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ABOUT COVER

Peer Review of *World Journal of Gastrointestinal Oncology*, Noha Elkady, MD, Assistant Professor, Department of Pathology, Faculty of Medicine Menoufia University, Shibin Elkom 32511, Egypt. drnohaelkady@gmail.com

AIMS AND SCOPE

The primary aim of *World Journal of Gastrointestinal Oncology* (*WJGO*, *World J Gastrointest Oncol*) is to provide scholars and readers from various fields of gastrointestinal oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including liver cell adenoma, gastric neoplasms, appendiceal neoplasms, biliary tract neoplasms, hepatocellular carcinoma, pancreatic carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc*.

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ORIGINAL ARTICLE

Retrospective Study

Assessment of programmed death-ligand 1 expression in primary tumors and paired lymph node metastases of gastric adenocarcinoma

Brendha Cação Coimbra, Marina Alessandra Pereira, Leonardo Cardili, Venancio Avancini Ferreira Alves, Evandro Sobroza de Mello, Ulysses Ribeiro Jr, Marcus Fernando Kodama Pertille Ramos

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Brendha Cação Coimbra, Marina Alessandra Pereira, Ulysses Ribeiro Jr, Marcus Fernando Kodama Pertille Ramos, Department of Gastroenterology, Instituto do Cancer, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Faculdade de Medicina, Universidade de São Paulo, São Paulo 01246000, Brazil

Leonardo Cardili, Venancio Avancini Ferreira Alves, Evandro Sobroza de Mello, Department of Pathology, Instituto do Cancer, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo 01246000, Brazil

Corresponding author: Marina Alessandra Pereira, MSc, PhD, Research Scientist, Department of Gastroenterology, Instituto do Cancer, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Faculdade de Medicina, Universidade de São Paulo, Av Dr Arnaldo, 251, São Paulo 01246000, Brazil. marina.pereira@hc.fm.usp.br

Abstract

BACKGROUND

Anti-programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) immunotherapy has demonstrated promising results on gastric cancer (GC). However, PD-L1 can express differently between metastatic sites and primary tumors (PT).

AIM

To compare PD-L1 status in PT and matched lymph node metastases (LNM) of GC patients and to determine the correlation between the PD-L1 status and clinicopathological characteristics.

METHODS

We retrospectively reviewed 284 GC patients who underwent D2-gastrectomy. PD-L1 was evaluated by immunohistochemistry (clone SP142) using the combined positive score. All PD-L1+ PT staged as pN+ were also tested for PD-L1 expression in their LNM. PD-L1(-) GC with pN+ served as the comparison group.

RESULTS

Among 284 GC patients included, 45 had PD-L1+ PT and 24 of them had pN+. For comparison, 44 PD-L1(-) cases with pN+ were included (sample loss of 4 cases). Of the PD-L1+ PT, 54.2% (13/24 cases) were also PD-L1+ in the LNM. Regarding

PD-L1(-) PT, 9.1% (4/44) had PD-L1+ in the LNM. The agreement between PT and LNM had a kappa value of 0.483. Larger tumor size and moderate/severe peritumoral inflammatory response were associated with PD-L1 positivity in both sites. There was no statistical difference in overall survival for PT and LNM according to the PD-L1 status (P = 0.166 and P = 0.837, respectively).

CONCLUSION

Intra-patient heterogeneity in PD-L1 expression was observed between the PT and matched LNM. This disagreement in PD-L1 status may emphasize the importance of considering different tumor sites for analyses to select patients for immunotherapy.

Key Words: Gastric cancer; Lymph node; Programmed death ligand 1; Stomach neoplasms; Immunohistochemistry; Metastasis

Core Tip: This is a retrospective study comparing programmed death-ligand 1 (PD-L1) expression in primary tumors (PTs) and matched lymph node metastases (LNM) of gastric cancer patients who underwent D2-gastrectomy. Among 284 patients, 24 were PD-L1 positivity in PT and had LNM. Among patients with PD-L1 positive in PT, 54.2% were also positive for PD-L1 in LNM. Considering the PD-L1 negative patients in PT, 9.1% of had PD-L1 positive in LNM. Accordingly, the intra-patient heterogeneity in PD-L1 expression between the PT and matched LNM found in our study may emphasize the importance of considering the site of tumor sample examined when selecting patients for immunotherapy.

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INTRODUCTION

Gastric cancer (GC) is a globally distributed disease, ranking worldwide as the fifth most common cause of cancer and fourth in mortality[1]. Surgery remains the main treatment modality, and the addition of perioperative or adjuvant chemotherapy improves the survival of advanced resected cases[2]. Different conventional chemotherapy combination schemes have been used according to the patient's tolerance and availability of drugs[3].

Recently, immunotherapy with checkpoint inhibition through the blockade of the programmed death protein 1 (PD-1)/programmed death ligand 1 (PD-L1) has emerged as a promising modality in several tumors, including GC[4,5]. The PD-1/PD-L1 pathway is a key in the negative regulation of cell-mediated immune responses. Anti-PD-1/PD-L1 antibodies prevent inhibitory effects of the PD-1/PD-L1 pathway and enhance T cell function, demonstrating robust antitumor responses[5-7].

PD-L1 expression, assessed with immunohistochemistry, has been used as marker to predict the therapeutic effect of anti-PD-1 or anti-PD-L1 immunotherapy in GC, as well as patients' prognosis[8-10]. However, GC is a highly heterogeneous disease from the morphological and molecular standpoints[11], with both intra-tumoral and intra-patient [between the primary tumor (PT) and its metastatic sites] variability[11-14]. Recent clinical studies reported that although patients with PD-L1+ tumors demonstrate higher rates of response to anti-PD-1/PD-L1 immunotherapy, those with PD-L1(-) tumors may also benefit from anti-PD-1/PD-L1 immunotherapy[4,7,15-17].

Indeed, the evaluation of PD-L1 is mostly based on PTs. However, immune checkpoints may express differently between primary and metastatic tumors, and this difference may have an impact on the selection of patients for therapy [13,18,19]. Thus, this study aimed to compare PD-L1 expression in PTs and its respective lymph node metastases (LNM) in patients with GC and to determine the association between PD-L1 status and clinicopathologic characteristics.

MATERIALS AND METHODS

Patients

We performed a retrospective review of all patients with GC who underwent gastrectomy with curative intent at a reference Cancer Center between 2009 and 2016, from a prospective collected medical database. Inclusion criteria were: (1) Gastric adenocarcinoma; (2) D2 lymphadenectomy; (3) R0 resection; and (4) Formalin-fixed paraffin-embedded tissue blocks available for analysis. Patients with remnant GC, palliative resections, and systemic metastatic disease were excluded.

Clinical data included sex, age, body max index (BMI) (kg/cm²), hemoglobin (g/dL), neutrophil-lymphocyte ratio (NLR), American Society of Anesthesiologists Classification, and Charlson-Deyo Comorbidity Index (CCI) excluding cancer as a comorbidity.

All patients underwent total or subtotal gastrectomy with D2 lymph node dissection based on the guidelines of the Japanese Gastric Cancer Association and Brazilian Gastric Cancer Association guidelines[2,20]. The tumor stage was defined according to the 8th edition of the TNM, as proposed by the International Union Against Cancer[21]. Surgical specimens were evaluated by histopathological criteria according to the College of American Pathologists protocol[22]. Postoperative follow-up appointments were performed once every three months in the first year and every 6 months in the following years. Physical examination and laboratory tests were performed on patient reassessments. Imaging and upper gastrointestinal endoscopy were selectively performed. Loss of follow-up was characterized as consecutively missed medical appointments for more than 12 months.

Sample selection and pathological analysis

First, PD-L1 expression was assessed in all PTs. After that, we selected all PD-L1+ patients in the PT site (PD-L1+ PT) who had LNMs (pN+). Patients with PD-L1(-) negative in the PT [PD-L1(-) PT] and pN+ were selected as the comparison group in the proportion of 1:2. The LNM from both groups were also evaluated for PD-L1 status by immunohistochemical (IHC) staining. Inclusion of PD-L1(-) PT cases was performed chronologically and sequentially.

Hematoxylin and eosin-stained slides were reviewed by a pathologist to select representative tumor areas from PT and LNM. The most representative LNM from each patient was selected for analysis, and the entire section was subjected to IHC staining. The lymph nodes evaluated were all regional lymph nodes, located in the greater or lesser gastric curvature.

The PT was evaluated through the tissue microarray construction (TMA) construction, using a precision mechanized system (Beecher Instruments, Silver Springs, MD, United States), with three tumor tissue cores from each patient. The TMA blocks were cut into 4 μm sections and submitted to IHC staining.

For IHC analysis of PD-L1 expression, sections were dewaxed, rehydrated, and submitted to heat-induced antigen retrieval using a citrate buffer. Endogenous peroxidase was blocked, and slides were incubated with primary antibody anti-PD-L1 (clone SP142) overnight at 4 ˚C. Avidin-biotin-free short polymer-based peroxidase (Novolink Polymer Detection System, Novocastra, Newcastle, United Kingdom) was used for amplification. Reaction products were visualized with diaminobenzidine, and sections were counterstained with Harris's Hematoxylin. Microscopic analysis was carried out by conventional light microscopy.

PD-L1 expression was evaluated in PT and LNM using the combined positive score (CPS)[23], which is defined based on the number of tumor cells and immune cells staining for PD-L1, divided by the total number of viable tumor cells, multiplied by 100. CPS ≥ 1 was defined as PD-L1+.

Statistical analysis

The χ^2 test or Fisher test (for nominal variables) and *t*-test or Mann-Whitney *U* (for continuous variables) was performed to assess the association of clinicopathological parameters with PD-L1 status. The analysis of the concordance between PD-L1 status in PT and LNM was performed using the Kappa coefficient. The Kappa coefficient was interpreted according to the Landis and Koch criteria, classified as poor (< 1%), slight (1%-20%), fair (21%-40%), moderate (41%-60%), substantial (61%-80%), almost perfect (81%-100%). Disease-free survival (DFS) was calculated from the date of surgery to the date of relapse or death. Overall survival (OS) was defined as the time between surgery and death of any cause. DFS and OS were estimated using the Kaplan-Meier method, and differences in survival were compared using the Log-Rank test. Statistical analyses were performed using the Statistical software package SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL). Statistical significance was defined as $P < 0.05$.

RESULTS

During the selected period, 284 patients were eligible for the study. Among them, 45 (15.8%) patients were positive for PD-L1 in the PT. Of these, 24 had lymph node metastasis (pN+) and were included for evaluation (PD-L1+ PT group). Among the remaining 239 patients with PD-L1(-) in the PT, 48 with pN+ were selected for comparison. Sample loss occurred in 4 cases, and the final control group was composed of 44 patients with PD-L1(-) PT. Microscopic findings of PT and LNM are showed in Figure 1.

In the PD-L1+ PT group, 54.2% (13/24 cases) were also PD-L1+ in the LNM. Regarding patients with PD-L1(-) PT, 9.1% (4/44) had PD-L1+ LNM (Table 1). The agreement between PT and LNM had a kappa value of 0.483. As for PD-L1 expression in LNM, most cases showed weak to moderate expression intensity, with a predominance of expression in both immune and tumor cells (15 cases). Only two patients exhibited PD-L1+ exclusively in immune cells in the LNM.

Clinical characteristics of GC according to PD-L1 expression in PT and LNM

Comparing groups according to PD-L1 status, there were no differences regarding sex, age, BMI, NLR, and CCI between both groups from PT and LNM. Seric hemoglobin levels were lower in patients with PD-L1+ PT. The clinical and surgical characteristics of the PD-L1(-) and PD-L1+ groups for PT and LNM are summarized in Table 2.

Table 1 Programmed death-ligand 1 expression in primary tumor site and metastatic lymph node

Primary tumor	Total	PD-L1 negative	PD-L1 positive	*P* value
	n = 68 (%)	*n* = 44 (%)	*n* = 24 (%)	
Lymph node				< 0.001
PD-L1 negative	51 (75)	40 (90.9)	11 (45.8)	
PD-L1 positive	17 (25)	4 (9.1)	13 (54.2)	
Total (%)	100.0	64.7	35.3	

PD-L1: Programmed death-ligand 1.

Table 2 Clinical characteristics according to the positivity for programmed death-ligand 1 in the primary tumor and lymph node metastasis

Variables	Primary tumor		*P* value	Lymph node		*P* value
	PD-L1(-), *n* = 44 (%)	PD-L1+, *n* = 24 (%)		PD-L1(-), *n* = 51 (%)	PD-L1+, *n* = 17 (%)	
Sex			0.417			0.558
Female	14 (31.8)	10 (41.7)		19 (37.3)	5 (29.4)	
Male	30 (68.2)	14 (59.3)		32 (62.7)	12 (70.6)	
Age (yr)			0.721			0.127
mean (SD)	60.7 (11.8)	61.7 (12.3)		59.8 (12.4)	64.9 (9.7)	
BMI (kg/cm²)			0.717			0.271
mean (SD)	24.3 (5.6)	23.9 (3.9)		24.6 (5.2)	23.0 (4.5)	
Hemoglobin (g/dL)			0.008[a]			0.148
mean (SD)	12.4 (2.1)	10.8 (2.5)		12.1 (2.2)	11.1 (2.7)	
Neutrophil-lymphocyte ratio			0.703			0.879
mean (SD)	3.30 (4.43)	3.72 (4.21)		3.39 (4.69)	3.31 (3.08)	
CCI			0.974			0.539
CCI 0	31 (70.5)	17 (70.8)		35 (68.6)	13 (76.5)	
CCI ≥ 1	13 (29.5)	7 (29.2)		16 (31.4)	4 (23.5)	
Tumor site			0.635			0.317
Lower	25 (56.8)	15 (62.5)		32 (62.7)	8 (47.1)	
Middle	12 (27.3)	6 (25)		11 (21.6)	7 (41.2)	
Upper	4 (9.1)	3 (12.5)		5 (9.8)	2 (11.8)	
All	3 (6.8)	0 (0)		3 (5.9)	0 (0)	
Type of resection			0.323			0.122
Subtotal	22 (50)	9 (37.5)		26 (51)	5 (29.4)	
Total	22 (50)	15 (62.5)		25 (49)	12 (70.6)	

[a]$P < 0.05$.

PD-L1: Programmed death-ligand 1; BMI: Body mass index; CCI: Charlson-Deyo Comorbidity Index.

Pathological characteristics of GC according to PD-L1 expression in PT and LNM

PD-L1+ groups in both PT and LNM were significantly associated with larger lesions and moderate/severe peritumoral inflammatory response (Table 3). PD-L1+ PT was associated with venous invasion ($P = 0.017$) and PD-L1+ LNM with a higher number of retrieved lymph node ($P = 0.035$). There were no differences between PD-L1+ and PD-L1(-) groups regarding the grade of histological differentiation, pT, and pTNM. Pathological characteristics are summarized in Table 3.

Table 3 Pathological characteristics according to the positivity for programmed death-ligand 1 in the primary tumor and lymph node metastasis

Variables	Primary tumor		*P* value	Lymph node		*P* value
	PD-L1(-), *n* = 44 (%)	PD-L1+, *n* = 24 (%)		PD-L1(-), *n* = 51 (%)	PD-L1+, *n* = 17 (%)	
Tumor size (cm)			**0.011**			**0.005**
mean (SD)	5.8 (3.3)	8.1 (3.6)		5.9 (3.3)	8.7 (3.5)	
Lauren type			0.952			1
Intestinal	18 (40.9)	10 (41.7)		21 (41.2)	7 (41.2)	
Diffuse/mixed	26 (59.1)	14 (58.3)		30 (58.8)	10 (58.8)	
Grade of histological differentiation			0.438			0.449
Well/mod differentiated	15 (34.1)	6 (25)		17 (33.3)	4 (23.5)	
Poorly differentiated	29 (65.9)	18 (75)		34 (66.7)	13 (76.5)	
Lymphatic invasion			0.176			1
No	14 (31.8)	4 (16.7)		14 (27.5)	4 (23.5)	
Yes	30 (68.2)	20 (83.3)		37 (72.5)	13 (76.5)	
Venous invasion			**0.017**			0.262
No	28 (63.6)	8 (33.3)		29 (56.9)	7 (41.2)	
Yes	16 (36.4)	16 (66.7)		22 (43.1)	10 (58.8)	
Perineural invasion			0.417			0.558
No	14 (31.8)	10 (41.7)		19 (37.3)	5 (29.4)	
Yes	30 (68.2)	14 (58.3)		32 (62.7)	12 (70.6)	
Peritumoral inflammatory response			**< 0.001**			**0.011**
Absent/mild	32 (72.7)	6 (25)		33 (64.7)	5 (29.4)	
Moderate/severe	12 (27.3)	18 (75)		18 (35.3)	12 (70.6)	
pT status			0.734			0.268
pT1/T2	8 (18.2)	3 (12.5)		10 (19.6)	1 (5.9)	
pT3/T4	36 (81.8)	21 (87.5)		41 (80.4)	16 (94.1)	
No. of lymph nodes			0.964			**0.035**
mean (SD)	42.3 (13.9)	42.5 (19.4)		40.1 (14.3)	49.4 (18.7)	
No. of positive lymph nodes			0.961			0.486
mean (SD)	7 (6.4)	6.9 (6.9)		6.7 (6.1)	7.9 (7.7)	
pN status			1			0.879
pN1	9 (20.5)	5 (20.8)		11 (21.6)	3 (17.6)	
pN2	18 (43.2)	11 (45.8)		23 (45.1)	7 (41.2)	
pN3	16 (36.4)	8 (33.3)		17 (33.3)	7 (41.2)	
pTNM status			1			0.299
I/II	9 (20.5)	5 (20.8)		12 (23.5)	2 (11.8)	
III/IV	35 (79.5)	19 (79.2)		39 (76.5)	15 (88.2)	

P values in bold are statistically significant. PD-L1: Programmed death-ligand 1.

Postoperative and follow-up

The mean length of hospital stay was 11 d (SD = 8.6, range 4-45 d). A total of 41 patients received some preoperative or postoperative chemotherapy treatment, but none of them received immunotherapy. After a median follow-up of 39.2 months, 30 patients had a recurrence and 37 died. Estimated DFS and OS for the entire study population were 68.4% and 55.6%, respectively.

Regarding the PT, there was no statistical difference in DFS rate according to PD-L1(-) and PD-L1+ status (37.9% *vs* 48%, respectively; $P = 0.336$); and for OS rate (37.5% *vs* 52.2%, respectively; $P = 0.166$). Considering the LNM, the median DFS was 26.4 and 26.3 months for PD-L1+ and PD-L1 negative cases ($P = 0.995$). No difference was observed for OS between PD-L1+ and negative groups ($P = 0.837$). The median OS was 33.3 months and 38.5 months for PD-L1(-) and PD-L1+ in LNM, respectively.

DISCUSSION

In the present study, we found a moderate concordance of PD-L1 expression between PT and paired LNM in patients with GC. As PD-L1 testing is currently aimed at selecting patients for immunotherapy, LNM assessment has the potential to increase the number of candidates by 10%. Further, PD-L1+ in both PT and LNM were associated with larger tumor size and moderate/severe peritumoral inflammatory response.

Although immunotherapy has emerged as a breakthrough in several malignancies, clinical indication of anti-PD-1/PD-L1 is limited to a small subset of patients, including patients with GC[7]. Food and Drug Administration (FDA) recently extended approval of pembrolizumab treatment for patients with unresectable or metastatic, microsatellite instability-high (MSI-H), or mismatch repair deficient (dMMR) solid tumors that progressed despite prior treatment[24]. Tumors expressing PD-L1 CPS ≥ 1 as determined by an FDA-approved test, exhibiting disease progression or after two or more therapeutic attempts were also included in recent approval[4].

As PD-L1 staining has proven to be decisive for immunotherapy indication, the expression of PD-L1 has started to be widely investigated in different types of tumor and sites. Recent studies have emphasized the heterogeneity of PD-L1 expression in tumor samples, showing a significantly higher accuracy of resection samples when compared to isolated biopsies[25,26]. Similarly, we also observed heterogeneity when correlating expression from different sites (PT *vs* metastatic site), showing that not only tumor samples - but also the site of evaluation - can have differences in expression.

This correlation of PD-L1 expression has been previously evaluated in other tumors[27-30]. A recent systematic review and meta-analysis extensively summarized the discordant status between PT and distant metastasis for nine different cancer types including non-small cell lung cancer, breast cancer, colorectal cancer (CRC), kidney renal clear cell carcinoma, head and neck squamous cell carcinoma, bladder cancer, melanoma, synovial sarcoma, and cervical cancer [27]. It was reported a pooled discordance rate for PD-L1 of 22% when comparing sites. Moreover, the PD-L1 conversion rate from PT to the metastatic site was 41% from positive to negative, whereas that from negative to positive was 16%, encouraging an evaluation of both PT and metastatic sites for better treatment eligibility[27].

In fact, the results regarding PD-L1 when evaluated in PT and metastasis, present variable results in the studies. An overall similar prevalence in PD-L1 expression between PT and LNM has been reported in CRC. The PD-L1 overexpression was more common along with a higher tumor mutational burden (TMB). Interestingly, a TMB-high status was significantly more frequent in lymph node than in PT and distant sites[29]. Differences between MMR status have also been previously described. MSI and MMR are also pivotal in immunotherapy eligibility since anti-PD-L1 therapy has shown benefits for patients with dMMR or MSI-H tumors[24,31]. A previous study found a high concordance rate between primary CRCs and matched metastatic lesions, describing some changes from MSI-H in primary CRC to MSS when peritoneal and ovarian metastases, suggesting the need for a new biopsy to evaluate MSI-H/dMMR status when an anti-PD-1 therapy is planned[32].

A good agreement of PD-L1 expression between the PT and LNM (90.9%) was reported in patients with esophageal cancer who previously received neoadjuvant chemotherapy. Among the 35 patients with PT PD-L1(-), 3 had positive expression in the LNM, contributing to the possibility of positivity amongst patients with negative PT site[33].

Some authors suggest that microenvironment characteristics may contribute to the differences in expression between PT and LNM, such as infiltration of cytotoxic T lymphocytes were considered to play a pivotal role in higher PD-L1 positivity by tumor positive score status found in metastatic sites[28].

In a study that evaluated the expression of PD-L1 in paired sample of 47 breast tumor and axillary LNM samples, PT and matched lymph node showed a positive PD-L1 rate of 29.8% (14/47) and 14.8% (7/47), respectively. When comparing matched sites, amongst the 14 patients with positive PD-L1(-) PT, 50 were also positive in the paired LNM. All 33 patients with PD-L1(-) PT showed negative PD-L1 expression in paired LNM[30]. Similarly, another study showed high fidelity across the matched primary and metastatic samples from treatment-naive patients, as 94% (16/17) of cases showed concordant staining between the PT and metastatic tumor. However, as for tumor-associated inflammatory cells, a gain of PD-L1 immune stromal positivity was observed in the site of metastasis in five cases (4 nodal and 1 lung metastasis)[34].

In GC, data regarding the concordance rate between PD-L1 in primary GC and matched regional LNM are limited. Gao *et al*[35] demonstrated a higher PD-L1 expression rate in LNM than in PT (45.4% *vs* 38.7%, $P = 0.005$). Also, similar to our findings, PD-L1 expression was inconsistent in PT and LNM from the same patient. This suggests that, when evaluated only one site exclusively, false-negative results may contribute to the inaccuracy of using PD-L1 as a predictor of response to anti-PD-1/PD-L1 immunotherapy[35].

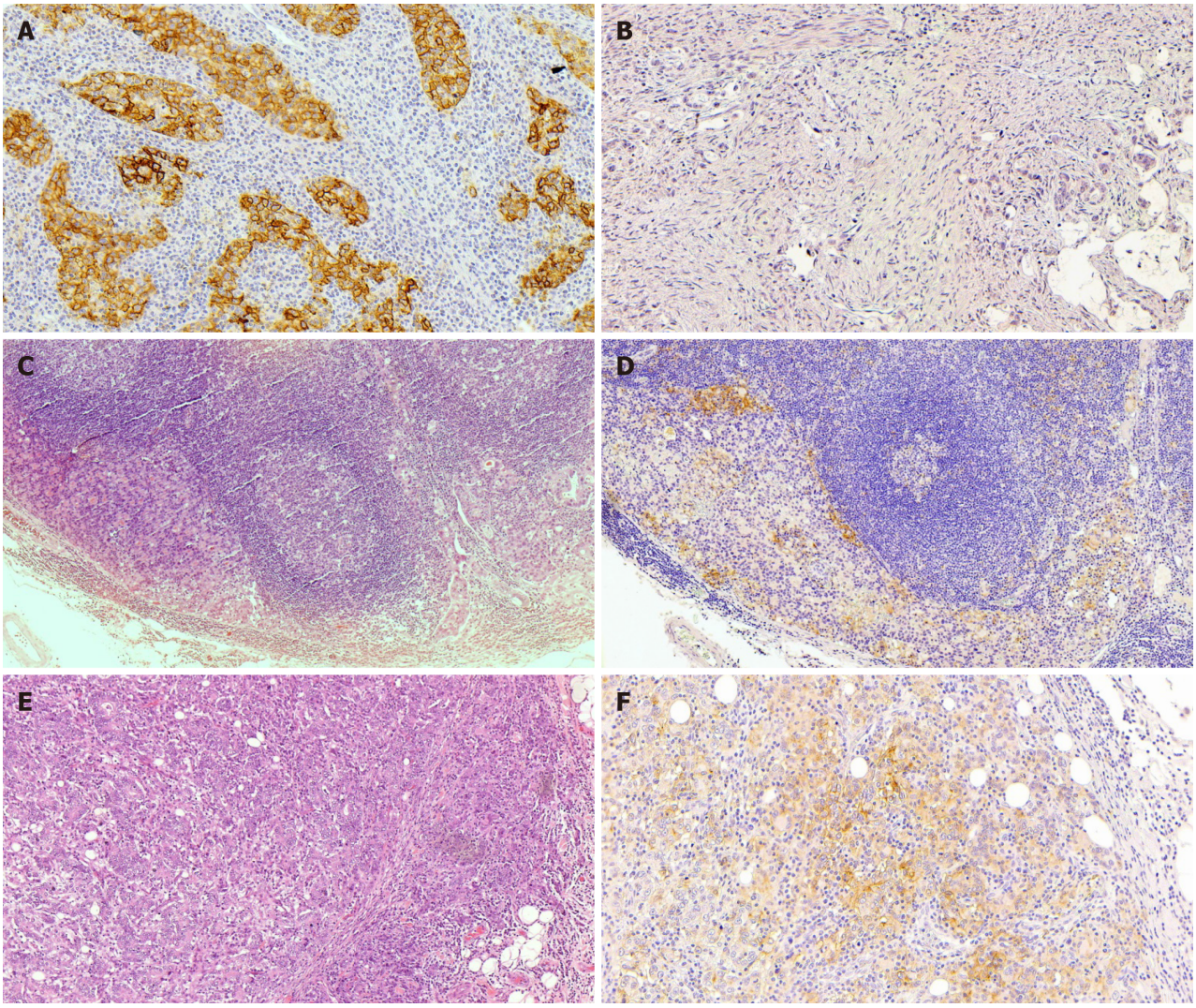

Figure 1 Microscopic findings of primary tumor and metastatic lymph nodes in cases with gastric adenocarcinoma. A: Gastric adenocarcinoma with positive staining for programmed death-ligand 1 (PD-L1), original magnification, 20 ×; B: Gastric adenocarcinoma PD-L1 negative, original magnification, 20 ×; C: Lymph node with adenocarcinoma metastasis stained with hematoxylin eosin (HE), original magnification, 10 ×; D: Positive PD-L1 staining in the metastatic lymph node showed in "C", from a case with PD-L1 negative in gastric tumor, original magnification, 10 ×; E: Lymph node metastasis in HE staining, original magnification, 10 ×; F: Positive staining for PD-L1 in the same lymph node, from a case with primary tumor also positive for PD-L1, original magnification, 20 ×.

Differences have also been reported in relation to PD-L1 positivity in tumor cells and tumor-infiltrating immune cells (TIC) between PT and a subset of paired LNM. Svensson *et al*[9] demonstrated that PD-L1 expression TIC was significantly higher in LNM compared to PT (54.4% *vs* 41.2%)[9]. In our study, PD-L1 expression was evaluated using the CPS. Therefore, we did not examine this difference in relation to the positivity pattern separately.

Interestingly, in addition to the heterogeneity of expression, some findings observed when evaluating PD-L1 expression in distant metastases beyond the PT and LNM suggest that tumor cells acquire PD-L1 expression during disease progression. Liu *et al*[14] showed up to 33% discordance of PD-L1 CPS between primary GC and LNM and/or distant metastasis, and the proportion of PD-L1 positive tumor cells increased from primary GC (26%) to LNM (42%) and was highest in distant metastasis (75%).

In a study that compared PD-L1 expression in paired baseline primary and baseline metastatic tumors from 62 patients with gastroesophageal adenocarcinoma, a spatial heterogeneity was noted in PT, which were PD-L1 positive, but frequently PD-L1 negative in paired metastases[13]. They found that 36 PT were PD-L1+, compared with 18 metastatic tumors. Baseline paired primary and metastatic tumor PD-L1-status were 61% concordant (38/62). Among the 26 PD-L1(-) in PT, 23 (88%) remained PD-L1(-) in the metastatic tumor. In contrast, of 36 PD-L1+ PT, only 15 (42%) remained PD-L1+ in the metastatic tumor[13]. In our study, the overall agreement rate was 77.9% (53/68). Conversely, the disagreement was 22.1% (15/68). Of the 24 PD-L1+ PTs, only 13 (54%) were positive on paired LNM. While in the 44 PD-L1(-) cases in PT, 9 were PD-L1+ in LNM (9.1%).

Thus, as the benefit of targeted therapies is limited to a restricted group of patients, improve assessment of PD-L1 expression may be important to broaden the therapeutic indication and evaluate the results obtained with the treatment in clinical trials. The PD-L1 positivity seen at the metastatic site could explain the survival benefit seen in some PT PD-L1(-) patients who received immunotherapy in clinical trials[4,17]. At the same time, since PD-L1 status in PT did not

show a clear dependence on metastatic site, these results may also provide explanation for lack of benefit of immune checkpoint inhibitor therapy in some metastatic patients, despite positive PD-L1 scoring in PT. Thus, evaluation of intra-patient heterogeneity of PD-L1 expression may better predict which patients are most likely to benefit from therapy.

The present study has limitations. The included sample size is relatively small, which limits the assessment of survival outcomes and clinical characteristics associated with PD-L1 status for both sites. We evaluated PD-L1 expression in the PTs and a single lymph node for each patient as a first assessment, limited by sample availability and feasibility. Furthermore, despite suggesting a potential increase in the patient that could benefit from immunotherapy by evaluating PD-L1 beyond the PT site, we cannot determine whether there will actually be a survival improvement in these cases, as our patients were not treated with immunotherapy with anti-PD-1/PD-L1.

On the other hand, to our knowledge, this study is the first to address the intra-patient heterogeneity of PD-L1 expression in paired PT and LNM in western patients with GC who underwent a curative intent gastrectomy. Our study demonstrated that PD-L1 positivity rate was varied between PT and LNM, and the agreement across tumor sites is not necessarily the same. Accordingly, as in our cohort of patients without PD-L1 expression in the PT were positive in LNM in 9.1% of the cases, we suggested that biopsies of primary GC and metastatic sites should be tested before considering treatment options. In our study, lymph node testing could increase the number of potential candidates for immunotherapy based on PD-L1 expression, which may be important to expand the immune checkpoint inhibitor therapy indication.

CONCLUSION

Intra-patient heterogeneity of PD-L1 expression were observed between the primary GC and matched LNM. The heterogeneity in PD-L1 status may emphasize the importance of considering the site of tumor sample examined when selecting patients for immunotherapy, since it could influence the role of PD-L1 as a predictive biomarker for response to immune checkpoint inhibitors.

ARTICLE HIGHLIGHTS

Research background

Programmed death ligand 1 (PD-L1) expression is a potential biomarker for response to immune checkpoint inhibitors in some tumors, including in gastric cancer (GC). However, many biomarkers exhibit heterogeneity in GC, and intra-patient heterogeneity of PD-L1 expression may influence its role as predictive biomarkers.

Research motivation

Data regarding the concordance rate between PD-L1 in primary GC and matched regional lymph node metastasis (LNM) are limited.

Research objectives

This study aimed to compare PD-L1 expression in paired primary tumor (PT) and LNM from patients with GC. Clinicopathological characteristics and prognosis according to PD-L1 status were also evaluated.

Research methods

We retrospectively reviewed 284 GC patients who underwent D2-gastrectomy. PD-L1 was evaluated by immunohistochemistry (clone SP142). PD-L1 status was defined as positive using the combined positive score ≥ 1. PD-L1+ in PT staged as pN+ were also tested for PD-L1 expression in their LNM. PD-L1(-) GC with pN+ served as the comparison group.

Research results

Among 284 patients, 24 were PD-L1 positivity in PT and had LNM. PD-L1+ in both PT and LNM were associated with larger tumor size and moderate/severe peritumoral inflammatory response. Among patients with PD-L1 positive in PT, 54.2% were also positive for PD-L1 in LNM. Considering the PD-L1 negative patients in PT, 9.1% of had PD-L1 positivity in LNM. The agreement between PT and LNM had a kappa value of 0.483 (moderate concordance). There was no difference in overall survival for PT and LNM according to the PD-L1 status.

Research conclusions

Our findings demonstrated that the expression of PD-L1 in the PT and LNM of patients with GC demonstrated discordance, and the heterogeneity observed between the sites evaluated may impact the use of PD-L1 as predictive biomarkers of response to immune checkpoint inhibitors.

Research perspectives

The intra-patient heterogeneity in PD-L1 status may emphasize the importance of considering the site of tumor sample examined when selecting patients for immunotherapy, and this difference in PD-L1 status between PT and matched LNM may be evaluated in future trials to justify the response observed in some PD-L1 negative cases in PT.

FOOTNOTES

Author contributions: Coimbra BC, Pereira MA, and Ramos MFKP contributed to the study design and data retrieval; Coimbra BC, Pereira MA, Alves VAF, Ribeiro U Jr, and Ramos MFKP were involved in the critical analysis; Coimbra BC and Pereira MA drafted the manuscript; Cardili L and de Mello ES participated in the laboratory techniques and pathological analysis; Alves VAF and Ribeiro U Jr supervised the project; Alves VAF, Ribeiro U Jr, and Ramos MFKP reviewed the manuscript; Ramos MFKP implemented the research.

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Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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Country/Territory of origin: Brazil

ORCID number: Brendha Cação Coimbra 0000-0002-3181-7430; Marina Alessandra Pereira 0000-0002-6865-0988; Leonardo Cardili 0000-0001-9673-4030; Venancio Avancini Ferreira Alves 0000-0001-5285-4460; Evandro Sobroza de Mello 0000-0002-4383-2910; Ulysses Ribeiro Jr 0000-0003-1711-7347; Marcus Fernando Kodama Pertille Ramos 0000-0003-0200-7858.

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