RESEARCH ARTICLE

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Assessment of Intratumoural and Stromal Infiltrating Lymphocytes in the Various Subtypes of Breast Carcinoma Patients who have Received Neoadjuvant Chemotherapy

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Abstract

Background: Breast cancer comprises a highly heterogeneous subset of tumours that respond well to Neoadjuvant Chemotherapy (NAC). Tumour Infiltrating Lymphocytes (TIL) act as a means to an end by shedding light on the treatment response as well as predictive factors to the clinicopathological features for the same. Therefore, this article attempts to shift the attention to the relevance of TIL in the aforementioned aspects by bringing to notice the contrasting traits displayed by them in the different immunohistochemical subtypes of breast carcinoma. **Materials and methods:** 75 triple-negative breast cancer (TNBC) patients, 25 human epidermal growth factor receptor (HER2BC) positive patients and 77 hormone receptor (HRBC) positive breast cancer patients were included in this study who received NAC before surgical excision of the tumour which was then stained using routine Haematoxylin and Eosin techniques. Standardised guidelines were used to evaluate TIL in the stroma and the tumour. **Results:** In TNBC, a significant association between Intratumoural (IT) TIL (p=0.0288) and Intrastromal (IS) TIL (p=0.0250) with pathological complete response (pCR). IS TIL and age at operation (p=0.0494) showed significant values but no correlation was found with IT TIL. In HER2BC, IS TIL revealed a significant association with the tumour response(p=0.0229). A strong association was found between IT TIL and the age of menopause(p=0.0441). In HRBC, no significant associations were found between IT and IS TIL scores and the clinicopathological features. **Conclusion:** The predictive factors of TIL and complete response post-neoadjuvant chemotherapy can be a strong indicative factor for immunohistochemical markers. It also helps throw light on further studies which can be carried out to determine the clinicopathological features and TIL correlation in the various subtypes of breast carcinoma.

Keywords: Tumour-Infiltrating Lymphocytes- neoadjuvant therapy- breast neoplasms- triple negative breast cancer

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Introduction

Breast Cancer is known for its diverse immunohistochemical sub-typing which provides insight into the growth pattern, clinicopathological features and prognosis of the same (Dai et al., 2015). The heterogeneous tumour displays a varied biological behaviour and in turn, requires a different therapeutic approach (Blows et al., 2010). concerning these marked mannerisms. Through this, the tumour microenvironment plays an influential role in the prognosis. The cell infiltrates which mainly consist of lymphocytes admixed with plasma cells, histiocytes, neutrophils and mast cells show variation according to the subtype of cancer (Segovia-Mendoza and Morales-Montor, 2019).

Neoadjuvant Chemotherapy is one such therapeutic approach employed to downstage the tumour and reduce the tumour burden and is preferred in human epidermal growth factor receptor 2 (HER2) positive and Triple Negative breast carcinoma (TNBC) subtypes (Brenton et al., 2005). since they have been proven to entail a worse prognosis. Contemporary works are being focused on the prediction potential of Tumour Infiltrating Lymphocytes (TIL) in regard to their response to neoadjuvant chemotherapy in the distinct subtypes of breast cancer patients (Subbiah et al., 2017).

The host response has also been shown to vary according to the type and time of initiation of chemotherapy. A study carried out be Pelekanou et al., (2017). Al studied the stromal TIL in breast cancer patients treated with neoadjuvant chemotherapy and deduced a higher TIL count in post-chemotherapy in comparison to

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pre-chemotherapy samples with an increase in the TIL count proving to have a 5-year longer recurrence-free survival rate (Pelekanou et al., 2017).

Another such comparison in the association of stromal tumour infiltrating lymphocytes before and after neoadjuvant chemotherapy in TNBC and HER2-enriched breast cancer was undertaken by Ochi et al., (2019). Those who found a low TIL count before neoadjuvant chemotherapy in TNBC and HER2-enriched Breast Cancer indicated a low likelihood of pCR in these individuals. They also found TNBC with Residual Disease to show both low counts of TIL before and after neoadjuvant chemotherapy which predicted a shorter recurrence-free survival (Ochi et al., 2019).

A study carried out by Lv et al., (2011) in Northeast China focused on determining the predictive role of the molecular subtypes in the tumour microenvironment post-neoadjuvant chemotherapy. It was found that the TNBC subtype followed by the HER2 positive subtype had the highest pCR rates and was most sensitive to Neoadjuvant Chemotherapy.

This is indicative of the need for further studies to be mainly targeted at understanding the tumour microenvironment and the role it plays with the varied breast cancer subtypes in response to neoadjuvant chemotherapy to evaluate the effectiveness in the prediction of Clinicopathological features and treatment response.

Materials and Methods

The following observational study was conducted on archival material after Institutional Ethics Committee approval was obtained. 180 patients with FNAC/Core Needle Biopsy confirmed breast cancer patients were included who had received neoadjuvant chemotherapy before surgical excision of the tumour. The post-surgically resected tumour specimens were sectioned and stained using routine Haematoxylin and Eosin techniques. The authors excluded patients who had inflammatory or benign lesions of the breast.

The total 180 specimens were categorised into five groups based on their Immunohistochemical classification:

Group A: Triple Negative Breast Cancer. (TNBC)

Group B: Human Epidermal Growth Factor Receptor 2 (HER-2/neu positive) Enriched Breast Cancer. (HER2BC)

Group C: Hormonal Receptor-Positive Breast Cancer (ER positive, PR positive or Both). (HRBC)

Group D: Triple Positive Breast Cancer. (TPBC)

Group E: Luminal B Breast Cancer.

Pathological complete response (pCR) was defined as there being no invasive and no in situ residuals in the breast and nodes according to Gunter von Minckwitz et al., 2012 Pathological complete response for each subtype was determined by the means of the above definition (von Minckwitz et al., 2012).

The evaluation and analyses of the IT and IS TIL were done according to the guidelines put forward by Salgado et al., (2015). Depending on the percentage, the evaluation was done as High-grade (50 - 90% TIL) or Low-Grade (0 – 10% TIL) (Intermediate grade: 20 - 40% TIL. For the intermediate group different areas were evaluated at higher magnification) (Salgado et al., 2015). For the above, 2 slides from the same tumour at two different levels were evaluated and the average value of the 2 slides was taken as the final value. Depending on the evaluation of TIL in stroma and tumour cells, a comparison between the clinicopathological and pCR for each breast cancer subtype was made.

Statistical Analysis

Data was collected and entered by categorising the patients according to their breast cancer subtype and features. Univariate analysis was conducted by carrying out chi-squared tests on a 2x2 contingency table and data was analysed on InStat software with a p-value < 0.05 considered significant with a 95% confidence interval.

Results

Out of the total count, 179 patients were female and 1 patient was male. The patients were subdivided according to the immunohistochemical status and the treatment response (i.e. pCR) for each subgroup was found to be as given in Table 1.

Out of the 75 TNBC patients, 27 patients (i.e. 36% of TNBC patients) attained pCR. Followed by which 18 out of the 25 HER2BC patients (i.e. 72% of HER2BC patients) attained pCR. In HRBC - 10 patients (i.e. 12.99% of HRBC patients) out of 77 were found to have achieved pCR after being treated with Neoadjuvant Chemotherapy. pCR was not attained by any of the patients from Group D (TPBC) and Group E (Luminal B Breast Cancer). The individualised IT and IS TIL count according to breast cancer subtype is as given in Table 2.

While comparing the IS and IT TIL values with their

Table 1. Treatment Response Found in the Five Subtypes of Breast Cancer

Group	Subtype	Number of patients (x)	Percentage (x/180)*100	Number of patients who attained pCR (y)	Percentage of patients who attained pCR (y/180)*100	Percentage of patients who attained pCR with respect to individual subtype (y/x)*100
A	Triple Negative Breast Cancer	75	41.67%	27	15.00%	36.00%
B	HER-2 Positive Breast Cancer	25	13.89%	18	10.00%	72.00%
C	Hormone Receptor Positive Breast Cancer	77	42.78%	10	5.56%	12.99%
D	Triple Positive Breast Cancer	2	1.11%	0	0.00%	0.00%
E	Luminal B Breast Cancer	1	0.55%	0	0.00%	0.00%
Total :		180	100%	55	30.56%	-

Table 2. TIL Score and Classification According to a Subtype of Breast Cancer

Group	Subtype	Number of patients	Intratumoural		Intrastromal	
			High	Low	High	Low
A	TNBC	75	36 (48.00%)	39 (52.00%)	57 (76.00%)	18 (24.00%)
B	HER-2 Positive Breast Cancer	25	10 (40.00%)	15 (60.00%)	22 (88.00%)	03 (12.00%)
C	HRBC	77	17 (22.08%)	60 (77.92%)	23 (29.87%)	54 (70.13%)
D	TPBC	2	02 (100.00%)	00 (00.00%)	02 (100.00%)	00 (00.00%)
E	Luminal B Breast Cancer	1	01 (100.00%)	00 (00.00%)	01 (100.00%)	00 (00.00%)
Total :		180	66	114	105	75

corresponding breast cancer Immunohistochemical subtype, it was found that TNBC (p=0.0008) showed a highly significant association with the IS and IT TIL score followed by HER2BC (p=0.0012) exhibiting the same, whereas HRBC (p=0.3582) did not show a significant relation with the TIL score. Group D and E were not statistically analysed due to the low number of patients in both groups (Table 3).

Furthermore, the breast cancer subtypes were individually separated and the IT and IS TIL scores were compared and contrasted with the clinicopathological features as follows:

Group A - TNBC (n = 75, All-Female)

1. Intratumoural TIL

A significant association was found between the pathological complete response (p=0.0288) and the IT TIL in the TNBC subtype as shown in Table 4. No correlation was found between any of the clinicopathological features in the TNBC subtype, on equating them with the IT TIL counts.

2. Intrastromal TIL

There was an association found between the IS TIL and Age (p=0.0494) in the TNBC subtype. A significant association was also observed between the pathological complete response (p=0.0250) and the TNBC IS TIL as seen in Table 5. No other such relation was seen between the clinicopathological features on equating them with the IS TIL counts in the TNBC subtype.

Group B - HER2BC (n = 25, All-Female)

1. Intratumoural

There was a significant association found between IT TIL and menopausal status (p=0.0441) in the HER2BC subtype - Table 6. There was no other correlation between any of the clinicopathological features and treatment outcome while equating them with the IT TIL counts in the HER2BC subtype.

2. Intrastromal

A significant association was found to be observed between the pathological complete response (p=0.0229) and the HER2BC IS TIL - Table 7. No other correlation between the clinicopathological features was found in the HER2BC subtype while equating them with the IS TIL count.

Group C - (n = 77; Female = 76, Male = 1)

1. Intratumoural

There was no correlation found between any of the clinicopathological features and treatment outcomes in the HRBC subtype on equating them with the IT TIL count.

2. Intrastromal

No correlation was obtained on equating the Intrastromal TIL count with the clinicopathological features and treatment outcomes in the HRBC subtype.

Table 3. Univariate Analysis Concerning TIL Score and Subtype

Group	Subtype	TIL	Intratumoural	Intrastromal	chi - square	p - value	Odds Ratio	Confidence Interval
A	TNBC	High	36 (48.00%)	57 (76.00%)	11.319	0.0008	0.2915	0.1451 - 0.5854
		Low	39 (52.00%)	18 (24.00%)				
B	HER-2 Positive Breast Cancer	High	10 (40.00%)	22 (88.00%)	10.503	0.0012	0.09091	0.02137 - 0.3867
		Low	15 (60.00%)	3 (12.00%)				
C	HRBC	High	17 (22.08%)	23 (29.87%)	0.8443	0.3582	0.6652	0.3216 - 1.376
		Low	60 (77.92%)	54 (70.13%)				
D	TPBC	High	02 (100.00%)	2 (100.00%)	-	-	-	-
		Low	00 (00.00%)	0 (00.00%)				
E	Luminal B Breast Cancer	High	01 (100.00%)	01 (100.00%)	-	-	-	-
		Low	00 (00.00%)	00 (00.00%)				

Table 4. Univariate Analysis of Intratumoural TIL with Clinicopathological Features in TNBC

Sr. No.	Parameter		Intratumoural TIL		chi square	p value	Odds Ratio	Confidence Interval
			High (n=36)	Low (n=39)				
1	Age at Operation	≤ 52 years	26 (72.22%)	20 (51.28%)	2.635	0.1046	2.47	0.9433 - 6.468
		> 52 years	10 (27.78%)	19 (48.72%)				
2	Menopause	Premenopausa l	19 (52.78%)	18 (46.15%)	0.117	0.7323	1.304	0.5258 - 3.234
		Postmenopaus al	17 (47.22%)	21 (53.85%)				
3	Tumour Size	≤ 4.5 cm	24 (66.67%)	25 (64.10%)	0.05434	0.8157	1.12	0.4317 - 2.905
		> 4.5 cm	12 (33.33%)	14 (35.90%)				
4	Lymph Node Status	Not Involved	16 (44.44%)	20 (51.28%)	0.1302	0.7182	0.76	0.3061 - 1.887
		Involved	20 (55.56%)	19 (48.72%)				
5	Perinodal Spill	Negative	28 (77.78%)	32 (82.05%)	0.03005	0.8624	0.7656	0.2462 - 2.381
		Positive	08 (22.22%)	07 (17.95%)				
6	Tumour Emboli	Negative	20 (55.56%)	26 (66.67%)	0.5623	0.4533	0.625	0.2452 - 1.593
		Positive	16 (44.44%)	13 (33.33%)				
7	Pathological Complete Response	pCR	18 (50.00%)	9 (23.08%)	4.779	0.0288	3.333	1.237 - 8.980
		Non-pCR	18 (50.00%)	30 (76.92%)				

Discussion

Breast cancer is known for its diverse tumour characteristics by its molecular sub-typing and though there have been multiple studies (Lv et al., 2011; Ochi et al., 2019). Carried out on its relevance in characterising the prognosis for the same, there has been a lack of emphasis on its association with clinicopathological features. This study set out to compare and contrast the IT and IS TIL in the various subgroups of breast carcinoma to come to a consensus on their relative effectiveness in governing the tumour microenvironment. It also dealt with bridging the gap between the individual subtypes and their clinicopathological features.

The cohort was first studied by classifying them into subgroups based on their immunohistochemical status and their treatment response was determined. Out of all the subtypes included in this study, the TNBC and HER2BC patients showed a higher percentage of pathological complete response which was credited to the fact that TNBC and HER2BC are highly immunoreactive tumours (Loi, 2013) and have a higher chance of improving the therapeutic response when compared to HRBC.

The IT and IS TIL scores when correlated with the breast cancer subtypes showed a very high significance with the TNBC(p=0.008) and HER2BC(p=0.012) subtypes of breast cancer showing that the scores obtained on evaluation (i.e. either high or low TIL) could be of importance in representing these subtypes.

This study also dealt with the individual subgroups of breast cancer and compared them with the clinicopathological features in Intratumoural and Intrastromal TIL.

In Group A - TNBC, an association was found between the IS TIL and age at operation (p=0.0494). This is

Table 5. Univariate Analysis of Intrastromal TIL with Clinicopathological Features in TNBC

Sr. No.	Parameter		Stromal TIL		chi square	p value	Odds Ratio	Confidence Interval
			High (n=57)	Low (n=18)				
1	Age at Operation	≤ 52 years	39 (68.42%)	07 (38.89%)	3.863	0.0494	3.405	1.133 - 10.231
		> 52 years	18 (31.58%)	11 (61.11%)				
2	Menopause	Premenopaus al	30 (52.63%)	07 (38.89%)	0.5569	0.4555	1.746	0.5923 - 5.147
		Postmenopau sal	27 (47.37%)	11 (61.11%)				
3	Tumour Size	≤ 4.5 cm	37 (64.91%)	12 (66.67%)	0.01859	0.8915	0.925	0.3015 - 2.838
		> 4.5 cm	20 (35.09%)	06 (33.33%)				
4	Lymph Node Status	Not Involved	25 (43.86%)	11 (61.11%)	1.013	0.3141	0.4972	0.1684 - 1.468
		Involved	32 (56.14%)	07 (38.89%)				
5	Perinodal Spill	Negative	44 (77.19%)	16 (88.89%)	0.5528	0.4572	0.4231	0.08582 - 2.086
		Positive	13 (22.81%)	02 (11.11%)				
6	Tumour Emboli	Negative	34 (59.65%)	12 (66.67%)	0.06522	0.7984	0.7391	0.2426 - 2.252
		Positive	23 (40.35%)	06 (33.33%)				
7	Pathological Complete Response	pCR	25 (43.86%)	02 (11.11%)	5.026	0.025	6.25	1.312 - 29.763
		Non-pCR	32 (56.14%)	16 (88.89%)				

Table 6. Univariate Analysis of IT TIL with Clinicopathological Features in HER2BC

Sr. No.	Parameter		Intratumoural TIL		chi square	p value	Odds Ratio	Confidence Interval
			High (n=10)	Low (n=15)				
1	Age at Operation	≤ 52 years	08 (80.00%)	06 (40.00%)	2.442	0.1181	6	0.9315 - 38.647
		> 52 years	02 (20.00%)	09 (60.00%)				
2	Menopause	Premenopaus al	06 (60.00%)	02 (13.33%)	4.052	0.0441	9.75	1.381 - 68.815
		Postmenopau sal	04 (40.00%)	13 (86.67%)				
3	Tumour Size	≤ 4.5 cm	07 (70.00%)	09 (60.00%)	0.007234	0.9322	1.556	0.2835 - 8.535
		> 4.5 cm	03 (30.00%)	06 (40.00%)				
4	Lymph Node Status	Not Involved	05 (50.00%)	10 (66.67%)	0.1736	0.6769	0.5	0.09697 - 2.578
		Involved	05 (50.00%)	05 (33.33%)				
5	Perinodal Spill	Negative	09 (90.00%)	11 (73.33%)	0.6098	0.2604	3.273	0.3083 - 34.742
		Positive	01 (10.00%)	04 (26.67%)				
6	Tumour Emboli	Negative	10 (100.00%)	11 (73.33%)	1.5	0.2206	8,217	0.3931 - 171.77
		Positive	00 (00.00%)	04 (26.67%)				
7	Pathological Complete Response	pCR	08 (80.00%)	10 (66.67%)	0.0744	0.785	2	0.3035 - 13.179
		Non-pCR	02 (20.00%)	05 (33.33%)				

Table 7. Univariate Analysis of IS TIL with Clinicopathological Features in HER2BC

Sr. No.	Parameter		Stromal TIL		chi square	p value	Odds Ratio	Confidence Interval
			High (n=22)	Low (n=03)				
1	Age at Operation	≤ 52 years	12 (54.55%)	02 (66.67%)	0.1574	0.6915	0.6	0.04715 - 7.635
		> 52 years	10 (45.45%)	01 (33.33%)				
2	Menopause	Premenopausal	07 (31.82%)	01 (33.33%)	0.002785	0.9579	0.9333	0.07192 - 12.113
		Postmenopausal	15 (68.18%)	02 (66.67%)				
3	Tumour Size	≤ 4.5 cm	14 (63.64%)	02 (66.67%)	0.01052	0.9183	0.875	0.06809 - 11.245
		> 4.5 cm	08 (36.36%)	01 (33.33%)				
4	Lymph Node Status	Not Involved	12 (54.55%)	03 (100.00%)	0.7734	0.3792	0.1701	0.007852 - 3.684
		Involved	10 (45.45%)	00 (00.00%)				
5	Perinodal Spill	Negative	17 (77.27%)	03 (100.00%)	0.02367	0.8777	0.4545	0.02017 - 10.242
		Positive	05 (22.73%)	00 (00.00%)				
6	Tumour Emboli	Negative	18 (81.82%)	03 (100.00%)	0.6494	0.4203	1	0.02547 - 13.541
		Positive	04 (18.18%)	00 (00.00%)				
7	Pathological Complete Response	pCR	18 (81.82%)	00 (00.00%)	5.178	0.0229	28.778	1.248 - 663.51
		Non-pCR	04 (18.18%)	03 (100.00%)				

attributable to the fact that 39 out of the 46 patients in this group were below the age of 52 years and had a high concentration of intrastromal TIL and with increasing age, the immune response decreases (Montecino-Rodriguez et al., 2013). There was no such significant correlation found in the IT TIL.

A significant association was seen between TIL and pathological response in TNBC patients. IT TIL (p=0.0288) and IS TIL (p=0.0250) rejected the null hypothesis at a 95% confidence interval, concluding that a high IT TIL score and a high IS TIL score is associated with the pathological complete resolution of the tumour.

In Group B - HER2BC, there was an association found between the IT TIL and menopause(p=0.0441). This is credited to the fact that 13 out of the 17 patients in this group were postmenopausal and had a low concentration of IT TIL and postmenopausal women have a decreased immune response (Gameiro et al., 2010). There was no such significant correlation found in the IS TIL.

The IS TIL in HER2BC also revealed a significant association with the tumour response(p=0.0229) exhibiting pathological complete resolution in 18 out of the 25 patients.

In Group C - HRBC, no significant associations were found between IT and IS TIL scores and the clinicopathological features.

As a result, the above provides sufficient data on the difference between the IT and IS TIL and their importance in predicting the treatment response after neoadjuvant chemotherapy in the individual subtypes of breast carcinoma. It also helps throw light on further studies which can be carried out to determine the

clinicopathological features and TIL correlation.

In conclusion, The tumour immunogenicity plays a vital role in governing the prognosis and response to neoadjuvant chemotherapy. There is a stark difference between the IT and IS TIL found in the individual subtypes of breast carcinoma, opening up new approaches to understanding the interaction between them.

Abbreviations

TNBC: Triple-negative breast cancer

TPBC: Triple positive breast cancer

HER2BC - Human epidermal growth factor receptor 2 breast cancer

HRBC: Hormone-receptor breast cancer

TILs: Tumour infiltrating lymphocytes

NAC: Neoadjuvant chemotherapy

pCR: Pathological complete response

Author Contribution Statement

RR was the principal investigator and was invested in the majority of the data collection, analysis and curating of the final manuscript. SRK provided invaluable guidance and expertise in the analysis of tumor-infiltrating lymphocytes (TILs) and served as the overseer of the project's analysis. SJB, in their role as the Onco-surgeon, provided crucial post-surgically resected breast tumor specimens for the project. DAM assisted with the statistical analysis. NJP facilitated the immunohistochemical (IHC) marking for the project, ensuring accurate interpretation of the histological data. RG played a pivotal role as the overseeing oncologist for the neoadjuvant chemotherapy administered to the patients. All authors read and provided their approval for the final manuscript, signifying their contribution to the completion of the research.

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Approval by Scientific Body

This research was approved by the Institutional Ethics Committee of Krishna Institute of Medical Sciences, Karad (Reference number - KIMSDU/IEC/02/2019).

Data Availability

The data that supports the findings of this study are available upon reasonable request from the corresponding author in accordance with the data-sharing policies of the Institutional Ethics Committee of Krishna Institute of Medical Sciences, Karad.

Conflict of Interest

The authors declare no conflicts of interest that could have influenced the results or interpretation of the findings presented in this manuscript.

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