Original Article

CD-10: An Emerging Biomarker in Prognostication of Infiltrating Duct Carcinoma Breast

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ABSTRACT

Background: All carcinomas of the breast can be classified based on the hormone receptors on cells and also by cell of origin. Generally most of health setup in developing countries carries out assessment of tumour behavior for breast cancer based on three parameters: tumour size, lymph node status, and histological grade. A few better established medical institutes have added immunohistochemistry to forecast the tumour outcome and guide the clinicians for better management of such patients. In carcinoma breast prognostic panel consisting of Estrogen Receptor (ER), Progesterone Receptor (PR) & Her2neu is being commonly used for prognostication of the Infiltrating duct carcinomas (IDC). With new breakthrough in the field it has become clear that stroma also plays a significant role in the spread of carcinoma breast and the proliferation rate of tumors has been shown to be a good predictor of aggressiveness of the tumor, hence new markers such as Ki67gene over expression and CD 10 have been added to the armament for prognostication . The aims of this study are to estimate the frequency of expression of stromal CD10 in invasive breast carcinomas and also to assess prognostic significance of stromal CD10 marker.

Methods: In this study, we examined 20 cases of invasive breast carcinoma. Apart from routine H&E stain, the selected cases were stained with concurrent immunohistochemical prognostic panel (ER, PR, Her2neu) and CD10 stromal marker, to characterize and to identify prognostic markers that can identify tumors with more aggressive behavior.

Result: We found 06 cases were of the triple-negative phenotype (ER, PR, Her2neu) and all exhibited strong CD10 stromal positivity. The majority of these tumors were grade III, IDC. There were positive associations with larger size, pushing margins, poorer Nottingham Prognostic Index (NPI). In all tumors, we considered tumor size, lymph node stage, and hormone receptors as the most useful prognostic markers and found that the traditional markers indicating poor outcome correlated well with CD 10 stromal positivity in 80% cases.

Conclusion: In the present study all triple-negative cases with positive nodal status , larger tumor size and higher grade, the hormonal expression revealed a strong correlation with CD 10 marker which appears to be a very strong potential and the most useful emerging prognostic marker.

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Introduction

Carcinoma breast is a growing menace world over and is taking its toll relentlessly. It is the leading cause of death in Indian women. It is estimated that about 1, 15,000 new patients are added every year and there are approximately 53,000 deaths.[1] Human breast carcinomas represent a heterogeneous group of tumors that are diverse in behavior, outcome, and response to therapy. Workers from all over the globe are trying to come up with novel strategies for early diagnosis as well as to find markers for better prognostication. Traditionally, prognostication in breast cancer relied on the clinicopathological parameters and individual molecular markers such as hormone receptors. All carcinomas of the breast can be classified based on the hormone receptors on cells and also by cell of origin. Assessment of tumor behavior for any breast cancer has been based on three parameters: tumor size, lymph node status, and histological grade. Authors have suggested many tools which have been have been used to improve the predictive value of the above individual factors; these include TNM staging and Nottingham prognostic index (NPI). To these, hormonal status of patients is added to pronounce the prognosis of these cases. These include Estrogen receptor (ER), Progesterone receptor (PR), and Her2neu. [2]

With new research in the field it has become clear that stroma also plays a significant role in the spread of carcinoma breast and the proliferation rate of tumors has been shown to be a good predictor of aggressiveness of the tumor, hence new markers such as Ki67gene over expression and CD 10 have been added to the armament for prognostication of these tumours. Although breast cancer is an epithelial malignancy, never the less the stroma has a key role in its development and pathogenesis. The scientists have dwelled upon this fact and are trying to establish that stromal markers may become novel factors in assessing the prognosis of invasive breast cancer. These have not been studied extensively till date. More recently, a combination of CD10 with the established four markers (ER, PR, Her2neu and Ki67) has been shown to have a strong prognostic impact that is similar to that of gene expression assays described by many studies. Expression of CD10 has been sometimes observed in the stromal cells of invasive ductal carcinoma, but its clinical significance has never been studied. It has been recently documented that CD-10 is present on the stromal cells in some cases of carcinoma breast. It has also been postulated that CD-10 is upregulated in these cases.

CD10 is a cell surface neutral endopeptidase that is not consistently expressed in the stromal cells of the normal breast. Normally the basal cells are ER negative but they are CD-10 positive and therefore CD-10 is used as a marker for basal cells. CD-10 is a 90- to 110-kDa cell surface zinc dependent metalloproteinase which is known as “Common Acute Lymphoblastic Leukaemia Antigen” (CALLA). It is now known that CD-10 is constantly expressed by the myoepithelial cells of the human breast during development and after maturation. It is considered a specific and useful marker for myoepithelial cells. [3]

The aims of this study are to estimate the frequency of expression of stromal CD10 in invasive breast carcinomas and also to assess prognostic significance of stromal CD10 marker.

Material and Methods

This study investigated 20 cases of invasive breast carcinoma obtained from patients presenting from January to December 2015. Patient’s clinical history and tumor characteristics were assessed in a uniform fashion. A scheme for prognostication was formulated to follow up these cases for assessing a disease free interval (DFI) and to detect the overall survival (OS). The DFI was defined as the interval (in months) from the date of the primary surgery to the first loco-regional recurrence or distant metastasis. The OS was the time, in months, from the date of the primary surgery to the time of breast cancer-related death. The NPI was calculated by using the following equation: NPI = 0.2 tumor size (cm) + grade (1–3) + lymph node score (1–3). [4] A total of 20 cases of infiltrating carcinoma of breast were included in the study. Representative sections were taken and Hematoxylin and Eosin (H&E) staining was done (Fig1). Immunohistochemistry was performed with ER (Fig3), PR(Fig4), Her2neu(Fig5), and CD10(Fig2), stromal marker. Stromal expression of CD10 (>10% stromal positivity was considered positive) in IDC was noted and was statistically analyzed with different known traditional prognostic markers of breast carcinoma.

Result

In the current study, 20 cases of IDC were analysed for 3 traditional markers ER,PR and Her2neu and we correlated them with CD10 stromal positive cases. We encountered a triple-negative phenotype in 06 cases. All these patients were >40 years of ageThe majority (95%) of tumors were ductal carcinoma of no special type (duct/NST) Table 2 shows the main features of triple-negative tumors compared with nontriple-negative tumors concerning different clinicopathological variables and biomarkers used in the current study. Triple-negative phenotype was associated with larger size, grade 3 tumors, and pushing margin. Significant association was found with lymph node

status. The stromal invasion seen by H&E stained section and positive CD 10 status was well delineated in all triple negative phenotypes (Figs. 1 -5).

We found 06 cases were of the triple-negative phenotype (ER, PR, Her2neu) and all exhibited strong CD10 stromal positivity. The majority of these tumors were grade III, IDC. There were positive associations with larger size, pushing margins, poorer Nottingham Prognostic Index (NPI). In all tumors, we considered tumor size, lymph node stage, and hormone receptors as the most useful prognostic markers and found that the traditional markers indicating poor outcome correlated well with CD 10 stromal positivity in 80% cases.

Table: 1.Relation of CD10 with Age, Tumour size, Lymphnode (LN) metastasis and HPE Grade

S. No	Parameter	No: of Cases	CD10 +ve	CD10-ve
1.	Age >40 years	20	11	09
2.a.	Tumour size<5cms	04	03	01
2.b.	Tumour size>5cms	16	10	06
3.a.	Axillary LN +ve	10	08	02
3.b.	Axillary LN-ve	10	03	07
4.a	HPE Grade I	01	00	01
4.b.	HPE Grade II	07	05	02
4.c.	HPE Grade III	12	09	03

Table:2 Comparison of CD10 with traditional IHC Panel

S. No	IHC	No: of Cases	CD-10 Positive	CD10 Negative
1.a	ER +ve	14	06	08
1.b	ER-ve	06	06	00
2.a	PR+ve	05	04	01
2.b	PR-ve	15	07	08
3.a	Her2neu +ve	14	10	04
3.b	Her2neu -ve	06	06	00
4.	Triple –ve	06	06	00

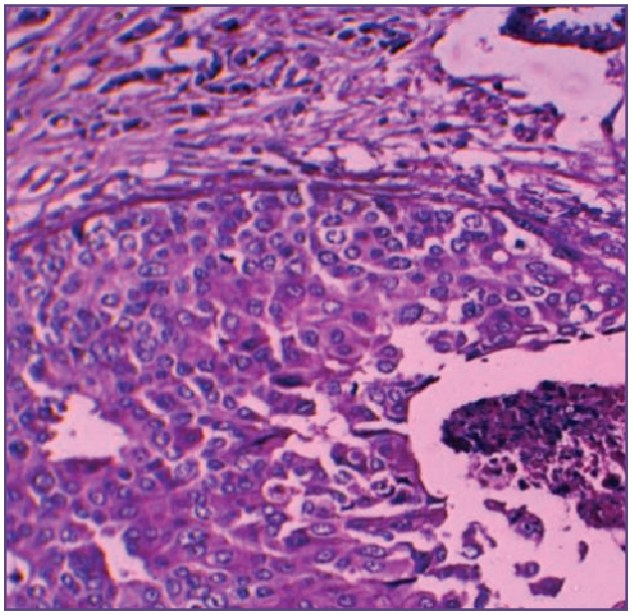

Fig. 1: Stromal Infiltration H & E(40x)

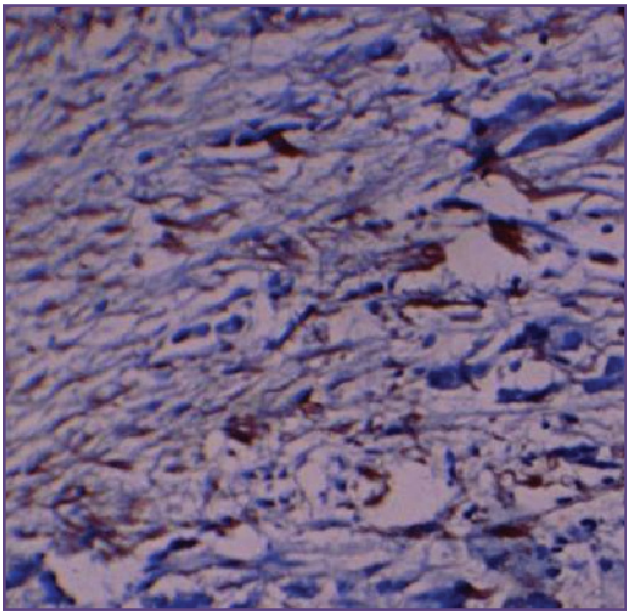

Fig. 2: Stromal Infiltration CD-10 (10x)

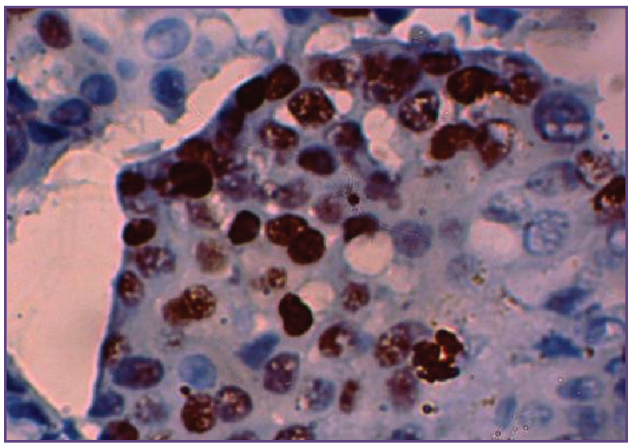

Fig. 3: ER positive (40x)

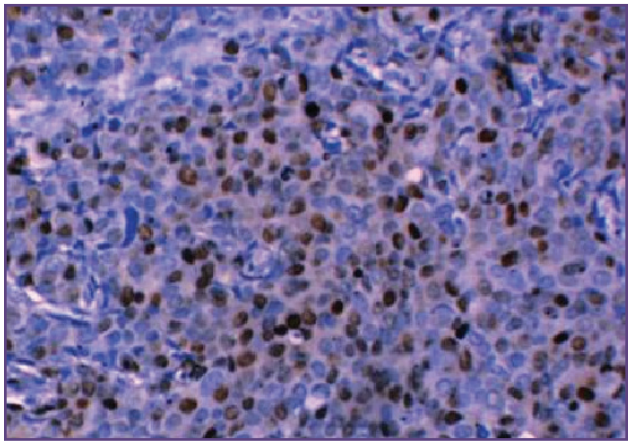

Fig. 4: PR positive (10x)

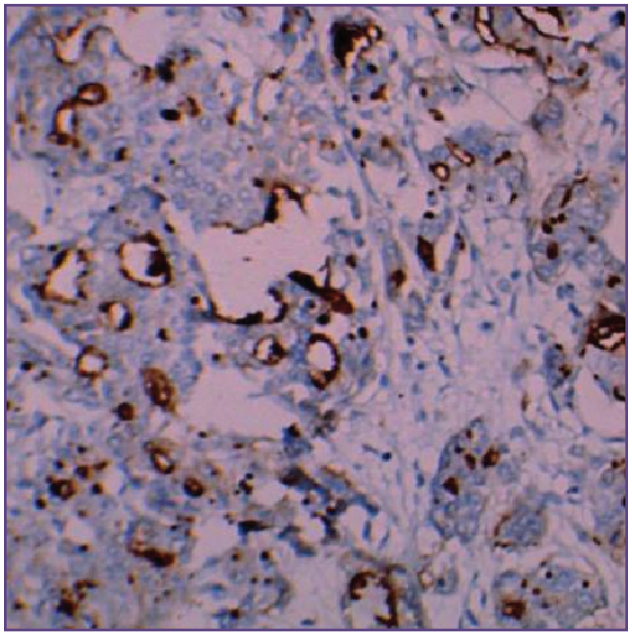

Fig. 5: Her2neu positive (40x)

Discussion

Proliferation of stromal cells is a common feature in cancer invasion and metastasis. CD10 is a zinc-dependent peptidase (metalloproteinase), surface neutral endopeptidase which degrades a variety of bioactive peptides. Earlier studies suggested that CD10 expression in tumor stroma is associated with biological aggressiveness of the tumor. To date, only one study has addressed the clinical significance of stromal CD10 expression in IDC. [5]

In the present study, we used immunohistochemical staining to investigate the expression of CD10 in the stroma of IDC. Normal myoepithelial cells lining the acinar and ductal structures in normal parenchyma adjacent to the tumor were used as positive internal control. According to the scoring system adopted, stromal CD10 expression was found in 80% of the cases (including 25% weakly positive and 55% strongly positive specimens) which is a significant frequency. Makretsov et al. found stromal CD10 expression in 79% of IDC. [6] Masaki et al. proposed that the CD10 expression to be considered positive when more than 10% of the stromal cells in vicinity of the neoplastic epithelial cells, were positive. Based on this criterion, they detected stromal CD10 expression in 19% of IDC. [7] In this study we had 45% cases showed CD10 positivity in stroma surrounding the sheets of neoplastic epithelial cells.

We in this study also observed that the lymph node metastasis correlates significantly with stromal CD10 positivity; therein the CD-10 is maximally positive in 08 out of 10 cases having lymph node metastasis which is in concordance with the study by Cui yazhon et al who reported this biomarker as positive in cases associated with lymphnode metastasis. [8] Our study shows that out of the 20 cases included, 12 were grade III and out of these 09 exhibited strong CD-10 positivity which is in agreement with the findings reported by Nikita A Makretstov et al in a study that was done on 438 cases and exhibited the maximum CD-10 positivity in grade III cases. We found that other than grade III tumours (60%), there were Grade II cases (35%) were also CD-10 positive. Stromal expression of CD10 was found to be significantly associated with increasing tumor grade .According to a study by Makretsov et al. percentage positivity of strong CD10 increased from 29% to 59% in grade I to grade III. Our results also showed very similar trends. One case in our study was of IDC with extensive in situ component. Extensive in situ component was clearly highlighted by strong positivity of CD10 in myoepithelial cells. The same case also showed foci of strong stromal CD10 positivity indicating invasion. [6] Martinez et al. proved in their study that there is a relationship between the presence

of extensive intraductal component and the risk of local recurrence for patient with IDC treated with conservative surgery and radiation surgery. [9]

We in this study concentrated on the most commonly used traditional biomarkers in vogue; those are ER, PR and Her2neu and their relation with CD10 stromal marker. The hormone receptor-negative group includes absent hormone receptor (ER & PR) and absent Her2neu expression called as triple-negative subtype of IDC. These tumours have been reported to have very poor prognosis. In our study we found 14 cases were ER Positive (Fig3) and Her2neu positive (Fig5). All 06 ER negative and Her2neu negative cases correspondingly showed CD10 stromal positivity (Fig2). Only few studies are available which have emphasized that extensive intraductal CD 10 positive component constitutes a very important predictive factor for local recurrences. One such study showed statistically significant correlation between strong CD10 staining and ER negativity. We also report strong positivity for the CD 10 marker in all 06 ER negative cases. Therefore in our study, CD10 was found to have good correlation with ER negativity; however, it was not statistically significant probably due to less number of cases in our study. Makretsov et al. showed statistically significant correlation between strong CD10 positive staining and ER negativity. [6]

Same study also reported no statistical significance between stromal CD10 expression and PR status. This is in agreement with our study, where CD10 was found to have good negative correlation with PR status. In this study we found only 07 mild CD10 positivity in 15 PR negative cases whereas 05 PR positive cases showed CD10 positivity in 04 cases.

Conclusion

Though this was a pilot study the follow up was possible in two cases that are disease free for last 10 months. Another 03 patients who were triple negative are also doing well for last 06 months. None of the CD10 positive cases have shown any evidence of recurrence till now.

When we stratified the cases in the present study all triple-negative cases with nodal status positive, larger tumor size and higher grade, the hormonal expression revealed a strong correlation with CD 10 marker which appears to be a very strong potential and the most useful prognostic marker.

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Competing Interests

None declared

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