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Prevalence and prognostic value of the programmed cell death-ligand 1 (PD-L1) expression among tumor samples from cervical cancer patients

Prevalência e valor prognóstico da expressão do ligante de morte celular programada 1 (PD-L1) em amostras tumorais de pacientes com câncer do colo do útero

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ABSTRACT

Objectives: This study aimed to investigate the programmed cell death-ligand 1 (PD-L1) expression in a cohort of cervical cancer (CC) patients evaluating its prognostic significance. **Methods:** All patients diagnosed at Brazilian National Cancer Institute (INCA), in 2011, with invasive CC, squamous cell carcinoma (SCC) or adenocarcinoma (ADC) were retrospectively included. Clinical and treatment data were collected and PD-L1 expression was evaluated according to the percentage of viable tumor cells showing staining. The survival analysis was performed using the Kaplan Meier method. **Results:** In total, 152 patients (105 SSC and 47 ADC) were included and the mean age was 52.4 years (±14.4). According to the International Federation of Gynecology and Obstetrics (FIGO) 2009, 84.2% had locally advanced disease (IB2-IVA). PD-L1 expression was considered positive (=1%) in 53.3% of the cases. After adjustment, the multivariable analysis confirmed that SCC (p=0.026) and tumor size >4cm (p=0.023) were independently associated with PD-L1 expression. There were no significant differences in disease-free survival (DFS), disease-specific survival (DSS) and overall survival (OS) comparing the subgroups with distinct PD-L1 expression. **Conclusion:** In this cohort, PD-L1 expression was not associated with DFS, DSS and OS.

Keywords: Immune checkpoint inhibitors; Uterine cervical neoplasms; Survival.

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RESUMO

Objetivos: Este estudo teve como objetivo investigar a expressão do ligante de morte celular programada 1 (PD-L1) em uma coorte de pacientes com câncer do colo do útero (CC) avaliando sua significância prognóstica. **Métodos:** Todos os pacientes diagnosticados no Instituto Nacional do Câncer (INCA), em 2011, com CC invasivo, carcinoma espinocelular (CEC) ou adenocarcinoma (CDA) foram incluídos retrospectivamente. Os dados clínicos e de tratamento foram coletados e a expressão de PD-L1 foi avaliada de acordo com a porcentagem de células tumorais viáveis mostrando coloração. A análise de sobrevida foi realizada pelo método de Kaplan Meier. **Resultados:** No total, foram incluídos 152 pacientes (105 CEC e 47 CDA) e a média de idade foi de 52,4 anos (±14,4). De acordo com a Federação Internacional de Ginecologia e Obstetrícia (FIGO) 2009, 84,2% apresentavam doença localmente avançada (IB2-IVA). A expressão de PD-L1 foi considerada positiva (=1%) em 53,3% dos casos. Após o ajuste, a análise multivariada confirmou que CEC (p=0,026) e tamanho do tumor >4cm (p=0,023) estavam independentemente associados à expressão de PD-L1. Não houve diferenças significativas na sobrevida livre de doença (SLD), sobrevida específica da doença (SED) e sobrevida global (SG) comparando os subgrupos com expressão distinta de PD-L1. **Conclusão:** Nesta coorte, a expressão de PD-L1 não foi associada a SLD, SED e SG.

Descritores: Inibidores do *checkpoint* imunológico; Neoplasias cervicais uterinas; Sobrevivência.

INTRODUCTION

Cervical cancer (CC) represents the fourth most commonly diagnosed cancer among females worldwide, with an estimated incidence of 570,000 new cases and about 311,000 deaths in 2018.[1] Low- and middle-income countries account for approximately 85% of the new CC cases and deaths.[1] According to the Brazilian National Cancer Institute (INCA), 16,590 new CC cases are estimated for 2021 in Brazil, being the third most commonly diagnosed cancer in women in the country.[2] Although preventive strategies are cardinal in CC, early diagnosis and effective treatment are primordial for morbidity and mortality reduction.

The immune system has a close and complex interaction with cancer cells and the tumor microenvironment. Although the defense mechanism works targeting and destroying non-native and nonfunctional cells, malignant cells can have a variety of scaping mechanisms.[3,4] These multifactorial and entangled interconnections opened space for the development of immunotherapies.[5]

Recently, translational research showed that there are in cervical cancer immune subgroups associated with immune modulatory alterations, genetic and epigenetic events, prognosis and treatment response. These findings might help predict ideal candidates to receive specific therapies, including immunotherapies.[6]

The better understanding of the immune system and its elements, the programmed cell death protein 1 (PD-1) and the programmed cell death-ligand 1 (PD-L1) has risen important targets for cancer therapy.[7] Anti-PD-1 and anti-PD-L1 immunotherapies have been successfully used in many cancer types.[8-12]

The presence of PD-L1 in the CC cells and in the microenvironment has been previously reported and opened the investigation of immune checkpoint inhibitors in CC.[13-15] Initial results were controversial but recently some promising recent clinical studies,[16-18] led to the approval by Food and Drug Administration (FDA) of pembrolizumab in recurrent or metastatic CC patients with disease progression on or after chemotherapy whose tumors express PD-L1.[18] Not to mention, positive results demonstrating overall survival (OS) benefit, regardless of PD-L1 status, from the phase 3 trial investigating the PD-1 inhibitor cemiplimab in monotherapy compared to chemotherapy in patients with recurrent or metastatic CC, previously treated with chemotherapy, were announced.[19]

These data indicate a potential role for anti-PD-1 and anti-PD-L1 agents in CC. In this context, translational data is warranted.

OBJECTIVES

The current study aimed to investigate the PD-L1 expression in a cohort of CC patients evaluating its prognostic significance.

METHODS

Study design and study population

This is a cohort with retrospective data and patients were identified through an internal database. All patients diagnosed at INCA from 2011 January to December with invasive CC and histologic subtypes of squamous cell carcinoma (SCC) or adenocarcinoma (ADC) and with available archived formalin-fixed paraffin-embedded (FFPE) blocks were included. Clinical and treatment data were collected from electronic hospital records and medical charts.

The study was approved by the Ethics in Human Research Committee of the Brazilian National Cancer Institute (CEP-INCA), Rio de Janeiro, Brazil, under the number CAAE 17361013.8.0000.5274, and conducted following the good clinical practice guidelines.

Pathological review and immunohistochemistry (IHC)

The cases were reviewed by an experienced pathologist who selected the punch area with at least 80% of malignant cells and at least 0.5cm^2 of tissue available for the tissue microarray (TMA). The TMA was built using standard procedures, extracting whenever possible, three punches from the primary cancer of each patient selected. In some cases, only one or two samples were extracted, due to the scarcity of material.

IHC was performed using the rabbit anti-human PD-L1 monoclonal antibody (clone 29E.2A3, BioLegend, dilution 1:400), adhering to the general guidelines recommended by the BioLegend protocol. Appropriate positive and negative controls were included. The PD-L1-stained TM slides were analyzed and imaged using a bright-field microscope (Olympus BX50; Olympus, Center Valley, PA, USA). A minimum of 100 viable tumor cells in the PD-L1-stained slide is required for the specimen to be considered adequate for PD-L1 evaluation.

The immunostaining scores were classified according to the percentage of viable tumor cells showing staining for PD-L1: negative, ≥1 to 49%, ≥50 to 100%.The sample with the highest staining was selected to represent the patient with multiple samples. Receiver-operating characteristic (ROC) curve analysis was used to define the optimal cutoff value of the PD-L1 expression that could be associated with survival time. Besides that, other cutoff values were used to better explore the data.

Statistical analysis

A descriptive study was carried out, using the mean and standard deviation for continuous variables and the distribution of absolute and relative frequency for categorical variables. The Kolmogorov-Smirnov test was used to verify standard normal distribution assumptions. The chi-square test was used to compare the two diagnostic groups (SCC and ADC) regarding the study variables.

The survival analysis was performed using the Kaplan Meier method, aiming to identify possible differences between the groups, according to PD-L1 expression. Disease-free survival (DFS) was defined as the time between the end of treatment and the confirmation of recurrence, metastasis or death. Only patients who had no event for at least 90 days after the end of treatment were included in the DFS analysis. For disease-specific survival (DSS), only deaths caused by CC were considered events. OS was defined as the interval between the date of diagnosis and the date of death. Patients who completed five years of follow-up without any event and those lost during the follow-up were censored. The diagnosis of local recurrence, metastasis and death were the outcomes considered. The log-rank test was used to analyze the difference in survival between the groups. Cox regression analysis was performed using the Enter method, to estimate the association between PD-L1 expression and outcomes. The effect of PD-L1 on survival was estimated by the hazard ratio (HR) and its 95% confidence interval (95%CI). A *p*-value of 0.05 or less was considered to indicate statistical significance. The software SPSS version 21.0 (IBM, Sao Paulo) was used for statistical analysis.

RESULTS

Baseline characteristics

In total, 152 patients (105 SSC and 47 ADC) of the entire cohort were included. The mean age was 52.4 years (± 14.4) and 55.3% of the patients were never smokers. According to The International Federation of Gynecology and Obstetrics (FIGO) 2009, 84.2% had locally advanced disease (IB2-IVA). Regarding the first treatment, 23% of the patients were submitted to surgery and 14.7% of them had lymph node metastases and 17.6% had lymphovascular space invasion; 75.8% of the entire group received pelvic radiotherapy with concomitant chemotherapy in 62.8% (Table 1).

All cases with a PD-L1 expression of ≥1% were considered positive, according to the cutoff defined by the ROC curve analysis (Figure 1 and Supplementary Table 1). PD-L1 expression was considered positive (≥1%) in 53.3% of the cases. PD-L1 was expressed between 1 to 49% and 50 to 100% of the tumor cells in 42.1% and 11.2% of the cases, respectively (Table 1).

Factors associated with PD-L1 expression ≥1%

According to the univariate analysis PD-L1 expression was significantly associated with the histology (*p*=0.005) and the tumor size (*p*=0.006) (Table 2). After adjustment, the multivariable analysis confirmed that SCC (*p*=0.026) and tumor size >4cm (*p*=0.023) were independently associated with PD-L1 expression (Table 3).

Patients with tumors showing PD-L1 expression (≥1%) did not show differences in OS compared to those without PD-L1 expression, with a 5-year survival rate of 49.4% (±5.6) for PD-L1 positive and 52.0% (±6.1) for those negative (adjusted HR 0.9, 95%CI 0.6-1.5, *p*=0.744) (Table 5). Similar trends were observed for DFS and DSS (Tables 4 and 5).

Even when stratifying as negative, positive 1 to 49% and ≥50% of tumor cells expressing PD-L1, there were no significant differences in 5-year OS rate - 52.0% (±6.1), 45.1% (±6.4) versus 64.7% (±11.6), respectively (*p*=0.371), DSS - 56.4% (±6.1), 49.8% (±6.5) versus 64.7 (11.6), respectively (*p*=0.560) and DFS - 57.3% (±7.8), 58.0% (±8.8) versus 75.0% (±12.9), respectively, *p*=0.425 (Supplementary Table 2).

Table 1. Epidemiological characteristics and morphological findings of cervical cancer cases.

Variables	Values	
Age at diagnosis (years), mean (±SD)	52.4	(±14.4)
Smoking, N (%)		
Yes (current or former)	68	(44.7)
Never	84	(55.3)
Histology, N (%)		
Squamous cell carcinoma	105	(69.1)
Adenocarcinoma	47	(30.9)
Clinical stage at diagnosis (FIGO, 2009), N (%)		
IA - IB1	24	(15.8)
IB2 - IVA	128	(84.2)
Tumor size (cm), N (%)		
≤4	57	(38.8)
>4	90	(61.2)
Surgery, N (%)		
Yes	35	(23.0)
No	117	(77.0)
Lymph node metastases, N (%)*		
Yes	5	(14.7)
No	29	(85.3)
Lymphovascular space invasion, N (%)*		
Yes	6	(17.6)
No	28	(82.4)
Chemotherapy, N (%)		
Yes	93	(62.8)
No	55	(37.2)
Pelvic radiotherapy, N (%)		
Yes	113	(75.8)
No	36	(24.2)
Brachytherapy, N (%)		
Yes	91	(61.5)
No		
Tumor differentiation, N (%)	57	(38.5)
Poorly differentiated	18	(12.2)
Moderately differentiated	93	(63.3)
Well differentiated	36	(24.5)
PD-L1 expression, N (%)		
Negative	71	(46.7)
≥1 to 49%	64	(42.1)
≥50 to 100%	17	(11.2)
Total		152

SD = Standard deviation; *In 34 patients who had surgery (whose data were available); Missing values: Tumor size = 5, Chemotherapy = 4, Pelvic radiotherapy = 3, Brachytherapy = 4, Degree of tumor differentiation = 5.

Table 2. PD-L1 expression according to selected factors.

Variables	Positive (≥1%)	Negative (<1%)	OR (95%CI)	*p*-value
Age at diagnosis (years), mean (±SD)	53.3 (±14.4)	51.4 (±14.3)	1.0 (1.0-1.0)	0.413
Smoking, N (%)				0.119
Yes (current or former)	41 (50.6)	27 (38.0)	1.7 (0.9-3.2)	
Never	40 (49.4)	44 (62.0)	Ref.	
Histology, N (%)				0.005
Squamous cell carcinoma	64 (79.0)	41 (57.7)	2.8 (1.4-5.6)	
Adenocarcinoma	17 (21.0)	30 (42.3)	Ref.	
Clinical stage at diagnosis (FIGO, 2009), N (%)				0.091
IB2 - IVA	72 (88.9)	56 (78.9)	2.1 (0.9-5.3)	
IA - IB1	9 (11.1)	15 (21.1)	Ref.	
Tumor size (cm), N (%)				0.006
>4	57 (71.2)	33 (49.3)	2.6 (1.3-5.0)	
≤4	23 (28.7)	34 (50.7)	Ref.	
Tumor differentiation, N (%)				0.818
Poorly differentiated	9 (11.1)	9 (13.6)	Ref.	
Moderately differentiated	53 (65.4)	40 (60.6)	1.3 (0.5-3.6)	
Well differentiated	19 (23.5)	17 (25.8)	1.1 (0.4-3.5)	
Total	81 (53.3)	71 (46.7)		152

SD = Standard deviation; Statistically significant p-values are highlighted in bold; OR = Odds ratio; 95%CI = 95% Confidence interval.

Table 3. Independent variables associated with PD-L1 expression ≥1%.

Variables	aOR (95%CI)	*p*-value
Histology		
Squamous cell carcinoma	2.3 (1.1-4.9)	0.026
Adenocarcinoma	Ref.	
Tumor size (cm)		
>4	2.3 (1.1-4.5)	0.023
≤4	Ref.	

aOR = Adjusted odds ratio; 95%CI = 95% Confidence interval.

Table 4. 5-year survival according to PD-L1 expression ≥1%.

PD-L1 expression	5-y disease-free survival (±SD)	5-y disease specific survival (±SD)	5-y overall survival (±SD)
Negative	57.3 (7.8)	56.4 (6.1)	52.0 (6.1)
Positive (≥1%)	62.4 (7.4)	53.1 (5.7)	49.4 (5.6)
Log-rank	0.436	0.812	0.763
Overall	60.0 (5.4)	54.7 (4.2)	50.7 (4.1)

SD = Standard deviation.

Table 5. Risk of progression/relapse or death according to the expression of PD-L1 ≥1%.

Survival	HR (95%CI)	*p*-value	aHR* (95%CI)	*p*-value
Disease-free survival				
Negative	Ref.		Ref.	
Positive (≥1%)	0.8 (0.4-1.5)	0.436	0.6 (0.3-1.2)	0.177
Disease specific survival				
Negative	Ref.		Ref.	
Positive (≥1%)	1.1 (0.7-1.7)	0.812	0.9 (0.6-1.5)	0.717
Overall survival				
Negative	Ref.		Ref.	
Positive (≥1%)	1.1 (0.7-1.7)	0.763	0.9 (0.6-1.5)	0.744

HR = Hazard ratio; aHR = Adjusted hazard ratio; *Adjusted for age (continuous) and clinical stage.

DISCUSSION

PD-L1 is the main ligand of the PD-1 in T-lymphocytes and their interaction suppresses the proliferative and effector responses of T-cells.[3,4] The expression of PD-L1 and its correlation with prognosis has been studied in many types of cancer and an attempt to correlate them in CC has also been carried out. Until now, however, there are not many published trials regarding this issue, and so, the prognostic value of PD-L1 in CC is still conflicting.

The current data is one of the largest series regarding the expression of PD-L1 and its association with survival. It was not observed relation between PD-L1 positivity (defined as ≥1%) and survival, even after elevating the cutoff of PD-L1 expression to 50%. In 2009, Karim et al.[20] analyzed 115 patients FIGO stage I-II treated with surgery (88 SCC and 26 ADC), showing PD-L1 expression in tumor cells in only 19% (23% in SCC and 8% in ADC), not correlating with survival. Enwere et al. (2017)[21] demonstrated in 120 patients (106 SCC and 14 ADC) with CC stage I-IVA, treated with chemoradiation, a PD-L1 expression (defined as ≥1%) of approximately 88% with no correlation with survival, even when a median PD-L1 expression per tumor area was used as a continuous variable. High PD-L1 expression was seen in 55% of SCC and 9% ADC.[21] In another paper, Feng et al. (2018)[22] showed PD-L1 expression (defined as >5%) in 32.4% of 219 SCC, FIGO stages I-IVA, treated with mixed approaches and no correlation with survival. Kawachi et al. (2018)[23] showed PD-L1 expression (defined as >5%) in 17.3% of 127 invasive ADC patients FIGO stage I-II treated with surgery. For survival and correlation analyses, patients were divided into two groups using the median PD-L1 expression as a cutoff, and no correlation was found (p=0.095). A recent meta-analysis, gathering data from 7 retrospective trials with 783 patients, evaluated the correlation of PD-L1 expression with progression-free survival (PFS) and OS. Although PFS did not achieve significance (HR 2.07, 95%CI 0.52-8.23, p=0.3), the authors found a significant worse OS for those considered PD-L1 positive compared to negative tumors as defined by each paper (combined HR 2.52, 95%IC 1.09-5.83, p=0.03).[24]

The current findings are in line with the literature that shows SCC consistently associated with a higher expression of PD-L1 in retrospective series compared to ADC.[14,21] The correlation of large tumors with PD-L1 expression is a new finding of our study. Although this finding is not well understood, same correlation can be seen in other tumor sites.[25]

The use of TMA in this cohort must be addressed. The main criticism of TMA technique is the limited amount of tissue analysed, which may not be representative of the whole specimen. Because of the intratumoral heterogeneity in malignant epithelial tumors this may pose a significant problem.[26] The limited number of ADC and tumors smaller than 4cm must be also considered when evaluating the current results and associations.

Although it was not the scope of this research, it is noteworthy that PD-L1 is also controversial as a predictive biomarker for immune checkpoint inhibitor response in CC. The United States Food and Drug Administration (FDA) approved pembrolizumab for CC tumors with combined positivity score (CPS) equal or more than 1%, but it is clear that checkpoint inhibitors have also demonstrated activity in negative CPS tumors.[27] Predictive markers of the immunotherapy response are still not well understood. Some biomarkers have shown a clear benefit for immunotherapy, such as unstable microsatellites and tumors with high tumor mutation. In CC, PD-L1 expression was correlated in the Keynote-158 study (pembrolizumab),[17,18] but it was not correlated in others such as Checkmate-358 (monotherapy with nivolumab or the combination of nivolumab/ipilimumab)[28] and balstilimab/zalifrelimab studies.[29] Recently, the Empower study demonstrated the benefit of second-line cemiplimab over physician-choice chemotherapy in a large population not selected for PD-L1 status.[19] Our research aimed to investigate the expression of PD-L1 in a cohort of patients with CC evaluating its prognostic significance. The search for predictive biomarkers beyond PD-L1 expression is an area of ongoing research.

CONCLUSION

The results indicated that in this large series, SCC and tumor size >4cm were independently associated with PD-L1 expression. Even when stratifying as negative, positive 1 to 49% and ≥50% of tumor cells expressing PD-L1, there were no significant differences in DFS, DSS and OS. Studies to identify specific populations that can benefit from immunotherapy in this unmet medical need are extremely important today.

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SUPPLEMENTARY MATERIAL

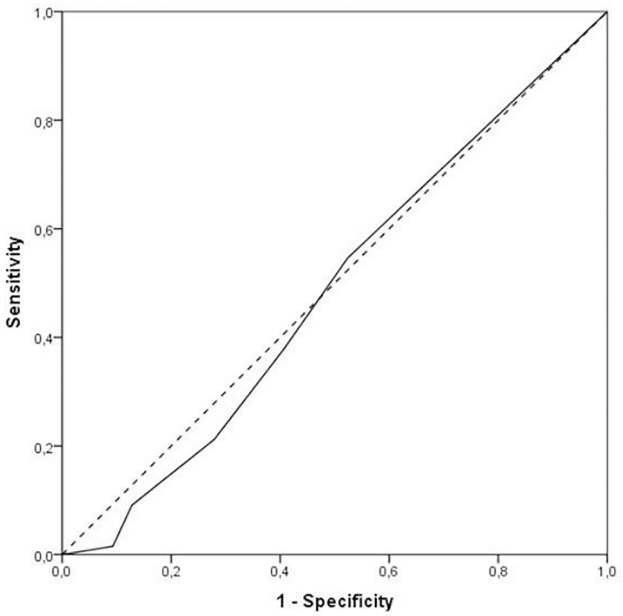
Supplementary Table 1. Discrimination performance as measured by the area under the curve (AUC) for the expression of PD-L1 ≥1%.

Performance measures	Value	Lower limit – Upper limit
Sensitivity	0.545	0.418 - 0.669
Specificity	0.477	0.368 - 0.587
Positive predictive value	0.444	0.338 - 0.574
Negative predictive value	0.577	0.450 - 0.681

Supplementary Table 2. Analysis of 5-year survival according to cutoff points for PD-L1 expression.

PD-L1 expression	5-y disease-free survival (±SD)	5-y disease specific survival (±SD)	5-y overall survival (±SD)
Negative	57.3 (7.8)	56.4 (6.1)	52.0 (6.1)
1-49%	58.0 (8.8)	49.8 (6.5)	45.1 (6.4)
50-100%	75.0 (12.9)	64.7 (11.6)	64.7 (11.6)
Log-rank	0.425	0.560	0.371
<50%	57.7 (5.8)	53.4 (4.4)	48.9 (4.4)
≥50%	75.0 (12.9)	64.7 (11.6)	64.7 (11.6)
Log-rank	0.203	0.372	0.240

SD = Standard deviation.



Supplementary figure 1. Area under the curve (AUC) for the expression of PD-L1=1%.