PD-L1 EXPRESSION IN TRIPLE-NEGATIVE BREAST CANCER: A CROSS-SECTIONAL STUDY IN A POLISH POPULATION

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Triple-negative breast cancer (TNBC) accounts for 15-20% of all breast carcinomas and represents an aggressive variant with a high mortality rate. PD-L1 is a protein that plays a pivotal role in suppressing the adaptive immune system. It has become a central target of the immunotherapy approach. Determining the PD-L1 status can identify TNBC patients who may benefit from targeted therapy. This study was performed to estimate the prevalence of PD-L1 expression among Polish TNBC patients. A total of 123 patients with TNBC were tested for PD-L1 expression using immunohistochemical studies. The PD-L1-positive tumors were found in 55 patients (45%), while PD-L1-negative tumors were found in 68 patients (55%). The PD-L1 positive tumors included 17 patients (31%) with the expression covering up to 1% of tumor area, 23 patients (42%) covering 2-5%, 8 patients (14%) covering 6-10% and 7 patients (13%) covering more than 10% of tumor area. The PD-L1 negative tumors included 17 patients (25%) with the expression covering less than 1% of tumor area and 51 patients (75%) with a complete lack of expression. There were no significant differences between the groups with different status of PD-L1 and the clinical tumor and lymph node stages as well as the patients’ age.

Key words: TNBC, PD-L1, breast cancer, triple negative.

Introduction

Breast cancer (BC) is the second leading cause of cancer death in women [1]. According to the diversity of clinical approaches to different subtypes, proper classification into subtypes determines treatment decisions. Based on intrinsic gene expression profiling, BC can be divided into five major subtypes: luminal A, luminal B, normal breast-like subtype, HER2-positive BC, and triple-negative breast cancer (TNBC) [2].

TNBC accounts for 15-20% of all breast carcinomas and represents a particularly proliferative and aggressive variant with a high mortality rate [3, 4, 5, 6]. The term “triple-negative” refers to the cancer testing negative for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) [7, 8, 9].
Programmed death ligand-1 (PD-L1/CD274), the ligand for the PD-1 receptor, is a transmembrane protein that plays a pivotal role in suppressing the adaptive immune system. PD-L1 is expressed on the surface of lymphocytes, macrophages, dendritic cells and certain solid tumor cells [10]. Because upregulation of PD-L1 on tumor cells often allows cancers to evade the host immune system, the PD-L1–PD-1 axis has become a central target of the immunotherapy approach [11, 12, 13].

Historically, BC was not considered as immunogenically active. In TNBC, however, the emerging data led to approvals of several immunotherapeutics. One of them, atezolizumab is particularly effective in the PD-L1-positive subgroup. Hence, determining the PD-L1 status can identify TNBC patients who may benefit from targeted therapy. This study was performed to estimate the prevalence of PD-L1 expression among Polish TNBC patients.

Material and methods

The study was performed on triple-negative breast cancer (negative for estrogen receptors, progesterone receptors, and excess HER2 protein, according to ASCO/CAP guidelines) routinely diagnosed by pathologists. All studied patients were diagnosed in the Oncology Centers in 2018. Key exclusion criteria were: insufficient material and/or extensive necrotic changes within surgical biopsy material. A total of 123 patients were enrolled in this study. All of the collected tissue sections were processed according to the standard diagnostic protocol. Briefly, collected tissue sections were fixed in 10% buffered formalin for 24 hours at room temperature. After fixation, the sections were dehydrated in ethyl alcohols (80-99.8%), cleared in xylenes (I-IV), and embedded in paraffin. Then preliminary evaluation of tissue samples according to hematoxylin and eosin staining was performed by two independent pathologists [14]. For the sake of this study representative material from tumors was selected for routine (eg. ER, PR, HER2) and additional immunohistochemical studies. The immunohistochemical studies of PD-L1 expression were performed using anti-PD-L1 antibody (clone SP142, Ventana Medical Systems). For the immunohistochemical staining, we used the original protocol provided by Ventana. Finally, the brown color as a result of the histochemical reaction product was considered as observed in the site of the presence of the searched antigen.

The pathologists who were evaluating the immunohistochemical expression of the examined antigen worked independently, and were blinded to the patients’ data and tissue characteristics. The protein expression was evaluated using a light microscope at 20× original objective magnification. The detailed scoring algorithm for PD-L1 expression has been described elsewhere [15, 16]. Briefly, tumor-infiltrating immune cells were scored as a percentage of tumor area that is occupied by PD-L1 positive cells. Tumor-infiltrating immune cells are immune cells present in the intratumoral and contiguous peritumoral stroma that include macrophages, lymphocytes, neutrophils, and dendritic cells. The case was considered as positive if the tissue sample exhibits ≥ 1% of tumor-infiltrating immune cells with PD-L1 expression (Fig. 1). Furthermore, PD-L1 positive tumors were divided into four more specific subgroups to assess the distribution of expression. The first subgroup included tumors with the PD-L1 expression covering 1% of tumor area, the second subgroup from 2% to 5%, the third from 6% to 10%, and the fourth more than 10%. In turn, PD-L1 negative tumors were divided into two subgroups, which included tumors with the PD-L1 expression covering less than 1% of tumor area and tumors with a complete lack of PD-L1 expression. The scoring system is summarized in Table I.

Results

The average age of patients was 58 years (min/max = 31/88 years). The clinical-stage distributions were cT1 in 35 patients, cT2 in 59 patients, cT3 in 11 patients, cT4 in 8 patients, and in 10 cases clinical data were unavailable. The clinical lymph node stage distributions were cN0 in 64 patients, cN1 in 40 patients, cN2 in 4 patients, cN3 in 5 patients. In 10 cases clinical data were unavailable. 84 tissue samples were obtained from core-needle biopsies and 39 were obtained from surgically resected specimens. PD-L1-positive tumors were found in 55 patients (45%), while PD-L1-negative tumors were found in 68 patients (55%) (Fig. 2A). The PD-L1 positive
tumors included 17 patients (31%) with the expression covering up to 1% of tumor area, 23 patients (42%) covering from 2% to 5%, 8 patients (14%) covering from 6% to 10% and 7 patients (13%) covering more than 10% of tumor area (Fig. 2B). The PD-L1 negative tumors included 17 patients (25%) with the expression covering less than 1% of tumor area and 51 patients (75%) with a complete lack of expression (Fig. 2C). There was no significant difference between the groups with different status of PD-L1 and the clinical tumor stage. 52% of patients with PD-L1 positive tumors were in cT2, 34% in cT1, 8% in cT3, and 6% in cT4 (Fig. 2D), whereas 52% of patients with PD-L1 negative tumors were in cT2, 29% in cT1, 11% in cT3, and 8% in cT4 (Fig. 2E). There was no significant difference between the status of PD-L1 and the clinical lymph node stage (p = 0.1090). 66% of patients with PD-L1 positive tumors had no lymph node metastases (cN0), and 34% had lymph node metastases (cN1, cN2, cN3) (Fig. 2F), whereas 51% of patients with PD-L1 negative tumors had no lymph nodes metastases (cN0), and 49% had lymph nodes metastases (cN1, cN2, cN3) (Fig. 2G). Moreover, there was no significant difference between the status of PD-L1 and the patients’ age (p = 0.4407). The average age of PD-L1 positive patients was 57 years (min/max = 31/82 years). The average age of PD-L1 negative patients was 59 years (min/max = 32/88 years) (Fig. 3).

Discussion

This is the largest study of PD-L1 prevalence in an unselected, consecutive cohort of Polish TNBC patients. The expression of PD-L1 on tumor cells and/or tumor-infiltrating immune cells is well established as a biomarker of response to anti-PD-1/PD-L1 therapy [17]. To minimize false negative results (according to the inappropriate pre-analytical phase), we analyzed the PD-L1 status on recent biopsy specimens.

Our results indicate that a significant proportion of TNBC Polish patients are positive for PD-L1 expression. Therefore, it seems rational to perform routine testing in each individual with TNBC. We found no relationship between PD-L1 expression and age or stage of the disease. It is a strong indication for routine evaluation of PD-L1 status in TNBC patients.

Based on our results we advise against using clinical characteristics to either select or exclude patients from testing for PD-L1 expression.

It is challenging to turn the PD-L1 assay into a dichotomous result, namely: “positive” or “negative”. In different reported series, the cutoff for positive PD-L1 staining ranges from 1% to 50% of the tumor area, making it complicated to compare the results across studies [18, 19]. In TNBC, the threshold is set at 1% and does not integrate staining intensity as a part of the scoring algorithm [20]. In our cohort, 25% of patients designated as PD-L1-negative showed PD-L1 expression (expression covering less than 1% of tumor area) and 31% of PD-L1-positive patients did not exceed 1% of stained area of the tumor. These results indicate that there is a considerable risk of assigning the patient to potentially harmful medical intervention. The IHC procedure needs to be performed with standardized amplification and detection systems, since both may dramatically affect the percentage of labelled cells, and eventually, patients’ outcomes.

The published results of the Impassion130 trial established the benefit of adding an immune checkpoint inhibitor (ICI) to standard chemotherapy as a first line treatment of metastatic PD-L1-positive TNBC [20]. In this group, addition of atezolizumab to nab-paclitaxel led to significantly prolonged progression-free survival (7.5 months vs. 5.0 months) and overall survival (25.0 months vs. 15.5 months). This study included 369 PD-L1-positive patients who constituted 40.9% of the study population, which is in accordance with our results. The strong treatment response together with notable prevalence of PD-L1 positivity provide a rationale for PD-L1 testing in metastatic TNBC.

Since primary tumors present a more immunogenic phenotype in comparison to metastatic cases, early-stage breast cancer appears to be even more appealing for the implementation of immunotherapy [21].
Fig. 2. A) The expression of PD-L1 in triple-negative breast cancer. B) PD-L1 positive tumors with expression covering up to 1%, 2%-5%, 6%-10% and more than 10% of tumor area. C) PD-L1 negative tumors with expression covering less than 1% of tumor area and with a complete lack of expression. D) Clinical stage distributions of PD-L1 positive tumors. E) Clinical stage distributions of PD-L1 negative tumors. F) Clinical lymph node stage distributions of PD-L1 positive tumors. G) Clinical lymph node stage distributions of PD-L1 negative tumors; (−) negative, (+) positive.
Given the encouraging results from other non-metastatic malignancies [22, 23, 24, 25], various ICIs are currently being tested for TNBC in the neoadjuvant setting [3].

A clearly positive response to ICIs has also been demonstrated among PD-L1 negative patients with non-small cell lung cancer (NSCLC) and kidney cancer [26, 27, 28]. These tumors are characterized by relatively high mutational and neoantigen loads, which result in a higher likelihood of response to PD-1 or PD-L1 inhibitors. Likewise, patients with TNBC that progressed on neoadjuvant chemotherapy might benefit from adjuvant ICI regardless of their PD-L1 status. Current clinical trials are investigating this possibility [3]. As PD-L1 expression is not ideal in selecting patients for anti-PD-1/PD-L1 therapy, a range of alternative biomarkers are now being assessed to predict immunotherapeutic efficacy in TNBC including tumor mutational burden (TMB), microsatellite instability (MSI), gene signatures, tumor-infiltrating lymphocytes (TILs), and mismatch repair (MMR) deficiency [3]. It remains to be elucidated whether a multidimensional immunogram will outcompete the current PD-L1-based unidimensional immunogram in predicting treatment efficacy.

**The authors declare no conflict of interest.**

The technical aspects of this work was financially supported by scientific grant Roche Polska Sp. z o.o.

**References**


![Fig. 3. Status of PD-L1 and patient’s age](image_url)


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