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## Program Death Ligan-1 (PD-L1) Expression in Invasif Breast Carcinoma of No Special Type

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### Abstract

Breast carcinoma is the most frequently cancer in women (24%) and the main cause of cancer death in women worldwide. Based on data from globocan 2018 shows the incidence of breast cancer is around 2.08 million (11.6%) which is the second rank of all cancers after lung cancer with a mortality rate of 626.6 thousand (6.6%). However, prognosis of the breast carcinoma is influenced by several factors, including tumor histology grade and Tumor Infiltrating Lymphocytes (TILs). PD-L1 expression has been investigated as a potential biomarker and immune checkpoint to assess the response of various types of cancer. This study aims to determine PD-L1 expression in Invasive Breast Carcinoma of No Special Type (IBC-NST) grades 1,2 and 3. This study used a sample of 80 cases of paraffin block for IBC-NST patients from 2017 to 2020. There were 17 samples (21.3%) with grade 1, 32 samples (40%) with grade 2, and 31 samples (38.8%) with grade 3. The number of samples with positive PD-L1 expression were 63 samples, and 17 samples of negative PDL-1 expression were obtained. In the PD-L1 negative group, from a total of 17 samples, 4 samples were grade 1, 10 samples were grade 2, and 3 samples were grade 3. In the PD-L1 positive group, from a total of 63 samples, 13 samples with grade 1, 22 with grade 2, and 28 samples with grade 3. Based on the Chi-square test, p value = 0.115 ( $p > 0.05$ ). The proportion of PD-L1 expression was higher at higher grades. There was no significant difference in PD-L1 expression in IBC-NST grade 1, grade 2 and grade 3.

**Keywords:** invasive breast carcinoma; PD-L1; grade.

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## **1. Introduction**

Breast carcinoma is the most frequently cancer in women (24%) and the main cause of cancer death in women worldwide. It is the second most common cancer overall in men and women (11.6%). The incidence rate has increased in the development countries in recent decades [1]. Based on data from Globocan 2018, the incidence of breast cancer is around 2.08 million (11.6%) which is the second rank of all cancers after lung cancer with a mortality rate of 626.6 thousand (6.6%) [2]. Data from the Ministry of Health of the Republic of Indonesia in 2013 shows the prevalence of breast cancer is 61.682 (0.5%) [3]. Data in the Anatomical Pathology Laboratory, Dr. Wahidin Sudirohusodo there were 136 cases in 2018 and 162 cases in 2019 with breast carcinoma. However, breast carcinoma can develop at any age, from childhood to old age [4]. Invasive breast carcinoma (IBC) is a malignant neoplasm originating from the mammary gland epithelium, while invasive breast carcinoma of no special type (IBC-NST) is a group of IBC that cannot be classified morphologically into a specific histological type. The majority of breast carcinomas are IBC-NST, where the prognostic characteristics and management are same as with the other variant or slightly worse. The prognosis of breast carcinoma is influenced by several factors, including tumor histology grade and TILs [1]. PD-L1 expression has been investigated as a potential biomarker and immune checkpoint to assess the response of various types of cancer [5]. PD-L1 is located on the membranes of various cell types, and overexpressed in the tumor cells [6]. PD-L1 inhibits T cell function by binding to its receptor on T cells, PD-1. The interaction of PD-L1 and PD-1 inhibits the activation and proliferation of T cells, inhibits the function of T cells to produce cytokines and kill the tumor cells [7]. Based on the importance in clinical application and their role in biological concepts, it is necessary and important to determine the expression of PD-L1 in IBC-NST, especially in grade 1, 2 and 3.

## **2. Material and Methods**

### **2.1 Collection of Samples**

In this study, we collected 80 paraffin block samples of patients with IBC-NST from the Anatomical Pathology Laboratory of Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital during the period 2017 to 2020.

### **2.2 Immunohistochemistry Staining**

Unstained slides were made from paraffin blocks and PD-L1 immunohistochemistry staining was performed. In each case, slides were made from paraffin blocks then cut with a 3  $\mu$ m thick microtome. The cut in the water bath was taken using a poly-L-lysine slide, then deparaffinized. Immunohistochemical staining using mouse monoclonal antibody, clone 22C3 (DAKO) with 1:50 dilution. PD-L1 was colored brown completely or partially circled on the cell membrane. The results of immunohistochemical staining were assessed by two pathologists. PD-L1 expression was calculated based on the percentage of tumor cells stained at any intensity by immunohistochemical staining. Score 0 = 0%, score 1 = 1-9%, score 2 = 10-49%, score 3 = 50-100%. PD-L1 expressions divided in two group, were negative if they were not colored (score 0), and positive if they were colored with any intensity (scores 1, 2, and 3) [8].

### 2.3 Data Processing

The data in this study were processed using SPSS 20 for Windows software. Descriptive statistical techniques used to describe the characteristic of the basic data obtained in the form of frequency distribution, range and average of age. Chisquare test were used to determine PD-L1 expression in positive and negative group based on IBC-NST grade.

## 3. Results

### 3.1 Patients Characteristics

Of the 80 samples, there were 11 samples in the <40 years age category and 69 samples in the 40 year age category with a mean of age 50.63 years, a minimum age was 28 years and a maximum age was 70 years. There were 17 samples (21.3%) with grade 1, 32 samples (40%) with grade 2, and 31 samples (38.8%) with grade 3. The number of samples with positive PD-L1 expression were 63 samples, and 17 samples of negative PD-L1 expression were obtained (Table 1).

### 3.2 PD-L1 Based on Histopathological Grade

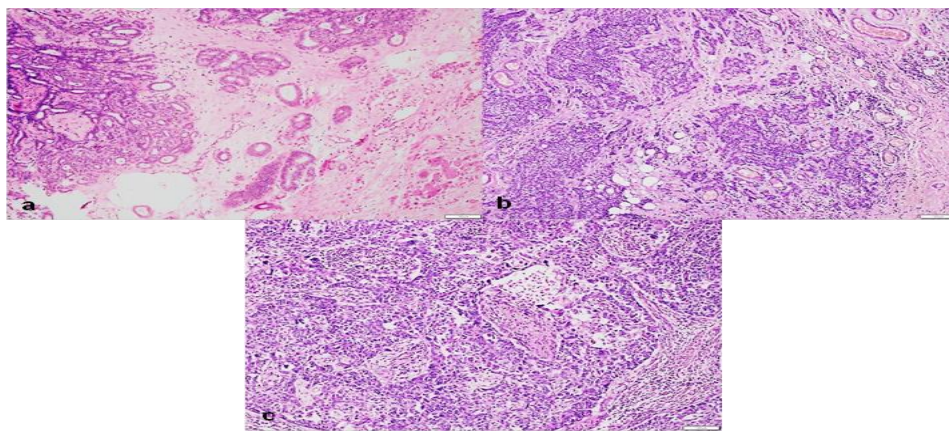
In the PD-L1 negative group, from a total of 17 samples, 4 samples were grade 1, 10 samples were grade 2, and 3 samples were grade 3. In the PD-L1 positive group, from a total of 63 samples, 13 samples with grade 1, 22 with grade 2, and 28 samples with grade 3. Based on the Chi-square test, p value = 0.115 ( $p > 0.05$ ) (Table 2). Histopathological and PD-L1 expression staining were observed in Figure 1 and Figure 2 respectively.

**Table 1:** Sample Characteristics.

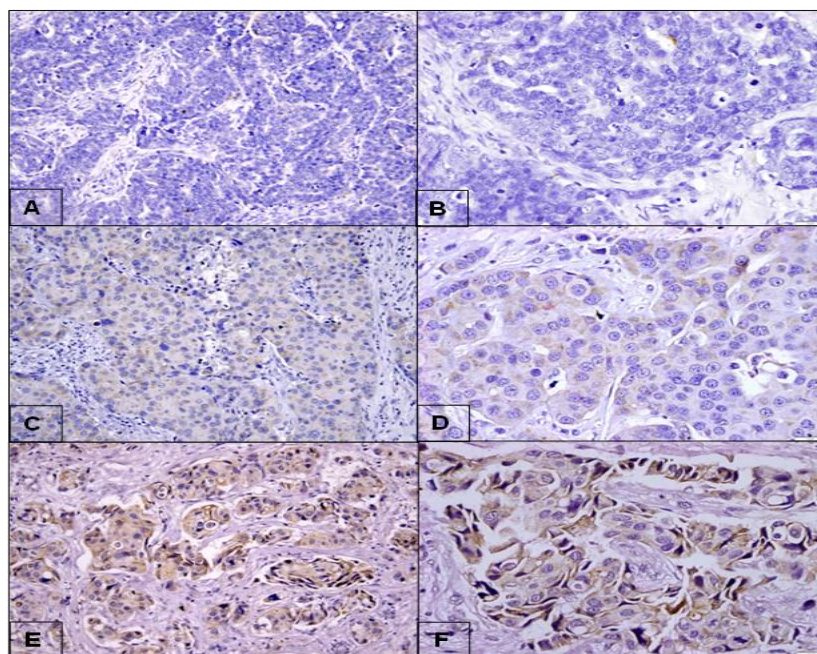
Characteristics	N	Minimum	Maximum	Mean	%
<b>Age (old)</b>	80	28	70	50,63	
< 40 years	11				13.8
≥ 40 years	69				86.3
<b>Histopathological Grade</b>					
Grade 1	17				21.3
Grade 2	32				40.0
Grade 3	32				38.8
<b>PD-L1 Expression</b>					
Positive	63				78.8
Negative	17				21.3

**Table 2:** PD-L1 Expression Based on Histopathological Grade.

		Histopathological Grade			Total n (%)	P
		Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)		
PD-L1 Expression	Negative	4 (23.5)	10 (31.3)	3 (9.7)	17 (21.3)	0,115
	Positive	13 (76.5)	22 (68.8)	28 (90.3)	63 (78.8)	
Total		17 (100)	32 (100)	31 (100)	80 (100)	
Chi-Square						



**Figure 1:** Histopathological Grade. A. Grade 1. B. Grade 2. C. Grade 3 (Objective 200x and 100x).



**Figure 2:** PD-L1 expression. A-B. Negative staining. C-F. Positive staining (Objective 200x and 400x).

#### **4. Discussion**

Of the 80 IBC-NST samples, all of them were female with the age  $\geq 40$  years (86.3%) more than the age  $<40$  years group (13.8%). This is in accordance with the results of the SEER program (Surveillance, Epidemiology, and End Results) in 2009 to 2018 conducted by NCI (National Cancer Institute), the number of breast cancer patients ranged from 20 to 34 years old (1.9%), 35 to 44 years old (8.2%), 45 to 54 years old (19.2%), 55 to 64 years old (25.6%), 65 to 74 years old (26.0%), 75 to 84 years old (13.7%), and  $>84$  years (5.4%). This is in accordance with the tendency of breast carcinoma as a malignancy of epithelial origin in general whose their incidence increases with age [9]. PD-L1 is located on the membranes of various cell types such as hematopoietic and nonhematopoietic cells. The amount of PD-L1 in these cells was very low, whereas in tumor cells it was overexpressed [6].

PD-L1 expression is divided into constitutive expression and inducible expression, which is dependent on extrinsic or intrinsic stimulus. Constitutive expression is induced by dysregulation of oncogenes or tumor suppressor gene signaling pathways, through abnormal activation of transcription factors, or by genomic alteration or gene amplification. Meanwhile, inducible expression is regulated by inflammatory signals from tumor cells or other immune cells, such as APCs and T cells, in the tumor microenvironment [10].

In this study, the expression of PD-L1 in grade 1, 2 and 3 was generally positive. There were 76.5%, 68.8% and 90.3%, respectively. With the Chi-square test, the p value = 0.115, which indicates that there was no significant difference in the expression of PD-L1 in each grade, where the expression of PD-L1 is not affected by histopathological grade.

The host immune system and tumor cells that persist in the elimination phase will enter the equilibrium phase which is a latent period due to incomplete tumor destruction. Tumor cells that cannot be eliminated are tumor cells that genetically unstable and rapidly mutate [11]. In this phase, tumor cells can avoid immune cells by expressing PD-L1. The regulation of PD-L1 expression is influenced by constitutive and inducible expression induced by various factors. Where constitutive expression is induced by dysregulation of signal transduction components in tumor cells, and inducible expression is induced by a number of inflammatory cytokines. In breast carcinoma constitutive expression of PD-L1 are induced by PTEN and EGFR, while inducible expression are induced by TNF- $\alpha$  and EGF [10]. In addition, the molecular mechanism of PD-L1 expression in cancer cells occurs at the level of genomic amplification, epigenetic regulation, transcriptional regulation, posttranscriptional regulation, translational regulation, and posttranslational modification [10,12].

This is in line with the research conducted by He and his colleagues and Zhou and his colleagues where PD-L1 expression is not related to grade, lymph node status and HER2 expression [13,14].

The variability of antibody clones, scoring methods and cut off positive expressions in various studies gives different positive rates between 1.7% to 80%. The PD-L1 assessment standard has not yet been found in breast carcinoma, so the results of the PD-L1 study still unclear [13].

In the study of Zhou and his colleagues PD-L1 with high expression was found in the Triple Negative Breast

Cancer (TNBC) molecular subtype, indicating that PD-L1 immune target therapy may benefit for patients with TNBC compared to other subtypes [14].

## 5. Conclusion

In this study, we conclude that the proportion of PD-L1 expression was higher at higher grades. There was no significant difference in PD-L1 expression in IBC-NST grade 1, grade 2, and grade 3.

## 6. Suggestions

PD-L1 expression in this study can be used as additional data in the development of the immunotherapy in IBC-NST. It is necessary to conduct further research on breast carcinoma samples by assessing the expression of PD-L1 based on molecular subtypes, particularly the Triple Negative Breast Cancer molecular subtype.

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