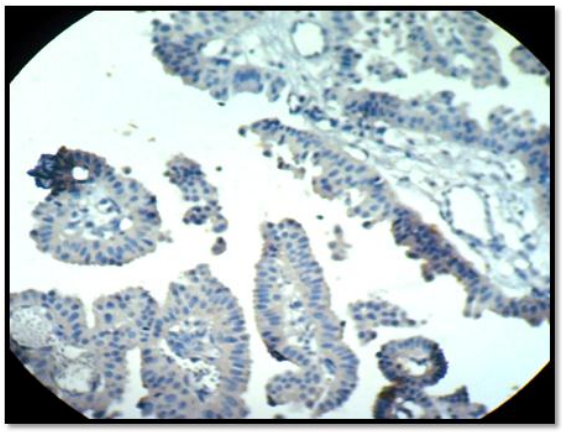

Figure 9: Atypical papilloma – IHC 40x.

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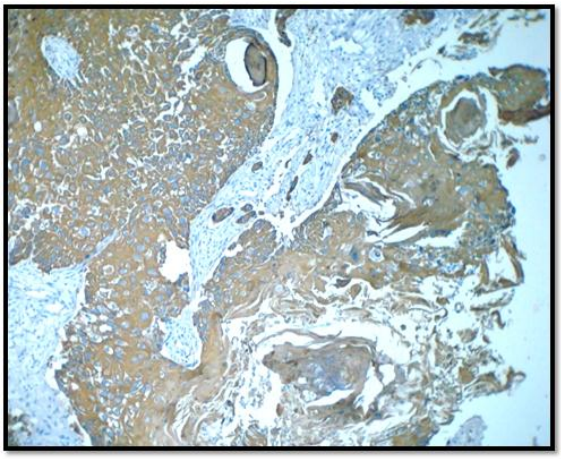

Figure 10: Metaplastic carcinoma IHC CK 5/6 10x

Table 1: Benign breast lesions – CK 5/6 expression

Benign breast lesions						
Histological diagnosis	**No. Of cases**	**Age**	**Ck 5/6 expression**			
			Positive	**Staining index**	**Negative**	**Staining index**
Fibroadenoma	5	20-42	5/5 – 100%	6+	0	-
Fibrocystic disease	5	24-40	5/5 – 100%	6+	0	-
Papilloma	4	30-70	2/4 – 50%	6+	2/4 – 50%	1+
Usual ductal hyperplasia	4	17-38	4/4 – 100%	6+	0	-
Atypical ductal hyperplasia	2	55-75	0	-	2/2 – 100%	1+

Table 2: CK 5/6 expression in breast carcinoma.

Breast carcinoma	**No of cases**	**Age**	**Grade**	**Lymph node status**	**Cytokeratin staining**	
					Positive	**Negative**
IDC NOS	33	32-80	I -12(36.36) II- 11(33.33) III – 10(30.30)	Reactive 12(36.36) Positive 21(63.63)	4/33	29/33
Metaplastic	2	38-45		Reactive	1/2 (50%)	1/2 (50%)
Papillary	2	70-80		Negative	0	2
Mucinous	1	52		10/13 positive	0	1
Medullary	1	70		Reactive	1	0
Cribriform	1	55		3/6 positive	0	1

Table 3: Comparison of CK 5/6 positive and CK 5/6 negative cases

Parameters	**CK 5/6 positive cases**	**CK 5/6 negative cases**
Incidence	6/40 - 15%	34/40 - 85%
Age	35-70 yrs avg 47.66 yrs	32-80 yrs Avg 56.20 yrs
Size	2.5 to 4.5 avg 3.5 cm	1.5 to 8 cm Avg 4.1 cm
Grade	100% grade III	I -12/34-41.37% II-11/34-37.93% III-6/34- 20.68%
Lymph node status	4 positive 66.66% 2 reactive 33.33%	Positive – 55.88% Reactive – 44.11%

DISCUSSION

The normal resting breast tissue is composed of luminal cells which express CK 8/18, CK 7, CK 19. The basal / myoepithelial cells express CK 5/6, CK 14, CK 17 and smooth muscle actin. A small subset of cells, comprising less than 5% of entire population express CK 5. These cells are dispersed in the inner layer of ductal system and differentiate into myoepithelial or glandular cells via intermediary cells.

The proliferated luminal cells in the benign lesions show a large number of CK 5/6 positive cells because of proliferation of both glandular and basal cells. Most malignancies are derived from differentiated glandular cells and do not reveal immunohistochemical staining on the CK 5/6 grading, thus explaining its negativity in most lesions of atypical hyperplasia and ductal carcinoma in situ. In the present study, all cases of fibroadenomas and fibrocystic disease showed positive immunoreactions with high staining index which is in concordance with Bhalla et. Al study. Papillary lesions account for between 3 – 5 % all core needle biopsy diagnosis and include broad spectrum of entities including benign intraductal papilloma with or without epithelial hyperplasia, benign papilloma with atypia of limited or greater extent, papillary DCIS, intracystic

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papillary carcinoma and invasive papillary carcinoma. Correct categorization of papillary lesions remains a challenge for pathologists and this is especially so on core biopsies because of limited material and fragmentation of tissue cores. Many studies have shown CK 5/6 to be a useful adjuvant to morphology in distinguishing atypical papillary lesion from benign papillomas. In the present study, two cases were positive for CK 5/6 indicating a benign proliferation and 2 cases were negative for CK 5/6 indicating atypical papillary proliferation. Shah VI et al.[8] concluded immunohistochemistry (CK 5/6, calponin, p 63) increases accuracy of diagnosis of papillary lesions on core biopsies.

The treatment and prognosis of ADH and DCIS differs significantly from UDH, but morphology often have overlapping features, thus giving rise to interobserver variability. The diagnosis of UDH can be assigned in cases of intraductal proliferation where they are diffusely positive for CK 5/6 immunostain because of proliferation of both luminal and myoepithelial cells. In case of ADH and DCIS, the intraductal proliferation is clonal and composed of only luminal cells and no myoepithelial cells, thus negative for CK 5/6. Only the retained myoepithelial cells layer in the periphery stains positive for CK 5/6. In the present study, 4 cases of UDH were positive for CK 5/6 with high staining index and 2 cases of atypical ductal hyperplasia were negative for CK 5/6, which is in concordance with Sharon Nofech – Mozes et al,[9] study. Many studies have concluded that CK5/6 may enhance the ability to differentiate between benign and malignant epithelial proliferations.[10] Nayak A et al,[11] have suggested that antibodies for CK5/6 and HMWK (34bE12) may be useful in determining the presence of DCIS at surgical margins even in the event of severe cautery artifact.

Breast cancer represents morphologically, molecularly and prognostically heterogeneous group of tumours. In order to describe the prognosis of individual patient more exactly, the histological classification of breast cancer by World Health Organization could be combined by DNS-microarray analysis in order to determine the molecular subtypes are potentially predictive of patterns of response to specific therapeutic agents. For instance, luminal A tumors are expected to be sensitive to endocrine therapy, HER-2– overexpressing tumors can be targeted with monoclonal antibodies against HER-2 or HER-2 tyrosine kinase inhibitors, and basal-like cancers may respond to specific therapeutic regimens and inhibitors of the poly (ADP-ribose) polymerase (PARP)enzyme.[11] However, gene expression microarrays have not become a routine practice in pathology laboratories yet. In contrast, immunohistochemistry (IHC) nowadays is a routine investigation. IHC markers like expression of oestrogen (ER) and progesterone (PR) receptors, and HER-2 protein can be used as surrogates for DNA-microarrays in subtyping the breast cancer. Breast cancer was classified in four molecular subtypes of which basal like breast cancer, a major subset of triple group has been the focus of extensive research. Basal-like tumors typically show high expression of genes characteristic of the basal epithelial cells of the normal mammary gland, including stratified epithelial cytokeratins, such as cytokeratins.[5,14,15] and 1712. The expression of basal markers has been reported in 2-18% of ductal invasive carcinoma cases, and in 25% of G3 breast carcinoma.[1] Metaplastic or anaplastic carcinomas, widely recognized as tumours with an unfavourable prognosis, are basal-like breast cancers. In the present study the incidence of BLBC is 15%. Basal-like breast cancers account for around 15% of all invasive ductal breast cancers of no special type with a higher prevalence among African-American women.[12] Age is one of the most important validated prognostic factors for breast cancer.[13] The characteristic feature of BLBC is early age of onset below 50 years. In the present study 4 cases were below 50yrs and 2 cases were above 50 yrs. Conventional histopathological as well as molecular studies of breast cancers with "basaloid" differentiation have shown that basal-like tumors are often high grade, have areas of necrosis, may have a typical or an atypical medullary phenotype.[12] In the present study all cases were histological grade 3 with areas of necrosis and were 3cm or more in size. In a study by Bhalla et al,[6] all malignant cases showing a positive immunoreaction for cytokeratin 5/6 were three centimeters or more in size, showed necrosis on gross examination and were grade III tumors with a high mitotic index. Similar findings were reported by Lakhani SR.[14]

In the present study, four cases out of the six exhibited metastatic lymph node involvement. Matt van de Rijn 15 observed that expression of basal type cytokeratins in node negative breast carcinoma was a prognostic factor independent of tumor size and tumor grade. It was associated with significantly shorter survival, but held no predictive value in patients with known lymph node metastases. On the contrary, it was report 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. Tot T reported CK 5/6 positivity in 25% of the typical, 43% of the atypical and 20% of the metastatic medullary carcinomas 6. In the present study,1 case of medullary carcinoma is reported positive for CK 5/6.

William D Foulkes et al,[16] and Sunil R Lakhani et al,[14] have studied ck5/6 expression in BRCA 1 breast cancers and found significant correlation. It is important given that mutation testing is very expensive and carries implications both for the individual and their family. Unlike expression profiling, the use of cytokeratin markers and ER assessment could be easily incorporated in the vast majority of pathology laboratories, providing a widely available and relatively inexpensive tool with which to identify patients for referral for genetic testing.

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Table 4: Comparison study with the Present study.

PARAMETERS	PRESENT STUDY 2013	BHALLA et .al 2010
Incidence	6/35 = 17.5%	24%
Size	>3 cm	>3 cm
Histological grade	6/6 Grade III	6/6 Grade III
Lymph node metastases	4/6	4/6

CONCLUSION

From this study we conclude that Ck 5/6 is a useful antibody frequently applied to help differentiate UDH from ADH and DCIS, Intraductal papilloma from intraductal papillary carcinoma, CK 5/6 positivity imply a 'basal like' molecular phenotype and signify poor prognosis. These tumors require an aggressive intervention. The patients with this subtype of breast cancer, along with their first degree relatives must be subjected for BRCA1 mutation testing.

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