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Original Research Paper

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ASSOCIATION BETWEEN IMMUNOHISTOCHEMICAL EXPRESSION OF PROGRAMMED DEATH – LIGAND 1 (PD-L1) AND CLINICOPATHOLOGICAL PARAMETERS IN INVASIVE BREAST CARCINOMA

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ABSTRACT Programmed Death-Ligand 1 (PD-L1) overexpression as a predictive biomarker for anti-PD-1/PD-L1 therapy effectiveness has been observed in various human malignancies and contributes to poor prognosis. However, this is still controversial in breast malignancy. This cross-sectional analytic study of 37 invasive breast carcinoma patients' formalin-fixed paraffin-embedded blocks was conducted to assess the association between immunohistochemical expression of PD-L1 and various clinicopathological parameters. PD-L1 was expressed in 48.6% of the invasive breast carcinoma specimens. PD-L1 positive expression was significantly associated with larger tumor size, higher histological grade, high stromal TILs, and aggressive molecular subtype, such as triple negative breast cancer. The association of PD-L1 expression with adverse clinicopathological characteristics indicates that PD-L1 expression can be considered as a prognostic biomarker for invasive breast carcinoma, and may be helpful in selecting potential patients for anti-PD-1/PD-L1 therapy.

KEYWORDS : invasive breast carcinoma, PD-L1, immunohistochemical

Introduction

Breast cancer is the main malignancy and cause of death in women in the world, including in Indonesia.[1,2] Breast cancer represents various entities with different morphology and natural behaviors. This heterogeneity causes the existing management to remain unsatisfactory, likely due to the lack of effective indicators to predict disease courses and the existence of chemoresistant breast carcinoma, such as the triple negative breast cancer (TNBC) subtype.[3]

Programmed cell death protein 1 (PD-1) is expressed on the surface of T cells and binds to one of its ligands, Programmed death-ligand 1 (PD-L1), which is expressed in tumor cells.[4] The interaction of these two proteins in the tumor will affect the anti-tumor immune response by causing T cells exhaustion and dysfunction so that tumor cells can evade the immune system, proliferate and metastasize.[5] Inhibition of the PD-1/PD-L1 pathway with monoclonal antibodies against PD-1 or PD-L1 is a promising therapeutic approach and is currently being explored in many types of human malignancies.[4] Although breast carcinoma has not been considered an immunogenic tumor, the presence of tumor infiltrating lymphocytes (TILs) along with tumor cells raises the thought that breast carcinoma has immune response defect and the question of whether the immunotherapy is also beneficial for patients with breast carcinoma.[6,7]

PD-L1 overexpression has been observed in various human malignancies and previous studies have shown that PD-L1 expression contributes to a poor prognosis.[8] However, this is still unclear in breast malignancy. Several studies have also reported different results regarding the association of PD-L1 expression with various clinicopathological features of breast cancer.[9-13] The application of PD-L1 immunohistochemistry is relatively new in Indonesia, especially in Medan, where studies of PD-L1 expression are limited and is still focused on NSCLC with respect to therapeutic interests. Therefore, this study aimed to evaluate the immunohistochemical expression of PD-L1 in invasive breast carcinoma and analyze its association with various clinicopathological parameters in invasive breast carcinoma.

Material and methods

Sample selection

This cross-sectional analytic study was conducted on 37 formalin-fixed paraffin-embeded tissue blocks of invasive breast carcinoma cases in Department of Anatomical Pathology, Universitas Sumatera utara/H. Adam Malik Hospital. All samples were obtained through surgical procedures, 7 (18.9%) cases from incisional biopsy and 30 (81.1%) cases from mastectomy.

Histology evaluation

Clinical data were obtained from medical records or pathology/radiology archieves consisting of age, tumor size, lymph node involvement, and immunohistochemical profiles. Immunohistochemical profiles based on ER, PR, HER2, and Ki-67 status were categorized according to St. Gallen International Conference in 2013.[14]

Histology type, grade, and TILs were determined through examination of Hematoxylin and Eosin stained slides. Histological types were divided into no special type and special type. Histological grade was determined using the method by Patey & Scarff and Bloom & Richardson modified by Elston and Ellis.[15] TILs was reported for the stromal compartment using the International TILs Working Group 2014 recommendation.[16]

Immunohistochemistry protocol and interpretation

Immunostaining was done using BOND-MAX Fully Automated IHC (Leica Biosystems). The tissue sections were deparaffinized and rehydrated. Endogenous peroxidase was blocked with peroxide block then followed by the application of antigen retrieval, PD-L1 (clone MD21R, Medaysis, CA) rabbit monoclonal antibody, post primary and polymer. The reaction was visualized with diaminobenzidine and counterstained with Hematoxylin followed by dehydration, clearing, and mounting. The antibody was optimized with known positive control using placenta tissue.

PD-L1 expression was determined independently by three observers using Histo-score (H-score), with a range of possible scores from 0 to 300. H-score 0-99 was considered negative/low,

while 100-300 was considered positive expression.[10]

Statistical analysis

Inter-rater reliability (IRR) in determining histological type, grade, and biomarker expression was calculated with Fleiss κ using Microsoft Excel. Data compilation and analysis were done using SPSS version 22.0 (SPSS Inc., Chicago). Analysis were performed using independent sample Student t, Chi square, Fisher's Exact, Mann-Whitney, and Pearson's correlation test with significance p-value < 0.05.

Results

Immunohistochemical expression of PD-L1 and clinicopathological parameters

Clinicopathological characteristics were presented in Table 1. The mean age of invasive breast carcinoma patients was 52.46 years. *IRR* revealed an almost perfect agreement in determining histological type and grade (κ = 0.85 and 0.83, respectively) and substantial agreement in determining stromal *TILs* and *PD-L1* expression (κ = 0.79 and 0.78, respectively).

Significantly larger tumor size and higher degree of stromal TILs in tumors with PD-L1 positive were compared to those with PD-L1 negative/low. PD-L1 expression (Figure 1) showed a significant association with larger tumor size, higher grade, high stromal TILs, and aggressive molecular subtype (Table 1). In addition, we also found that PD-L1 expression was negatively associated to ER (prevalence ratio = 3.167; p = 0.026) and PR (prevalence ratio = 4.574; p = 0.001) status.

Table 1. PD-L1 expression and clinicopathological parameters of patients with invasive breast carcinoma

Variable	Number of cases n = 37		Positive n = 18		Negative/low n = 19		P
	n	%	n	%	n	%	
Tumor size							
Mean ± SD (cm)	4.46 ± 1.59		5.19 ± 1.51		3.76 ± 1.36		0.005*
≤2 cm	4	10.8	2	11.1	2	10.5	0.021*[a]
2,1-5 cm	19	51.4	5	27.8	14	73.7	
>5 cm	14	37.8	11	61.1	3	15.8	
Lymph node involvement							
Negative	20	54.1	7	38.9	13	68.4	0.072
Positive	17	45.9	11	61.1	6	31.6	
Histological type							
No special type	24	64.9	10	55.6	14	73.7	0.209
Special type	13	35.1	8	44.4	5	26.3	
Histological grade							
Grade 1	2	5.4	0	0	2	10.5	0.001*[,b]
Grade 2	20	54.1	6	33.3	14	73.7	
Grade 3	15	40.5	12	66.7	3	15.8	
Stromal TILs							
Mean ± SD (%)	27.03 ± 18.91		40.28 ± 16.49		14.47 ± 10.78		<0.001*
Low (<50%)	31	83.8	12	66.7	19	100	0.008*
High (≥50%)	6	16.2	6	33.3	0	0	
Immunohistochemical profile							
Luminal A	13	35.1	1	5.6	12	63.2	0.001*[,c]
Luminal B	12	32.4	8	44.4	4	21	
HER2-enriched	7	18.9	5	27.8	2	10.5	
TNBC	5	13.5	4	22.2	1	5.3	

* p<0,05
[a] mean rank PD-L1 positive 22.81; PD-L1 negative/low 15.39
[b] mean rank PD-L1 positive 24.17; PD-L1 negative/low 14.11
[c] mean rank PD-L1 positive 24.89; PD-L1 negative/low 13.42

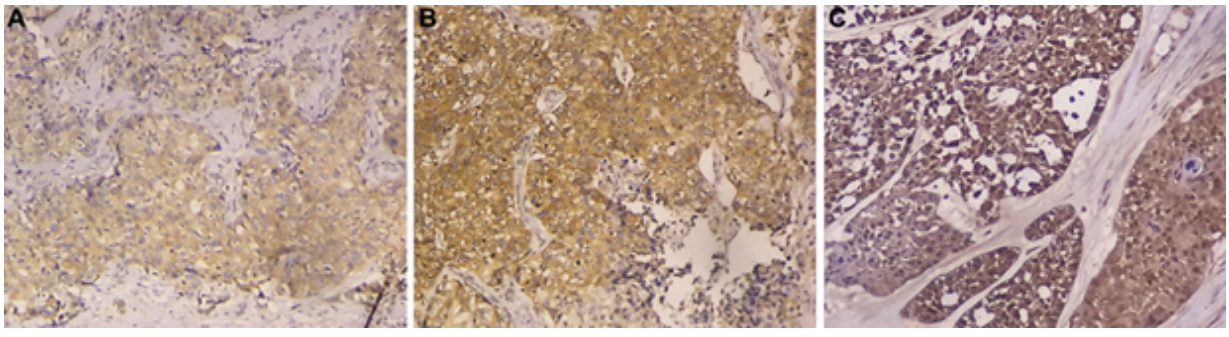

Figure 1. Immunohistochemical expression of PD-L1. A, Weak. B, Moderate. C, Strong.

Correlation of PD-L1 expression score with degree of stromal TILs

PD-L1 expression score and degree of stromal TILs showed linear correlation (Figure 2). This significant positive correlation between *PD-L1* expression and degree of stromal *TILs* showed that the higher PD-L1 expression, the higher degree of stromal TILs in invasive breast carcinoma patients (r 0.799; p <0.001).

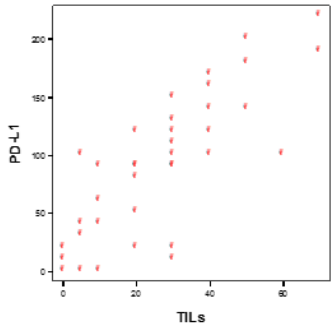

Figure 2. Scatter plot of PD-L1 expression score and degree of stromal TILs.

Discussion

The development of monoclonal antibodies against PD-1 or PD-L1 is one of the latest breakthroughs in oncology. This therapy causes a long-term response in several types of malignancies, especially in tumor with higher PD-L1 expression.[17] In this study, the percentage of invasive breast carcinoma with PD-L1 positive was in the range of 21.7% -56.6% of cases reported in several studies.[9-13] The activation of PD-1/PD-L1 pathway causes suppression of anti-tumor adaptive responses resulting in evasion of tumor cells from the immune system which leads to tumor progression.[18] This is evidenced by a significant associatiation of PD-L1 expression with larger tumor size and higher histological grade, which is also in line with Muenst, et al. (2014), Qin, et al. (2015), Baptista, et al. (2016).[9,10,12] However, there was no significant association between PD-L1 expression and lymph node involvement. This is in line with several studies,[9,13] but contrary to other studies.[10-12,19]

In line with the study of Qin, et al. (2015) and Li, et al. (2016), tumors expressing PD-L1 tended to have aggressive immunohistochemical profiles.[9,11] This was evident from the large proportion of TNBC, followed by HER2-enriched, which showed positive PD-L1 expression. Although Muenst, et al. (2014) found no association of immunohistochemical profiles with PD-L1 expression, the results of his study and several other studies found that PD-L1 was positively associated with HER2 and Ki-67, but negatively related to ER and PR.[9,10,11,13] We also found a significant negative association of PD-L1 expression with ER and PR. This finding further supports the assumption that PD-L1 plays a role in breast cancers that do not develop through hormonal pathway.

In this study, the degree of TILs in tumors with PD-L1 positive was found to be higher than in those with PD-L1 negative/low. The PD-L1 expression score was found to be positively correlated with the degree of stromal TILs, which is in line with Wimberly, et al. (2015), Thompson, et al. (2016), and Kitano, et al. (2017).[20-22] The association between TILs and PD-L1 in various types of tumors leads to the emergence of allegations that these two factors are biologically interrelated.[23]

Most studies have confirmed the association of TILs with a better prognosis, but this is contrary to the expression of PD-L1 which is more often associated with a poor prognosis.[13] Specific subsets of TILs seem to have a role in this problem. Park, et al. (2016) and Wang, et al. (2017) reported that positive expression of PD-L1 and CD8 in some TNBC were associated with a better prognosis.[13,24] Meanwhile, Tawfik, et al. (2018) and Li, et al. (2016) reported that PD-L1 expression was associated with CD68+ cells and FOXP3+ T reg, respectively.[25,11] As previously known, CD8+ T cells have the ability to kill cancer cells, while T reg cells can suppress proliferation and cytokines secretion of effector T lymphocyte which associated with poor prognosis.[11,26,27]

Tumors can be PD-L1 positive or negative through several biological processes that can be categorized as induced PD-L1 expression by the presence of T cells (PD-L1+ TILs+), absence of T cell leading to no reactive PD-L1 expression (PD-L1- TILs-), constitutive or oncogene-induced PD-L1 expression (PD-L1+ TILs-), and genetic events that preclude PD-L1 expression upon T cell infiltration (PD-L1- TILs+).[28,29] This theory can explain the findings of PD-L1 positive tumors with a low degree of TILs in this study, where adaptive resistance is not always a trigger factor for PD-L1 expression, but can also be caused by other factors i.e. loss of PTEN, activation of the PI3K pathway, JAK2 or EGFR mutation.[28] This group highlights that PD-L1 positivity alone cannot be considered a predictive factor for the response of anti-PD-1/PD-L1 therapy because, without TILs in the tumor, it is unlikely that PD-1/PD-L1 inhibition will cause T cell response to cancer.[29]

A number of clinical trials on anti-PD-1/PD-L1 are ongoing, both as single or combination therapy, in invasive breast carcinoma, especially TNBC, because PD-L1 is more likely to expressed in this subtype. TNBC is generally regarded as the most 'inflamed' breast cancer, although there are significant differences between each TNBC subtype.[30] Based on gene expression analysis, Lehmann, et al. (2011) found 6 subtypes that showed unique biological properties, one of which was immunomodulatory subtype.[31] Meanwhile, other classifications proposed by Burstein, et al. (2014) and Jézéquel, et al. (2015), included two basal-like subtypes that were differentiated based on their immune activation.[32,33] Both of these classifications showed a better prognosis for basal-like subtype with activated/high immune responses. So far, TNBC heterogeneity has not been taken into account in ongoing clinical trials and this is one of the challenges in developing a new therapeutic strategy for TNBC.[30]

All similarities and differences between this study and other studies are somewhat due to the absence of validated examination methods, differences in the types of antibodies and the interpretation of PD-L1 expression.[28] Until now, FDA only approved three PD-L1 antibodies for several types of malignancies, especially NSCLC, and each is accompanied by its own platform and interpretation cut-off.[34] The high cost of these three antibodies and the absence of a comprehensive consensus make it difficult to generalize the assessment of PD-L1 expression. However, Karnik, et al. (2018), found similar if not identical performance of three PD-L1 antibodies (Dako, BioCare, Ventana). These findings offer an opportunity for the examination of PD-L1 expression in other organs and for other less expensive PD-L1 antibodies.[35] Given the concerns regarding the analytic and clinical validity of PD-L1 testing, there is still a possibility that the negative result with one antibody can turn positive using a different method and antibody.

The results of this study are expected to provide an overview of invasive breast carcinoma patients who are potential for anti-PD-1/PD-L1 immunotherapy. Examination of PD-L1 status can also be considered to predict the prognosis of breast carcinoma. Evaluation of specific subsets of TILs and its association with PD-L1 should be investigated further in order to understand the clinical impact of their existence and open up the possibility of other targeted therapy.

Conclusion

Immunohistochemical expression of PD-L1 was significantly associated to several clinicopathological parameters, specifically larger tumor size, higher histological grade, high stromal TILs, and aggressive molecular subtype. Increased PD-L1 expression score was also correlated linearly with increased stromal TILs degree.

Competing interests:

The authors declare that they have no conflict of interest

Ethical approval:

Health Research Ethical Committee, Universitas Sumatera Utara, Medan, Indonesia approved this study (No. 94/TGL/KEPK FK USU-

RSUP HAM/ 2019).

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