(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 6, Issue 1 , Page No: 310-322
January-February 2023

IJMSCR

Programmed Cell Death Ligand-1 And Tumour Infiltrating Lymphocytes, In Invasive Ductal Carcinoma Breast Pre-And-Post Neoadjuvant Chemotherapy

[1]Dr. Jhansi Jayachandran, [2]Dr. Pampa Ch Toi, [3]Dr. Sree Rekha Jinkala, [4]Dr. Kadambari Dharanipragada, [5]Dr. Smita Kayal

[1]Former Junior Resident, [2]MD Professor, [3,5]MD Additional Professor, [4]MS Professor and HOD,
[1,2,3]Department of Pathology, [4]Department of Surgery, [5]Department of Medical Oncology
Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry-605006, India.

***Corresponding Author:**
Dr. Pampa Ch Toi
Professor
Department of Pathology, JIPMER, Puducherry-605006, India

Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Aims: We aim to study the Expression of Programmed cell Death Ligand 1 (PDL1) and CD8+ tumour-infiltrating Lymphocytes (TILs) in patients with invasive ductal carcinoma (IDC) breast, pre and post neoadjuvant chemotherapy (NACT).

Methods and Results: Immunohistochemistry was performed for PDL-1 and CD8+T cells on 73 pre-chemotherapy biopsies diagnosed with IDC breast and on 33 post NACT mastectomy cases. We assessed PDL-1 expression (categorical variable) and CD8+ TILMolecular subtyping of IDC breast was done in 71/73 of pre-NACT biopsy .The study population comprised 40 luminal, 18 TNBC and 13 HER2neu positive cases. Of the 33 post NACT mastectomy cases, there was no response in 15, partial response in 12 and complete response in 6. There was a statistically significant reduction of PDL1 expression in post -NACT patients from 49% in pre-NACT biopsy to 15% post-NACT. The mean Peritumoral CD8+TIL in post-NACT Specimens (20%) was significantly higher than that in pre-chemotherapy biopsies (5 %) (p=0.03), the difference being significantly higher in luminal subtype type (p=0.01)). However, there was no statistically significant increase in intra-tumoral CD8+ TILs post-NACT(p=0.93). There was no significant association between trends of change in PDL1 and CD8+TIL. Though only 18% of the patients attained pCR, NACT significantly reduced PDL-1 expression and increased CD8+TILs.

Conclusion: we conclude that PDL1 positivity and CD8+TIL count could be helpful to predict chemo-responsiveness and help select patients who need additional targeted therapy.

Keywords: Programmed cell death ligand-1, tumour-infiltrating Lymphocytes, Neoadjuvant chemotherapy, Breast cancer

Introduction

Breast cancer is the leading cancer among females worldwide and constitutes 9% to 30% of all new cancers globally. [1,2] The age-adjusted incidence in India is 25.8/ lakh, which is much lower than 95/ lakh in the UK, but the mortality rate is 12.7/lakh in India, which is on par with 17.1/lakh in the UK.[3,4]

The tumour and host immune system interact in the tumour microenvironment. Mismatch of immune interplay could cause immune evasion, dissemination, relapse, and metastasis. Therefore protective tumour immunity needs to be strengthened with advanced therapy, including immunotherapy.

Tumour infiltrating lymphocytes (TIL) indicate immune responsiveness to tumour neoantigens, a

concept first described in melanoma. TILs can be either intratumoral or peritumoral.[5]

Programmed cell death ligand-1 (PDL1) is a transmembrane protein, generally expressed in antigen-presenting cells (APC) to induce apoptosis of self-reacting lymphocytes and maintain peripheral T cell tolerance. Various tumours utilize this mechanism to suppress tumour immunity.[6] As host immunity mounts an immune response against tumour cells, they express ligands like PDL1 that bind to receptors like PD 1 on the lymphocytes, thereby causing their apoptosis, leading to immune escape. The natural course of disease and response to treatment differ due to imbalances in these factors. Chemotherapy causes rapid death of the proliferating tumour mass, releasing tumour death signals from the dying cells causing activation of APC, releasing IL 1 beta, thus recruiting more TIL and activating both innate and adaptive immunity.[7,8]

Hence, chemotherapy may lower the level of PDL1 expression due to rapid reduction of the tumour mass and increase the TIL.[8] Probably chemotherapy reduces the tumour load, and the rest is taken care of by the strengthened immune system, which works unopposed, as chemotherapy eliminates the immunosuppressive arm. Therefore, we can assume that a significant response predictor is pre-existing lymphocytic infiltrate in breast carcinoma.

Immunogenic tumours can place tumour danger signals, activating the host immune system. Nevertheless, the exact influence of neoadjuvant chemotherapy (NACT) on PD-L1 and TILs' expression is yet to be explored.

This study aims to establish the parameters related to tumour immunity, namely PDL1 expression and CD8+ TIL in both pre-NACT biopsy and the residual tumour in post-NACT MRM specimens and assess their changes between paired pre and post-NACT samples wherever available.Very few studies exist highlighting the effect of chemotherapy on these parameters.

Methodology:

This analytical study of 2 years duration from January 2016 to January 2018 was carried out in the Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India, after the approval from the Institute's Scientific Advisory and Ethical Committees(JIP/IEC/2017/0431). We retrospectively evaluated Seventy-three (73) cases with diagnostic core biopsy reported as invasive ductal carcinoma breast, NOS (IDC-NOS) during this period. Of them, 33 underwent modified radical mastectomy (MRM) and post-neoadjuvant chemotherapy (post-NACT).

The clinical details such as age, menopausal status, clinical stage and pathological details like histological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor (Her-2) status were noted from the medical records available in the Hospital Information System.

For the 33 cases that had undergone NACT followed by MRM, details of chemotherapy regimen, cycles and response were noted from the data in the Hospital Information system and Medical Oncology Department. All the patients were treated with an anthracycline-based chemotherapy regimen. Response post-NACT was classified into three types-pathological complete response (pCR), partial response and no response. pCR is defined as the absence of tumour cells or persistent in-situ disease and negative axillary lymph node status. Some patients with ER+ breast cancer received tamoxifen, and Her-2+ breast cancer received trastuzumab.

IHC staining procedure

The core biopsy of 73 cases and the tumour bed of post-NACT MRM specimen (33/73) were fixed in 10% neutral buffered formalin (NBF) and embedded in paraffin. For IHC staining, we used sections of 4-micron thickness. Primary antibodies used were PDL1 (1:200; E3L1N; Cell signalling technology) and CD8 (prediluted; EP150Y; Dako). For chromogenic visualization, we used, Horseradish peroxide/ DAB. We performed IHC by the HRP polymer technique.

Interpretation of IHC for PDL-1

For evaluating PDL1, we selected three invasive hotspots taken as an area of maximum PDL1 intensity, and the average score was given . PDL1 positivity was scored according to Allred score, with the intensity ranging from 0-3(four categories) in tumour cells, tumour-infiltrating lymphocytes and stromal cells, [% (0 to 5) + intensity (0 to3)]. Cut-off for positivity ≥ 3.[9]They were estimated

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved

independently by two investigators. Sections of the human placenta were used as positive controls. PDL1 showed both cytoplasmic and membranous positivity.(Fig:1A-1D)

Evaluation of CD8+ TILs

For evaluating CD8+ TILs, three invasive hotspots were selected. CD8+ TIL was counted in intratumoral and peritumoral areas as a percentage of space occupied by TILs.[10]The average count of three hotspots was taken as the final value.(Fig:2A-2D)

Statistical analysis:

We performed all statistical analyses using IBM SPSS version 2.0 software. The distribution of categorical variables such as gender, stage, histological subtype, PD-L1 expression status, treatment outcomes was expressed as percentage(%) and frequency. The distribution of continuous variables such as age and TILs expression was defined as mean with standard deviation or median based on the data distribution. The comparison of the TIL score and change in the level of TIL, pre and post-NACT, between the categorical variables, including pCR, was carried out using independent student t-test or Mann Whitney u-test and paired t-test, respectively.

The comparison of the PDL1 expression and change in the PDL1 levels between the categorical variables, including pCR, was carried out using the Chi-square test and McNemar's test. The relationship between the PDL1 and TILs was carried out using the Kruskal Wallis test. All statistical analysis was carried out at a 5% significance level, and a P-value < 0.05 was considered statistically significant.

Results:

The Clinical and pathological parameters :

The cases of IDC-NOS varied from 24 to 84 years of age, with a median age of 49. 53 %(39/73) of patients were younger than 50 years of age. Menopausal data were available for 64/73 cases, and 53% (34/64) were premenopausal. The clinical-stage, retrieved for 65 /73 patients, showed that 46% (30/65) were of clinical stage 4 . Pathological details were available for all 73 cases. The tumours' grading was according to the Nottingham modification of the Bloom Richardson system, of which 59%(43/73) were of grade 2. The molecular subtype was done based on the ER, PR, and HER2 reports available, where 56%(40/71) were of luminal (A and B) subtypes(Table 1)

Association of PDL1 positivity with clinical and pathological parameters :

PDL1 scoring on the pre-NACT biopsy was positive in 29%(21/73) and negative in 71%(52/73). There was a significant association between PDL1 positivity in those above 50 years (p=0.02) and clinical stage4(p=0.04). However, there was no significant association between PDL1 positivity and menopausal status, histological grade or molecular subtype(Table 2)

PDL1 positivity with pathological response post-NACT

MRM was performed in 33 cases post-NACT, of which 6 attained pCR, 12 showed partial response, and 15 had no response. The pathological response had no significant impact on the reduction of PDL1 post-NACT(Table 3)

Impact of clinical and pathological parameters on change in PDL1, post-NACT

There was no significant association between PDL1 positivity and response to chemotherapy in 33 patients(post-NACT) with MRM, of whom 16 were positive and 17 were negative pre-NACT. While 5/33 were positive, 28/33 were negative for PDL1 post-NACT. There was a statistically significant reduction in PDL1 expression post-NACT (p=0.001).

PDL1 expression showed a significant reduction (p<0.01)in those above 50 years of age, while those below 50 years did not show a decrease. Post-menopausal cases had a significant reduction of PDL1expression (p= 0.03), while there was no significant decrease in the premenopausal group.

Stage 4 disease had a significant reduction of PDL1 expression (p=0.03), while those with stage 3 had no significant reduction. There was no substantial reduction in PDL1 expression in relation to the grade and molecular subtype of the tumour. (Table 4)

Association of TIL with clinical and pathological parameters

Median TIL in the intratumoral and peritumoral compartment were 10%, with a range of 0 to 90%.

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved

There was no statistically significant association between TIL (peritumoral TILwas taken) and any clinical parameter such as age, menopausal status and clinical tumour stage, although stage 1 had the highest median TILs of 35%. There was no significant association between the histological grade and TIL, whereas the triple-negative molecular subtype had a significantly higher TIL count of 45% (p=0.03). (Table 5)

Of the 33 cases with post-NACT MRM, there was no significant association between high TIL and treatment response. However, analyzing the change, median peritumoral TIL pre-NACT was 5%, and post-NACT was 20%. There was a significant increase in TIL(peritumoral) post -NACT(p=0.03) while no substantial change in intratumoral TILs as the median intratumoral TIL pre-NACT was 2% and post-NACT was 5%.

Impact of clinical and pathological parameters on change in TIL, post-NACT

Only the luminal subtype showed a statistically significant impact(p=0.01) (Table 6). There was no significant association between PDL1 and high TILs with each other or with their trend.

Table 1: Clinical and pathological parameters of the cases

Parameters	Frequency (%)
Age (n=73)	Mean - 48.9; Median - 49 (Range-24-84)
<50 years	39 (53)
>50 years	34 (47)
Menopausal status (n=64)	
Postmenopause	30 (41)
Pre menopause	34 (47)
Clinical stage (n= 65)	
1	2 (3)
2	19 (29)
3	14 (22)
4	30 (46)
Grade (n=73)	
1	19 (26)
2	43 (59)
3	11 (15)
Molecular subtype (n=71)	
Luminal A and B	40 (55)
Her2 rich	13 (18)
TNBC	18 (25)

Volume 6, Issue 1; January-February 2023; Page No 310-322

© 2023 IJMSCR. All Rights Reserved

Table 2: Association of PDL1 positivity with clinical and pathological parameters			
Data	Positive (N=21)	Negative (N=52)	P value (significant < 0.05)
Age(n=73)			
<50 (N=39)	7 (18%)	32 (82%)	**0.02**
>50 (N=34)	14 (41%)	20 (59%)	
Menopause (n=64)			
Yes (N=30)	10 (33%)	20 (67%)	0.38
No (N=34)	8 (24%)	26 (77%)	
Clinical stage (n=65)			
1 (N=2)	0 (0%)	2 (100%)	**0.04**
2 (N=19)	1 (5%)	18 (95%)	
3 (N=14)	6 (43%)	8 (57%)	
4 (N=30)	11 (37%)	19 (63%)	
Grade (n=73)			
1 (N=19)	7 (37%)	12 (63%)	0.55
2 (N=43)	12 (29%)	31 (71%)	
3 (N=11)	2 (18%)	9 (82%)	
Molecular subtype (n=71)			
Luminal A and B (N=40)	10(25%)	30(75%)	0.57
Her2rich (N=13)	4(31%)	9(69%)	
TNBC (N=18)	7(39%)	11(61%)	

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved

Table 3: Association of PDL1 positivity with pathological response post-NACT			
Response	Positive (N=16)	Negative (N=17)	P value (significant <0.05)
Complete (N=6)	1 (17%)	5(83%)	0.14
Partial (N=12)	6(50%)	6(50%)	
No (N=15)	9 (60%)	6 (40%)	

Table 4: Impact of clinical and pathological parameters on change in PDL1, post-NACT			
Data	Pre NACT	Post NACT	P value (significant <0.05)
Age(n=33)			
<50 (N=18)	5	3	0.72
>50 (N=15)	11	2	<0.01
Menopause (n=28)			
Yes (N=9)	7	1	0.03
No (N=19)	6	3	0.51
Clinical stage (n=28)			
3 (N=5)	2	1	1.00
4 (N=23)	11	3	0.03
Grade (n=33)			
1 (N=7)	6	1	0.06

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved

2 (N=22)	9	3	0.10
3 (N=4)	1	1	1.00
Molecular subtype (n=33)			
Luminal A and B (N=19)	9	4	0.18
Her2 rich (N=5)	2	0	0.50
TNBC (N=9)	5	1	0.22
Pathological response (n=33)			
Complete (N=6)	1	0	1.00
Partial (N=12)	6	2	0.22
No (N=15)	9	3	0.11

Table 5: Association of TIL with clinical and pathological parameters

Data	Median TIL	Range	P value (significant < 0.05)
Age(n=73)			
<50 (N=39)	20	0-90	0.64
>50 (N=34)	5	0-90	
Menopause (n=64)			
Yes (N=30)	8	0-90	0.83
No (N=34)	18	0-90	
Clinical stage (n=65)			
1 (N=2)	35	20-50	0.59
2 (N=19)	5	0-90	

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved

3 (N=14)	20	0-80	
4 (N=30)	8	0-90	
Grade (n=73)			
1 (N=19)	5	0-70	0.54
2 (N=43)	13	0-90	
3 (N=11)	20	0-90	
Subtype (n=71)			
Luminal A and B (N=40)	5	0-90	**0.03**
Her2rich (N=13)	5	0-70	
TNBC (N=18)	45	0-90	

Table 6: Impact of clinical and pathological parameters on change in TIL, post-NACT Table 7: Impact of clinical and pathological parameters on change in TIL, post-NACT

Data	Pre NACT	Post NACT	P value (significant <0.05)
Age(n=33)			
<50 (N=18)	8	20	0.09
>50 (N=15)	5	20	0.16
Menopause (n=28)			
Yes (N=9)	2	20	0.45
No (N=19)	5	20	0.08

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved

Clinical stage (n=28)			
3 (N=5)	2	20	0.46
4 (N=23)	5	20	0.08
Grade (n=33)			
1 (N=7)	2	20	0.46
2 (N=22)	10	20	0.17
3 (N=4)	2	8	0.58
Subtype (n=33)			
Luminal A and B (N=19)	2	20	**0.01**
Her2 rich (N=5)	10	20	0.46
TNBC (N=9)	20	20	0.83
Response			
Complete (N=6)	10	20	0.78
Partial (N=12)	8	18	0.21
No (N=15)	5	30	0.75

Images:

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved

Fig1:1A- Invasive carcinoma breast on core biopsy.1B-IHC -PDL1 score 7 (PDL1 – E3L1N)1C -PDL1 score 5 (PDL1 -E3L1N).1D-PDL1 score 0 (PDL1 -E3L1N)

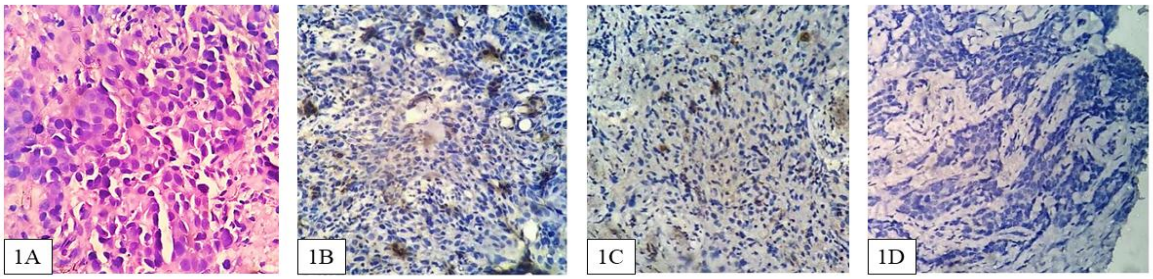
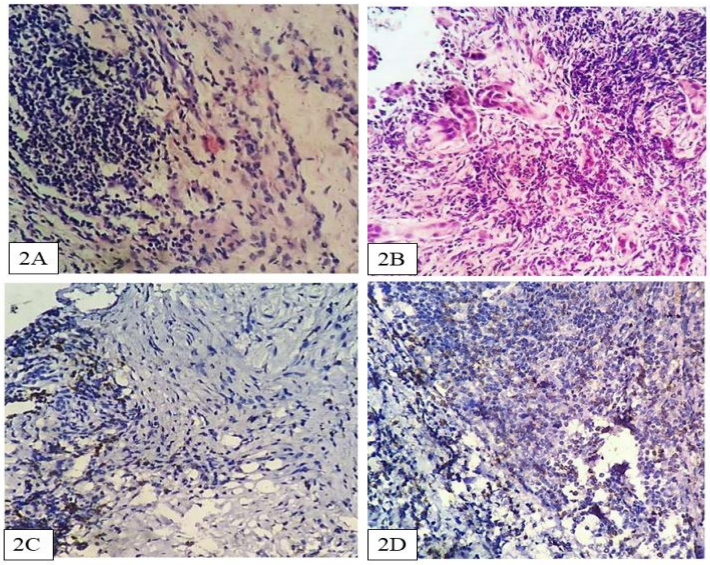


Fig2:2A-Invasive carcinoma breast- core biopsy, with increased TIL.1B-Invasive carcinoma, breast- MRM, with increased TIL,1C-TIL – peritumoral 40% (IHC CD8+) ,1D- TIL – peritumoral 90% (IHC CD8+)



Discussion:

Response to chemotherapy varies from tumour to tumour, based on tumour immunogenicity. The expression of PD-L1 and TILs, in pre-NACT IDC, may help to predict responsiveness to chemotherapy. The sample size of 73 in the current study was comparable with similar studies identifying the association of PDL1 with tumour characteristics ranging from 44 to 105. [11-14] However, only 33 had NACT followed by MRM, reducing the cohort size, wherein pre-and-post-NACT changes could be compared.PDL1 expression was positive in 29%(pre-NACT), comparable to findings by Wimberly et al., and Ghebeh et al., who reported 30% and 34% positivity, respectively.[12,13] PDL1 expression decreased significantly, post-NACT from 48% to 15%, which agrees with limited studies on the effect of chemotherapy on PDL1. Pelekanou et al., found that PDL1 expression was significantly lower in post-NACT samples with a P-value of 0.001.[11]

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved

PDL1 expression was highly heterogeneous throughout the breast tumour. Though the clone used is known to show membranous positivity, we found cytoplasmic positivity also, which is described in the literature.[9,15] It was challenging to assess the expression of PDL1 by stromal cells and lymphocytes along with epithelial cells separately. Based on a study by Wimberly et al., that showed no significant difference between epithelial and stromal expression, we scored PDL1 expression in epithelial, lymphocytes and stromal areas together.[2]

In the present study, those above 50 years(comprising 53%) showed significant association with PDL1 positivity and significant reduction of PDL1 post-NACT. There are conflicting studies between PDL1 positivity and age. In some, a lower age correlated with PDL1 positivity,[16] while others showed no correlation.[9,12,13,17]The difference in the age group expressing PDL1 positivity can reflect existing host immunity, which is age-dependent.

Menopause as a reflection of the patient's hormonal milieu was assessed for association with PDL1 expression, whereas menopausal status did not affect PDL1 expression. However, there was a significant reduction in PDL1 expression in post-menopausal patients. Botti et al., observed that premenopausal women had a significant association with PDL1 expression.[15] Menopausal women, expected to be elderly and with the most negligible hormonal influence on breast cancer, are expected to have higher PDL1 expression, not observed in either study.

Larger tumour size and regional nodal metastasis are expected to evade the host tumour immunity and hence are expected to be positive for PDL1 expression. We found the predicted association between stage 4 tumours and PDL1 expression with a significant reduction in PDL1 expression post-chemotherapy as also observed by Schütz et al.[18] However, Bae et al. found an association between PDL1 in cases with no nodal metastasis and lower tumour stage where only 13.5% were positive for PDL1.[9]

There was no significant association between PDL1 and tumour grade in the current study though Bae et al., stated a significant association.[9] A higher sample size could probably solve the disparity.

TNBC are highly immunogenic and proliferating tumours, and associations between PDL1 positivity and TNBC have been reported. Of 71 pre-NACT cases where molecular subtyping was available, 18/71(24%) were TNBC, of which 7/18(39%) were positive for PDL1; however, the association between them was not statistically significant, which is in contrast with a study by Kitano et al., and Li et al., who found a significant association between PDL1 expression and TNBCs.[19,20] Mittendorf et al., found a significantly higher PDL1 expression in TNBC than non-TNBC.[21] However, Mori et al., reported that 42% of TNBC expressed PDL1.[16]There was also no statistically significant reduction of PDL1, post-NACT, amongst TNBCs in our study.

We observed a pCR in 18% of cases with a significant reduction in PDL1 expression, while a similar study reported a pCR of 35%.[22] Sixty per cent of cases with no response were positive for PDL1, pre-NACT. However, no significant association between PDL1 expression and response to chemotherapy was observed.

We assessed CD8+TIL count pre-NACT and its change with chemotherapy, and only peritumoral TILs were of significant interest. It was not easy to assess TIL in partial and complete response cases, post-NACT, due to necrosis; hence, we chose sections showing the least necrosis areas.

There was a significant increase in peritumoral CD8+TILs, post-NACT, from 5% to 20%, which agrees with Pelekanou et al., where higher TIL counts were observed in post-chemotherapy compared to pre-chemotherapy.[11]

There was no significant association between age, clinical stage, pathological stage or the grade and TILs in this study, as observed in other studies.[11,23] However, Al-Saleh et al., found higher grade tumours to have significantly higher TILs.[22]

ER-negative tumours and TNBCs analyzed separately showed statistically higher TILs as observed by Luen et al., in ER-negative tumours[23] and Garcia-Teijido et al. and Hartkopf et al. in TNBCs.[24,25] We found a statistically significant increase in TIL post-NACT in the luminal subtypes.

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved

pCR cases had the highest TIL count of 10%; however, the association between pathological response and TIL was not statistically significant in our study. Literature states a significant association between higher TIL and pCR.[12,22] Garcia-Tejido et al., observed pCR in 74% of lymphocyte-predominant breast cancer.[24] TILs have been known to predict pCR, pre-NACT and predict survival post-NACT.[21]With only 33 patients in the present study receiving chemotherapy, plotting association could not yield the expected outcome.

Median TIL in PDL1 positive cases was 10% while12.5% in PDL1negative patients; however, the association was insignificant. An association between higher TIL score and PDL1 positivity is due to the release of interferon-gamma by the lymphocytes that upregulate PDL1 expression in the tumour cells.[9,11,14,16,19,26,27] .A larger sample size could give a better association between TIL and PDL1.

Low pCR suggest that chemotherapy alone may not be sufficient to nullify the effect of PDL1, though it reduces it. Hence, PDL1 expression may be routinely tested and immunotherapy along with chemotherapy to bring about pCR. CD8+TILs can identify immunogenic tumours that would respond to chemotherapy. In setups where IHC is not affordable, Haematoxylin and Eosin based TIL scores may be helpful. Further research can open doors for the new generation of targeted therapy and immunotherapy.

References:

1. Behera P. Epigenetics changes in breast cancer: Current aspects in India. J. Bioeng. Biomed. Sci. 2017;7:223.doi:10.4172/2155-9538.1000223
2. Sun Y-S, Zhao Z, Yang Z-N, Xu F, Lu H-J, Zhu Z-Y, et al. Risk Factors and Preventions of Breast Cancer. Int J Biol Sci. 2017;13(11):1387–97.
3. Olsen AH, Lynge E, Njor SH, Kumle M, Waaseth M, Braaten T, et al. Breast cancer mortality in Norway after the introduction of mammography screening. Int J Cancer. 2013;132(1):208–14.
4. Malvia S, Bagadi SA, Dubey US, Saxena S. Epidemiology of breast cancer in Indian women: Breast cancer epidemiology. Asia Pac J Clin Oncol.2017;13(4):289–95.
5. Sinn H-P, Kreipe H. A Brief Overview of the WHO Classification of Breast Tumors, 4th Edition, Focusing on Issues and Updates from the 3rd Edition. Breast Care. 2013;8(2):149–54.
6. Mohammed AA. Predictive factors affecting axillary lymph node involvement in patients with breast cancer in Duhok: Cross-sectional study. Annals of Medicine and Surgery. 2019; 44:87-90.
7. Tang P, Tse GM. Immunohistochemical Surrogates for Molecular Classification of Breast Carcinoma: A 2015 Update. Arch Pathol Lab Med. 2016;140(8):806–14.
8. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. Sci Transl Med. 2016;8(328):328rv4-328rv4.
9. Bae SB, Cho HD, Oh M-H, Lee J-H, Jang S-H, Hong SA, et al. Expression of Programmed Death Receptor Ligand 1 with High Tumor-Infiltrating Lymphocytes Is Associated with Better Prognosis in Breast Cancer. J Breast Cancer. 2016;19(3):242-251
10. McIntire PJ, Zhong E, Patel A, Khani F, D, AlfonsoTM, Chen Z, et al.Hotspot enumeration of CD8+ tumour-infiltrating lymphocytes using digital image analysis in triple-negative breast cancer yields consistent results.Hum Pathol.2019;85:27-32
11. Pelekanou V, Carvajal-Hausdorf DE, Altan M, Wasserman B, Carvajal-Hausdorf C, Wimberly H, et al. effect of neoadjuvant chemotherapy on tumour-infiltrating lymphocytes and PD-L1 expression in breast cancer and its clinical significance. Breast Cancer Res. 2017;19(1):91. doi: 10.1186/s13058-017-0884-8. Erratum in: Breast Cancer Res. 2017 Sep 25;19(1):109. PMID: 28784153; PMCID: PMC5547502.
12. Wimberly H, Brown JR, Schalper K, Haack H, Silver MR, Nixon C, et al. PD-L1 Expression Correlates with Tumor-Infiltrating Lymphocytes and Response to Neoadjuvant Chemotherapy in Breast Cancer. Cancer Immunol Res. 2015;3(4):326–32.
13. Ghebeh H, Mohammed S, Al-Omair A, Qattant A, Lehe C, Al-Qudaihi G, et al. The B7-H1 (PD-L1) T Lymphocyte-Inhibitory Molecule Is Expressed in Breast Cancer Patients with Infiltrating Ductal Carcinoma: Correlation with

Volume 6, Issue 1; January-February 2023; Page No 310-322

© 2023 IJMSCR. All Rights Reserved

Important High-Risk Prognostic Factors. Neoplasia. 2006;8(3):190–8.
14. Wu Z, Zhang L, Peng J, Xu S, Zhou L, Lin Y, et al. Predictive and prognostic value of PDL1 protein expression in breast cancer patients in neoadjuvant setting. Cancer Biol Ther. 2019;20(6):941–7.
15. Botti G, Collina F, Scognamiglio G, Rao F, Peluso V, De Cecio R, et al. Programmed Death Ligand 1 (PD-L1) Tumor Expression Is Associated with a Better Prognosis and Diabetic Disease in Triple-Negative Breast Cancer Patients. Int J Mol Sci. 2017;18(2):459. doi:10.3390/ijms18020459. PMID: 28230773; PMCID: PMC5343992
16. Mori H, Kubo M, Yamaguchi R, Nishimura R, Osako T, Arima N, Okumura Y, Okido M, Yamada M, Kai M, Kishimoto J. The combination of PD-L1 expression and decreased tumour-infiltrating lymphocytes is associated with a poor prognosis in triple-negative breast cancer. Oncotarget. 2017;8(9):15584-92
17. Gatalica Z, Snyder C, Maney T, Ghazalpour A, Holterman DA, Xiao N, et al. Programmed Cell Death 1 (PD-1) and Its Ligand (PD-L1) in Common Cancers and Their Correlation with Molecular Cancer Type. Cancer Epidemiol Biomarkers Prev. 2014;23(12):2965–70.
18. Schütz F, Stefanovic S, Mayer L, von Au A, Domschke C, Sohn C. PD-1/PD-L1 Pathway in Breast Cancer. Oncol Res Treat. 2017;40(5):294–7.
19. Kitano A, Ono M, Yoshida M, Noguchi E, Shimomura A, Shimoi T, et al.Tumour-infiltrating lymphocytes are correlated with higher expression levels of PD-1 and PD-L1 in early breast cancer. ESMO Open. 2017;2(2):e000150.
20. Li Z, Dong P, Ren M, Song Y, Qian X, Yang Y, Li S, Zhang X, Liu F. PD-L1 Expression Is Associated with Tumor FOXP3(+) Regulatory T-Cell Infiltration of Breast Cancer and Poor Prognosis of Patient. J Cancer. 2016;7(7):784-93. doi: 10.7150/jca.14549. PMID: 27162536; PMCID: PMC4860794.
21. Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, et al. PD-L1 Expression in Triple-Negative Breast Cancer. Cancer Immunol Res. 2014;2(4):361–70.
22. Al-Saleh K, Abd El-Aziz N, Ali A, Abozeed W, Abd El-Warith A, Ibraheem A, et al. Predictive and prognostic significance of CD8+ tumour-infiltrating lymphocytes in patients with luminal B/HER 2 negative breast cancer treated with neoadjuvant chemotherapy. Oncol Lett. 2017;14(1):337–44.
23. Luen SJ, Salgado R, Fox S, Savas P, Eng-Wong J, Clark E, et al. Tumour- infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: a retrospective analysis of the CLEOPATRA study. Lancet Oncol.2017;18(1):52–62.
24. García-Teijido P, Cabal ML, Fernández IP, Pérez YF.Tumour-Infiltrating Lymphocytes in Triple Negative Breast Cancer: The Future of Immune Targeting. Clin Med Insights Oncol. 2016;10s1:CMO.S34540.
25. Hartkopf AD, Taran F-A, Wallwiener M, Walter CB, Krämer B, Grischke E-M, et al. PD-1 and PD-L1 Immune Checkpoint Blockade to Treat Breast Cancer. Breast Care.2016;11(6):385–90.
26. Beisecker AE, Cook MR, Hayes J, Brecheisen M, Helmig L, Hyland S, et al. Side Effects of Adjuvant Chemotherapy: Perceptions of Node-Negative Breast Cancer Patient Psychooncology1997;6: 85–93.
27. Stanton SE, Disis ML. Clinical significance of tumor-infiltrating lymphocytes in breast cancer. J Immunother Cancer. 2016;4(1):59.doi: 10.1186/s40425-016-0165-6.PMID: 27777769; PMCID:PMC5067916.

Volume 6, Issue 1; January-February 2023; Page No 310-322
© 2023 IJMSCR. All Rights Reserved