RESEARCH ARTICLE

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Expression of PD-L1 in Locally Advanced Breast Cancer and Its Impact on Neoadjuvant Chemotherapy Response

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Abstract

Objectives: Programmed cell death-ligand 1 (PD-L1) is a new target in breast cancer (BC) and its impact on neoadjuvant chemotherapy (NACTH) response is still unclear. The aim of this study was to investigate the prevalence of PD-L1 in locally advanced invasive BC of different molecular subtypes and to elucidate its relation to tumor-infiltrating lymphocytes (TILs) density, established clinicopathological factors, pathological therapy response after neoadjuvant chemotherapy and patients' outcome. **Materials and Methods:** One hundred and five cases of locally advanced invasive BC were enrolled in our study. Cases were classified into five molecular subtypes according to the Immuno-histochemical data. PD-L1 immunostaining was analyzed for all studied cases and its expression was correlated with TILs density, histopathologic parameters, BC molecular subtypes, Pathological therapy response, 7-years disease-free survival (DFS) and overall survival (OS). **Results:** PD-L1 was expressed in 32.4% of the studied locally advanced BC cases. It showed a significant correlation with old age group (p= 0.010), high tumor grade (p= 0.046) and high pretherapy TILs density (p= <0.001). PD-L1 expression was higher in HER2/neu-enriched group (45.5%) followed by TNBC (44.4%). There were no significant relations between PD-L1 expression and DFS, OS as well as pathological therapy response, although, it revealed more expression in cases with complete and marked therapy response. Conclusion: In spite our results fail to prove that PD-L1 is a bad prognostic biomarker in locally advanced BC, but they indicate PD-L1 could be a new target for the treatment of patients with high grade breast carcinoma and TNBC group.

Keywords: PD-L1- Advanced- BC- TILs- NACTH

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Introduction

Breast cancer is a complex disease whose progression is difficult to predict. As a result, treatment is not as customized as it should be. Gene expression studies have identified five molecularly distinct subtypes of breast cancer that have prognostic value across multiple treatments and can predict distinct clinical outcomes (Abo-elazm et al., 2018).

These subtypes differ in clinical outcome; HER2-enriched and TNBC subtypes are hormone receptor negative and have a poorer prognosis with shorter survival times than other types (Hennigs et al., 2016). Luminal breast cancers, on the other hand, are distinguished by the expression of hormone receptor(s), with luminal B tumors having a shorter survival time and worse outcomes than luminal A tumors, which have the longest survival time. The main biological difference between luminal A and B tumors is the proliferation signature, which includes genes like MKI67 (encoding Ki-67), which is more prevalent in luminal B tumors (Park et al., 2018).

High TILs density has been associated with favorable clinical outcomes in various solid tumors, including breast cancer (Salgado et al., 2015). Large breast cancer clinical trials have validated the prognostic and predictive significance of TILs, particularly in TNBC and HER2-enriched molecular subtypes. These studies principally scored TILs on hematoxylin and eosin stained (H&E) sections, with occasional use of immunohistochemistry (IHC) and immune gene signatures (Savas et al., 2016; Buisseret et al., 2017).

Immune response is a complex phenomenon characterized by a balance of activator and inhibitor pathways that regulate TILs activity. This balance may be upset in certain pathological conditions, such as cancer, where immune system suppression promotes tumor progression. The PD1 (Programmed Cell Death 1) - PDL1 (Programmed Cell Death Ligand 1) pathway is one important inhibitor. PD1 is a cell surface membrane protein expressed by a variety of immune cells, including T-cells; it is activated by its ligands PDL1 and PDL2, which are expressed by antigen-presenting cells such as macrophages and B-cells. After being activated by its ligands, PD1 inhibits lymphocyte activation and promotes T-regulatory cell development and function, allowing the immune response to be terminated through induction of apoptosis, reduction of proliferation, and inhibition of cytokine secretion (Hinshaw et al., 2019).

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The use of immunotherapy in combination with chemotherapy has been approved as a first-line therapy for advanced metastatic BC cases (Franzoi et al., 2021). However, TILs are important biomarker in predicting the response of BC to immunotherapy alone or in combination with chemotherapy (Karn et al., 2020).

Neoadjuvant chemotherapy is increasingly used to induce tumor shrinkage, allowing smaller surgical resection, eliminating clinically silent micro-metastases, and providing prognostic information based on the extent of pathologic response. Pathologic complete response (pCR) predicts excellent survival while residual disease (RD) is associated with higher but variable risk of recurrence depending on the molecular subtype (Wimberly et al., 2015; Pelekanou et al., 2017).

Pretreatment immune infiltration in breast cancer predicts both for better prognosis, with or without adjuvant therapy, and for greater sensitivity to chemotherapy reflected by the higher pCR rates in immune rich cancers (Loi et al., 2013; Nummer et al., 2013). Many studies have shown high TILs count is associated with higher pCR rate after neoadjuvant chemotherapy (Yamaguchi et al., 2012; Seo et al., 2013; Denkert et al., 2015). High TILs count in residual disease is also associated with better survival (Ono et al., 2012; Lee et al., 2013).

Materials and Methods

Patients and methods

This retrospective study was carried out on one hundred and five cases of women patients diagnosed as locally advanced invasive breast carcinoma (Stages IIB, IIIA, IIIB & IIIC) according to TNM staging system. Cases were retrieved from the Pathology Department, National Cancer Institute (NCI), Cairo University, throughout the period from January 2012 to December 2015. Follow up time was up to 117.4 months with a median period of 73.5 months. All the included patients received neoadjuvant chemotherapy. To avoid effect of NAC on PD-L1 expression that might occur, we used in our study biopsy material before NAC and surgery.

Microscopic review of the cases for confirming the diagnosis and tumor grading were assessed according to World Health Organization (WHO) Classification of Breast Tumors, Fifth Edition, 2019 (Rakha et al., 2019). Pathologic stage was determined by examining the excised specimens, according to tumor-node-metastasis (TNM) classification of the American Joint Committee on Cancer (AJCC), 8th edition (Giuliano et al., 2017).

Data of ER, PR, and Her2 were all reviewed and reported according to the updated American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) 2018 guidelines (Wolff et al., 2018; Allison et al., 2020)

Immunohistochemistry (IHC)

Sections of 4 μm were cut from the paraffin-embedded tissues and placed onto positive charged slides. Standard immunostaining was done using BenchMark ULTRA (Ventana) autostainer according to the manufacturer's instruction. Primary monoclonal antibodies (ready-to-use) were used as follows: Rabbit monoclonal antibodies against PD-L1 (RBT-PDL1), Cat No (BSB 2651). Tissue sections from normal human placental tissue was used as a positive control for PD-L1.

PD-L1 IHC analysis

The percentage of PD-L1 expression was calculated by using high power field (400x) in whole core biopsy. In tumor cells (TC), by dividing total number of positive TC (membranous staining) over total number of TC. In tumor-infiltrating immune cells (IC), it was assessed as the proportion of tumor area occupied by PD-L1-positive immune cells of any intensity in any cell compartment. The total percent of PD-L1 expression in tumor-infiltrating immune cells and invasive tumor cells (TCIC) was also calculated as the number of those cells showing PD-L1 staining divided by the total number of invasive tumor cells. Percentage 1% or greater was considered positive (Guo et al., 2020).

TILs density (pre & post-NACTH) assessment

Assessment changes in stromal TILs between paired pre-NACTH and post-NACTH samples was done according to Pelekanou and colleagues' study. Stromal TILs scores were defined as the percentage of tumor stroma area that was occupied by mononuclear inflammatory cells. Five HPFs with the highest TILs infiltration were chosen and the mean of the five fields was used to express the density of TILs (percent), (Pelekanou et al., 2017).

Receiver Operating Characteristics (ROC) curve was done to estimate the best cut off point of pre-therapy TILs as well as the change in TILs after therapy. Cut off values were calculated as 37.5% for pre-therapy TILs and 0.5% for TILs change. In all pre-therapy samples, TILs ≤ 37.5% was defined as a low- density infiltration and > 37.5% as a high-density infiltration. Changes in TILs pre-therapy and post-therapy were calculated through the formula: Post-therapy TILs – Pretherapy TILs/ Pretherapy TILs TILs change ≤ 0.5% was defined as a low change and > 0.5% as a high change.

NACTH pathological assessment

Neoadjuvant chemotherapy response was assessed pathologically in tumors and lymph nodes of surgical specimens by MD Anderson (Residual Cancer Burden) calculator (http://www3.mdanderson.org). Residual cancer burden (RCB) is evaluated from the two-dimensional diameter of the primary tumor from the resected specimen, the numbers of positive lymph nodes, the proportion of primary tumor beds containing invasive cells, the percentage of in situ component and the maximum diameter of axillary lymph node metastases after NACTH (Symmans et al., 2007).

Statistical Methods

IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 24, was used to analyze the data (SPSS Inc., Chicago, IL). The standard chi-squared (Fisher's exact) test was used to determine the relationship between categorical variables. Using a logistic regression

model, multivariate analysis was performed on variables that were statistically significant on a univariate level to identify independent prognostic factors and to eliminate the effect of confounders. A Cox regression model was used to calculate the hazard ratio (HR) and its 95 percent confidence interval, and survival curves were plotted using Kaplan–Meier estimates. For survival endpoints, DFS and OS were used. A p-value of 0.05 or less was considered statistically significant.

Results

Clinicopathological findings

Detailed clinical and pathologic features are shown in ‹Table 1. Follow up time was up to 117.3 months with a median period of 73.5 months (range, 26.3–117.3 months). For the whole group, 7-years disease free (DFS) survival was 69.5% and the overall survival (OS) was 77.5%.

Based on immunohistochemical criteria for defining breast cancer molecular subtypes (Rakha et al., 2019); our cases were classified into five molecular subgroups as follows: Luminal A (ER+, PR+, HER2- and Ki67% < 20%) = group 1 (19 cases; 18.1%), Luminal B-Her2 negative (ER+, PR- or low, HER2- and Ki67% ≥ 20%) = group 2 (41 cases; 39.0%), Luminal B-Her2 positive (ER+, HER2+, PR any and Ki67 any) = group 3 (25 cases; 23.8%), HER2-enriched (ER-, PR- and HER2+) = group 4 (11 cases; 10.5%) and TNBC = group 5 (9 cases; 8.6%).

Expression of PD-L1 and its correlation with clinicopathologic characteristics

Thirty-four cases (32.4%) expressed PDL1. Out of which, twenty cases showed positive expression in both TCs and TILs, ten cases showed expression in TILs only while the remaining four cases showed expression in TCs only.

We found a strong association between PD-L1 protein expression with old age group (>55 years), (p value= 0.010) and high tumor grade (p value = 0.046) [Table 2]. No significant correlation found with other variables.

As regard the relation between PD-L1 expression and different BC molecular subtypes; HER2/neu-enriched group showed the highest expression (45.5%), followed by TNBC group (44.4%), then luminal B-Her2 negative and luminal B-Her2 positive (31.7% and 31.6%, respectively). Luminal A group expressed 24.0% only. However, this difference was statistically non-significant (p value = 0.686).

TILs density and its relation to PD-L1 expression

In the present cohort study, most of cases (71 cases; 67.6%) showed pre-NACTH low TILs density, while thirty-four cases (32.4%) revealed pre-NACTH high TILs density. Also, sixty-five cases (61.9%) showed high TILs change, while 40 cases (38.1%) revealed low TILs change after NACTH. PD-L1 expression revealed strong association with pre-NACTH high TILs density (P= <0.001) while it did not reveal any significant relation with TILs change (Table 2).

Multivariate analysis revealed that pre-therapy TILs density is an independent prognostic factor affecting PD-L1 expression (p= <0.001; OR=14.640, 95%CI 5.409 to 39.623).

Representative examples of BC cases with expression of PD-L1, TILs and NACTH response are shown in Figures 1 and 2.

NACTH pathological response and its relation to PD-L1 expression and TILs density change

Pathological therapy response was assessed in tumors and lymph nodes of all studied cases according

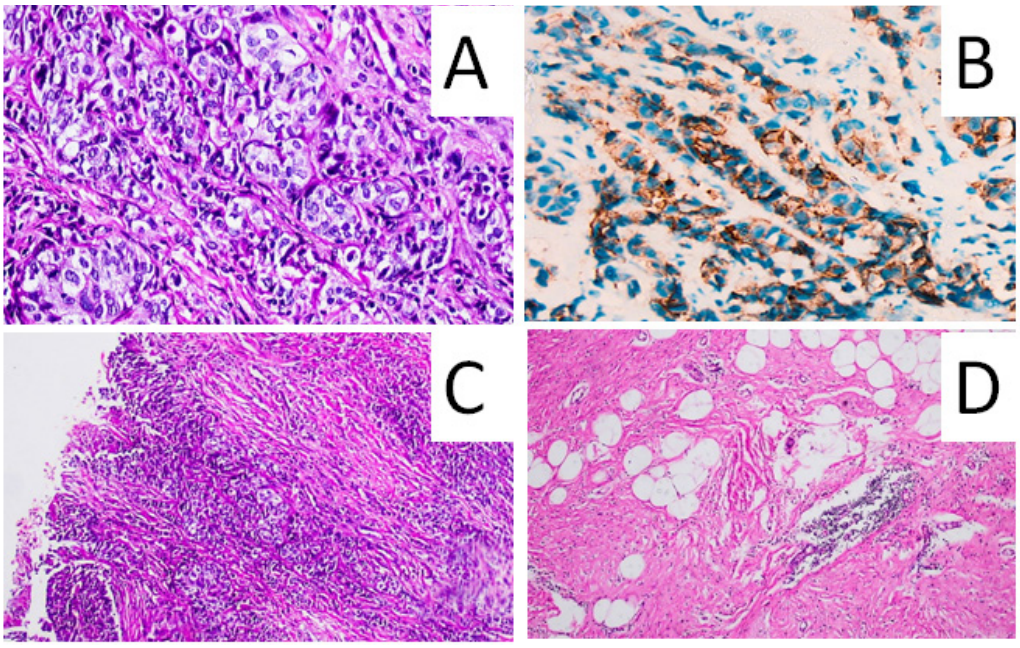

Figure 1. Example of Immunohistochemical Staining Results. A, Hematoxylin and Eosin image of a case high grade invasive duct carcinoma (x400); B, Immunostaining image of PD-L1 positive membranous expression in tumor cells (x400); C, Hematoxylin and Eosin image showing pretherapy high TILs density (x200); D, Hematoxylin and Eosin image showing complete therapy response (x200).

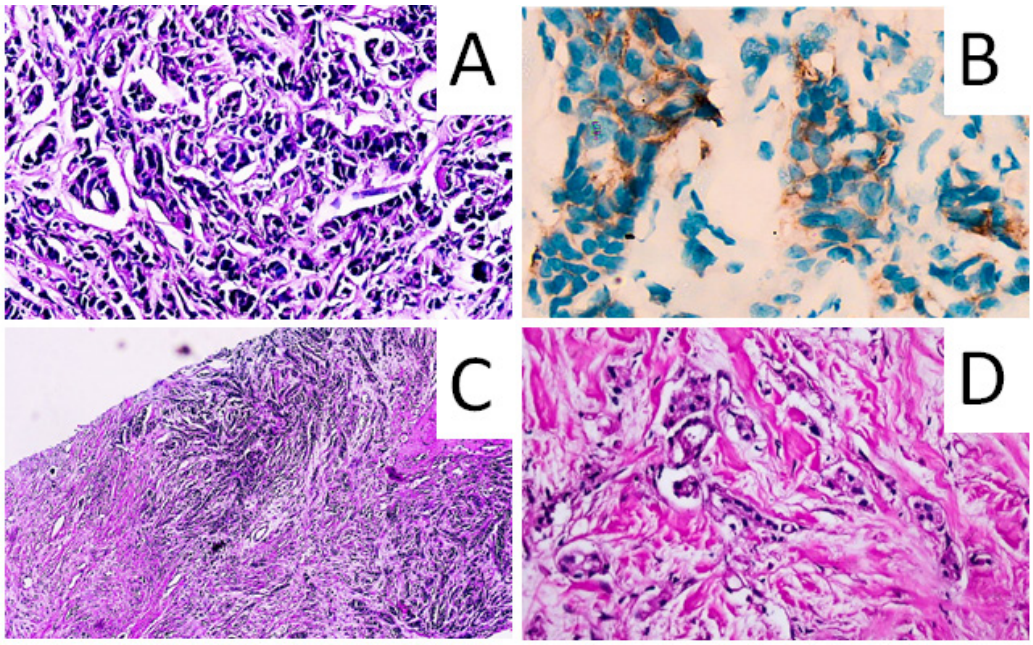

Figure 2. Another Example of Immunohistochemical Staining Results. A, Hematoxylin and Eosin image of a case high grade invasive duct carcinoma (x400); B, Immunostaining image of PD-L1 positive membranous expression in tumor cells (x400); C, Hematoxylin and Eosin image showing pretherapy high TILs density (x200); D, Hematoxylin and Eosin image showing moderate therapy response (RCB-II) (x400).

to Residual Cancer Burden system (RCB). The cases were categorized into four groups; RCB-0 (complete response), RCB-I (marked response), RCB-II (moderate response) and RCB-III (poor response) (Table 1). There was no statistically significant relation between PD-L1 expression and the pathological NACTH response. While pathological NACTH revealed significant relation to TILs density change after therapy (p=<0.001) (Table 2).

Example of BC case with complete therapy response revealed the change in TILs density is shown in Figure 3.

The relations of PD-L1, TILs, pathological NACTH response with OS and DFS

PD-L1 expression did not reveal significant relations with DFS and OS. However, there were better estimates for PD-L1 negative cases than PD-L1 positive cases (73.5% versus 67.6%, respectively for DFS), (82.2% versus 71.6%, respectively for OS).

DFS and OS estimates were significantly different among cases with high TILs change versus cases with low TILs change. The differences were statistically significant (P value=0.032 for DFS), (P value=0.003 for OS).

Pathological therapy response was strongly associated with DFS and OS. Where, they revealed better estimates in cases with complete, marked, and moderate response than cases with poor response (P=0.006 for DFS & P=<0.001) (Figures 4A and 4B).

Multivariate analysis revealed that PD-L1 and PR status were the only two independent factors affecting disease-free survival (P=0.031; HR=2.798, 95% CI 1.100

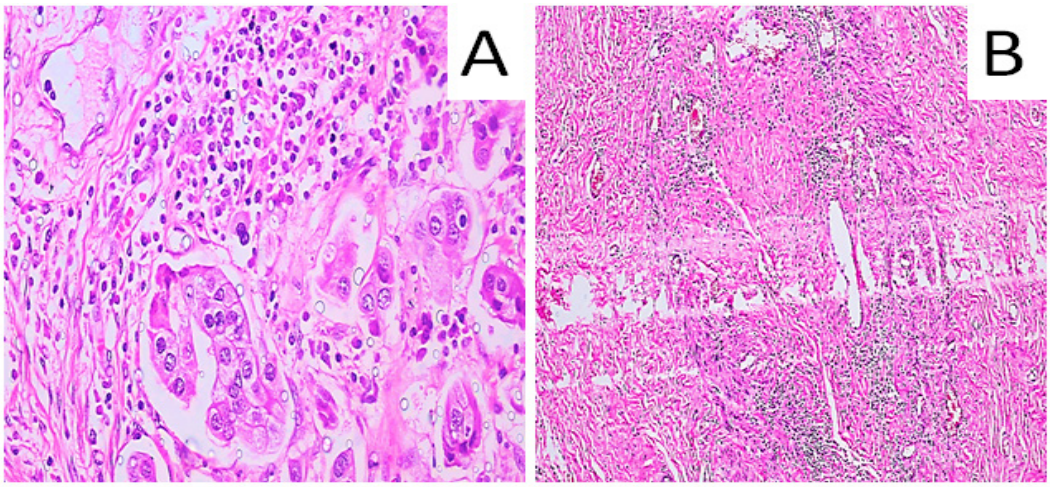

Figure 3. Example of TILs Density Change. A, Hematoxylin and Eosin image of a case grade 2 invasive duct carcinoma (x400) showing moderate TILs density (pretherapy); B, Hematoxylin and Eosin image of the same case (x200) after NACTH showing complete therapy response and dense TILs density (post-therapy).

Table 1. Clinicopathologic Characteristics of the Studied Cases (no. =105).

Patients' characteristics	No. (%) Total=105
Mean age	55±10.7 years [range 29-79]
≤ 55 years	59 (56.2)
>55 years	46 (43.8)
Histopathologic type	
Invasive duct carcinoma	95 (90.5)
Invasive lobular carcinoma	5 (4.8)
Mixed invasive duct and invasive lobular carcinoma	4 (3.8)
Invasive micropapillary carcinoma	1 (0.9)
Tumor grade	
I	6 (5.7)
II	90 (85.7)
III	9 (8.6)
Clinical TNM staging	
Clinical tumor size pretherapy (cT) (median=6 cm)	
cT2	27 (25.7)
cT3	65 (61.9)
cT4	13 (12.4)
Clinical lymph node status pretherapy (cN)	
cN0	27 (25.7
cN1	26 (24.8)
cN2	33 (31.4)
cN3	19 (18.1)
Clinical stage	
IIB	26 (24.8)
IIIA	54 (51.4)
IIIB	7 (6.7)
IIIC	18 (17.1)
Pathological TNM staging	
Residual tumor size post therapy (ypT) (median=2.5 cm)	
ypT0	22 (21.0)
ypTis	5 (4.8)
ypT1	30 (28.6)
ypT2	30 (28.6)
ypT3	15 (14.3)
ypT4	3 (2.9)
Lymph node status post therapy (ypN)	
ypN0	38 (36.2)
ypN1	23 (21.9)
ypN2	26 (24.8)
ypN3	18 (17.1)
Pathological stage	
0	16 (15.2)
IA	15 (14.3)
IB	1 (1.0)
IIA	21 (20.0)
IIB	4 (3.8)
IIIA	29 (27.6)
IIIB	1 (1.0)
IIIC	18 (17.1)

Table 1. Continued

Patients' characteristics	no. (%) Total=105
Metastatic sites (no.=28 cases)	
Bone	14 (50.0)
Lung	8 (28.6)
Liver	3 (10.7)
Contralateral Axillary L.Ns	2 (7.2)
Skin nodules	1 (3.6)
Surgical procedure	
Modified radical mastectomy	92 (87.6)
Conservative breast surgery	13 (12.4)
Adjuvant chemotherapy	
Yes	86 (81.9)
No	19 (18.1)
Radiotherapy	
Yes	85 (81.0)
No	20 (19.0)
Hormonal therapy	
Yes	86 (81.9)
No	19 (18.1)
ER status	
Positive	79 (75.2)
Negative	26 (24.8)
PR status	
Positive	73 (69.5)
Negative	32 (30.5)
HER2/neu status	
Positive	36 (34.3)
Negative	69 (65.7)
KI-67 LI (no.=86 cases)	
≥20%	19 (22.0)
<20%	67 (78.0)
Molecular subtypes	
Luminal A	19 (18.1)
Luminal B-Her2 negative	41 (39.0)
Luminal B-Her2 positive	25 (23.8)
Her2/neu-enriched	11 (10.5)
TNBC	9 (8.6)
PD-L1 status	
Positive	34 (32.4)
Negative	71 (67.6)
Tumor-infiltrating lymphocytes (TILs) density	
Pretherapy TILs density	
High	34 (32.4)
Low	71 (67.6)
TILs density change (post-therapy)	
High	65 (61.9)
Low	40 (38.1)
Residual Cancer Burden (RCB)	
RCB-0	16 (15.2)
RCB-I	9 (8.6)
RCB-II	25 (23.8)
RCB-III	55 (52.4)

Table 2. The Relation between PD-L1, Clinico-Pathological Variables, Breast Cancer Molecular Subtypes, TILs Density and NACT Response.

Variables	PDL1		p value
	Negative (n=71)	Positive (n=34)	
Age (years)			0.01
≤ 55	46 (78.0)	13 (22.0)	
>55	25 (54.3)	21 (45.7)	
Histopathologic type			0.112
Invasive duct carcinoma	62 (65.3)	33 (34.7)	
Other types	9 (90.0)	1 (10.0)	
Tumor grade			0.046
I	5 (83.3)	1 (16.7)	
II	63 (70.0)	27 (30.0)	
III	3 (33.3)	6 (66.7)	
Clinical TNM staging			
Clinical tumor size pretherapy (cT)			0.646
cT2	19 (70.4)	8 (29.6)	
cT3	42 (64.6)	23 (35.4)	
cT4	10 (76.9)	3 (23.1)	
Clinical lymph node status pretherapy (cN)			
cN0	17 (63.0)	10 (37.0)	0.33
cN1	18 (69.2)	8 (30.8)	
cN2	20 (60.6)	13 (39.4)	
cN3	16 (84.2)	3 (15.8)	
Clinical stage			
IIB	16 (61.5)	10 (38.5)	0.455
IIIA	35 (64.80	19 (35.2)	
IIIB	5 (71.4)	2 (28.6)	
IIIC	15 (83.3)	3 (16.7)	
Pathological TNM staging			
Residual tumor size post therapy (ypT)			0.661
ypT0	13 (59.1)	9 (40.9)	
ypTis	3 (60.0)	2 (40.0)	
ypT1	19 (63.3)	11 (36.7)	
ypT2	22 (73.3)	8 (26.7)	
ypT3 & T4	14 (77.8)	4 (22.2)	
Lymph node status post therapy (ypN)			0.165
ypN0	23 (60.5)	15 (39.5)	
ypN1	14 (60.9)	9 (39.1)	
ypN2	18 (69.2)	8 (30.8)	
ypN3	16 (88.9)	2 (11.1)	
Pathological stage			0.09
yp stages 0 & I	21 (65.6)	11 (34.4)	
yp stage II	13 (52.0)	12 (48.0)	
yp stage III	37 (77.1)	11 (22.9)	
ER status			0.779
Positive	17 (65.4)	9 (34.6)	
Negative	54 (68.4)	25 (31.6)	
PR status			0.232
Positive	19 (59.4)	13 (40.6)	
Negative	52 (71.2)	21 (28.8)	
HER2/neu status			0.773
Positive	46 (66.7)	23 (33.3)	
Negative	25 (69.4)	11 (30.6)	
Molecular subtypes			0.686
Luminal A	13 (68.4)	6 (31.6)	
Luminal B-Her2 negative	28 (68.3)	13 (31.7)	
Luminal B-Her2 positive	19 (76.0)	6 (24.0)	
Her2/neu-enriched	6 (54.5)	5 (45.5)	
TNBC	5 (55.6)	4 (44.4)	
Tumor-infiltrating lymphocytes (TILs) density			
Pretherapy TILs density			<0.001
High	10 (29.4)	24 (70.6)	
Low	61 (85.9)	10 (14.1)	
TILs density change (post-therapy)			0.205
High	41 (63.1)	24 (36.9)	
Low	30 (75.0)	10 (25.0)	
Residual Cancer Burden (RCB)			0.447
RCB-0	10 (62.5)	6 (37.5)	
RCB-I	5 (55.6)	4 (44.4)	
RCB-II	15 (60.0)	10 (40.0)	
RCB-III	41 (74.5)	14 (25.5)	

to 7.120, and P= 0.047; HR=2.270. 95% CI 1.1010-5.100, respectively).

Discussion

The success of ER+/PR+ and HER2 targeted therapies has shifted researchers' focus to the triple negative disease. However, PD-L1 targeted therapies may be useful for those who have developed resistance to current hormone and HER2 directed therapies (Sanilmanejad et al., 2019). As a result, we investigated the role of PD-L1 in locally advanced BC of various molecular subtypes, as well as its relationship to clinicopathologic parameters, TILs density, and NACTH response.

The status of PDL-1 in BC has been reported in various studies with varying results. This could be due to differences in antibody clones and approved assays with varying degrees of sensitivity and reproducibility. The expression of PD-L1 in both tumor and immune cells was examined in our study, and 32.4% of our cases had PD-L1 expression. Wimbery et al., (2015) and Chen et al., (2017) reported a high frequency of PD-L1 expression in advanced breast carcinomas, which is consistent with our findings (49% and 30%, respectively). While Kitano et al., (2017) and Guo et al., (2020) investigated the frequency of PD-L1 expression in early-stage breast carcinomas, they found PD-L1 expression rates of 10% and 13%, respectively. PD-L1 expression in tumor cells was very low (1.9%) in Berckelaer and colleagues' study, but it was much higher (43%) in TILs (Van Berckelaer at al., 2019). Furthermore, Gatalica and colleagues discovered

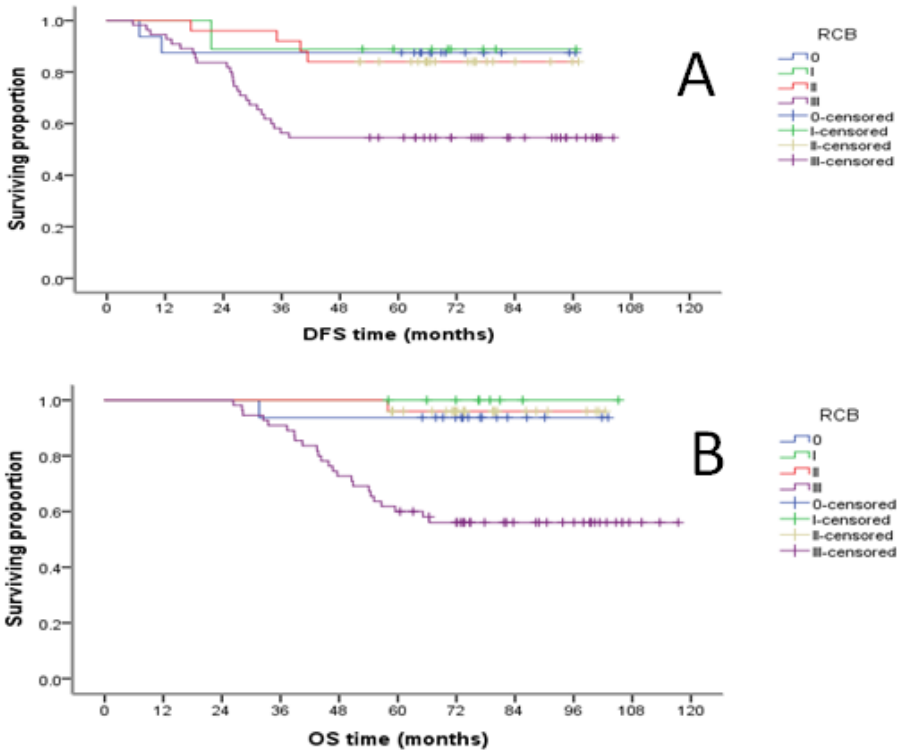

Figure 4. A, Association of RCB with patients' survival . A) Kaplan-Meier curves of disease-free survival between cases with different RCB categories; B, Kaplan-Meier curves of overall survival between cases with different RCB categories.

a high rate of PD-L1 expression (45%) after studying 116 BC cases (Gatalica et al., 2014). This is because PD-L1 expression in tumor cells is strangely associated with aggressive biological behavior of the tumor and a poor prognosis (Wu et al., 2019).

In our study, we discovered that PD-L1 expression differed between molecular subtypes. The Her2-enriched group had the highest PD-L1 expression (45.5%), followed by the TNBC group (44.4%), and the Luminal A group had the lowest (24%). Unfortunately, the difference between subtypes was not statistically significant. This could be due to the small sample size of each molecular subtype. Similarly, Gatalica et al., (2014) and Kim et al., (2017) discovered high PD-L1 expression in both HER2 positive and TNBC subtypes when compared to luminal subtypes.

On the other hand, Tsang and colleagues studied 1091 BC patients. PD-L1 expression was higher in the luminal A subtype (34.1%) than in the other BC subtypes (Tsang et al., 2017). We recommend that larger samples of luminal A subtypes be studied to draw conclusions about the efficacy of immunotherapy in this specific group of patients.

In the present study, the PD-L1 expression revealed strong association with old age group (>55 years) and high tumor grade. The same results were reported by the studies of Kitano et al., (2017), Okabe et al., (2017) and Guo et al., (2020). It has been documented that PD-L1 expression is associated with poor prognostic factors, including high grade, large tumor size and positive lymph node metastasis.

We also found that PD-L1 expression is strongly associated with the pre-NACTH tumor infiltrating lymphocytes (TILs). PD-L1 showed marked expression in tumors with high density TILs than those with low density TILs. This is in concordance with Pelekanou et al., (2017) and Kitano et al., 2017 results.

In spite PD-L1 expression in our study did not reveal significant relation to pathological therapy response and this was in concordant with Oner and colleagues study results (2021), other studies reported a significant association between PD-L1 expression and higher pathologic response to neoadjuvant chemotherapy, where it can act as a promising immune marker to predict neoadjuvant chemotherapy response in patients with breast cancer (Ahmed et al., 2020; Du et al., 2020).

While Pelekanou and colleagues (2017) found that higher TILs density change correlated with higher rates of pathological complete response (pCR). The same finding was observed in our study.

Regarding survival, our results revealed strong association between patient's outcomes (DFS & OS) and TILs density change after NACTH as well as pathological therapy response, where they showed better estimates with high TILs change and complete therapy response. The same results were reported by Lee et al., (2020); Wang and Mao (2020) that using different systems for accurate evaluation of pathological response after therapy and reported that pathologic complete response is a validated and valuable surrogate prognostic factor of survival after therapy.

Unfortunately, we could not prove in our study any significant relation between PD-L1 expression and OS or DFS. In a large metanalysis study (2,546 women) done by Zhang et al., (2017), showed that PD-L1 overexpression was associated with worse prognosis and shorter overall

survival. While in another study, PD-L1 positivity was also associated with poor DFS, but there was no effect on OS (Kim et al., 2017).

I conclusion, according to our study, we found that a new biomarker PD-L1 may be useful in stratifying patients with locally advanced breast cancer and identifying those who may benefit from immunotherapy, especially in cases with high-grade BC, when we used a 1% cutoff value.

Author Contribution Statement

All authors contributed to this study. Preparation, data collection, review of the slides and analysis were performed by Dr Mustafa A. Hussein. Monitoring of data collection, interpretation of results, revision and guidance were done by Dr Hoda Ismail and Dr Akram Nouh. The preliminary draft of the manuscript was written by Dr Mustafa A. Hussein. All authors revised and commented on primary version of the manuscript and approved the final one.

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Ethical approval

The study was approved by the Institutional Review Board (IRB) no. IRB00004025 of National Cancer Institute (NCI), Cairo University. Oral and written informed consents were obtained from all patients or from their eligible relatives.

Availability of data

The datasets are available from the corresponding author on reasonable request.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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