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PD-L1 expression in carcinoma of the esophagogastric junction is positively correlated with T-cell infiltration and overall survival

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ABSTRACT

Introduction: In recent years antitumor immunity and inhibition of checkpoint molecules, such as PD-1 and PD-L1, have emerged as potential therapeutic strategies in advanced stages of various malignancies. We investigated PD-L1 expression in adenocarcinomas of the esophagogastric junction and correlated the results with densitiy of intratumoral T-lymphocytes.

Methods: Immunohistochemical staining for PD-L1 was carried out on 135 samples using a tissue microarray. Scoring was done according to the combined positivity score.

Results: 48.1% of tumors (65 cases) showed PD-L1 positivity with a score ≥ 1 while 51.9% were PD-L1 negative (70 cases). A positive correlation between PD-L1 negativity and mucinous and poorly cohesive carcinoma could be shown ($p = 0.043$), while no association existed for either gender, T-stage, N-stage, grading, surgical resection status, neoadjuvant therapy, distant metastases, lymphovascular or perineural invasion. No correlation of PD-L1 expression and overall survival could be detected ($p = 0.497$). Again, when stratified according to presence or absence of neoadjuvant therapy, no survival differences could be shown for either group ($p = 0.540$ and $p = 0.736$). When PD-L1 expression was correlated with density of tumor-infiltrating T-lymphocytes a positive correlation between PD-L1 positivity and denser T-cell infiltration could be shown ($p = 0.001$). Concerning overall survival, in PD-L1 negative cases, denser CD8-positive T-cell infiltrates were associated with prolonged survival times ($p = 0.045$). No differences could be shown for PD-L1 positive cases or CD103-positive T-cells.

Conclusion: PD-L1 expression is frequent in esophagogastric adenocarcinoma and - when combined with dense CD8 infiltration – PD-L1 negativity correlates with prolonged overall survival.

1. Introduction

Recently, so called checkpoint inhibitors have emerged as new targets in cancer immunotherapy, especially in advanced and metastatic disease as well as a therapeutic option for patients not eligible for first-line multidrug chemotherapy [29,37]. One of the most widely known molecules is the programmed death ligand-1 (PD-L1) which is crucial for T-cell regulation [3]. Its activation in malignant tumors reduces the host's immune response, especially the activity of cytotoxic T-cells, lowers cytokine production and enables tumor cells to escape immune surveillance, leading to tumor progression and metastases [29,30]. Inhibition of PD-L1 via different immune checkpoint inhibitors (*i.e.* pembrolizumab or atezolizumab) induces restoration of the antitumor response and has been shown to prolong progression-free and overall survival in different types of epithelial malignancies [11,31,34] although other studies reported shortened survival in patients with higher expression of PD-L1 [1,6,19] or could show no association at all [47].

Concerning adenocarcinomas of the esophagogastric junction, only few studies addressing PD-L1 expression and its clinical and prognostic relevance are available in the literature [17,23], while most studies focus on gastric carcinomas or squamous cell carcinomas of the esophagus. We therefore aimed to assess PD-L1 expression in a larger cohort of esophagogastric adenocarcinomas and correlate expression with various clinicopathological characteristics as well as with overall and disease free survival.

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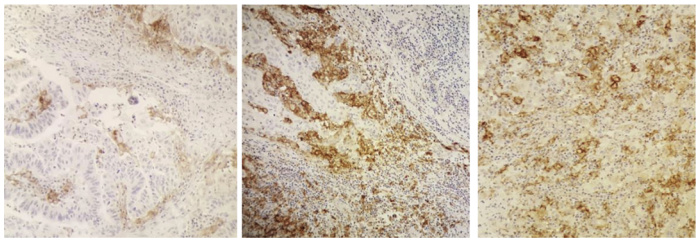

Fig. 1. Immunohistochemical staining for PD-L1 showing positive staining in immune cells (left) with predominantly cytoplasmic positivity and tumor cells (right) with membranous staining. Middle: Case with positive staining in both tumor and immune cells. Magnification 200×.

2. Materials and methods

2.1. Selection of cases and tissue samples

135 samples from formalin-fixed, paraffin-embedded (FFPE) tissue containing adenocarcinomas of the esophagogastric junction (GEJ) were included in the present study. All cases were collected as part of routine clinical care at the University Hospital of Schleswig-Holstein, Campus Luebeck during 1997-2013. Resection specimen were used for analysis when available, in cases with complete tumor regression after neoadjuvant therapy pretherapeutic biopsies were used instead. All analyses performed were in accordance with the Declaration of Helsinki and had been approved by the local Ethics Committee beforehand (reference number 14–242 A).

2.2. Tissue microarray construction

Tissue microarrays were constructed using the manual QuickRay® Kit (Unitma, Seoul, Korea) in accordance with the manufacturer's instructions, including appropriate positive and negative control samples (tonsil, kidney parenchyma). One core per tumor (measuring 2 mm) was incorporated in the microarray. All cases were examined beforehand and areas with a high count of vital tumor cells and adjacent tumor-associated stroma were selected.

2.3. Immunohistochemistry and evaluation of staining

Immunohistochemical analysis was performed according to a standard three-step immunoperoxidase technique using the anti-PD-L1 antibody clone QR1 (quartet immunodiagnostics, Berlin, Germany), dilution 1:100.

Immunohistochemical staining was evaluated for both tumor cells and tumor-infiltrating immune cells as described previously [20,35]. Concerning tumor cells only membranous staining (complete or incomplete) was considered to be positive, regardless of staining intensity. For immune cells (lymphocytes, macrophages and dendritic cells) both cytoplasmic and membranous staining was considered positive. For all cases the combined positivity score (CPS) was calculated and a Cut-off ≥1 was used to determine positivity [35]. Density of intratumoral T-lymphocytes was determined with antibodies against CD8 and CD103 – results were taken from one of our previous studies using a three-tier system for assessing the density: no infiltrates, scattered infiltrates or dense infiltrates. Cases were evaluated by two pathologists (blinded to all clinical information), in cases were evaluation differed a consensus was reached by reviewing these cases together.

2.4. Data analysis and statistics

All statistical analysis was conducted using SPSS Version 22 (IBM, Ehringen, Germany). For correlation of PD-L1 expression and tumor-infiltrating lymphocytes as well as clinicopathological characteristics χ^2-test was used. Survival differences (overall and disease-free survival) were visualized with Kaplan-Meier estimates including Log rank test. Additionally, Cox regression analysis was performed to test for independence. Hazard ratios as well as 95%-Confidence Intervals (95%-CI) were calculated. (A p-value < 0.05 was considered statistically significant for all tests.

3. Results

3.1. PD-L1 staining and CPS scores

Overall, 65 cases (48.1%) showed PD-L1 staining in tumor and/or immune cells (CPS score ≥1), while 70 cases (51.9%) were completely negative. Staining was primarily detected in immune cells in 49 cases (36.3%) with only a minority (16 cases) showing PD-L1 staining additionally in tumor cells (11.85%).

Concerning the CPS score in positive cases, scores varied from 1 to 80 with a mean value of 3.9 (standard deviation 11.2). Representative examples of PD-L1 staining in both tumor and immune cells are shown in Fig. 1.

3.2. Correlation with clinical characteristics

Correlation with a variety of clinicopathological features showed that PD-L1 positivity was associated with histologic tumor type in our study. PD-L1 positive cases showed a significantly higher proportion of tubular adenocarcinomas while PD-L1 negative cases were more often of mucinous or poorly cohesive type ($p = 0.043$). Characteristic features of the study cohort are briefly summarized in Table 1. In addition, there was a trend towards a lower rate of lymphovascular invasion in PD-L1 positive tumors, although no significance could be shown ($p = 0.089$). No association with other factors, such as gender, tumor stage, grading, perineural invasion, completeness of resection or the presence of metastases could be shown (p-values shown in Table 1).

3.3. Correlation with overall survival as well as 1-year, 3-year and 5-year survival

Correlation of PD-L1 expression and overall survival revealed no significant differences in survival times for patients with CPS-scores ≥1 or < 1 ($p = 0.497$). Mean survival times for PD-L1 positive cases were 60.54 months (standard deviation 8.46; 95% confidence interval 43.95–77.12 months), for PD-L1 negative cases 67.97 months (standard deviation 10.93; 95% confidence interval 46.55–89.4 months). 1-year, 3-year and 5-year survival could be determined at 63%, 46% and 22% for PD-L1 positive cases and at 48%, 34% and 26% for PD-L1 negative cases. Appropriate survival curves are shown in Fig.2.

3.4. Correlation with neoadjuvant therapy

Overall, 52 patients (38.5%) received neoadjuvant therapy prior to tumor resection (consisting primarily of a combination chemotherapy including cisplatin and 5-fluoruracil). No significant difference could be detected between PD-L1 expression and the administration of neoadjuvant therapy ($p = 0161$; Table 1). Additionally, survival times did not

Table 1
Clinicopathologic characteristics of the study population (135 patients) including important demographic information. Absolute numbers are given. Bold lettering in p-values indicates a statistically significant difference. LVSI: lymphovascular space invasion.

Characteristic	PD-L1 negative	PD-L1 positive	p-value
Sex			
Male	57	59	*0.119*
Female	13	6	
pT			
(low)			
pT0	0	3	*0.428*
pT1a			
pT1b			
pT2			
(high)			
pT3			
pT4a			
pT4b	2	0	
pN			
pN0	23	31	*0.239*
pN1	13	13	
pN2	16	11	
pN3	18	10	
Grading			
G1	1	0	*0.113*
G2	12	18	
G3	34	20	
Not applicable	23	27	
Surgical resection			
Complete	60	61	*0.122*
Incomplete	10	4	
LVSI			
Present	42	29	*0.089*
Absent	28	35	
Perineural invasion			
Present	19	29	*0.167*
Absent	51	35	
Distant metastases			
Present	12	12	*0.841*
Absent	58	53	
WHO classification			
Tubular	44	49	***0.043***
Poorly cohesive	11	5	
Mucinous	7	0	
Papillary	1	1	
Undifferentiated /Other	7	10	
Neoadjuvant therapy			
Yes	23	29	*0.161*
No	47	36	

differ significantly between PD-L1 positive and negative cases when viewing patients with or without neoadjuvant therapy separately ($p = 0.540$ for cases without neoadjuvant therapy and $p = 0.736$ for patients with neoadjuvant therapy). Mean survival times and appropriate survival curves are shown in Table 2 and Fig. 3.

3.5. Correlation between PD-L1 expression and density of CD8- and CD103-positive tumor-infiltrating lymphocytes

Including results from one of our previous studies concerning density of tumor-infiltrating T-lymphocytes, we correlated PD-L1 expression with CD8- and CD103-density. A positive correlation between both densitiy of CD8- and CD103-positive lymphocytes and PD-L1 positivity could be shown ($p = 0.001$). Concerning overall survival, the combination of both parameters (PD-L1 expression and density of T-cell infiltration) could show no significant differences in patients' survival in PD-L1 positive cases (p-values 0.542 and 0.924 for CD8- and CD103-positive lymphocytes, respectively). For PD-L1 negative cases, a difference in overall survival could be detected in relation to densitiy of CD8-positive lymphocytes ($p = 0.045$). Mean survival for patients with dense infiltrates was almost four times longer than for patients without T-lymphocytes (mean survival 97.21 months vs. 28 months; standard deviations 17.96 vs. 11.92; 95% confidence intervals 62.52–132.93 vs. 4.63–51.37). No such difference could be shown for CD103 ($p = 0.289$). Appropriate survival curves are depicted in Fig. 4.

3.6. Cox regression analysis

Cox regression analysis was performed for density of tumor-infiltrating lymphocytes as well as for PD-L1 expression (positive vs. negative) taking into account the following clinicopathologic features: sex, pT stage, nodal stage, grading, completeness of surgical resection and distant metastases. For CD8-positive lymphocytes, we found a Hazard ratio of 0.664 (95%-CI 0.4463-0.951) with a p-value of 0.026. For CD103-positive lymphocytes no independence could be shown (Hazard ratio 0.780; 95%-CI 0.541–1.125; $p = 0.184$). Concerning PD-L1 we calculated a Hazard ratio of 0.934 (95%-CI 0.584–1.496) with a p-value of 0.778.

4. Discussion

Studies concerning PD-L1 expression in adenocarcinomas of the esophagogastric junction are still scarce and more often than not, studies analyse both esophagogastric and gastric carcinomas together. Positivity rates vary but approximately half of the carcinomas are reported to be PD-L1 positive when applying the commonly used CPS score [7,17,27]. This is in line with our results, where 48.1% of cases were PD-L1 positive, applying a Cut-off ≥1. Additionally, most gastric and GEJ carcinomas are reported to show PD-L1 expression predominantly in tumor-associated immune cells and stroma (44%) with only a minority also showing PD-L1 expression on tumor cells (12%) [5,40]. This is in line with our results where 36.3% of cases showed PD-L1 staining primarily in immune cells with only a minority (11.85%) showing PD-L1 staining additionally in tumor cells.

Studies concerning the relationship between PD-L1 expression and clinicopathologic characteristics found that in gastric carcinomas, higher PD-L1 expression was associated with advanced tumor stage, deeper infiltration and tumor size as well as lymph node and distant metastases [9,14,24,32,33,39,41,49]. No such results could be shown in our study, on the contrary, the only difference detected was a slight trend ($p = 0.089$) towards a higher rate of lymphovascular space invasion in PD-L1 negative cases. Additionally, we found that PD-L1 negativity was associated with mucinous and poorly cohesive histologic type ($p = 0.043$). Whether this might hint at a more aggressive clinical course has been extensively debated but no satisfactory consensus has been reached [18,26,38]. Nevertheless, as these groups contain small case numbers (n = 7 for mucinous carcinomas; n = 16 for poorly cohesive carcinomas) results should be interpreted with caution and may not hold true for larger cohorts.

Throughout the literature, studies addressing the prognostic value of PD-L1 expression in gastric carcinomas and adenocarcinomas of the esophagogastric junction show conflicting results. While some studies report no impact on patients' survival at all [2,10,14,41,48], others report prolonged or shortened overall and tumor-free survival with most studies showing worst survival times in cases with high PD-L1 expression and only a few reporting improved survival in PD-L1-positive tumors [5,12,15,21,22,27,36,42,44]. In our study, we could demonstrate no correlation between PD-L1 expression and overall survival (p = 0.497) although survival times in PD-L1 negative cases were marginally longer than in PD-L1 positive cases (67.97 months vs. 60.54 months). These results are in line with previously published data were either no impact on survival or longer survival in PD-L1 negative cases was reported [2,10,14,41,48].

We also calculated survival differences in relation to PD-L1

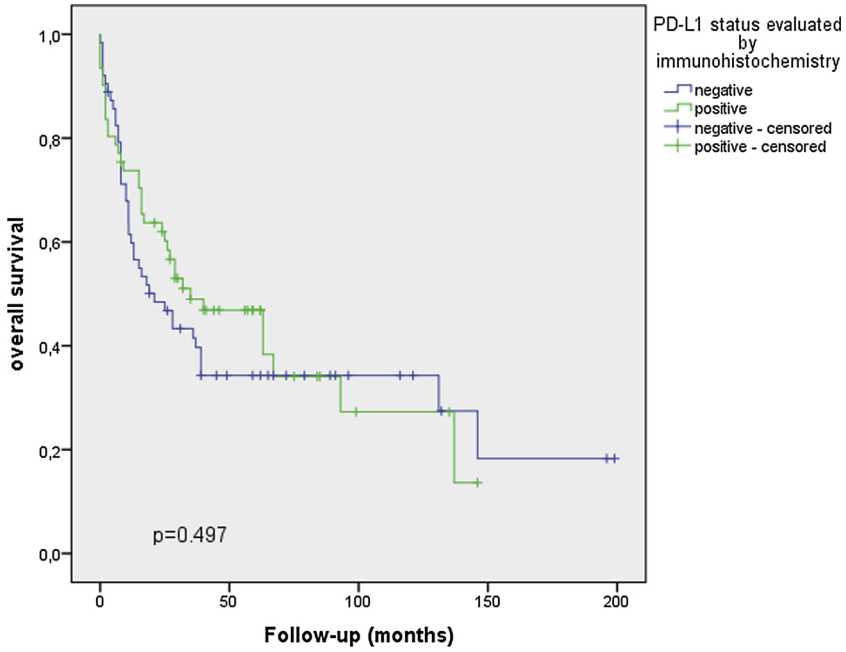

Fig. 2. Kaplan-Meier curve showing patients' overall survival in relation to PD-L1 positivity or negativity with a *p*-value of 0.497.

Table 2
Relationship between overall survival and PD-L1 expression stratified by neoadjuvant therapy. Survival times are given in months with standard deviation (SD), 95% Confidence Interval.

	average survival	SD	95% CI	p-value
No neoadjuvant treatment				
PD-L1 positive	59.58	10,26	39.46-79.7	*0.736*
PD-L1 negative	68.06	12,2	44.14-91.97	
Neoadjuvant treatment				
PD-L1 positive	46.91	9,6	28.09-65.74	*0.540*
PD-L1 negative	40.06	9,62	21.21-58.91	

expression separately for patients with or without neoadjuvant therapy. Again, no significant differences could be detected in either group ($p = 0.736$ and $p = 0.540$; Fig. 3). This is in line with results from a previous study where no significant survival differences could be detected concerning PD-L1 expression in cases with or without neoadjuvant therapy [10].

Recently, some studies reported an association of PD-L1 expression with density of tumor-infiltrating T-lymphocytes, especially CD8-positive lymphocytes, and showed either prolonged or shortened survival times with the majority of studies reporting longest overall and disease-free survival in patients with high CD8 count and low PD-L1 expression [4,8,13,16,25,28,33,40,43,46]. Following these observations, we correlated PD-L1 expression with density of CD8-positive tumor-infiltrating lymphocytes, using results from one of our previous studies and found that PD-L1 positivity was correlated with increased densitiy of tumor-infiltrating T-lymphocytes ($p = 0.001$). This is in line with results from other studies where PD-L1 expression was also positively correlated with T-cell infiltration [40,45]. Even the often reported effect on patients' survival could be reproduced in our current study, showing that denser CD8-infiltrates and PD-L1 negativity increased survival times significantly ($p = 0.045$) [4,40,45]. We additionally performed Cox regression analysis and could verify CD8-positivity as an independent prognostic factor with a Hazard ratio of 0.664 (95%-CI 0.4463-0.951) and a *p*-value of 0.026, irrespective of patients' sex, tumor stage, grading or completeness of resection.

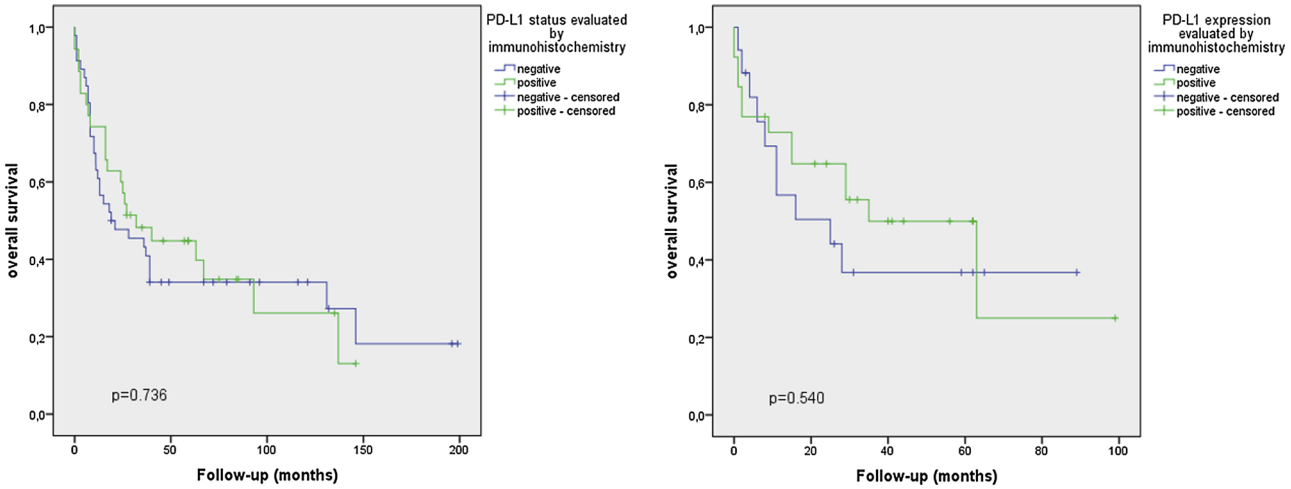

Fig. 3. Kaplan-Meier curves showing overall survival for patients with or without neoadjuvant therapy in relation to PD-L1 positivity or negativity. Left: Patients without neoadjuvant therapy ($p = 0.736$). Right: Patients with neoadjuvant therapy ($p = 0.540$).

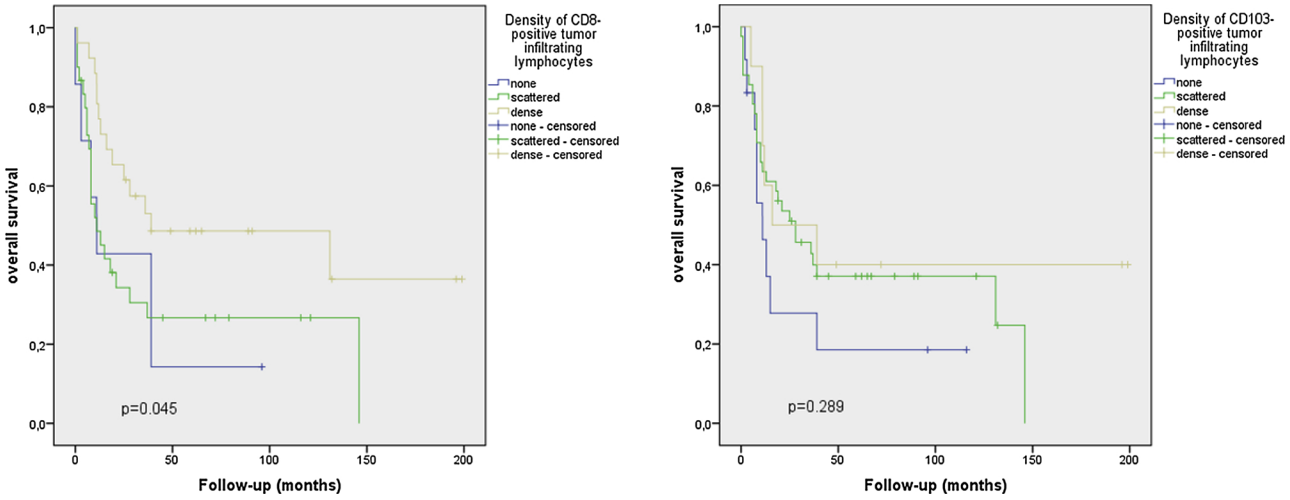

Fig. 4. Kaplan-Meier curves showing overall survival for patients with PD-L1 negativity in relation to density of tumor-infiltrating T-lymphocytes. Left: CD8-positive lymphocytes ($p = 0.045$). Right: CD103-positive lymphocytes ($p = 0.289$).

In conclusion, our study adds to the understanding of the importance of PD-L1 expression in adenocarcinomas of the esophagogastric junction as we could demonstrate and verify that PD-L1 expression is frequent and might – especially in conjunction with tumor-infiltrating T-cells – provide an easy and reliable prognostic tool to identify subgroups with significant survival differences. This could help to further select patients who might benefit from new targeted therapies or more aggressive therapy options.

Declaration of interest

None.

Author contributions

JK and CT performed morphological and immunohistochemical studies, analyzed the data and wrote the manuscript. PLK, UW and RH contributed cases and clinical data and revised the manuscript. All authors read and approved the final version of the manuscript.

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