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Expression of PD-L1 and Relationship with Tumor-Infiltrating Lymphocytes and Metastasis in Serous Ovarian Carcinoma

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Abstract

Ovarian serous carcinoma is the most common ovarian malignancy in women, and most of them are diagnosed at an advanced stage and have metastasis. With current therapy, the 5-year mean survival rate is still not satisfying and opening a new approach. Immunotherapy using anti-PD-L1 is widely used in various types of malignancies. The use of PD-L1 in serous ovarian carcinoma is still quite limited. This study aimed to explore the potential of immunotherapy by assessing the expression of PD-L1 in serous ovarian carcinoma. Immunohistochemical performed to analyze PD-L1 dan compared with metastases incidence and tumor immune response. PD-L1 expression appeared to be stronger in high-grade serous carcinoma than low-grade serous carcinoma, but there was no statistical difference (p 0.539). There was a correlation between PD-L1 and metastasis incident (p 0.015) in high-grade serous ovarian carcinoma. There was a significant correlation between PD-L1 and tumor-infiltrating lymphocytes. PD-L1 in serous carcinoma has a strong relationship with TILs, opens up the possibilities of using anti-PD-L1 on serous carcinoma. PD-L1 expression in high-grade serous carcinoma has a relationship with the incidence of metastases.

Keywords: PD-L1; Ovarian Cancer; LGSC; HGSC; Metastasis; TILs.

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1. Introduction

Ovarian carcinoma is the 7th most common tumor occurring in women. Ovarian carcinoma alone ranks third after cervical cancer and uterine cancer [1]. The prevalence of ovarian cancer worldwide is 9.2 / 100,000 people. The number of new cases reaching 295,414 and occupying the highest position causes mortality in ovarian malignancies [2,3]. About 90 percent of ovarian malignancies are epithelial carcinomas, and about 70 percent of them are serous carcinomas. Most of these tumors were diagnosed end-stage with a 5-year survival rate of only 29% [4]. The cause of mortality is most often due to complications due to metastases, such as cachexia and multiple organ dysfunction syndromes [5,6]. Ovarian cancer therapy that uses for end-stage is platinum-based chemotherapy combined with anti-vascular endothelial growth factor antibodies. With the current therapy, 5-year mean survival rate is around 41% [7]. In several previous studies, it was found that the role of the immune system, especially Tumor-infiltrating lymphocytes(TILs), has a positive effect on prognosis. The existence of TILs has a positive effect on progression-free survival and overall survival [8–10]. The presence of TILs in ovarian cancer is related to the presence of the PD-L1 protein expressed by tumor cells [11]. PD-L1 expression itself has a relationship with grading and type of serous carcinoma [12]. PD-L1 is a protein typically found in solid tumors and works by inhibiting the immune system response to tumor cells. PD-L1 is a protein expressed on the tumor cell membrane and works by inhibiting T cell activity [11,13,14]. This protein is regulated by several pathways, namely Stat1, PI3k-AKT, and SHP2 [15]. Anti PD-L1 itself has been used for immunotherapy for lung carcinoma, colorectal adenocarcinoma, head, and neck squamous cell carcinoma.[16]–[18].In another study, CD8 + in TILS facilitated metastasis involving IL-9 / IL-10 and PD-L1 pathways.[19].In animal trials, using a combination of anti-PD-L1, LAG3, and CTL4 to prevent metastasis has shown unsatisfactory results.[20]. The use of immunotherapy in ovarian cancer is relatively new and still requires further investigation. This study aimed to explore the potential use of immunotherapy in metastatic dan non-metastatic serous carcinoma by assessing PD-L1 expression and their immune response role.

2. Material and Methode

2.1. Sample

This retrospective study is based on a series of 62 formalin-fixed and paraffin-embedded serous ovarian cancer specimens based on WHO classification of malignant tumor. Tissue was derived from patients with primary ovarian cancer diagnosed at Hassanudin University Hospital, Wahidin Sudirohusodo Hospital, and Sentra Diagnostik Patologia Makassar between 2018-2020. Two gynecological pathologists confirmed all histological diagnoses. Inclusion criteria were there is sufficient tumor material, clinical data (age at diagnosis), and metastatic status

2.2. Immunohistochemistry

Histological sections of 3 mm were taken from each group of ovarian cancer tissue. Positive controls for primary antibodies use lymph nodes tissue. The tissue section was incubated with phosphate buffer saline instead of the primary antibody as negative controls. The primary antibodies were proceeded with working dilution 1:50 (Monoclonal Rabbit Anti-PD-L1, Clone 28-8, Dako). The immunohistochemical procedures were

performed based on the manufacturer's protocols. The positive expression will appear brown stained on the membrane and cytoplasm of tumor cells. Immunohistochemistry was assessed based on the intensity and proportion of membranous or cytoplasmic staining in the tumor cell. Scored as follows : 0, negative; 1, weak staining intensity ≤10% or high staining intensity <1%; 2, moderate staining intensity >10% or high staining intensity 1-10%; 3, high staining intensity >10%. All slides were scored twice by two independent gynecological pathologists.

2.3. TILs Scoring

The TILs scoring method used is based on the percentage of the number of stromals filtered by lymphocytes compared to the total stromal among tumors on the Hematoxilin Eosin Stain stains. TILs outside of the tumor border and TILs in tumor zones with crush artifacts, necrosis, regressive hyalinization as well as in the previous core biopsy site will be exclude. All mononuclear inflammatory cells were assessed, including lymphocytes and plasma cells, while granulocytes and other PMN leukocytes were not assessed. Scored as follows <5%, 5-10%, and >10%.[11,13]

2.4. Statistical Analysis

All clinicopathological data obtained from patients (age, morphology, grading, and metastasis) proceeded with univariate analysis. Numerical and proportion data are presented as mean and percentages, respectively. Chi-square test or Fisher's exact test used to assess the relationship between categorical variables. Numerical variables were tested with the T-test to evaluate differences between groups of variables.

Table 1: The Relationship between PD-L1 Expression with the Clinicopathological Parameters and TILs

Chlinico-pathological data	Total n(%)	PD-L1				p-value
		Negative n(%)	Low n(%)	Moderate n(%)	High n(%)	
Age						
<55	43 (69.4%)	27 (65.9%)	11 (84.6%)	3 (75.0%)	2 (50.0%)	0.477[a]
≥55	19 (30.6%)	14 (34.1%)	2 (15.4%)	1 (25.0%)	2 (50.0%)	
Diagnosis						
LGSC	11 (17.7%)	9 (22.0%)	1 (7.7%)	0 (0.0%)	1 (25.0%)	0.539[a]
HGSC	51 (82.3%)	32 (78.0%)	12 (92.3%)	4 (100.0%)	3 (75.0%)	
TILs						
<5%	13 (21.0%)	1 (2.4%)	5 (38.5%)	4 (100.0%)	3 (75.0%)	
5-10%	24 (38.7%)	16 (39.0%)	7 (53.8%)	0 (0.0%)	1 (25.0%)	<0.001[a]
>10%	25 (40.3%)	24 (58.5%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	

[a] = Fisher's exact test

3. Result

Sixty-two patients with serous carcinoma were eligible for the analysis. The clinical characteristic of the patients is shown in table 1. The sample's mean age was 49 years old, and the median age was 50 years old (range, 23-77 years old). 11 (17.7%) sample diagnosed Low-Grade Serous Carcinoma (LGSC) dan 51(82.3%) diagnosed with High-Grade Serous Carcinoma (HGSC). The other clinicopathological data were metastatic status, that metastatic found in 34(54.8%) sample and 28(45.2%) has no metastatic. (Tabel 1)

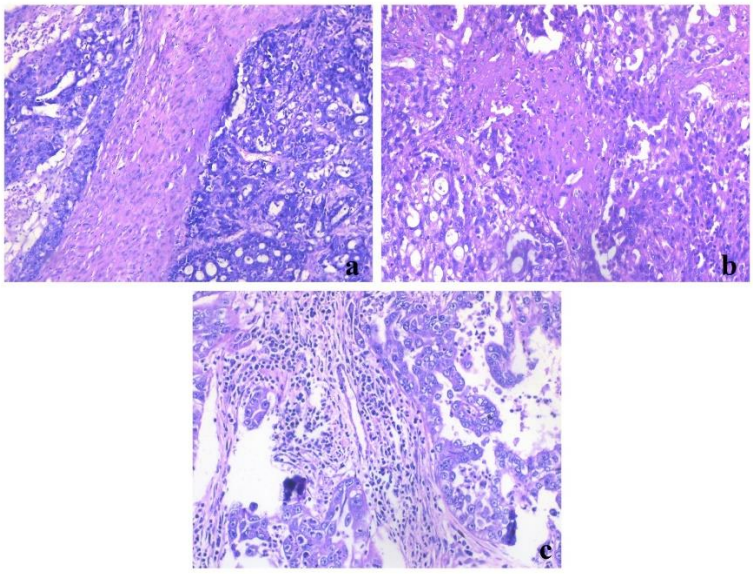

Figure 1: Hematoxylin and eosin staining, objective magnification 20x. a. Low TILs (<5%). b. Moderate TILs (5-10%). c. High TILs (>10)

Using prior threshold based on the intensity and percentage tumor stained, negative PD-L1 stain found in 41 (66.1%) samples, low PD-L1 stain was found in 13 (21%) sample, moderate and high PD-L1 stain both found in 4 (6.5%) samples. We have classified TILS into three categories, Low (<5%); Moderate (5-10%); High (>10%); stromal tumor had infiltrated by mononuclear immune cell (lymphocyte and plasma cell). Low TILS was found in 13 (21%) samples, moderate TILS found in 24(38.7%) samples, and high TILS found in 25(40.3%) samples. (Tabel 1)

Table 2: The relationship between PD-L1 Expression on LGSC and HGSC with Metastatic Incidence

		PD-L1				
	Total n(%)	Negative n(%)	Low n(%)	Moderate n(%)	High n(%)	p-value
LGSC						
Non-Metastatic	8 (72.7%)	7 (77.8%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0.491[a]
Metastasis	3 (27.3%)	2 (22.2%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	
HGSC						
Non-Metastatic	20 (39.2%)	9 (28.1%)	8 (66.7%)	3 (75.0%)	0 (0.0%)	0.015[a]
Metastasis	31 (60.8%)	23 (71.9%)	4 (33.3%)	1 (25.0%)	3 (100.0%)	

a = Fisher's exact test

There was a significantly different correlation between PD-L1 and metastatic groups in serous carcinoma (p = 0.029, Chi-squared test). Interestingly, there was no correlation between PD-L1 and metastatic in LGSC groups (p = 0.491), but the correlation was found in HGSC (p = 0.015) (Tabel 2). Furthermore, there was a strong significant correlation between TILS and PD-L1 groups (p = <0.001, Chi-square test). There was no statistically significant difference observed between age, diagnosis, and PD-L1 status. There was no statistically significant difference between TILS and metastatic status (p = 0.803). (Tabel 1)

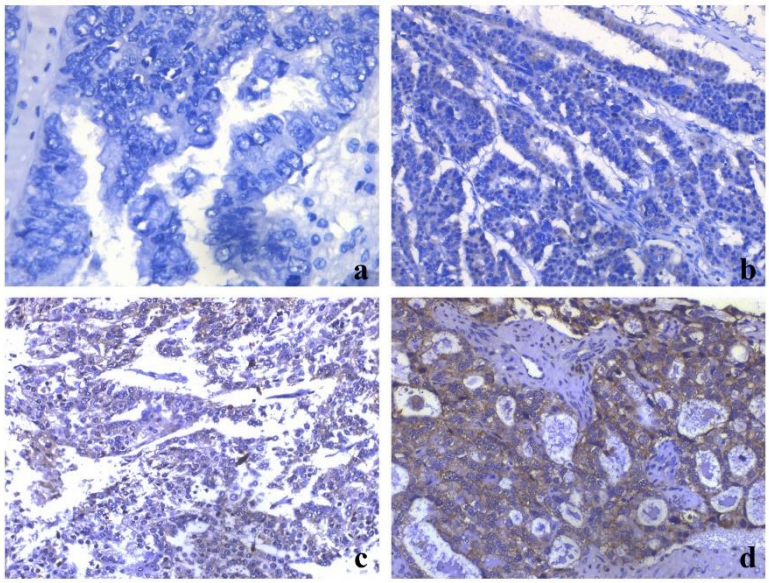

Figure 2: PD-L1 Staining. a. Negative stain for anti-PD-L1. b. Weak staining for anti-PD-L1. c. Moderate staining for anti-PD-L1. d. High staining for anti-PD-L1

Even though there was no statistical difference between PD-L1 status and diagnosis, according to this study, we found that 10 (91%) samples of LGSC have negative or low PD-L1 status, and only 1(9%) sample has high PDL1 status. There were eight samples with moderate and high PDL1 status, seven (87.5%) diagnosed by HGSC, and 1(12,5%) was diagnosed by LGSC. In general, moderate-high PD-L1 expression is more likely found in HGSC. (Tabel 1)

4. Disscussion

In this study, 54 (87%) samples showed negative or low PD-L1 expressions, while 8 (13%) samples were stained moderate-high. The results obtained in other studies found that PD-L1 expression in serous carcinoma was present in 44-63% of tumors [12,21]. Our results differ from previous studies, which may be due to several factors. The first factor is the use of the PD-L1 threshold in this study. We try to combine the intensity and number of expressed cells. The next factor that caused the difference was the PD-L1 clone that was used. The use of antibodies with different clones causes different epitopes recognized. However, there were studies with similar results with the PD-L1 expression results in HGSC of 13.2%[11].Further investigation is needed to

clarify their conflicting result. We did not find any significant statistical differences between PD-L1 expression with HGSC and LGSC (p = 0.539). This result is consistent with other reports that Type 1 ovarian epithelial tumors (clear cell carcinoma, endometrioid carcinoma, mucinous carcinoma, and LGSC) do not have PD-L1 expression differences with Type 2 ovarian epithelial tumor (HGSC)[12].Whereas in this study, there was no significant relationship between LGSC and HGSC on PD-L1. It may be because LGSC may exhibit BRAF mutation, but HGSC is more associated with TP53 mutation than BRAF mutation[22].Nearly every HGSC harbors a deleterious TP53 mutation[23,24].PD-L1 expression on LGSC affected by BRAF mutation than activating MEK, ERK, and HIF1α/2α pathways,[25].while PD-L1 expression on HGSC affected by decrease P53 than activating PI3K/AKT pathway[26].In other studies, it was also found that the BRCA1 / 2 mutation was associated with HGSC. Tumors with a BRCA1 / 2 mutation have a higher PD-L1 expression and a lower number of TILs[27]. In general, there are two regulatory mechanisms for PD-L1 that are innate immune resistance and adaptive immune resistance. In Innate immune resistance, PD-L1 is influenced by Stat1, PI3k-AKT, dan SHP2 signaling pathway. These three pathways when activated by the oncogenesis process will increase PD-L1 expression[28]. On the adaptive immune resistance pathway, INF-ɣ produced by CD8 + on TILs increases PD-L1 expression of tumor cells[29]. In innate immune resistance, the PD-L1 staining pattern is usually diffuse because it is influenced by the signaling pathway factor in the tumor. Whereas in adaptive immune resistance, the PD-L1 staining pattern is focal, where tumors around TILs will be more colored compare to the surroundings. In our study, we found that the staining was diffuse and the staining didn't affect by TILs. We did not find any focal stained tumor areas around the TILs, we suspect that PD-L1 in serous carcinoma is more influenced by endogenous pathway factors and not influenced by the INF- ɣ that produced by TILs. This is also evidenced by the strong correlation between PD-L1 and TILs, wherein an increase in PD-L1 expression causes a decrease in the number of TILs. We did not find an association between TILs in tumor tissue and the incidence of metastases. In other studies, CD8+ on TILs could promote the metastasis of ovarian cancer via IL-8/IL-10 and PD-L1[19]. We suspect that TILs are only a contributing factor for metastasis. The most common omental metastases come from epithelial ovarian cancer. The main cause of this metastasis due to the location of the ovary adjacent to the omentum. TILs on tumor tissue can affect metastasis and regulatory T cells mediate homeostatic peripheral tolerance by suppressing autoreactive T cells in the omentum. Metastasis can occur when the number of TILs is low with a large number of regulatory T cells[6]. Besides, metastasis occurs due to the loss of bonds between cells which is influenced by the loss of E-Cadherin[6,30]. In HGSC, it is generally caused by a P53 mutation, that will decrease E-cadherin expression with inhibition mTOR, Wnt/βcatenin, dan snail family protein signaling pathway[31]. Mutation of P53 will increase PD-L1 and decrease E-Cadherin. This is consistent with our findings that there is an association between PD-L1 and metastatic which may be due to the P53 mutation. In LGSC the PD-L1 activation pathway is due to a BRAF mutation that has no direct relationship with E-Cadherin. We found in this study that the incidence of metastasis in LGSC has no relationship with PD-L1.

5. Conclusion

PD-L1 expression varies widely in ovarian serous carcinoma, and high PD-L1 is more likely to occur in HGSC. PD-L1 in serous carcinoma has a strong relationship with immune response, thus allowing the use of anti-PD-L1 on serous carcinoma. PD-L1 expression in HGSC has a relationship with the incidence of metastases and open

possibility to use PD-L1 as a predictor of metastasis and prognosis, further research is needed.

6. Conflict of Interest

We have no Conflict of interest to declare

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