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Case Report

The Association of Tumor-Infiltrating Lymphocytes and Programmed Cell Death 1 Expression with the Incidence of Distant Metastasis in Triple-Negative Breast Cancer Subjects in Sanglah General Hospital, Bali, Indonesia

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Keywords
Triple-negative breast cancer · Programmed cell death-ligand 1 · Tumor-infiltrating lymphocytes · Distant metastasis

Abstract
The triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype with a high rate of distant metastasis. The tumor immunity microenvironment plays an important role, including tumor-infiltrating lymphocytes (TIL) and PD-1 (programmed cell death 1)/PD-L1 (programmed cell death-ligand 1), in promoting TNBC aggressiveness. This study aimed to determine the association of TIL and PD-L1 expression with the incidence of distant metastasis in TNBC. This study is a cross-sectional study involving TNBC subjects at Sanglah General Hospital, Denpasar, conducted in 2019. The parameters analyzed were the expression of TIL, PD-L1, and the incidence of distant metastasis. The expression of TIL was analyzed histopathologically while PD-L1 was measured with Ventana PD-L1 kit test. Subject characteristics were obtained from medical records. Data were collected and analyzed by SPSS 22.0. As many as 31 subjects with TNBC were included in this study, with 51.6% subjects with distant metastasis. The majority of subjects with distant metastasis had low TIL and low tumoral PD-L1 but high PD-L1 stromal in TIL. From statistical analysis, only PD-L1 stromal in TIL expression was associated significantly with distant metastasis (p = 0.043). In conclusion, there was a significant association between PD-L1 stromal in TIL and the incidence of distant metastasis in TNBC.

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Introduction

Breast cancer is still an emerging problem worldwide. In Indonesia, breast cancer is the most common cancer found in women [1]. The breast cancer subtype with a poor prognosis is the triple-negative breast cancer (TNBC). TNBC is most prevalence in a younger age, but aggressive, with a high degree of differentiation, and thus has a high incidence of metastasis. In addition, this subtype also showed no significant benefit from adjuvant therapy [2].

Recent studies have found a promising role to target the tumor immunity in breast cancer. The immune response is a complex phenomenon of the immunity activation and inhibition toward the tumor development, including tumor-infiltrating lymphocytes (TIL) and programmed cell death (PD). TILs are lymphocytes surrounding the tumor that directly fight tumor cells [3]. Contrary, the inhibition pathway that plays an important role is the PD-1/PD-L1 pathway. PD-1 is a protein on the membrane surface expressed by various immune cells including T cells. This protein is activated by ligands, namely PDL-1 and PDL-2, which are expressed by antigen-presenting cells such as macrophages or B cells. After binding to the ligand, PD-1 attenuates lymphocyte activation and triggers the development of T-regulatory cells, which in turn induces termination of the immune response [4].

The immune responses to cancer cells are important in TNBC progression or inhibition, and thus can be targeted for further drug development. The presence of TIL, for example, is associated with a reduced risk of recurrence, reduced mortality, increased pathologic complete response (pCR), and a better prognosis [2–4], while PD-L1 expression is reported to be correlated with a worse outcome. Therefore, this study aimed to determined the association of TIL and PD-L1 expression with the incidence of distant metastasis in TNBC.

Methods

This is a cross-sectional study to determine the association of TIL and PD-L1 expression with the incidence of distant metastases in TNBC subjects. This study has been approved by the Research Ethical Committee of Faculty of Medicine, Udayana University. All consented breast cancer women with triple-negative subtype admitted to the Sanglah General Hospital, Denpasar, Bali, Indonesia, in the period of September to December 2019, were included in this study. Subjects who had immunodeficiency disease and other malignancies were excluded from this study.

The parameters measured in this study were subjects' characteristic, TIL, PD-L1 expression, and the incidence of distant metastases. TIL, defined as lymphocytic cell populations in the tumor tissue area, was analyzed by histopathological examination. According to the classification of The International TILS Working Group, TIL was classified into high and low TIL. The PD-L1 expression was checked using the VENTANA PD-L1 (SP263) test. Subjects' characteristic and clinicopathological data included age, tumor size, stage, grading, and type of tumor histopathology. The incidence of distant metastases was scored based on the M1 criteria as per the guidelines of the American Joint Committee on Cancer Staging Manual 8th Edition. Data were collected and analyzed using SPSS 22.

Results

A total of 31 subjects were included in this study, with 15 subjects in non-metastasis and 16 subjects in metastasis group. The subjects were around 39–69 years old, which is shown in Table 1. The non-metastasis and metastasis groups had similar characteristics, where most

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Table 1. Data characteristics of subjects

Characteristics	Non-metastasis	Metastasis	*p*
Age			
<55 years	8 (25.8%)	10 (32.2%)	0.722
≥55 years	7 (22.6%)	6 (19.4%)	
Tumor size			
≤5 cm	4 (12.9%)	1 (3.2%)	0.172
>5 cm	11 (35.5%)	15 (48.4%)	
Histopathological type			
Invasive carcinoma of non-special type	12 (38.7%)	14 (45.2%)	0.796
Invasive lobular carcinoma	2 (6.5%)	1 (3.2%)	
Special type carcinoma	1 (3.2%)	1 (3.2%)	
Histological grade			
Low	7 (22.6%)	3 (9.7%)	0.135
High	8 (25.8%)	13 (41.9%)	
TIL			
Low	12 (48.0%)	13 (52.0%)	0.641
High	3 (50.0%)	3 (50.0%)	
PD-L1 stromal			
Low	12 (38.7%)	7 (22.6%)	0.043
High	3 (9.7%)	9 (29.0%)	
PD-L1 tumor			
Low	14 (45.2%)	15 (48.4%)	1.000
High	1 (3.2%)	1 (3.2%)	

subjects had tumor size more than 5 cm, high histological grade, and were categorized as invasive carcinoma of non-special type.

There were 25 out of 31 subjects classified to have low TIL (80.6%), where it was similarly found either in non-metastasis or metastasis group (48 vs. 52%). Thus, there was no statistically significant difference of TIL level between both groups ($p = 0.641$). In this study, PD-L1 expression was divided into PD-L1 at the surface of tumor tissue cells and PD-L1 on the TIL stromal. The majority of the subjects in non-metastasis and metastasis groups had either low PD-L1 at the surface of tumor tissue cells (93.5%) and low PD-L1 in the TIL stromal (61.3%). From the statistical analysis, there was a significant relationship between PD-L1 in the TIL stromal and the incidence of distant metastasis ($p = 0.043$). However, there was no significant relationship between PD-L1 in tumor cells ($p = 1.000$).

Discussion

Each year, about half a million women die from breast cancer and 150,000 of them are estimated to be TNBC cases, representing about 30% of deaths caused by breast cancer [2]. The failure of tumor eradication and the prognosis of breast cancer patients is associated with the spread of tumor cells outside the primary area or commonly known as metastasis. The epithelial-to-mesenchymal transition is a potential mechanism by which epithelial tumor cells acquire phenotypic properties that are more motile and invasive, in order to escape from the primary tumor [5]. Several studies have found that there were associations between TIL and PD-L1 with the epithelial-to-mesenchymal transition and angiogenesis, although there were no studies that explain the detailed molecular relationship [6].

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In this study, a total of 31 TNBC subjects were included. The majority of subjects were found in younger age (<55 years old, 58.1%). The results were similar to the prior existing studies. Dent et al. [7] found that TNBC tended to occur in premenopausal women or in younger age groups, compared to other subtypes of breast cancer. TNBC tended to occur or were diagnosed at a higher stage than non-TNBC [8]. Based on tumor histological grading, most patients in this study were found in high grade (67.7%). About 51.6% subjects were shown to have distant metastasis. Foulkes et al. [9] found that TNBC tend to spread to other organs, especially to the visceral organs, like lung and brain. Dent et al. [7] found that patients with TNBC had a relatively poor prognosis in the first 5 years with median time from diagnosis to death compared to non-TNBC patients (3.5 vs. 5.7 years).

In this study, most subjects had low TILs, both in non-metastasis and metastasis group. An increased proportion of TILs was correlated with a better prognosis, but somehow some studies showed contradictory results. TILs induced the migration of T cells to the tumor microenvironment. CD4 Th2 T cells expressed interleukin (IL)-10 and IL-6, which could inhibit the inflammatory process, while regulatory T cells could inhibit CD8 T cell function and prevent CD8 T cell migration to tumors [10].

PD-L1 expression could be found in both tumor cells and immune cells (the TILs). In this study, PD-L1 was expressed in low level either at the surface of tumor tissue cells or in the TILs. Specifically, in the metastasis group, PD-L1 stromal was significantly higher than in the non-metastasis group ($p = 0.043$). In the previous studies, Cimino-Mathews et al. [11] showed that the expression of PD-L1 in TIL was higher than that of PD-L1 in tumor cells (78 vs. 21%). Jilaveanu et al. [12] showed in renal cell carcinoma that PD-L1 expression in TIL was associated with poor prognosis and metastasis. A study by Doğukan et al. [13] showed that 37.7% positive PD-L1 expression in tumor cells was associated with a 31% increase rate of metastasis, but it was not statistically significant.

PD-L1 is expressed on T cells, B cells, macrophages, dendritic cells, and mesenchymal stem cells in various tumor cells. After binding to their ligands, PD-1 attenuates lymphocyte activation and triggers T-regulatory cell development, which in turn induces the termination of the immune response. The complex bond between PD-1 and its ligand (PD-L1 or PD-L2) functioned as a negative regulator of the immune system, delivering inhibitory signals, leading to decreased proliferation and activation of CD8+ T lymphocytes. The decrease in the number of CD8 + and NK T cells consequently increased breast cancer metastasis [14].

Conclusion

There was a significant association between PD-L1 stromal in TIL and the incidence of distant metastasis in TNBC.

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Statement of Ethics

This study has been approved by the Research Ethical Committee of Faculty of Medicine, Udayana University. Written informed consent to publish this case report was obtained from all the study participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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None.

Author Contributions

I Wayan Sudarsa, as the main and corresponding author, was involved in all parts of this article. The second author, I Putu Ari Gunawan, was involved in the data collection and analysis. The third author, Ida Bagus Tjakra Wibawa Manuaba, was involved in the discussion section.

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