Does tumor stroma ratio of breast cancer trucut biopsy determine response to neoadjuvant therapy?

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ABSTRACT

Aims and Objectives: We examined the prognostic value of Tumor stroma ratio (TSR) in breast tumor core biopsy (TCB) specimen to determine response to neoadjuvant therapy (NAT) prior to modified radical mastectomy (MRM).

Methods: This was a retrospective analysis of patients with breast cancer who underwent TCB before NAT between August 2016 and July 2018. TSR in TCB was studied independently by 2 pathologists (VM, VS) defined as stroma rich (TSR≤50%) or stroma poor (TSR>50%). MRM specimen of these patients were subsequently studied. Residual cancer burden (RCB) was calculated using the MD Anderson RCB calculator, categorized as complete (0), good (1) Partial (2) and no response (3). Statistical analysis was done to assess correlation of TSR to RCB.

Results: A total of 62 patients were analyzed. Mean(SD) age was 48(11) years. Twenty eight (45%) and 34 (55%) patients were stroma rich and stroma poor respectively. Twenty six (42%) patients were responders and 36 (58%) non-responders to NAT. Among stroma rich patients, only 3 (10%) were responders (Class 0 & 1) and 25 (90%) non-responders (Class 2&3) to NAT, among stroma poor patients 23 (68%) responded well and 11 (32%) did not. TSR had a moderate negative correlation with RCB (-0.6). On univariate analysis, only TSR had a significant effect on RCB class (<0.001).

Conclusions: TSR on TCB is a useful prognostic factor to determine response of breast carcinoma patients to neoadjuvant therapy. It is cost effective, simple and quick. Larger multi-centric studies would be useful to study its clinical implications.

KEY WORDS: Breast, neoadjuvant chemotherapy, residual cancer burden, tumor stroma ratio

INTRODUCTION

Breast Cancer is major cause of morbidity and mortality in India with age adjusted rates of 25.8 and a mortality of 12.7 per 100,000. Neoadjuvant therapy (NAT) is administered before the initiation of loco-regional treatment. Also termed as primary systemic therapy or preoperative therapy, the size of the primary tumor, the presence or absence of locally advanced disease and the hormone receptor status are the key factors in determining the relevance of NAT. Breast cancer is a heterogenous disease, it is imperative to look for response to treatment as it determines the disease free and overall survival. This is done by measuring the residual cancer burden (RCB) in post therapy specimens, which categorizes patients into responders (RCB 0- pathological complete response and RCB I-minimal residual disease) and nonresponders (RCB II –moderate residual disease and RCB III- extensive residual disease). The RCB is a numerical value generated by a webpage-based calculator from the MD Anderson Cancer Center, Houston, Texas, United States of America (www3.mdanderson.org/app/medcalc/index.cfm?pagename = jsconvert3).

This incorporates five parameters of prognostic significance, namely, the size of primary tumor bed, the percentage cellularity, the percentage of in situ component, presence or absence of nodal metastasis and the diameter of the largest node. The cross talk between the tumor and its stroma has gained momentum in recent times. The Tumor Microenvironment (TME) comprises of fibroblasts, cells of the immune system, blood vessels, adipose tissue, and products produced by these cells that enhance...
and promote their growth and spread. The Cancer associated Fibroblasts (CAF) are found at the invasive margins of tumor and produce tumor promoting growth factors, cytokines, and extracellular matrix (ECM) remodeling enzymes that play a role in dissemination of cancer by inducing vascular and cancer cell proliferation.[4-7] The cells of the immune system produce angiogenic factors accumulating in the hypoxic and necrotic areas around the tumor, thus enhancing tumor activity. The angiogenic factors produce vessels that are leaky promoting tumor progression. The fat cells produce adipokines which help in the release of fatty acids that act as food for the tumor cells thus promoting growth [Figure 1].[8-10]

The tumor stroma ratio (TSR) or the cancer percentage (CP) is the ratio of tumor cells to its microenvironment, emerging as a prognostic indicator, is known to influence overall and disease-free survival. It has known to be of prognostic significance in solid epithelial neoplasms such as breast, oral, esophagus, colon, and stomach.[4-6] Here we aimed to evaluate the role of TSR in determining response to NAT in breast carcinoma patients by assessing its correlation with RCB. To the best of our knowledge this has not been investigated before.

MATERIALS AND METHODS

A retrospective study was carried out on patients with Ductal Carcinoma breast (NOS) carcinoma who underwent trucut core biopsy before undergoing neoadjuvant therapy (with chemotherapy/trastuzumab/both) followed by MRM between August 2016 to July 2018. Baseline characteristics studied were age, menopausal status, hormone receptor, and Her2neu status. TSR in core biopsies were studied independently by 2 pathologists (VM, VS) on Hemotoxylin and Eosin (H and E) stained slides. The maximum stroma rich area was assessed with a 10X objective. Extra caution was taken to select an area which had cells all around the circumferential border. Stromal percentage was scored in multiples of 10 and was defined as stroma poor (TSR >50%) [Figure 2a] or stroma rich (TSR <50%) [Figure 2b].[7] MRM with axillary dissection specimen of these patients were studied in a blinded manner by the same 2 pathologists. Pathological data recorded were size of primary tumor bed [Figure 3a and b] (which was assessed from the detailed archives storing the gross description and further confirmed on microscopy), percentage cellularity [Figure 4], percentage of in situ component [Figure 5], nodal status and diameter of largest node. Residual cancer burden (RCB) was calculated using the MD Anderson RCB calculator which takes into consideration the aforementioned pathological characteristics and quantifies responsiveness to neo-adjuvant therapy.[5] A numerical RCB value is further stratified into RCB classes 0,1,2 and 3 which indicate complete, good, partial and no response, respectively. Classes 0 and 1 are considered as responders and classes 3 and 4 as non-responders.[5] Statistical analysis was done to assess the correlation of TSR to the final RCB, using SPSS version 20.

RESULTS

A total of 62 patients with complete data were analyzed. Only female patients were considered for study with mean (SD) age at the time of diagnosis being 48 (11) years. Twenty-seven (43%) patients were post-menopausal. Triple positivity, triple negativity, and non-triple negativity was seen in 5 (8%), 19 (31%), and
38 (61%) patients, respectively. Twenty-eight (45%) and 34 (55%) patients had stroma rich and stroma poor core biopsies respectively. Final pathological findings were as follows: Median (IQR) of: Tumor bed area was 1500 (2400) sq mm and percentage cellularity was 11% (10). Twenty-nine (47%) patients were node positive. Median (IQR) node number was 2 (4) and nodal diameter was 11.3 mm (10). Mean (SD) RCB was 2.12 (1.53), RCB class 0,1,2 and 3 was seen in 12 (19.3%), 14 (22.5%),15 (24.1%) and 21 (33.8%) patients, respectively. Totally, 26 (42%) patients were considered to be responders and 36 (58%) non-responders. Among stroma rich patients, only 3 (10%) were responders and 25 (90%) non-responders to NAT and among stroma poor patients 23 (68%) responded well and 11 (32%) did not [Tables 1 and 2]. On univariate analysis, only TSR correlated negatively with RCB (60% correlation, $P < 0.001$), none of the other factors determined RCB.

**DISCUSSION**

Malignant epithelial tumors consist of two components, tumor cells, and surrounding supporting framework that is the stroma. The stromal component has been an area of extensive research, as it plays a role in tumor motility, growth, invasiveness, angiogenesis, and metastasis. TSR is a surrogate marker contributing to tumor aggressiveness as it is shown that low stroma tumors (TSR >50%) were associated with a better prognosis and increased disease free survival and high stroma tumors (TSR <50%) with an adverse outcome.[4‑7] The role of TSR in the prognosis of epithelial malignancies has been investigated. Wang K et al. stated that stroma rich esophageal squamous cell carcinomas were associated with a poor outcome.[12] West NP et al. also postulated that low TSR was associated with poor cancer specific survival in colorectal carcinoma.[13] Stroma rich oral cancers had a poor outcome and could be used for prognostication as concluded by Almangush A et al.[14] Similarly in breast carcinoma, the amount of tumor associated stroma was found to be an independent risk factor for disease free overall survival.[5,15]

The unpredictable response to NAT is explained by the heterogeneity of the disease.[3,16] It is extremely important to evaluate the response to NAT as it helps to identify residual disease (RD) and predict distant relapse free survival. The

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**Table 1: Table depicting the relationship of TSR with RCB Class**

| RCB class | TSR Rich | TSR Poor |
|-----------|----------|----------|
| RCB 0     | 12 (19.3%) | 3 (0%)   |
| RCB 1     | 14 (22.5%) | 15 (24.1%) |
| RCB 2     | 15 (24.1%) | 21 (33.8%) |
| RCB 3     | 21 (33.8%) | 26 (42%)  |

**Table 2: Table depicting relationship of NAT with stromal cellularity**

| Response to NAT vs TSR | Stroma Poor | Stroma Rich |
|------------------------|-------------|-------------|
| Non-responders         | 32%         | 0%          |
| Responders             | 68%         | 10%         |

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**Figure 4:** Photomicrograph showing the residual cellularity separated by dense fibrosis unresponsive to therapy (H and E, ×400)

**Figure 5:** Photomicrograph showing the *in situ* component of tumor resistant to NAT (H and E, ×100)
Residual Cancer Burden (RCB) calculator incorporates routinely evaluated variables on the post treatment Modified Radical Mastectomy Specimens. These include primary tumor bed dimensions that were that were recorded after gross assessment of the sample and further confirmed on reviewing the microscopy, the percentage cellularity of the tumor bed, the percentage of in situ component, the nodal status and the diameter of the largest positive node. The RCB is tabulated as RCB I- pathological complete response, RCB II-minimal RD, RCB III- moderate RD, RCB III- extensive RD. RCB 0 and I were responders and RCB II and III non responders. In the present study we tried to analyze the significance of TSR on trucut biopsies in determining the response to NAT by evaluating RCB on post treatment surgical resections of the corresponding patients. It was inferred that a high tumor stroma ratio resulted in low residual disease and a low TSR was associated with a higher RCB class. The therapeutic drugs act on rapidly proliferating cells, hence more the cancer percentage, the better the response of tumor to the drugs. None of the other parameters studied (age, menopausal status, hormonal or Her2/neu status) correlated with the RCB. Hence TSR is a simple method, requiring no additional cost and can be incorporated in daily practice. This could potentially help oncologists to decide about further management at the level of breast cancer trucut biopsy itself and could be a potential tool to predict responsiveness to neo-adjuvant therapy. Larger studies are required to validate the association of TSR with RCB.

There is a bidirectional talk between the stroma and tumor cells. The stroma primarily composed of the adaptive and innate immune cells, fibroblasts, vascular, and lymphatic endothelial cells and the adipocytes, plays a role in tumor growth, progression, and epithelial mesenchymal transition. A stroma rich tumor has more of these constituents and less of tumor cells. The fibroblasts are either in a normal state or activated state. In breast cancers especially the stromas rich breast cancers 80% fibroblasts are in the activated state. Drugs inhibiting activation of fibroblasts and antifibrotic agents may reduces aggressiveness of the lesions. These may also reduce the collagen content of the microenvironment thus allowing better drug delivery. Antiangiogenic drugs can also be potential oncotargets in future. Administering anti TGF-β therapy could prevent development of osteolytic metastasis. Targeting the tumor microenvironment is a new concept in anti-cancer therapeutics still in trial phases and will require larger studies over longer periods to see its efficacy.

**CONCLUSION**

Tumor stroma ratio is a simple, useful, and practical parameter to predict residual cancer burden following neo-adjuvant therapy in breast cancer. It is worth studying this in multicentric studies with larger number of patients.

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**Conflicts of interest**
There are no conflicts of interest.

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