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The relation of CD3, CD4, CD8 and PD-1 expression with tumor type and prognosis in epithelial ovarian cancers

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ABSTRACT

Objectives: Ovarian cancer is a heterogeneous disease, where chronic inflammation plays a key role in carcinogenesis. In this study, it is aimed to analyze the relationship with prognosis and chemotherapy response to clinicopathological variables in epithelial ovarian cancers such as proliferation of PD-1 +, CD8 +, CD4 +, CD3 + T-lymphocytes infiltrating the tumor and tumor stroma.

Matrial and methods: Seventy-six cases diagnosed with primary epithelial ovarian tumor from biopsy or surgical resection materials were included in the study. Immunreactivity of CD3, CD4, CD8, PD1 was evaluated immunohistochemically in lymphocytes in tumor infiltrating lymphocytes and stromal lymphocytes.

Results: Seventeen (22.4%) of the cases were Type I, 59 (77.6%) of them were Type II ovarian carcinoma. PD-1 positivity was observed in stromal and intraepithelial lymphocytes in 22 (28.9%) of 76 cases. In the presence of PD-1 + T-lymphocytes that infiltrate tumor and stroma, disease-free survival are shorter (p = 0.037). The presence of stromal CD4 + and CD8 + T-lymphocytes was more common in late stage patients (p = 0.012, p = 0.036; respectively). The disease-free and overall survival rate was statistically significantly shorter in the presence of CD8 + T lymphocytes (p = 0.009, p = 0.003; respectively).

Conclusions: CD3, CD4 and CD8 may contribute to PD-1 mediated tumor control. Anti PD-1 therapy may be an alternative to chemotherapy in PD-1 positive patients. Identifying patients who do not respond to chemotherapy through PD-1 expression prior to immunotherapy will help develop potential personalized immunotherapy.

Key words: ovarian cancer; PD-1; CD3; CD4; stromal lymphocytes

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INTRODUCTION

Ovarian cancers are the second most common malignancy among gynecological malignancies, causing the most frequent death due to nonspecific symptomatology and the absence of effective diagnostic methods that provide early detection [1, 2]. According to global statistical data, approximately 295,414 new ovarian cancers and 184,799 deaths were reported worldwide in 2018 [3].

The immune system; it is considered to have a key role in carcinogenesis, especially the suspension of tumor development. The increased concentration of tumor infiltrating lymphocyte (TIL) is associated with good prognosis in various types of cancer [4]. Recent advances in immunotherapy, particularly immune checkpoint inhibitors (ICIs), have increased interest in the new treatment strategy and the immune status of the cancer microenvironment [5]. New treatment strategies are needed as current treatment methods are not sufficient to increase the survival rate of patients with ovarian cancer. Nowadays, there are many ongoing clinical studies on the effectiveness of ICIs in ovarian cancer [6].

The use of ICIs for cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), programmed cell death-1 (PD-1) receptor/programmed death-ligand 1 (PD) receptors in many cancer patients, particularly malignant melanoma, lung

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cancer, and bladder cancer has been shown to increase overall survival [7]. TIL's; CD4 + and CD8 + T-cells, natural killer (NK) cells, natural killer T lymphocytes (NKT), dendritic cells, CD3 + T-lymphocytes and CD20 + B-lymphocytes are involved in the antitumor immune response. These cells directly participate in the presentation of tumor antigens and/or the attack of tumor cells [3].

PD-1, also known as CD279, is mainly expressed through activated immune cells such as regulatory T (Treg)-cells and NK-cells. Thus, it prevents excessive immune response and protects normal cells from immune attack [8, 9]. PD-1 has two ligands, PD-L1 and PD-L2. In order for PD-1 to inhibit T-lymphocyte functions, it must be combined with PD-L1 and PD-L2 ligands. The ligand commonly found in tumor cells is PD-L1. PD-L1, expressed in the micro-environment of the tumor, suppresses the immune response developing against the tumor [10]. PD-L1 is thought to play a role in the immunological escape mechanism that causes tumor cell growth, proliferation and metastasis [2]. Cancer immunotherapy is due to the emergence of T-cells through immune checkpoint blockage and its functional role to eliminate tumor cells [8]. The expression of immune checkpoint molecules carries prognostic and predictive instructions.

In this study, it is aimed to analyze the relationship with prognosis and chemotherapy response to clinicopathological variables in epithelial ovarian cancers such as proliferation of PD-1 +, CD8 +, CD4 +, CD3 + T-lymphocytes infiltrating the tumor and tumor stroma, tumor type, tumor size, lymphovascular invasion, lymph node invasion, tumor stage.

MATERIAL AND METHODS

Patients

Seventy-six cases diagnosed with epithelial ovarian tumor from biopsy or surgical resection materials at Pamukkale University Faculty of Medicine, Department of Pathology, between 2011 and 2019 were included in the study. The cases were retrospectively reviewed.

The ethics approval of this study was accepted by the Non-Interventional Clinical Research Ethics Committee of Pamukkale University at the meeting dated 19.11.2019 and numbered 60116787-020/83868.

Epithelial ovarian tumors has been reclassified as type I and II tumors according to the new carcinogenesis model. Type I tumors originate from extra-ovarian lesions that can turn into malignant lesions; 1. clear cell carcinoma, seromucinous carcinoma, endometrioid carcinoma known to be associated with endometriosis 2. low grade serous carcinomas; 3. mucinous carcinoma and malignant brenner tumors. Type II tumors developed from intraepithelial lesions in the fallopian tube and divided into three groups: 1. high grade serous carcinoma, 2. carcinosarcoma, and 3. undifferentiated carcinoma (Fig. 1) [11]. The stage of the tumor was determined according to the International Federation of Gynecology and Obstetrics (FIGO) criteria [12]. While primary epithelial ovarian tumors were included in the study; secondary malignant neoplasms (metastases) and primary nonepithelial ovarian tumors were excluded. Patient information such as age, stage, and treatment information of the cases were obtained from the gynecology

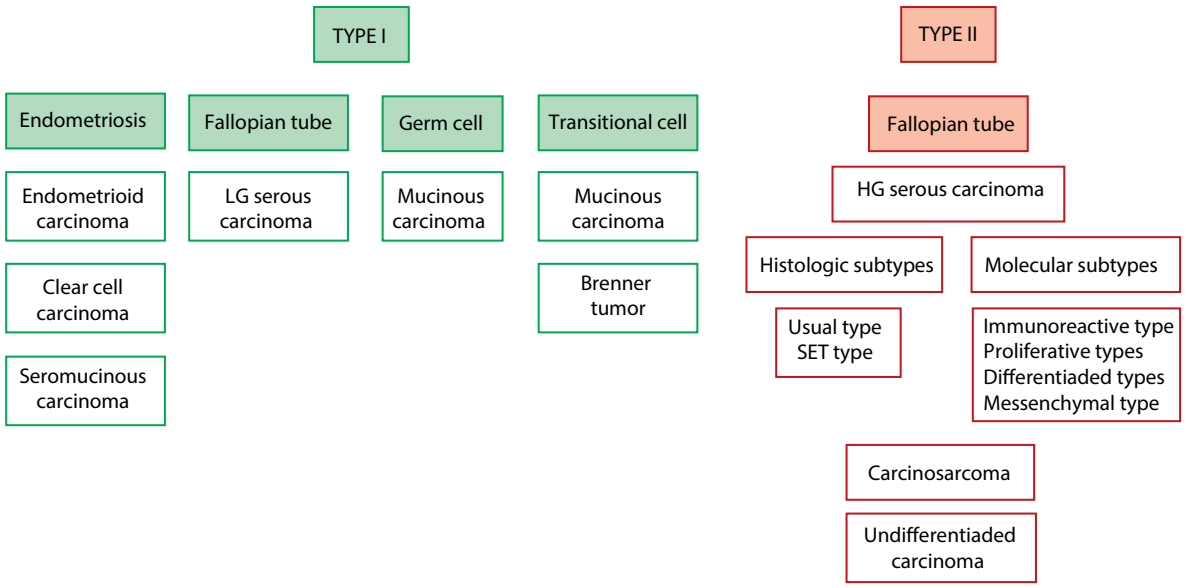

Figure 1. Expanded dualistic model of ovarian carcinogenesis. Ovarian carcinomas derive from endometrial tissue, fallopian tube tissue, germ cells, and transitional epithelium. Type I carcinomas comprise endometrioid, clear cell, LG serous, and mucinous carcinomas. Seromucinous carcinomas and malignant Brenner tumors are rare. It was recently proposed that seromucinous neoplasms be designated mixed Müllerian tumors. Type II carcinomas are largely composed of HG serous carcinoma, carcinosarcoma, and undifferentiated carcinoma. Transitional cell indicates metaplastic transitional epithelium at the tuboperitoneal junction; HG — high-grade; LG — low-grade; SET — solid pseudoendometrioid transitional [11]

department, data such as disease-free survival and overall survival were obtained from the patient files of the department of oncology.

While TAH + BSO + lymph node dissection was applied to 69 (90.8%) of the cases, 7 (9.2%) were performed biopsy. The mean age of the cases is 55 (24–81). Considered as older than 55, younger under 55. The mean tumor diameter is 0.9 cm (0.5–11.5). 42 (55.3%) of them are located bilaterally. 17 (22.4%) of the cases were Type I, 59 (77.6%) of them were Type II ovarian carcinoma. Their distribution is 59 (77.6%) high grade serous carcinoma, 8 (10.5%) borderline serous carcinoma, 4 (5.3%) clear cell carcinoma, 3 (3.9%) endometrioid carcinoma, 2 (2.6%) is in the form of mucinous carcinoma. In immunohistochemical analysis for ER 48/59 (82.7%), PR 33/52 (63.4%), C-erb 2 1/6 (16.6%), PAX8 14/15 (93.3%), WT1 39/71 (54.9%), p53 40/46 (86.9%), CEA 125 38/39 (97.4%) were positive in cases. Capsule invasion was present in 36 (47.4%) of the cases and lymphovascular invasion in 12 (15.8%). 23 (30.3%) of the intraabdominal fluid is malignant. 28 (36.8%) of the cases are early stage (I–II), 36 (47.4%) of the cases are late stage (III–IV). Recurrence was observed in 27 (35.5%) of the cases, and the disease-free survival was 29.6 ± 28.7 months. 67 (88.7%) of the cases died and the overall survival time was 34.8 ± 30.8 months.

Immunohistochemistry (IHC)

A tumor-rich paraffin block was selected for immunohistochemical examination. Ventana Benchmark XT™ fully automated staining device was used with the procedure suitable for sections of about 3–5 microns thick taken on lysine slide from selected paraffin blocks. CD3 (Dako, prediluted, ready-to-use antibody antibody; Rabbit Polyclonal Primary Antibody), CD4 (Dako, prediluted- auto-ready antibody; Mouse Monoclonal Primary Antibody, clone 4B12), CD8 (Dako, prediluted- auto-ready antibody; Mouse Monoclonal Primary Antibody, clone C8/144B), PD-1 (CD279) (Cell Marque, predilute-auto ready-to-use antibody; mouse monoclonal, clone: NAT105) antibodies were used for immunohistochemical staining. The *ultra*View Universal DAB detection kit is used for all staining. The primary antibody stage was omitted for negative control in immunohistochemical staining. For positive control, tonsil tissue for CD3, CD8, CD4 and placental chorionic villus for PD-1 were used. The slides examined were evaluated by two pathologists (FB, YAK) for the immunoreactivity of CD3, CD4, CD8, PD1, taking into account cytoplasmic and/or membranous staining in tumor infiltrating lymphocytes and stromal lymphocytes. Percentage ratio for CD3, CD4, CD8 T lymphocytes in lymphocytes infiltrating the tumor and stroma is given. Median value was taken as cutt-off. Above the median value was classified as high, below the median value was classified as low. For PD-1, it was evaluated as < 1% negative and ≥ 1% positive.

Statistical evaluation

Descriptive values of quantitative continuous variables (such as age) were examined using standard descriptive statistical methods (arithmetic mean, standard deviation, median, etc.). Categorical variables (asset frequencies) are given together with their frequencies and percentages in the total. Comparisons of categorical variables were made by Chi-square or Fischer's Exact Test, depending on the state of the case distributions. Kaplan Mayer test was performed for disease-free survival and overall survival.

RESULTS

PD-1 positivity was observed in stromal and intratumoral lymphocytes in 22 (28.9%) of 76 cases (Fig. 2A, B). PD-1 positivity in intratumoral lymphocytes was found to be higher in elderly patients than in younger patients ($p = 0.030$). Distribution of PD-1 positive cases by tumor types; 18/59 (30.5%) of our high-grade serous carcinoma cases, in 1/2 (50%) of mucinous carcinoma, 2/8 (25%) of borderline serous carcinoma, 1/4 (25%) of our clear cell carcinoma cases showed positive PD-1, in addition no PD-1 positivity was found in a patient with endometrioid carcinoma. 18/22 (81%) of PD-1 positive T-lymphocytes were seen in patients with type II ovarian carcinoma, 4/22 (19%) were seen in patients with type I ovarian carcinoma, this relationship was not statistically significant ($p = 0.500$). In PD-1 positive group, 17 (77.3%) of 22 cases were exitus ($p = 0.029$), and recurrence was detected in 6 (27.3%) ($p = 0.337$). In the presence of PD-1 + T-lymphocytes that infiltrate tumor and stroma, disease-free survival and overall survival are shorter than PD-1 negative cases ($p = 0.037$, $p = 0.063$; respectively). The relationship between the proliferation of stromal PD-1, CD8, CD4, CD3 T lymphocytes and clinicopathological variables is shown in Table 1.

High incidence of stromal CD3 + T-lymphocytes in early-stage ovarian carcinomas is associated with longer disease-free survival ($p = 0.087$). Also, high incidence of stromal CD3 + T-lymphocytes in Type I ovarian carcinomas is associated with longer disease-free survival ($p = 0.066$) (Fig. 2C, D). The presence of stromal CD3 + T-lymphocyte was found to be higher in living patients than in ex patients ($p = 0.093$). Stromal CD3+ T-lymphocytes was higher in PD-1 positive patients than PD-1 negative patients ($p = 0.012$) (Tab. 2). In the survival statistics, in the presence of PD-1 positive CD3 + T-lymphocyte, disease-free survival ($p = 0.032$), overall survival ($p = 0.063$) tends to be longer.

The disease-free and overall survival rate was statistically significantly shorter in the presence of CD8 + T-lymphocytes

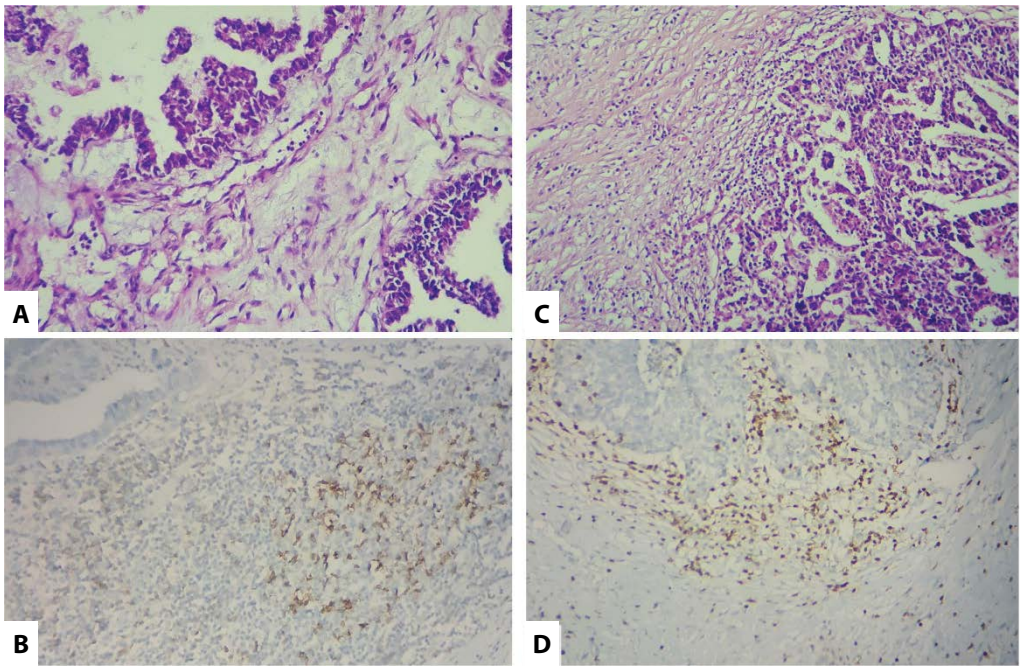

Figure 2A. Serous ovarian carcinoma, H-E, ×200; **B.** Stromal PD-1 + lymphocytes, IHK, ×200; **C, D.** High incidence of stromal CD3 + T-lymphocytes (C — H-E, ×200, D — CD 3 IHK, ×200)

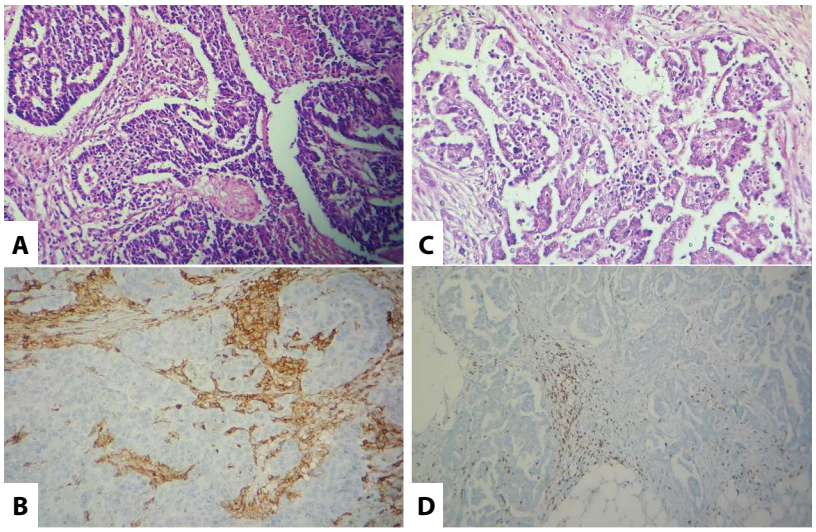

Figure 3A, B. The presence of stromal CD4 + T-lymphocyte was common in type II ovarian carcinomas (A — H-E, × 200, B. CD 4 IHK, ×200); **C, D.** The presence of stromal CD8 + T lymphocytes (C — H-E, ×200 D — CD 8 IHK, ×100)

(p = 0.009, p = 0.003; respectively). In patients receiving chemotherapy, the disease-free and overall survival rate was shorter in the presence of CD8 + T-lymphocytes (p = 0.569, p = 0.014; respectively).

The presence of stromal CD4 + T-lymphocyte above the median value was more common in type II ovarian carcinomas than in type I (p = 0.080) (Tab. 3). The presence of stromal CD4 + and CD8 + T lymphocytes was more common in late stage patients than in the early stage (p = 0.012, p = 0.036; respectively) (Fig. 3A–D). There was no statistically significant relationship between disease-free survival and overall survival in the presence of PD-1 positive CD4 + T-lymphocyte in survival statistics (p = 0.789, p = 0.863).

There was no statistically significant relationship between the proliferation of intratumoral PD-1, CD8, CD4, CD3 T-lymphocytes and clinicopathological variables.

There was no statistically significant relationship between ER, PR, C-erb 2, PAX8, WT1, p53, CEA 125 immuno-expression and CD3, CD4, CD8 and PD-1 expression.

Table 1. Relationship between proliferation of stromal PD-1, CD8, CD4, CD3 T-lymphocytes and clinicopathological variables

Stromal	CD3L	CD3H	CD4L	CD4H	CD8L	CD8H	PD-1L	PD-1H
				n/%				
Tumor diameter (n = 58)								
> 0.5 cm (n = 38)	8 (13.8)	30 (51.7)	20 (34.5)	18 (31.0)	19 (32.8)	19 (32.8)	26 (44.8)	12 (20.7)
< 0.5 cm (n = 20)	8 (13.8)	12 (20.7)	7 (12.1)	13 (22.4)	5 (8.6)	15 (25.8)	17 (29.3)	3 (5.2)
Histological grade (n = 76)								
Low (n = 17)	6 (7.9)	11 (14.5)	11 (14.5)	6 (7.9)	10 (13.2)	7 (9.2)	13 (17.1)	4 (5.3)
High (n = 59)	12 (15.8)	47 (61.8)	24 (31.6)	35 (46.0)	19 (25.0)	40 (52.6)	41 (53.9)	18 (23.7)
Stage (n = 64)								
I/II (n = 28)	8 (12.5)	20 (31.3)	19 (29.7)	9 (14.1)	15 (23.5)	13 (20.3)	20 (31.2)	8 (12.5)
III/IV (n = 36)	10 (15.6)	26 (40.6)	13 (20.3)	23 (35.9)	10 (15.6)	26 (40.6)	27 (42.2)	9 (14.1)
Lymphovascular invasion (n = 25)								
Yes (n = 12)	3 (12.0)	9 (36.0)	3 (12.0)	9 (36.0)	4 (16.0)	8 (32.0)	9 (36.0)	3 (12.0)
No (n = 13)	4 (16.0)	9 (36.0)	7 (28.0)	6 (24.0)	8 (32.0)	5 (20.0)	8 (32.0)	5 (20.0)
Capsule invasion (n = 51)								
Yes (n = 36)	9 (17.7)	27 (52.9)	15 (29.4)	21 (41.2)	16 (31.4)	20 (39.2)	28 (54.9)	8 (15.7)
No (n = 15)	6 (11.7)	9 (17.7)	8 (15.7)	7 (13.7)	6 (11.7)	9 (17.7)	11 (21.6)	4 (7.8)
Metastasis (n = 72)								
Yes (n = 45)	12 (16.7)	33 (45.8)	15 (20.8)	30 (41.7)	12 (16.7)	33 (45.8)	34 (47.2)	11 (15.3)
No (n = 27)	6 (8.3)	21 (29.2)	19 (26.4)	8 (11.1)	14 (19.4)	13 (18.1)	18 (25.0)	9 (12.5)
Intraabdominal fluid (n = 33)								
Benign (n = 10)	0 (0.0)	10 (30.3)	3 (9.1)	7 (21.2)	2 (6.1)	8 (24.2)	4 (12.1)	6 (18.2)
Malign (n = 23)	5 (15.2)	18 (54.5)	10 (30.3)	13 (39.4)	11 (33.3)	12 (36.4)	15 (45.5)	8 (24.2)
Nuks (n = 76)								
Yes (n = 27)	7 (9.2)	20 (26.3)	13 (17.1)	14 (18.4)	6 (7.9)	21 (27.6)	21 (27.6)	6 (7.9)
No (n = 49)	11 (14.5)	38 (50.0)	22 (29.0)	27 (35.5)	23 (30.3)	26 (34.2)	33 (43.4)	16 (21.1)
Ex (n = 75)								
Yes (n = 67)	18 (24.0)	49 (65.3)	32 (42.7)	35 (46.6)	26 (34.7)	41 (54.6)	50 (66.7)	17 (22.7)
No (n = 8)	0 (0.0)	8 (10.7)	3 (4.0)	5 (6.7)	3 (4.0)	5 (6.7)	3 (4.0)	5 (6.6)

Table 2. Intratumoral and stromal CD3, CD4, CD8 ratios in PD-1 positive and negative cases

		Low	High	p
CD3 intratumoral	PD-1 negative	20/54 37%	34/54 63%	0.416
	PD-1 positive	8/22 36%	14/22 64%	
CD4 intratumoral	PD-1 negative	12/54 22%	42/54 78%	0.695
	PD-1 positive	4/22 18%	18/22 82%	
CD8 intratumoral	PD-1 negative	27/54 50%	27/54 50%	0.279
	PD-1 positive	8/22 36%	14/22 64%	
CD3 stromal	PD-1 negative	17/54 31%	25/54 47%	0.012
	PD-1 positive	10/ 22 45%	12/22 55%	
CD4 stromal	PD-1 negative	29/54 53%	25/54 47%	0.947
	PD-1 positive	10/ 22 45%	12/22 55%	
CD8 stromal	PD-1 negative	27/54 50%	27/54 50%	0.468
	PD-1 positive	8/22 36%	14/22 64%	

Table 3. Intratumoral and stromal PD-1, CD3, CD4, CD8 ratios in Type 1 and Type 2 ovarian carcinomas

		Type I Ovarian Carcinoma n = 17 (22.4%)	Type II Ovarian Carcinoma n = 59 (77.6%)	p
PD-1 intratumoral	Positive negative	4 (5.3%) 13 (17.1%)	18 (23.7%) 41 (53.9%)	0.576
PD-1 stromal	Positive Negative	4 (5.3%) 13 (17.1%)	18 (23.7%) 41 (53.9%)	0.576
CD3 intratumoral	High Low	10 (13.2%) 7 (9.2%)	40 (52.6%) 19 (25%)	0.492
CD3 stromal	High Low	11 (14.5%) 6 (7.9%)	47 (61.8%) 12 (15.8%)	0.201
CD4 intratumoral	High Low	13 (17.1%) 4 (5.3%)	47 (61.8%) 12 (15.8%)	0.776
CD4 stromal	High Low	6 (7.9%) 11 (14.5%)	35 (46.1%) 24 (31.5%)	0.080
CD8 intratumoral	High Low	9 (11.8%) 8 (10.5%)	32 (42.1%) 27 (35.6%)	0.925
CD8 stromal	High Low	8 (10.5%) 9 (11.8%)	39 (51.4%) 20 (26.3%)	0.154

DISCUSSION

The presence of TILs in the intratumoral and stromal component of epithelial tumors of the ovary is an important prognostic factor. The diversity of stromal TILs with tumor cell proliferation, invasion, and matrix rearrangement that causes carcinogenesis resulting in different survival creates a tumor-specific microenvironment [13]. Tumor cells modify the tumor microenvironment, both to suppress T-cells and to stimulate tumorigenic inflammation [14]. Afterwards, PD1 becomes apparent in this process and limits T-cell activity in the tumor microenvironment [15]. In the study of Webb et al., PD-1 was positive in 22.1% of 489 ovarian cancers. PD-1 positivity was seen in 75 (38.5%) of 195 high-grade serous carcinomas, 22 (17.6%) of 125 endometrioid carcinomas, 11 (8.6%) of 128 clear cell carcinomas in this study, while 30 mucinous and 11 low-grade serous tumor was PD-1 negative [16]. In our study, PD-1 positivity was observed in 22 (28.9%) of 76 cases. PD-1 positivity was seen in 18 (30.5%) of 59 high-grade serous carcinomas, 1 (50%) of 2 mucinous carcinomas, 2 (25%) of 8 borderline serous carcinomas, 1 (25%) of 4 clear cell carcinomas, was not seen in endometrioid carcinoma in the current study.

Infiltration of PD-1 + lymphocytes is associated with distant metastasis, recurrence and poor prognosis in most tumor types [2, 17]. Wieser et al. found poor prognosis in PD-1 positivity at 170 cases of ovarian cancer series [18]. Similarly, in our study, the disease-free survival and overall survival duration were shorter at infiltration of stromal and intraepithelial PD-1 + lymphocytes.

Transformed tumor cells as a source of tumor-associated antigen or neoantigen may induce an immune response. After all, cytotoxic T-cells contribute to the elimination of tumor cells [19]. Zhang et al. (2003) reported that CD3 + T-lymphocytes show more expression in the advanced stages of serous ovarian carcinomas [20, 21]. In a recent study, higher intraepithelial CD3 and CD8 TIL scores were significantly associated with longer survival in univariate and multivariate analyzes [22]. In another study, no correlation was found in survival analysis with stromal TILs in ovarian cancer. Accordingly, the importance of evaluating TILs for each tumor type is emphasized [23]. In our study, high prevalence of stromal CD3 positive lymphocytes in Type I ovarian carcinomas and early stage patients was associated with good prognosis. Stromal CD3 positivity was higher in living patients than ex patients. CD3 positivity was higher in PD-1 positive group and was associated with good prognosis. Sato et al. [23] stated that the presence of CD8 + T-lymphocytes in ovarian tumors is associated with good prognosis. They reported that high expression of CD8 + T-lymphocytes in the tumor was observed, but not in the tumor stroma [23]. In another study, CD8 + T-lymphocyte infiltration was found to be associated with advanced stage, high tumor grade, and metastasis in the epithelial tumors of the ovary [24, 25], and therefore it was advocated to adversely affect the antitumor immune response [26]. According to the literature, there is a disagreement over the role of CD8 in the prognosis of ovarian cancer. In our study, the presence of stromal CD8 + TIL was more common in late stage patients than in the early stage. The presence of CD8 + T-lymphocytes has been associated with a worse prognosis.

Hamanishi et al. [27], showed high rates of CD4 + TILs in cases of ovarian cancer with better prognosis. These cells

can recognize cancer antigens and mature to type-1 helper cells (Th1). Although the mechanism is uncertain, it can activate M1 macrophages through interleukin-12 or interferon gamma secretion [27]. In studies conducted in different years, the presence of intraepithelial CD4 + T-lymphocytes has been associated with better survival [6, 28–30]. In our study, the presence of stromal CD4 + T-lymphocyte was more common in late stage patients than in the early stage. The presence of stromal CD4 T-lymphocyte was more prevalent in type II ovarian carcinomas than in type I. However, no significant relationship was found with the prognosis.

Cancer immunotherapy has been a controversial issue for years, but studies in this area reached a milestone in 2014. Antibodies specifically blocking PD-1 became available for melanoma in 2014 and went into use for non-small cell lung cancer (NSCLC) in the United States, the European Union and Japan, in 2015, primarily approved by the Food and Drug Administration (FDA) [31]. The FDA approved the use of anti-PD-1 antibody pembrolizumab for solid cancers with microsatellite instability (MSI)-H or mismatch repair (MMR) deficiency in May 2017 [6]. Currently, two classes of FDA-approved immunotherapy for clinical use are PD-1 / PD-L1 and CTLA-4 inhibitors [32]. New agents targeting other courses of the immune system are in the research phase [33]. Until now, single-agent PD-1 blockade has shown moderate activity in patients with ovarian carcinoma, with 15% and 8% response rates reported in nivolumab and pembrolizumab studies, respectively [19]. Phase II clinical study in ovarian cancer has shown that nivolumab, a PD-1 receptor blocker, is well tolerated and offers a 45% disease control rate [34]. A recent update to a patient cohort demonstrated the ongoing clinical benefit even after drug discontinuation. In addition, Pembrolizumab, a PD-1 blocker similar to Nivolumab, currently shows good tolerance and promising disease control on patients with ovarian cancer in early results. Therefore, it is important to identify biomarkers for immune checkpoint inhibitors in ovarian cancers. There are about 100 clinical trials testing PD-1 blockers, many of which focus on ovarian cancer [35]. PD-1 inhibition ensures proliferation of circulating tumor-specific CD8 + T-cells and reduces the functional depletion of specific T-cells [11]. To improve our current knowledge about the immunological environment of epithelial ovarian tumors, spesific immune cells need to be further investigated in different region tumors [3].

CONCLUSIONS

In our study, three important results were obtained. Firstly, PD-1 is positive in 28.9% of stromal and intraepithelial lymphocytes in our cases. CD3 positivity was higher in stromal and intraepithelial T-lymphocytes of these cases, which is associated with good prognosis. Second, infiltration of intraepithelial and stromal PD-1 + T-lymphocytes has been associated with poor prognosis. Third, stromal CD 4+ and CD 8+ T-lymphocytes are more common in late stages. In addition, the presence of CD 3 + T-lymphocytes is associated with good prognosis, while the presence of CD8 + T-lymphocytes is associated with poor prognosis. As a result, we think that CD3, CD4 and CD8 may contribute to PD-1 mediated tumor control. Anti PD-1 therapy may be an alternative to chemotherapy in PD-1 positive patients. Identifying patients who do not respond to chemotherapy through PD-1 expression prior to immunotherapy will help develop potential personalized immunotherapy and will help patients avoid unnecessary treatment.

Conflict of interest

There is no conflict of interest by the authors.

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REFERENCES

1. Jammal MP, Lima CA, Murta EF, et al. Is ovarian cancer prevention currently still a recommendation of our grandparents? Rev Bras Ginecol Obstet. 2017; 39(12): 676–685, doi: 10.1055/s-0037-1608867, indexed in Pubmed: 29179244.
2. Wang L. Prognostic effect of programmed death-ligand 1 (PD-L1) in ovarian cancer: a systematic review, meta-analysis and bioinformatics study. J Ovarian Res. 2019; 12(1): 37, doi: 10.1186/s13048-019-0512-6, indexed in Pubmed: 31039792.
3. Lima CA, Jammal MP, Etchebehere RM, et al. Lymphocytes in peritumoral stroma: evaluation in epithelial ovarian neoplasms. Immunol Invest. 2020; 49(4): 397–405, doi: 10.1080/08820139.2019.1637435, indexed in Pubmed: 31298603.
4. Ladányi A, Somlai B, Gilde K, et al. T-cell activation marker expression on tumor-infiltrating lymphocytes as prognostic factor in cutaneous malignant melanoma. Clin Cancer Res. 2004; 10(2): 521–530, doi: 10.1158/1078-0432.ccr-1161-03, indexed in Pubmed: 14760073.
5. Sawada M, Goto K, Morimoto-Okazawa A, et al. PD-1+ Tim3+ tumor-infiltrating CD8 T cells sustain the potential for IFN-γ production, but lose cytotoxic activity in ovarian cancer. Int Immunol. 2020; 32(6): 397–405, doi: 10.1093/intimm/dxaa010, indexed in Pubmed: 32009163.
6. Yamashita H, Nakayama K, Ishikawa M, et al. Relationship between microsatellite instability, immune cells infiltration, and expression of immune checkpoint molecules in ovarian carcinoma: immunotherapeutic strategies for the future. Int J Mol Sci. 2019; 20(20), doi: 10.3390/ijms20205129, indexed in Pubmed: 31623180.
7. Martin de la Fuente L, Westbom-Fremer S, Arildsen NS, et al. PD-1/PD-L1 expression and tumor-infiltrating lymphocytes are prognostically favorable in advanced high-grade serous ovarian carcinoma. Virchows Arch. 2020; 477(1): 83–91, doi: 10.1007/s00428-020-02751-6, indexed in Pubmed: 31980961.
8. Tu L, Guan R, Yang H, et al. Assessment of the expression of the immune checkpoint molecules PD-1, CTLA4, TIM-3 and LAG-3 across different cancers in relation to treatment response, tumor-infiltrating immune cells and survival. Int J Cancer. 2020; 147(2): 423–439, doi: 10.1002/ijc.32785, indexed in Pubmed: 31721169.
9. Kim KiH, Choi KUn, Kim A, et al. PD-L1 expression on stromal tumor-infiltrating lymphocytes is a favorable prognostic factor in ovarian serous carcinoma. J Ovarian Res. 2019; 12(1): 56, doi: 10.1186/s13048-019-0526-0, indexed in Pubmed: 31208449.
10. Aksu ÖB, Şengül Ş. Immune Checkpoints and Inhibitors. Journal of Ankara University Faculty of Medicine. 2020; 72(3): 262–267, doi: 10.4274/atfm.galenos.2019.24382.

11. Kurman RJ, Shih IM. The dualistic model of ovarian carcinogenesis: revisited, revised, and expanded. Am J Pathol. 2016; 186(4): 733–747, doi: 10.1016/j.ajpath.2015.11.011, indexed in Pubmed: 27012190.
12. Zeppernick F, Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer. Arch Gynecol Obstet. 2014; 290(5): 839–842, doi: 10.1007/s00404-014-3364-8, indexed in Pubmed: 25082067.
13. Okada Y, Yahata G, Takeuchi S, et al. A correlation between the expression of CD 8 antigen and specific cytotoxicity of tumor-infiltrating lymphocytes. Jpn J Cancer Res. 1989; 80(3): 249–256, doi: 10.1111/j.1349-7006.1989.tb02301.x, indexed in Pubmed: 2524461.
14. Ernst B, Anderson KS. Immunotherapy for the treatment of breast cancer. Curr Oncol Rep. 2015; 17(2): 5, doi: 10.1007/s11912-014-0426-9, indexed in Pubmed: 25677118.
15. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunol Rev. 2008; 224(1): 166–182, doi: 10.1111/j.1600-065X.2008.00662.x, indexed in Pubmed: 18759926.
16. Webb JR, Milne K, Nelson BH. PD-1 and CD103 are widely coexpressed on prognostically favorable intraepithelial CD8 T-Cells in human ovarian cancer. Cancer Immunol Res. 2015; 3(8): 926–935, doi: 10.1158/2326-6066.CIR-14-0239, indexed in Pubmed: 25957117.
17. Lamichhane P, Karyampudi L, Shreeder B, et al. IL10 release upon PD-1 blockade sustains immunosuppression in ovarian cancer. Cancer Res. 2017; 77(23): 6667–6678, doi: 10.1158/0008-5472.CAN-17-0740, indexed in Pubmed: 28993412.
18. Wieser V, Gaugg I, Fleischer M, et al. BRCA1/2 and TP53 mutation status associates with PD-1 and PD-L1 expression in ovarian cancer. Oncotarget. 2018; 9(25): 17501–17511, doi: 10.18632/oncotarget.24770, indexed in Pubmed: 29707124.
19. Mlynska A, Vaišnorė R, Rafanavičius V, et al. A gene signature for immune subtyping of desert, excluded, and inflamed ovarian tumors. Am J Reprod Immunol. 2020; 84(1): e13244, doi: 10.1111/aji.13244, indexed in Pubmed: 32294293.
20. Clarke B, Tinker AV, Lee CH, et al. Intraepithelial T-cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss. Mod Pathol. 2009; 22(3): 393–402, doi: 10.1038/modpathol.2008.191, indexed in Pubmed: 19060844.
21. Zhang L, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T-cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003; 348(3): 203–213, doi: 10.1056/NEJMoa020177, indexed in Pubmed: 12529460.
22. Wang Q, Lou W, Di W, et al. Prognostic value of tumor PD-L1 expression combined with CD8 tumor infiltrating lymphocytes in high grade serous ovarian cancer. Int Immunopharmacol. 2017; 52: 7–14, doi: 10.1016/j.intimp.2017.08.017, indexed in Pubmed: 28846888.
23. Sato E, Olson SH, Ahn J, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T-cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci USA. 2005; 102(51): 18538–18543, doi: 10.1073/pnas.0509182102, indexed in Pubmed: 16344461.
24. Doo DW, Norian LA, Arend RC. Checkpoint inhibitors in ovarian cancer: A review of preclinical data. Gynecol Oncol Rep. 2019; 29: 48–54, doi: 10.1016/j.gore.2019.06.003, indexed in Pubmed: 31312712.
25. Yildirim N, Akman L, Acar K, et al. Do tumor-infiltrating lymphocytes really indicate favorable prognosis in epithelial ovarian cancer? Eur J Obstet Gynecol Reprod Biol. 2017; 215: 55–61, doi: 10.1016/j.ejogrb.2017.06.005, indexed in Pubmed: 28601728.
26. Singh M, Loftus T, Webb E, et al. Minireview: Regulatory T Cells and Ovarian Cancer. Immunol Invest. 2016; 45(8): 712–720, doi: 10.1080/08820139.2016.1186689, indexed in Pubmed: 27420920.
27. Hamanishi J, Mandai M, Abiko K, et al. The comprehensive assessment of local immune status of ovarian cancer by the clustering of multiple immune factors. Clin Immunol. 2011; 141(3): 338–347, doi: 10.1016/j.clim.2011.08.013, indexed in Pubmed: 21955569.
28. Pinto MP, Balmaceda C, Bravo ML, et al. Patient inflammatory status and CD4+/CD8+ intraepithelial tumor lymphocyte infiltration are predictors of outcomes in high-grade serous ovarian cancer. Gynecol Oncol. 2018; 151(1): 10–17, doi: 10.1016/j.ygyno.2018.07.025, indexed in Pubmed: 30078505.
29. Le Page C, Marineau A, Bonza PK, et al. BTN3A2 expression in epithelial ovarian cancer is associated with higher tumor infiltrating T-cells and a better prognosis. PLoS One. 2012; 7(6): e38541, doi: 10.1371/journal.pone.0038541, indexed in Pubmed: 22685580.
30. Yang L, Wang S, Zhang Qi, et al. Clinical significance of the immune microenvironment in ovarian cancer patients. Mol Omics. 2018; 14(5): 341–351, doi: 10.1039/c8mo00128f, indexed in Pubmed: 30129640.
31. Iwai Y, Hamanishi J, Chamoto K, et al. Cancer immunotherapies targeting the PD-1 signaling pathway. J Biomed Sci. 2017; 24(1): 26, doi: 10.1186/s12929-017-0329-9, indexed in Pubmed: 28376884.
32. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer. 2016; 54: 139–148, doi: 10.1016/j.ejca.2015.11.016, indexed in Pubmed: 26765102.
33. Hamanishi J, Mandai M, Ikeda T, et al. Safety and antitumor activity of anti-PD-1 antibody, nivolumab, in patients with platinum-resistant ovarian cancer. J Clin Oncol. 2015; 33(34): 4015–4022, doi: 10.1200/JCO.2015.62.3397, indexed in Pubmed: 26351349.
34. Hamanishi J, Mandai M, Ikeda T, et al. Durable tumor remission in patients with platinum-resistant ovarian cancer receiving nivolumab. Journal of Clinical Oncology. 2015; 33(15_suppl): 5570–5570, doi: 10.1200/jco.2015.33.15_suppl.5570.
35. Normann MC, Türzer M, Diep LMy, et al. Early experiences with PD-1 inhibitor treatment of platinum resistant epithelial ovarian cancer. J Gynecol Oncol. 2019; 30(4): e56, doi: 10.3802/jgo.2019.30.e56, indexed in Pubmed: 31074244.