**Tumor budding in breast carcinoma- As a potential novel prognostic marker**

**Introduction:**

Cancer is the second most important cause of death worldwide and breast cancer (BC) is one among the leading causes of cancer deaths. BC is the most prevalent diagnosed cancer presently and is the fifth cause of cancer deaths with an approximately 2.3 million new cases across the world and 685,000 deaths according to GLOBOCON 2020. It has the high incidence rate and is significantly affecting women’s health. BC is a heterogenous tumor and owing to this characteristic there are molecular and histological subtypes of BC with varied biological behaviors and clinicopathological features and finally resulting in significant variation in prognosis. In spite of a promising favorable outcome the metastatic potential of BC is a major factor determining the aggressiveness and poor prognosis. Irrespective of its favorable overall survival rate, the recurrence rate of BC within fifteen years crosses 40%. Hence, identification, assessment and standardisation of various prognostic markers are important for better management, prognosis and in targeting therapeutic approaches.

**Tumor budding**

Tumor budding (TB) is a pathologic event associated with many solid malignancies and was first introduced in colorectal cancer (CRC). It is traditionally defined as single cells or clusters of less than five malignant cells at the invasive front of the tumor. It has been extensively studied in solid malignancies of head and neck, lung and gastric, esophageal and colorectal cancers. Tumor buds can be seen near the tumor margins at the invasive tumor front which are called peritumoral buds and inside the tumor mass which are termed as intratumoral buds. TB is an emerging promising prognostic biomarker in solid malignancies.

TB was defined in 1989 by Morodomi as a collection of isolated cancer cells without distinct structure. Since the cells seemed to bud out from the large malignant mass they were named as “tumor budding”. Additionally, to determine the degree of TB the tissue section was divided into four areas measuring 500 × 2,500 µm and the mean number of buds per area was calculated. TB was defined by the Japanese classification as an isolated single cell or cluster of less than five cells at the invasive front of the tumor. The International Tumor Budding Consensus Conference (ITBCC) established a standardized evidence- based scoring system for CRC TB and defined tumor bud as a single cell or a cluster of ≤ 4 tumor cells. A three- tier system was advocated to grade TB. TB is assessed in a field of area 0.785 mm2 of invasive front. Scores of 0–4 indicate low budding (Bd1), 5–9 indicate intermediate budding (Bd2), and ≥ 10 indicate high budding (Bd3).

The 2019 World Health Organization (WHO) classification of CRC included TB as a second main grading criterion. In addition, the importance of TB in prognosis of CRC was stressed upon by including this feature as an additional prognostic factor in TNM classification of 2017 and 2019 WHO classification. It is considered as an independent predictor of lymph node metastasis in patients with pT1 CRC. Above and beyond, TB is also considered as a novel prognostic marker independent of tumor grade and stage in gastric, esophageal, pancreatic and urinary bladder malignancies. But role of tumor budding in breast cancer is still not confirmed.

**Pathophysiology of tumor budding**

TB has been considered as indicator of cancer cell motility and a first step in metastasis cascade. As we all know the metastastic process begins with detachment of tumor cells from the tumor bulk, infiltration through the contiguous extracellular tissue into the blood vessels and propagate in the blood stream to remote sites where they extravasate and ultimately establish metastatic deposits. The pivotal step in process of metastasis is epithelial to mesenchymal transition (EMT) and the opposite process of mesenchymal to epithelial transition (MET). These processes are together labeled as epithelial mesenchymal plasticity and are seen normally in embryogenesis and wound healing. The cells of tumor buds have cancer stem cell properties because of their properties of migration and redifferentiation. This was supported by the strong expression of CD44 and ALDH1A1 in tumor buds. TB promote pro- tumor development in the tumor microenvironment than anti-tumor immune response. High TB is seen to be associated with low CD 8+ T lymphocyte index resulting in bad prognosis.

There are many signal transduction pathways that are the main regulators of EMT like SNAIL, ZEB, TWIST which in turn activate EMT- transcription factors. These EMT- transcription factors are activated by TGF- β-SMADS, WNT/β- Catenin signalling, epidermal growth factor/ fibroblast growth factor- receptor tyrosine kinase signalling, Notch signalling and MAPK pathways. TB along with EMT is strongly associated with tumor microenvironment. Tumor microenvironment is characterized by hypoxia, acidity, inflammation and immunospression. The immune cells in tumor microenvironment secrete cytokines and chemokines which play a key role in EMT process. Cancer cells interact with immune cells to induce cell plasticity, release immunosuppressive substances and establish an immunosuppressive microenvironment to promote invasion and metastasis. Thus, TB and tumor microenvironment have a strong relation in metastatic cascade. Tumor buds overexpress stem cell markers like ALDH1, CD44 and LGR5 which equip them with ability to self- renew at primary or metastatic sites.

**Detection of tumor bud**

TB can be detected on hematoxylin and eosin (H& E) and immunohistochemical staining like pan-cytokeratin. Studies have revealed that budded cells exhibited lower proliferation activity and this indicates that dissociation of tumor cells is not affected by increased tumor size, differentiation or proliferation activity. Diminished E- cadherin expression was found in patients with high grade TB and unusual expression of vimentin in those with low grade TB. The possible mimickers of tumor buds include inflammatory cells, multinucleated giant cells, fibroblasts, endothelial cells, smooth muscle cells and artifacts.

There are many differences in nomenclature and methods for detecting and describing tumor budding. This leads to lack of uniformity in reporting tumor budding. The method of counting tumor buds, power of objective used and area of field in different studies were different. This has given rise to qualitative heterogeneity in reporting tumor budding. These variations highlights the requirement for training and standardization of method of counting tumor buds. There is also need to pay attention to TB mimickers and train the pathologists to identify them. Standardization will eventually reduce inter-observer variability and make way for enabling a single observer to accurately identify tumor budding.

**Tumor budding in breast cancer**

There are many studies which show that in solid tumors high grade TB is associated with lymphovascular invasion and lymph node metastasis. In addition, high TB is also associated with short overall and cancer specific survival in breast cancer and other solid malignancies. It is well established that cells in TB undergo EMT and thus are more invasive and chances of metastasis is high leading to poor overall survival. As a result, inhibiting these tumor cells involved in early metastatic cascade would be of gigantic clinical value as metastasis is the major cause of death in approximately 30% of breast cancer patients. But in breast cancer the information about the role of tumor budding is limited.

Many studies have showed that high grade tumor budding is significantly associated with ER- positive tumors. It has been reported that estrogen is involved in EMT in breast cancer cell lines with stem cell properties and estrogen is also involved in tight junction disruption and enhanced cell motility. Thus, it was proposed that ER- positive tumors with high grade TB may undergo high degree of EMT and hence has more potential for metastasis. If this is proved with further studies that anti-estrogen therapy may help in decreasing the degree of TB. Other studies showed that high grade TB predicted worse disease- free survival in those with HER2 positive, luminal A and triple negative breast carcinomas. Many studies have proved TB as an independent factor predicting cancer specific survival, irrespective of tumor microenvironment and pathological features.

There are several studies which have confirmed the prognostic value of TB in solid tumors. But there are various different ways of describing tumor budding. Hence, there is a necessity for a standardized protocol to assess TB in malignancies. With reference to breast carcinoma, TB is rarely examined and thus the results from available studies have to validated and a standard universally accepted parameters for scoring and cut off criteria have to be framed so that TB can be included as an additional prognostic biomarker in pathology reports. It is the need of the hour to establish a consistent pathological criteria to identify and quantify TB to improve accuracy and repeatability. This will definitely add useful prognostic information to the clinician. TB is strongly associated with lymphnode involvement and lymphatic invasion which will be useful in predicting prognosis.

**Automation in tumor budding**

Many newer gene sequencing methods like Oncotype DX, Mammaprint and Endoprint have been adopted to help prognosticate breast carcinoma. But their main drawback is that these tests are exorbitantly expensive and highly impractical to be implemented in under developed and developing countries. In this view, TB can serve as a cost effective and easily reproducible prognostic biomarker. But since the reporting of TB in breast cancer is not standardized, its implementation is still under question. This problem of standardization can be solved by automated tissue microarray systems or automated tumor budding evaluation tools. These automated methods can help in detecting the exact number of tumor buds per image along with good correlation with the manual quantification. Thus, similar to other fields automation can prove its efficiency in TB in future.

**Conclusion:**

High tumor budding is associated significantly with lympho-vascular invasion, lymph node metastasis, primary tumor staging and finally prognosis. Criteria for evaluating the tumor buds requires to be standardized and should be incorporated as an additional new parameter in the reporting protocol for breast cancer. Thus, tumor budding may serve as a prerequisite benchmark in evaluation invasive breast cancer.

**References:**

1. Sung H., Ferlay J., Siegel R.L., Laversanne M., Soerjomataram I., Jemal A., Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 2021; 71:209–249. doi: 10.3322/caac.21660.
2. Kim J, Rhee Y Y, Bae J M, Kim J H, Koh S J, Lee H J et al. Composite scoring system and optimal tumor budding cut‑off number for estimating lymph node metastasis in submucosal colorectal cancer. BMC Cancer. 2022; 22:861.
3. Gujam F J A, McMillan D C, Mohammed Z M A, Edwards J, Going J J. The relationship between tumour budding, the tumour microenvironment and survival in patients with invasive ductal breast cancer. BJC.2015; 113: 1066-1074. Of
4. Huang T, Bao H, Meng YH, Zhu JL, Chu XD, Chu XL, Pan JH. Tumour budding is a novel marker in breast cancer: the clinical application and future prospects. Ann Med. 2022 Dec;54(1):1303-1312.
5. Xiang Z, He Q, Huang L, Xiong B, Xiang Q. Breast Cancer Classification Based on Tumor Budding and Stem Cell-Related Signatures Facilitate Prognosis Evaluation. Front Oncol. 2022 Jan 10;11:818869

C 6. Voutsadakis IA. Prognostic role of tumor budding in breast cancer. *World J Exp Med*

2018; 8(2): 12-17.

1. 5. (2015) 113, 1066–1074 Journal of Cancer (2015) 113, 1066–1074 British Journal of Cancer (2015) 113, 1066–1074