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Assessment of Inter-observer Reproducibility of the Residual Cancer Burden Index and Neoadjuvant Chemotherapy Response in Breast Carcinoma

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ABSTRACT

Background: Locally advanced breast cancers are nowadays treated with neoadjuvant chemotherapy (NAC) to downstage the tumor. One way to assess the NAC response is to use Residual cancer burden index (RCB).

The aim of the study was to assess inter-pathologist reproducibility of residual cancer burden index and to evaluate different clinico-pathological parameters that determine the pathological response.

Methods: In this retrospective cohort study, surgically excised specimens of 49 NAC treated breast cancer were examined by histopathology and immunohistochemistry over a period of one and a half years. Four pathologists with different levels of experience reviewed the reports (unaware of original RCB index) and the slides and assigned the RCB indices for each case. Clinical, histopathological and immunohistochemical parameters were evaluated. Fleiss-Kappa statistics were applied to assess interobserver reproducibility of RCB index.

Results: No significant relationship was observed between RCB index (of original report) with age, largest tumor dimension and the number of chemotherapy cycles. The RCB index of 49 cases as assigned by four pathologists was calculated by Fleiss-kappa statistics, which showed good overall agreement (82.6%).

Conclusion: It has been observed that there is no significant relation between pathological response to NAC with age, largest tumor dimension and the number of chemotherapy cycles of breast carcinoma. It can also be concluded that RCB can be reliably used to report the neoadjuvant chemotherapy treated specimens of breast cancer.

INTRODUCTION

Breast cancer is the most common cancer and a leading cause of death in women. Locally invasive breast cancers are often treated with neoadjuvant chemotherapy (NAC). Pathological complete response (pCR) is defined as the absence of invasive breast cancer (in breast as well as in nodes) after the completion of chemotherapy. Disease-free and overall survival depends on response to standard NAC in invasive breast cancer.[1] Invasive breast cancer shows different responses to NAC ranging from complete absence of disease to extensive residual disease. More than 15 grading systems including Miller-Payne system, AJCC staging system, residual cancer burden (RCB) system, Neo-Bioscore etc., have been proposed to evaluate the pathological response. Among them, RCB index developed by MD Anderson Cancer centre is most

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commonly used.[2-5] RCB can be calculated by a web-based calculator as score or category. It has four categories which include RCB 0 showing pathological complete response, RCB 1 showing minimal residual disease, RCB 2 showing moderate residual disease and RCB 3 showing extensive residual disease. The index is based on several histopathological parameters, such as two-dimensional size and cellularity of tumor bed, percentage of in situ cancer, number of involved nodes and diameter of largest metastasis. It provides more quantitative information about the response unlike other systems which are more descriptive.[2] However, there is concern regarding the subjectivity of this grading system specially in assessing cellularity.[3] To date, only few studies have verified the feasibility of RCB index in stratifying the prognosis of patients treated with NAC, with no study on Indian population.[4,5]

The aim of the present study was to assess the inter-observer agreement of RCB index when slides were examined by four pathologists with different levels of expertise in breast pathology. Several clinical and pathological parameters were also evaluated.

METHODS

Study design and participants

This retrospective cohort study was conducted on 49 cases of locally invasive breast cancer treated with standard regimen of NAC (consisting of anthracycline based chemotherapy regimen with or without additional taxane) and subsequently operated by modified radical mastectomy. The grossing, histopathological examination, immunohistochemistry interpretation and reporting were done according to standard protocol (described below). The residual cancer burden was reported according to RCB index as per institutional mandate. All relevant history of the patient, like age, menstrual status, breast radiology findings, pre-operative size of the tumor, number of chemotherapy cycles, trucut biopsy/Fine needle aspiration cytology findings, and molecular subtypes were recorded. All the patients meeting the above criteria, with all slides available for review and written consent for inclusion in the study were recruited as study participants.

Grossing methodology

During grossing, radiology findings were evaluated. The formalin fixed specimen was oriented and measured and all surfaces were inked with different colors. The specimens were serially sliced at 5 mm thickness exposing the largest cross-section of the tumor bed. The slices were serially placed on the table, palpated carefully to find the firm area or the presence of clips. When the tumor bed was identified, the size and distance from margins were documented. Those sections representing the full face of tumor bed were studied in multiple sections. A photograph of the tumor bed was taken and the diagrammatic map was made for the sections taken.

Microscopic evaluation

For the present study, four pathologists with different ages and experiences were recruited. Among them, one had 30 years of experience and others had 15 years, 10 years and 9 years of experience, consecutively. They were provided with published materials and web site instructions to calculate the RCB index. The pathologists reviewed the gross finding report and the archival slides of the cases (unaware of the original RCB score and category). They individually graded each case and assigned the RCB score and category using online RCB calculator.[2] (https://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3). The association between the original RCB index (issued by the department) with the age of the patient, largest tumor dimension, number of chemotherapy cycles and molecular subtypes were analysed and inter-pathologists agreement was evaluated.

RCB scoring parameters included the following: (1) Cellularity which is the percentage of the tumor bed area that contains malignant cells (invasive or in situ). Cellularity is assessed by comparison with the chart provided in the online calculator; (2) estimate of the percentage of the carcinoma in situ in the tumor bed; (3) two dimensions of the tumor bed containing residual cancer in millimiters; (4) the number of residual cancer positive lymph nodes; (5) the longest diameter of largest nodal metastatic deposit in millimetres. The RCB class 0 represents pCR and classes 1-3 represent the increasing extent of the residual cancer.

Statistical analysis

The relationship between the age of the patient, size of the tumor, number of chemotherapy cycles and molecular subtypes with RCB indices were evaluated by the Chi-square test. Continuous variables (e.g., age, tumor size and number of chemotherapy cycle) were divided into categories to use the Chi-square test for relationship analysis.

The inter-observer-reproducibility of RCB indices on 49 cases between four pathologists was calculated using Fleiss-kappa statistics by an online calculator.[6] Fleiss-kappa is a statistical measure for assessing the reliability of the agreement between a fixed number of raters when assigning categorical ratings to a number of items or classifying items.

Kappa values range from –1 to +1. The higher the value of kappa, the stronger the agreement, as follows: Kappa = 1, perfect agreement exists; Kappa = 0, agreement is the same as would be expected by chance; Kappa < 0, agreement is weaker than expected by chance, which rarely occurs.[6]

All statistical analyses were performed using SPSS16, at a P-value< 0.05.

RESULTS

In the present study, 49 patients were included (mean age of 52 years), out of whom 39(80%) were above 45 years. There was no statistically significant association between age and NAC response (P-value= 0.38). The mean tumor bed diameter was 4.8 cm. The size of the tumor bed dimension was divided into two groups: those less than or equal to 5 cm (26 cases, 53%) and those more than 5 cm (23, 47%). No statistical significance was found with the size of the tumor bed and response to NAC (P-value=0.8). There was no statistically significant correlation between neoadjuvant chemotherapy cycles (less than or more than 4 cycles) with RCB categories (P-value =0.49). We could retrieve the IHC data of 36 cases, among whom 20 (56%) cases were of Luminal subtype, 8 (22%) were Her 2 neu positive and 8 (22%) were Triple negative (Table 1).

The cases were reviewed independently by four pathologists without knowing the original diagnosis. Individual pathologist's diagnosis (RCB score), as the percentage of the total number of cases is depicted in Figure 1. In the analysis, the four pathologists were treated similarly and none of them was considered providing a reference score. The RCB index of all the cases as assigned by the four pathologists was statistically calculated by Fleiss-kappa statistics and the overall agreement was calculated to be 82.6%. The free marginal kappa was 0.76 and fixed marginal kappa was 0.71 (Figure 1).

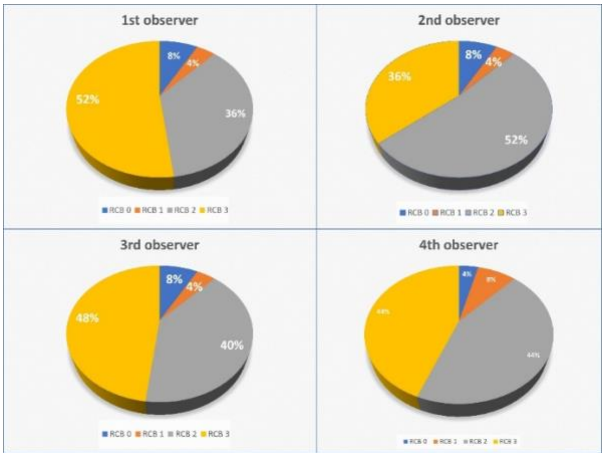

Figure 1. Individual pathologist's diagnosis (RCB score), as the percentage of 49 cases. Fleiss-kappa statistics showed the overall agreement of 82.6%. The free marginal kappa was 0.76 and the fixed marginal kappa was 0.71.

Table 1. Relationship between clinicopathological parameters of the breast carcinoma patients treated with NAC and RCB indices

Age (years) (n=49)	RCB 0 n (%)	RCB 1 n (%)	RCB 2 n (%)	RCB 3 n (%)	P value and χ^2 value
<45 (10), 20.4%	1 (10%)	1 (10%)	2 (20%)	6 (60%)	P= 0.38
>45 (39), 79.6%	1(2%)	2 (4%)	18 (46%)	18 (46%)	χ^2 =30.3
Size of tumor (mm) (n=49)					
<=50 (26), 53%	1(3.8%)	1(3.8%)	10(38.5%)	14(53.9%)	P= 0.84
>50 (23),47%	1(4.3%)	2(8.6%)	10(43.5%)	10(43.5%)	χ^2 =0.81
Number of chemotherapy cycles (n=49)					
<4 (26), 53%	1(3.8%)	1(3.8%)	12(46.1%)	12(46.1%)	P= 0.91
>4 (23),47%	1(4.3%)	2(8.6%)	8(34.7%)	12(52.3%)	χ^2 =0.49
Molecular subtypes(n=36)					
Luminal (20), 56%	0	0	8(40%)	12(60%)	
Her2neu (8), 22%	0	0	4(50%)	4(50%)	
Triple negative (8), 22%	2(25%)	0	4(50%)	2(25%)	

Chi-square test

The pairwise observer agreement between the pathologists ranged from 57.1 to 83.67% and the pairwise kappa value ranged from 0.43 to 0.78 (Table 2).

Table 2. Pairwise observer agreement and kappa values

Observer pairing	Percent overall agreement	Free marginal kappa
Observer 1 and observer 2	57.14%	0.43
Observer 1 and observer 3	67.35%	0.56
Observer 1 and observer 4	48.98%	0.32
Observer 2 and observer 3	83.67%	0.78
Observer 2 and observer 4	75.51%	0.67
Observer 3 and observer 4	75.51%	0.67

DISCUSSION

Many grading systems have been proposed to report post-neoadjuvant chemotherapy treated surgically excised specimens of breast cancer such

RCB index, Miller-Payne system, Residual Disease in Breast and Nodes, etc.[2,4,5] Among them, the RCB index formulated by MD Anderson Cancer Centre is the most relevant and commonly used.[2] Sahoo *et al.* have argued that almost all available post-NACT assessment methods at present are similar to each other except for RCB which is a web-based system[7]. Sejben *et al.* recommend the use of RCB index in histopathology, as this classification makes the best distinction in outcomes.[8] It has been thoroughly explained with directions on how to use this grading system and a freely accessible online calculator is available.[2]

Out of all our cases, 10 belonged to the age group less than 45 years and the rest were above this age. Twenty cases belonged to luminal subtype, 8 to Her2neu positive subtype and 8 to triple positive subtype. Also, 26 patients had received four cycles of neoadjuvant chemotherapy and the rest of them had received 5-8 cycles of the same chemotherapy. The largest tumor dimension was less than or equal to 50mm for 26 cases. All these parameters have shown that they have no significant impact on the response to chemotherapy as graded using RCB index. However, Lv *et al.* showed that high histological grade, negative HER2 status and lymph node metastasis, positive HER2 status, and taxane-based regimens were significantly associated with the achievement of pCR with NAC.[9] Tang *et al.* have demonstrated that among 84 patients of ages less than 45 years and 189 patients of more than or equal to 45years, 12 and 26 patients achieved pCR, respectively. The P-value was not statistically significant. Additionally, among 230 patients, with a tumor size less than 5 cm and 43 patients with a tumor size more than 5cm, 32 and 6 patients achieved pCR, respectively. The P-value was not statistically significant. Thus, similar to our study, they also concluded that age and tumor size have no effect on NAC response.[10] Our sample size was small and a larger cohort is needed to establish a statistical correlation between clinical parameters affecting the response to NAC.

Peintinger *et al.* found that accuracy and overall concordance for the agreement in the RCB score among pathologists is significant and highly reproducible.[2] In our study, the Fleiss-kappa statistics have shown that the percentage of overall agreement among the pathologists was 82.31%, where the free marginal kappa was 0.76 and fixed marginal kappa was 0.71. Thus, it can be inferred from the results that RCB has good inter-pathologist reproducibility for reporting post-neoadjuvant chemotherapy treated breast cancer specimens, and can be reliably used for the same purpose.

For proper use of the RCB grading system, a training programme is highly recommended for better understanding from fixation to microscopy. It is also recommended that the tumor bed area be marked with pin during trucut biopsy, as it is not often discernible during the grossing of specimens after chemotherapy. Radiography is required for the identification of the tumor bed area as per the protocol, with photography being available at the grossing station. It is often very difficult to reconstruct and measure the tumor bed area by microscopy alone. Hence, proper orientation of the area by drawing pictures and meticulous grossing are necessary steps which were followed in this study. The entire tumor bed area embedding had been followed as per protocol and lymphovascular invasion was not included in the calculation. It is also recommended that cellularity percentage images be displayed in the reporting room to reduce inter-observer variation in reporting. In this study, we observed that out of 8 cases who demonstrated triple negative status, 2 patients (25%) achieved pCR with NAC. In other molecular subtypes, none of the patients showed pCR. Jeon *et al.* foundthat pCR rates were significantly lower in hormonal receptor positive patients, i.e., 55.4% compared to pCR rates in hormonal receptor negative patients which was 77.5%.[11] Jin *et al.* reported that hormone receptor positive and HER2 low/negative has a pCR rate less than or equal to 8% whereas in cases of hormone receptor negative with HER2 low and HER2 negative have pCR rates of 21.95% and 23.64%, respectively.[12]

Apart from the small sample size, another major limitation of this retrospective study was that the pathologists were uninvolved in the grossing, which could have been a potential cause of variability among them (i.e., grossing of specimens and identification of the tumor bed might be different).

CONCLUSION

It has been observed that there is no significant association between RCB indices with age of the patient, largest tumor dimension, and number of chemotherapy cycles. It can also be concluded that RCB can be reliably used to report specimens of breast cancer patients who have received prior chemotherapy.

ETHICAL CONSIDERATIONS

Ethical clearance was taken from Institutional Ethics Committee and approval was given by West Bengal University of Health Sciences, India.

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CONFLICT OF INTEREST

No conflicts of interest exist regarding the publication of the present study.

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