ORIGINAL RESEARCH PAPER

Pathology

KEY WORDS: CD44; Cancer Stem Cells; TNBC-triple-Negative Breast Carcinoma; NTNBC-non Triple Negative Breast Carcinoma; IBC-Invasive Breast Carcinoma; IHC-Immunohistochemistry.

# ROLE OF CD 44 AS CANCER STEM CELL MARKER IN TRIPLE NEGATIVE AND NON -TRIPLE NEGATIVE INVASIVE BREAST CARCINOMA IN A TERTIARY CARE CENTRE – A COMPARATIVE STUDY

**Dr Nivedha J** Postgraduate Resident, Department of Pathology, BJGMC, Pune, Maharashtra, India.

**Dr Deepak Dangmali** Associate Professor & Guide, Department of Pathology, BJGMC, Pune, Maharashtra, India.

**Dr Sharada Rane** Professor & HOD, Department of Pathology, BJGMC, Pune, Maharashtra, India.

**ABSTRACT**

**Background:** Triple-negative breast carcinoma (TNBC) lacks estrogen receptor, progesterone receptor, HER2 expression and is associated with aggressive biological behavior with limited targeted treatment options. Cancer stem cells may contribute to tumor progression and treatment resistance. CD44 is a commonly studied cancer stem cell marker. This study compared CD44 expression in TNBC and non-triple-negative breast carcinoma (NTNBC). **Methods:** A comparative study was conducted in tertiary care centre in Pune from July 2023 to December 2024. 72 histopathologicaly confirmed invasive breast carcinoma of no special type cases were included, comprising 36 TNBC and 36 NTNBC cases. Representative tumor paraffin blocks were evaluated by immunohistochemistry for ER, PR, HER2 and CD44. CD44 positivity was defined as at least 2+ circumferential membranous staining in at least 10% of tumor cells. Associations were assessed using chi-square testing; $p<0.05$ was considered statistically significant. **Results:** CD44 positivity was observed in 20/72 cases (27.8%). Positivity was significantly higher in TNBC (16/36; 44.4%) than NTNBC (4/36; 11.1%; p=0.002). Among CD44-positive cases, all TNBC cases showed moderate-to-strong staining (2+, 75%; 3+, 25%), whereas NTNBC showed weaker staining (1+ in 100%, 2+ in 14% and 3+ in 0 cases); the difference in staining intensity was significant (p=0.001). CD44 expression was not significantly associated with age (p=0.152), laterality (p=0.231) or tumor grade (p=0.300). **Conclusion:** CD44 expression was substantially more frequent and stronger in TNBC than NTNBC in this study. These findings support a potential association between CD44 expression and the aggressive phenotype of TNBC.

## INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in women worldwide and remains a major cause of cancer-related mortality [1]. Triple-negative breast carcinoma (TNBC), defined by absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), represents a biologically aggressive group with fewer targeted treatment options than hormone receptor-positive or HER2-positive tumors. [2] Much research is going on in this category of breast cancer. One such research on the identification of cancer stem cell (CSC) niches in tumors playing a central role in tumorigenesis. [3,4]

Cancer stem cells (CSCs) are a subpopulation of tumor cells characterized by self-renewal and differentiation capacity and have been implicated in tumor initiation, metastasis and treatment resistance. [5] CD44 is a transmembrane glyco-protein involved in cell adhesion, migration and signaling and has been extensively investigated as a CSC-associated marker. [6,7] The CD44+/CD24 phenotype has been associated with enhanced invasive properties in breast cancer, while CD44 isoforms may show heterogeneous expression across breast cancer subtypes. [8,9]

This study investigates the expression of CD44, a cancer stem cell marker, in patients with TNBC and NTNBC- IBC at a tertiary care center. This comparison could provide insights into the aggressive nature of triple-negative breast cancer and its reliance on cancer stem cell pathways.

Higher CD44 expression in triple-negative IBC may highlight its potential as a therapeutic target, especially for this aggressive subtype with limited treatment options. Conversely, understanding CD44's role in non-triple-negative IBC could refine existing therapies by identifying patients who might benefit from targeted interventions. Overall, this research underscores the importance of targeted medicine to CD 44 and TNBC that might possibly improve the prognosis.

### Aim

Comparison of CD44 expression as a cancer stem cell marker in TNBC and NTNBC of IBC -NST.

### Objectives

To assess the role of CD44 expression in both groups (TNBC and NTNBC)

To determine its statistical association with histological grade of IBC- NST.

## MATERIALS AND METHODS

This was a comparative study conducted at a tertiary care centre in Pune during July 2023–December 2024. Ethical approval was obtained from the Institutional Ethical Committee. The study included 72 histopathologically confirmed (IBC-NST) cases, equally divided into 36 TNBC and 36 NTNBC cases.

Inclusion criteria were IBC-NST cases. Invasive breast carcinomas of other special types, cases treated with neoadjuvant chemotherapy and male breast carcinoma were excluded. Demographic and clinicopathological information were collected using a predefined case-record form. Breast biopsy, lumpectomy and modified radical mastectomy specimens were evaluated.

Representative paraffin blocks were subjected to immuno-histochemistry for ER, PR, HER2 and CD44. Sections of 4 μm thickness were stained using a polymer-HRP detection system with DAB chromogen.

Evaluation of Immunohistochemical expression ER, PR, Her 2 neu and CD44:

The samples were considered positive for ER or PR if ≥1% of tumor cell nuclei were immunoreactive. [10]

HER2/Neu reporting was done as per the standard criteria. [11]

CD 44 Assessment of positivity or negativity for CD44 was performed semi-quantitatively from immunohistochemically stained sections of tumor samples from patients. Tumor cells were localized using 10x objective and the level of positivity was graded for CD44 staining as 0 (negative), 1 (mild), 2 (moderate), and 3 (strongly positive). Positive staining for CD44 was defined as minimum 2+ circumferential

membranous staining in atleast 10% of the tumor cells.[12,13] Only membranous staining in tumor cells is scored and included in our study; stromal or non-membranous staining is not included.

Data were entered into Microsoft Excel and analyzed using SPSS version 22.0. Frequencies, percentages, means and standard deviations were calculated. Chi-square and Kappa tests were used where appropriate. A p-value <0.05 was considered statistically significant.

## RESULTS

The study included 72 cases, with equal numbers of TNBC and NTNBC. The most frequent age group overall was 41–50 years (22/72; 30.6%), followed by 61–70 years (19/72; 26.4%). TNBC was most frequent in the 41 to 50 year group, whereas NTNBC was most frequent in the 61 to 70 year group. Biopsy was the most common specimen type, accounting for 54.1% overall.

Laterality was nearly balanced: 37 cases (51.4%) involved the right breast and 35 (48.6%) the left. TNBC showed equal left- and right-sided distribution, while NTNBC showed a slight right-sided predominance. The association between tumor laterality and TNBC/NTNBC was not statistically significant (p=0.814)

**Table 1: Comparison Among the Study Population and Corresponding P value**

| | | Group | | Total | P value |
|---|---|---|---|---|---|
| | | NTNBC | TNBC | | |
| Type of specimen | Biopsy | 17 | 22 | 39 | 0.185 |
| | Lumpectomy | 4 | 4 | 8 | |
| | Modified Radical Mastectomy | 14 | 10 | 24 | |
| | Toilet Mastectomy | 1 | 0 | 1 | |
| | | | | | |
| Laterality | Left | 17 | 18 | 35 | 0.598 |
| | Right | 19 | 18 | 37 | |
| | | | | | |
| CD 44 | Negative | 32 | 20 | 52 | 0.002 |
| | Positive | 4 | 16 | 20 | |
| | | | | | |
| Grade of tumor | Grade 1 | 10 | 6 | 16 | 0.444 |
| | Grade 2 | 20 | 21 | 41 | |
| | Grade 3 | 6 | 9 | 15 | |
| | | | | | |
| Cd44 grading | 1+ | 2 | 0 | 2 | 0.001 |
| | 2+ | 2 | 12 | 14 | |
| | 3+ | 0 | 4 | 4 | |

**Table 2: Size of Tumor Amongst the Study Population-NTNBC and TNBC (N- Number of Cases)**

| Group | | N | Mean | SD | P value |
|---|---|---|---|---|---|
| Size of tumor | TNBC | 13 | 5.00 | 1.01 | 0.89 |
| | NTNBC | 20 | 2.53 | 1.26 | |

CD44 expression was detected in 20/72 cases (27.8%). TNBC showed significantly higher CD44 positivity than NTNBC: 16/36 (44.4%) versus 4/36 (11.1%), respectively (p=0.002). Among CD44-positive cases, all 16 TNBC cases showed moderate-to-strong expression: 12 (75.0%) were 2+ and 4 (25.0%) were 3+. In NTNBC, two cases (50.0%) were 1+ and two (50.0%) were 2+, with no 3+ cases. The difference in CD44 staining intensity was statistically significant (p=0.001).

**Table 3: CD 44 Status Amongst Various Parameters of Our Study**

| | | CD 44 | | Total | P value |
|---|---|---|---|---|---|
| | | Negative | Positive | | |
| Age (in years) | <=40 | 4 | 6 | 10 | 0.152 |
| | 41-50 | 16 | 6 | 22 | |
| | 51-60 | 13 | 4 | 17 | |
| | 61-70 | 16 | 3 | 19 | |
| | >70 | 3 | 1 | 4 | |
| | | | | | |
| Laterality | Left | 23 | 12 | 35 | 0.231 |
| | Right | 29 | 8 | 37 | |
| | | | | | |
| Grade of Tumor | Grade 1 | 14 | 2 | 16 | 0.300 |
| | Grade 2 | 28 | 13 | 41 | |
| | Grade 3 | 10 | 5 | 15 | |

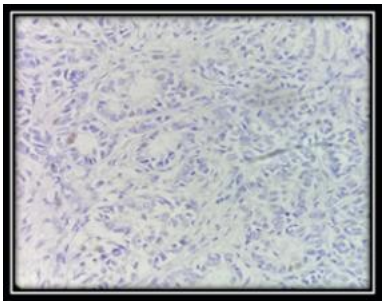


**Figure 1 : CD 44 – Negative (400X)**

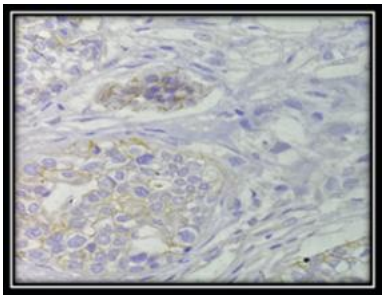


**Figure 2 : CD 44 – Positive 1+ (400X)**

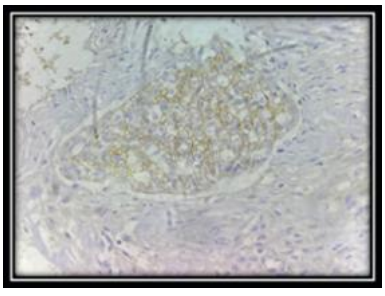


**Figure 3: CD 44 -Positive 2 + (400X)**

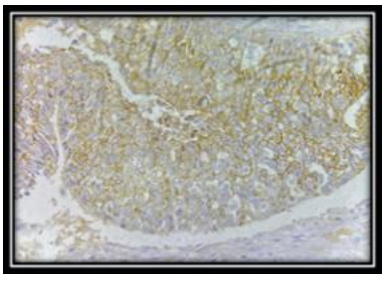


**Figure 4 : CD 44 positivity 3 + (400X):**

## DISCUSSION

Breast cancer is the most frequently diagnosed cancer in women globally and the primary cause of cancer-related deaths.[14] Triple Negative Breast Cancer (TNBC) is characterized by the lack of estrogen (ER), progesterone (PR), and HER2 receptors, is linked to a younger age at diagnosis and worse prognosis. [15] While targeted treatments are available for other breast cancer subtypes, no standard therapy exists for TNBC [16]. Ongoing research is focusing on cancer stem cells (CSCs), which are vital in tumor formation.

Treatment options have not yielded desirable outcomes for TNBC patients, necessitating the research on new prognostic parameters and therapeutic targets.[17]

Markers such as CD44, CD133, and ALDH1 are commonly used to identify CSCs.[18]

CD44, in particular, plays a key role in tumor growth, migration, and resistance to chemotherapy, with CD44 + cells in breast cancer exhibiting increased invasive abilities.[19]

Tomar et al. reported higher CD44 positivity in TNBC and an association with histological grade [15]. In the present study, CD44 was not significantly associated with grade (p=0.300), although positivity increased from 12.5% in grade 1 to 33.3% in grade 3. Age and laterality were also not significantly associated with CD44 expression (p=0.152 and p=0.231, respectively).

Al-Hajj et al. established the cancer stem cell concept in breast cancer, while Sheridan et al. demonstrated enhanced invasive properties in CD44+/CD24 breast cancer cells [20]. The present TNBC positivity rate (44.4%) was similar to the 40% reported by Punhani et al.; differences in populations, antibodies and scoring thresholds may explain variation between studies.[21]
Our results were comparable to research conducted by Almasi et al., Liu et al. and
Chekhun et al.[22,23,24]

The principal finding was significantly greater CD44 expression in TNBC than NTNBC. CD44 positivity was present in 44.4% of TNBC compared with 11.1% of NTNBC (p=0.002), and stronger staining was also concentrated in TNBC (p=0.001). This supports the concept that cancer stem cell-associated phenotypes may be enriched in biologically aggressive breast cancer.[25] In our study, CD44 expression and score distribution failed to show a significant correlation with tumor size and nodal spread. Our findings were parallel to observations made by Jang et al. 1 [26]

### Strengths and Limitations

Our study included a comparative study of 72 invasive breast carcinoma cases, divided into TNBC and NTNBC groups, allowing meaningful subgroup comparisons and enhancing the internal validity of the findings.

Also compared the CD 44 expression in both TNBC and NTNBC subtypes, providing valuable insight into the differential role of CD 44 as a cancer stem cell marker with potential prognostic and therapeutic implications.

The study had a relatively small sample size and was performed at a single centre. Clinical follow-up for recurrence, survival and treatment response was unavailable. Molecular subtyping of TNBC was not performed. CD44 was assessed semi-quantitatively by immunohistochemistry, with potential interobserver variability and functional assays were not performed to confirm stem-cell properties.

## CONCLUSION

CD44, a key breast cancer stem cell marker, has demonstrated a significant association with several prognostic factors in triple negative breast cancer. [27,28] In this cohort of 72 invasive breast carcinoma–NST cases, CD44 expression was significantly more frequent and stronger in TNBC than NTNBC. CD44 positivity occurred in 44.4% of TNBC and 11.1% of NTNBC cases (p=0.002), while stronger 2+/3+ staining was significantly enriched in TNBC (p=0.001). No significant association was demonstrated between CD44 expression and age, tumor laterality or histological grade. CD44 may therefore represent a useful marker for studying the CSC-associated biology of TNBC, but its prognostic and therapeutic value requires validation in larger, standardized, longitudinal studies.

### Declarations

Ethics Approval: Institutional Ethical Committee approval was obtained for the study.

Funding: No external funding.
Conflict of Interest: No conflict of interest.
Data Availability: Dataset maintained in institutional records; no public data-sharing statement was provided.

## REFERENCES

[1] World Health Organization. (2024, March 13). Breast cancer. https://www.who.int/news-room/fact-sheets/detail/breast-cancer.
[2] Yin, L., Duan, J. J., Bian, X. W., & Yu, S. C. (2020). Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Research, 22(1), 61.
[3] Oh, HJ., Kim, ST., Kim, HJ. *et al.* Multifaceted roles of CD44 in cancer progression and targeted therapeutic strategies. *Exp Mol Med* 58, 2451–2469 (2026)
[4] Chen, C., Zhao, S., Karnad, A., & Freeman, J. W. (2018). The biology and role of CD44 in cancer progression: Therapeutic implications. Journal of Hematology & Oncology, 11(1), 64.
[5] Ju, F., Atyah, M. M., Horstmann, N., Gul, S., Vago, R., Bruns, C. J., Zhao, Y., Dong, Q. Z., & Ren, N. (2022). Characteristics of the cancer stem cell niche and therapeutic strategies. Stem Cell Research & Therapy, 13(1), 233.
[6] Sneath, R. J., & Mangham, D. C. (1998). The normal structure and function of CD44 and its role in neoplasia. Molecular Pathology, 51(4), 191–200.
[7] Cabarcas SM, Mathews LA, Farrar WL. The cancer stem cell niche–there goes the neighborhood? Int J Cancer. 2011;129(10):2315–27
[8] Chen, C., Zhao, S., Karnad, A., & Freeman, J. W. (2018). The biology and role of CD44 in cancer progression: Therapeutic implications. Journal of Hematology & Oncology, 11(1), 64.
[9] Abdoli Shadbad M, Hosseinkhani N, Asadzadeh Z, Derakhshani A, Karim Ahangar N, Hemmat N, et. al. A Systematic Review to Clarify the Prognostic Values of CD44 and CD44+CD24- Phenotype in Triple-Negative Breast Cancer Patients: Lessons Learned and The Road Ahead. Front Oncol. 2021;11:689839. doi:10.3389/fonc.2021.689839.
[10] Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP Guideline Update. J Clin Oncol 2020;38:1346-66.
[11] Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. Arch Pathol Lab Med 2018;142:1364-82.
[12] Tomar, R., Rakheja, G., Verma, N., Thakur, S., Khurana, N., & Ghuliani, D. (2023). Role of CD44 as cancer stem cell marker in triple-negative breast cancer and its association with histological grade and angiogenesis. Indian Journal of Pathology and Microbiology, 66, 258–263.
[13] Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J., & Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. Proceedings of the National Academy of Sciences of the United States of America, 100(7), 3983–3988.
[14] World Health Organization. Global status report on cancer 2026: The Future We Choose Together. Geneva: World Health Organization; 2026
[15] Dogra, A., Doval, D. C., Sardana, M., Chedi, S. K., & Mehta, A. (2014). Clinicopathological characteristics of triple negative breast cancer at a tertiary care hospital in India. Asian Pacific Journal of Cancer Prevention, 15(24), 10577–10583.
[16] Al Saad, S., Al Shenawi, H., Almarabheh, A., Al Shenawi, N., Mohamed, A. I., & Yaghan, R. (2022). Is laterality in breast cancer still worth studying? Local experience in Bahrain. BMC Cancer, 22, 968.
[17] Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of Human Triple-Negative Breast Cancer Subtypes and Preclinical Models for Selection of Targeted Therapies. J Clin Invest 2011;121(7):2750–67. doi: 10.1172/JCI45014.
[18] Sharma, M., Sharma, J. D., Sarma, A., Ahmed, S., Kataki, A. C., Saxena, R., & Bhuyan, C. (2014). Triple negative breast cancer in people of North East India: Critical insights gained at a regional cancer centre. Asian Pacific Journal of Cancer Prevention, 15(11), 4507–4511.
[19] Chen, C., Zhao, S., Karnad, A., & Freeman, J. W. (2018). The biology and role of CD44 in cancer progression: Therapeutic implications. Journal of Hematology & Oncology, 11(1), 64.
[20] Sheridan, C., Kishimoto, H., Fuchs, R. K., Mehrotra, S., Bhat-Nakshatri, P., Turner, C. H., Goulet, R., Jr., Badve, S., & Nakshatri, H. (2006). CD44+/CD24 breast cancer cells exhibit enhanced invasive properties: An early step necessary for metastasis. Breast Cancer Research, 8(5), R59.
[21] Punhani, P., Ahluwalia, C., Agrawal, M., & Kandwal, P. (2024). Expression of CD44 in triple negative breast cancer and its correlation with prognostic parameters: Correlation of CD44 expression in TNBC. Archives of Breast Cancer, 11(4).
[22] Almási S, Nagy Á, Krenács T, Lantos T, Zombori T, Cserni G. The prognostic value of stem cell markers in triple-negative breast cancer. Pathol Oncol Res. 2023;29:1611365. doi: 10.3389/pore.2023.1611365.
[23] Liu YX, Wang KR, Xing H, Zhai XJ, Wang LP, Wang W. Attempt Towards a Novel Classification of Triple Negative Breast Cancer Using Immunohistochemical Markers. Oncol Lett. 2016;12(2):1240–56. doi: 10.3892/ol.2016.4778.
[24] Chekhun SV, Zadvorny TV, Tymovska YO, Anikusko MF, Novak OE, Polishchuk LZ. CD44+/CD24- markers of cancer stem cells in patients with breast cancer of different molecular subtypes. Exp Oncol. 2015;37(1):58-63.
[25] Wu, Z., Lu, J., Loo, A., Ho, N., Nguyen, D., Cheng, P. Y., Mohammed, A. I., & Cirillo, N. (2024). Role of CD44 in chemotherapy treatment outcome: A scoping review of clinical studies. International Journal of Molecular Sciences, 25, 3141.
[26] Jang MH, Kang HJ, Jang KS, Paik SS, Kim WS. Clinicopathological analysis of CD44 and CD24 expression in invasive breast cancer. Oncol Lett. 2016;12(4):2728-2733. doi:10.3892/ol.2016.4987
[27] Obidiro O, Battogtokh G, Akala EO. Triple Negative Breast Cancer Treatment Options and Limitations: Future Outlook. Pharmaceutics. 2023;15(7):1796. doi: 10.3390/pharmaceutics15071796.
[28] Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res. 2005;65(13):5506-11. doi: 10.1158/0008-5472.