Original Article

The Prevalence of PD-L1 Expression in Triple-Negative Breast Cancer Patients and Its Correlation with Survival Rates and Other Prognostic Factors: A Survival Analysis

Arefeh Izadi[1], Azar Naimi[2], Elham Amjadi[3], Dorsa Beheshtiparvar[1], Maryam Soltan[2]

[1]School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Pathology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Poursina Hakim Digestive Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: Triple-negative breast cancer (TNBC) is a leading cause of cancer-related mortality among women, with a poor prognosis. The programmed cell death 1 (PD-1) pathway has emerged as a potential immunotherapy target. This study aimed to assess PD-L1 expression in TNBC patients and its relationship with prognostic variables.

Materials and Methods: This cross-sectional study included 107 TNBC patients recruited between 2016 and 2020. Patient age, tumor grade, and Ki67 expression were obtained from pathology reports. Immunohistochemistry was utilized to determine PD-L1 status, and 2-year survival data were collected through telephone follow-up.

Results: PD-L1 expression frequency in TNBC patients was 76.6%. Grade 3 was the most common cancer grade, significantly more prevalent in the PD-L1 positive group ($P = 0.01$). High Ki67 expression (≥14%) was observed in 89% of patients, significantly higher in the PD-L1 positive group ($P = 0.003$). The 2-year survival rates for the PD-L1 positive and negative groups were 84.1% and 92%, respectively, with no significant difference between the groups ($P = 0.512$).

Conclusion: This study investigated PD-L1 expression prevalence in TNBC patients and its correlation with prognostic variables. PD-L1 expression was associated with higher tumor grade and elevated Ki67 expression, indicating a potential role in tumor aggressiveness. However, despite these associations, PD-L1 expression did not significantly impact the 2-year survival rate in TNBC patients. These results emphasize the complexity of the immune microenvironment in TNBC and the necessity for further research to elucidate the precise role of PD-L1 in disease progression and patient outcomes.

Keywords: Breast cancer, PD-L1, prognostic variables, triple-negative breast cancer, tumor-infiltrating lymphocytes

Address for correspondence: Dr. Maryam Soltan, Department of Pathology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: Maryam.soltan1367@gmail.com
Submitted: 01-Jan-2024; **Revised:** 29-May-2024; **Accepted:** 01-Jun-2024; **Published:** 23-Sep-2024

Introduction

Triple-negative breast cancer (TNBC), which lacks the expression of estrogen receptors, progesterone receptors, and human epidermal growth factor-2,[1] is the main cause of female mortality worldwide.[2] Targeted therapy for TNBC is difficult and leads to a worse prognosis.[3] It is well established that tumor-infiltrating lymphocytes (TILs) are essential for antitumor responses. The immune response of TILs is controlled by the programmed cell death 1 (PD-1) pathway, which consists of PD-1 and its ligands (PD-L1). Knowing how PD-L1 expression and prognostic variables interact in TNBC may help identify potential therapeutic targets and lead to better patient outcomes.

Access this article online

Quick Response Code:

Website: www.advbiores.net

DOI: 10.4103/abr.abr_2_24

How to cite this article: Izadi A, Naimi A, Amjadi E, Beheshtiparvar D, Soltan M. The prevalence of PD-L1 expression in triple-negative breast cancer patients and its correlation with survival rates and other prognostic factors: A survival analysis. Adv Biomed Res 2024;13:86.

TILs play an important role as the soldiers of anti-tumoral responses by detecting tumoral antigens and removing tumoral cells. Several human tumors express PD-1 or CD279 protein, which is an immune checkpoint pathway that is present on the surface of T-cells and suppresses the immune response of TILs.[4] Indeed, the interaction between PD-1 and its ligands (PD-L1 or CD274) prevents the destruction of cancer cells.[5] It has been reported that TILs are directly related to more survival, especially in TNBC.[6] So theoretically, targeting the PD-L1 pathway and subsequently, the high number of functional TILs promises more favorable outcomes. On the other hand, there is a correlation between the level of TILs and high grade of tumor, lack of hormone receptors and Ki67 overexpression.[7]

According to earlier research, the 5-year survival rate of TNBC patients dropped significantly from stage II to stage III (76%–45%), indicating that a timely diagnosis may increase the likelihood of a successful outcome.[8] Although many studies have been conducted on PD-1 and its effects on TNBC, there are still many disagreements about it. The aim of this study was to explore the prevalence of PD-1 expression in TNBC patients, examining its correlation with survival rates and other prognostic factors to determine its specific prognostic value.

Materials and Methods

Study design

In Isfahan, Iran, 107 women who were diagnosed with TNBC between 2016 and 2020 participated in this cross-sectional study.

Participant selection

TNBC patients who had high-quality, analyzable, paraffin-embedded tissues were included. Individuals with equivocal HER-2 in IHC staining and then negative result in fluorescence *in situ* hybridization were additionally included. All exclusion criteria were low-quality or suspicious specimens, patients who had received neoadjuvant therapy, or individuals with equivocal HER-2 in IHC staining and then positive result in fluorescence *in situ* hybridization. Patients who lacked contact information, had unclear patient follow-up status, unknown Ki67 status, or both were also removed.

Data gathering

Pathology results from Al-Zahra, Omid, and Poursina hospitals in Isfahan, Iran, were reviewed for inclusion in the study. Age, tumor grade, and Ki67 expression were among the demographic and clinical information taken from prior pathology reports. Immunohistochemistry (IHC) was used to ascertain the PD-L1 status. Data on 2-year overall survival were gathered via telephone follow-up.

Immunohistochemistry

An anti-PD-L1 monoclonal antibody from ZYTOMED in Germany was used for IHC staining. At a 100× objective lens magnification, three fields from regions with highest degrees of PD-L1 stainability (strong staining) were chosen, and the ratio of stained immune cells (cytoplasmic or membranous including lymphocytes and macrophages) and tumor cells to the total number of viable tumor cells was computed. Tumoral PD-L1 staining was designated as positive when clear membranous staining was present in at least 1% of tumor cells. The degree of staining was graded using the following scale: 0 (no staining), 1 (1%–5% staining), 2 (6%–50% staining), and 3 (>50% staining). Scores 1 to 3 were considered as positive and score 0 as negative. Tonsil tissue is our choice for control of PD-L1 in IHC staining which follicular macrophages (weak moderate staining) and reticulated crypt epithelial cells (strong staining) are considered as positive controls and superficial squamous epithelium, fibroblasts, and endothelium are considered as negative control.

Statistical analysis

IBM SPSS Statistics version 26.0.0.1 was used for data analysis. For non-numerical variables, descriptive statistics were presented as numbers and percentages. For numerical variables, they were presented as mean and standard deviation (median range − interquartile range). Independent *t*-test and Chi-square test were used to analyze age, Ki67 expression status, tumor grade, and survival rate. The significance level for all tests was set at 0.05.

Results

In the current investigation, 107 TNBC samples were examined using PD-L1 IHC. For PD-L1, 25 specimens from our patients were negative (score 0), while 82 of them tested positive (scoring 1–3) [Table 1]. The mean age in PD-L1-positive and PD-L1-negative groups was 50.6 ± 16.2 and 50.6 ± 13.2 years, respectively ($P = 0.98$), so the relationship between PD-L1 expression and age was not statistically significant. There was a significant difference between the frequency of various scores of PD-L1 expression in TNBC patients ($P = 0.0001$).

The most common grade of cancer among our patients was grade 3, but the difference was significant between PD-L1 positive and negative groups ($P = 0.01$). Eighty-nine percent of the patients had high Ki67 expression (≥14%) which was significantly more in PD-L1 positive group ($P = 0.003$). Totally, 92 patients had overall survival in 2 years while the percentage in PD-L1 positive and negative groups was 84.1% and 92%, respectively. The 2-year survival between the two groups was not significant ($P = 0.512$) [Table 2].

Table 1: Relative frequency of PD-L1 scores among TNBC patients

PD-L1 score	No. (Percentage)
0	25 (23.4%)
1	19 (17.8%)
2	34 (31.8%)
3	29 (27.1%)

Discussion

According to our research, 76.6% of the TNBC patient group exhibited relative PD-L1 expression. Furthermore, a clear distinction was observed between patients with PD-L1-positive and PD-L1-negative statuses concerning cancer grade and Ki67 expression. However, there was no difference detected in the 2-year survival rate between the PD-L1 positive and negative groups, indicating that PD-L1 expression may not be a significant prognostic factor in TNBC patient outcomes. The effect of anti-PD1 drugs on TNBC therapy approaches has been the subject of numerous earlier investigations. Through their interaction with PD-1, tumor-associated macrophages (TAMs) have the potential to be targeted as a practical strategy for controlling TNBCs, according to Santoni *et al*.[9] It is known that the JAK2/STAT1 pathway controls PD-L1 expression, which raises the possibility that JAK1/2 inhibitors may be useful.[10] In 2021, Vranic *et al*.[11] suggested that IHC PD-L1 identification as a first step toward immunotherapeutic treatment for TNBC has the potential to produce better results. Similar to this, Yeong *et al*.[12] advocated multiplex IHC/immunofluorescence as a cutting-edge method for more accurate PD-L1 detection than traditional IHC a year earlier. As a result, a more effective approach for TNBC control is revealed by analyzing PD-L1 expression using IHC and using current therapeutic drugs to suppress it.[13]

Divergent perspectives on the prognosis, overall survival (OS), and disease-free survival (DFS) of individuals exhibiting PD-L1 overexpression have been found in earlier studies. Numerous studies have shown a connection between increased PD-L1 expression and a better prognosis. For instance, Li *et al*.[14] showed that PD-L1-positive patients had better DFS. A study with 238 samples conducted in 2016 revealed that higher PD-L1 was associated with improved OS but not DFS.[15] Additionally, some investigations have confirmed that PD-L1 expression is advantageous for OS and DFS.[16] Contrarily, some investigators claim that elevated PD-L1 expression does not coincide with improved pathological and clinical characteristics despite the prognosis.[17] On the other hand, hypotheses assert that cancers with few TILs and PD-L1 expression have a bad prognosis.[18,19] One of the earlier studies came to the conclusion that the prognosis is bad if TILs (rather than tumor cells) had overexpressed PD-L1.[20] According to Cirqueira *et al*.'s[21] comprehensive review and meta-analysis, PD-L1 positive was linked to poor OS but did not significantly differ from DFS.

Table 2: Difference of variables between PD-L1 positive and PD-L1 negative group

Variable	PD-L1 positive (*n*=82)	PD-L1 negative (*n*=25)	*P**
Grade of cancer			0.01
1	1 (1.2%)	2 (8%)	
2	10 (12.2%)	8 (32%)	
3	71 (86.6%)	15 (60%)	
Ki67 expression			0.003
Low (<14%)	4 (4.9%)	7 (28%)	
High (>14%)	78 (95.1%)	18 (72%)	
Two-year overall survival			0.512§
Yes	69 (84.1%)	23 (92%)	
No	13 (15.9%)	2 (8%)	

*All of the analysis was done using the Pearson Chi-square test.
§Two-sided significance

We looked at 2-year overall survival in this trial and found no appreciable differences between the PD-L1 positive and PD-L1 opposing groups. Given the lack of agreement, additional research is required to determine the connection between PD-L1 expression and patients with TNBC's survival.

The link between TILs and PD-L1 expression has also been researched in the literature. In 2017, 215 TNBC samples with PD-L1 IHC showed a connection between PD-L1 and TIL predominance. They discovered that stromal TILs and PD-L1 are separate prognostic variables for TNBC patients.[22] This connection was corroborated in a comprehensive review and meta-analysis research by Lotfinejad *et al*.[23] Although we did not focus on TILs in the current investigation, earlier research has shown how important the relationship between TILs and PD-L1 expression is. We also looked into our patients' proliferation indexes. We discovered that PD-L1 positive patients had a considerably greater high proliferation index. Botti *et al*.'s findings[15], which discovered a connection between PD-L1 and Ki67 expression, agreed with our own. This fact has been supported by several investigations over time.[19,21,24]

The association between PD-L1 expression and tumor grade is controversial. Our results revealed a strong correlation, which is in line with Kitano's study, while other research has found the contrary.[17,25]

This study had strengths and limitations. The first limitation was the low number of samples, which could affect the generalizability of the data. However, considering the capacities and management priorities of laboratories and hospitals, we tried to collect the largest possible number of samples. The second difficulty was the small volume of samples to perform IHC. The reason was that most of the patients had undergone core needle biopsy. Third, due to the retrospective nature of the study, the patients did not remember some of the variables, and this caused data defects and failure to enter the study. Finally, because the patients underwent mastectomy in different centers, our data on lymph node involvement and disease stage were limited, and for this reason, we did not examine these two variables. The strengths of the study were obtaining the appropriate number of patients for examination despite the fact that TNBC is not such common, and also performing several times of IHCs on the samples to improve the quality of the examinations.

Conclusion

The relative frequency of PD-L1 expression in our patients

with TNBC was 76.6%. Also, we found that the grade of cancer and Ki67 expression were significantly higher in PD-L1 positive compared to PD-L1 negative patients. Two-year survival did not differ between the two groups.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Ethical considerations

The Ethics Committee of Isfahan University of Medical Sciences, Iran, approved the study protocol (IR.MUI.MED.REC.1400.477).

References

1. Hoda RS, Brogi E, Dos Anjos CH, Grabenstetter A, Ventura K, Patil S, *et al*. Clinical and pathologic features associated with PD-L1 (SP142) expression in stromal tumor-infiltrating immune cells of triple-negative breast carcinoma. Mod Pathol 2020;33:2221-32.
2. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, *et al*. Cancer treatment and survivorship statistics, 2016. Cancer J Clin 2016;66:271-89.
3. Den Brok WD, Speers CH, Gondara L, Baxter E, Tyldesley SK, Lohrisch CA. Survival with metastatic breast cancer based on initial presentation, de novo versus relapsed. Breast Cancer Res Treat 2017;161:549-56.
4. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, *et al*. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti–PD-1 therapy association of PD-1 and ligands with response to anti–PD-1. Clin Cancer Res 2014;20:5064-74.
5. John LB, Devaud C, Duong CP, Yong CS, Beavis PA, Haynes NM, *et al*. Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells anti-PD-1 therapy enhances tumor rejection by CAR T cells. Clin Cancer Res 2013;19:5636-46.
6. Denkert C, Loibl S, Noske A, Roller M, Muller B, Komor M, *et al*. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:105-13.
7. Loi S, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, *et al*. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol 2013;31:860-7.
8. Liu Y, Teng L, Fu S, Wang G, Li Z, Ding C, *et al*. Highly heterogeneous-related genes of triple-negative breast cancer: Potential diagnostic and prognostic biomarkers. BMC Cancer 2021;21:644.
9. Santoni M, Romagnoli E, Saladino T, Foghini L, Guarino S, Capponi M, *et al*. Triple negative breast cancer: Key role of tumor-associated macrophages in regulating the activity of anti-PD-1/PD-L1 agents. Biochim Biophys Acta Rev Cancer 2018;1869:78-84.
10. Chen M, Pockaj B, Andreozzi M, Barrett MT, Krishna S, Eaton S, *et al*. JAK2 and PD-L1 amplification enhance the dynamic expression of PD-L1 in triple-negative breast cancer. Clin Breast Cancer 2018;18:e1205-15.
11. Vranic S, Cyprian FS, Gatalica Z, Palazzo J. PD-L1 status in breast cancer: Current view and perspectives. Semin Cancer Biol 2021;72:146-54.
12. Yeong J, Tan T, Chow ZL, Cheng Q, Lee B, Seet A, *et al*. Multiplex immunohistochemistry/immunofluorescence (mIHC/IF) for PD-L1 testing in triple-negative breast cancer: A translational assay compared with conventional IHC. J Clin Pathol 2020;73:557-62.
13. García-Teijido P, Cabal ML, Fernández IP, Pérez YF. Tumor-infiltrating lymphocytes in triple negative breast cancer: The future of immune targeting. Clin Med Insights Oncol 2016;10:31-9.
14. Li X, Wetherilt CS, Krishnamurti U, Yang J, Ma Y, Styblo TM, *et al*. Stromal PD-L1 expression is associated with better disease-free survival in triple-negative breast cancer. Am J Clin Pathol 2016;146:496-502.
15. Botti G, Collina F, Scognamiglio G, Rao F, Peluso V, De Cecio R, *et al*. Programmed death ligand 1 (PD-L1) tumor expression is associated with a better prognosis and diabetic disease in triple negative breast cancer patients. Int J Mol Sci 2017;18:459.
16. Barrett MT, Lenkiewicz E, Malasi S, Basu A, Yearley JH, Annamalai L, *et al*. The association of genomic lesions and PD-1/PD-L1 expression in resected triple-negative breast cancers. Breast Cancer Res 2018;20:71.
17. Baptista MZ, Sarian LO, Derchain SF, Pinto GA, Vassallo J. Prognostic significance of PD-L1 and PD-L2 in breast cancer. Hum Pathol 2016;47:78-84.
18. Mori H, Kubo M, Yamaguchi R, Nishimura R, Osako T, Arima N, *et al*. The combination of PD-L1 expression and decreased tumor-infiltrating lymphocytes is associated with a poor prognosis in triple-negative breast cancer. Oncotarget 2017;8:15584-92.
19. Muenst S, Schaerli A, Gao F, Däster S, Trella E, Droeser R, *et al*. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat 2014;146:15-24.
20. Wang X, Liu Y. PD-L1 expression in tumor infiltrated lymphocytes predicts survival in triple-negative breast cancer. Pathol Res Pract 2020;216:152802.
21. Cirqueira MB, Mendonça CR, Noll M, Soares LR, de Paula Carneiro Cysneiros MA, Paulinelli RR, *et al*. Prognostic role of PD-L1 expression in invasive breast cancer: A systematic review and meta-analysis. Cancers 2021;13:6090.
22. AiErken N, Shi H-j, Zhou Y, Shao N, Zhang J, Shi Y, *et al*. High PD-L1 expression is closely associated with tumor-infiltrating lymphocytes and leads to good clinical outcomes in Chinese triple negative breast cancer patients. Int J Biol Sci 2017;13:1172-9.
23. Lotfinejad P, Asghari Jafarabadi M, Abdoli Shadbad M, Kazemi T, Pashazadeh F, Sandoghchian Shotorbani S, *et al*. Prognostic role and clinical significance of tumor-infiltrating lymphocyte (TIL) and programmed death ligand 1 (PD-L1) expression in triple-negative breast cancer (TNBC): A systematic review and meta-analysis study. Diagnostics 2020;10:704.
24. Huang X, Wang X, Qian H, Jin X, Jiang G. Expression of PD-L1 and BRCA1 in triple-negative breast cancer patients and relationship with clinicopathological characteristics. Evid Based Complement Alternat Med 2021;2021:5314016.
25. Pang J-MB, Castles B, Byrne DJ, Button P, Hendry S, Lakhani SR, *et al*. SP142 PD-L1 scoring shows high interobserver and intraobserver agreement in triple-negative breast carcinoma but overall low percentage agreement with other PD-L1 clones SP263 and 22C3. Am J Surg Pathol 2021;45:1108-17.