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ORIGINAL RESEARCH ARTICLE

Frequency and clinicopathologic associations of microsatellite instability and PD-L1 expression in Vietnamese patients with gastric cancer

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ABSTRACT

Background: Our study aimed to determine the frequency of microsatellite instability (MSI) and characterize the associations between MSI status, PD-L1 expression, and clinicopathological features in Vietnamese patients with gastric cancer. **Methods:** We performed a retrospective cohort study that analyzed 87 patients with gastric cancer who underwent gastrectomy from January 2020 to March 2023. MSI status was assessed by immunohistochemistry for mismatch repair proteins. PD-L1 expression was evaluated by tumor proportion score (TPS) and combined positive score (CPS). Associations between MSI, PD-L1, and clinicopathologic factors were analyzed. **Results:** MSI-high (MSI-H) was identified in 13.8% of tumors and significantly associated with intestinal subtype, moderate differentiation, necrosis, tumor-infiltrating lymphocytes, and PD-L1 positivity. Lymphatic invasion correlated with increased TPS. Intestinal classification correlated with higher CPS. **Conclusion:** MSI-H identifies a subset of gastric cancers with distinct features. PD-L1 expression is associated with aggressive disease parameters. Biomarker-based stratification may guide personalized therapy.

Keywords: gastric cancer; microsatellite instability; PD-L1; tumor microenvironment; biomarkers; Vietnam

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1. Introduction

Gastric cancer is a significant cause of cancer mortality worldwide, representing a substantial global health burden[1]. Surgery remains the only curative treatment, yet recurrence rates are high even after complete resection[2]. There is a need to understand the molecular characteristics of gastric cancer better to guide personalized therapy and improve patient outcomes.

Microsatellite instability (MSI) is an important molecular subtype of gastric cancer caused by impaired DNA mismatch repair. MSI tumors have distinctive clinicopathologic features and improved prognosis compared to microsatellite stable (MSS) gastric cancers[3]. MSI status may also predict response to immune checkpoint inhibitors, as demonstrated in other gastrointestinal cancers[4,5]. However, data on the prevalence and characteristics associated with MSI in gastric

cancer remain limited, particularly in the Vietnamese population.

Programmed death ligand-1 (PD-L1) is another promising biomarker indicating a potential immunotherapy response. PD-L1 expression correlates with MSI status and favorable prognosis in gastric cancer[6–8]. However, the relationship between PD-L1 and clinicopathologic factors is not fully defined. Furthermore, the combined positive score (CPS) may provide a more comprehensive assessment of PD-L1 expression compared to tumor proportion score (TPS) alone[9,10].

Therefore, this study aimed to determine the frequency of MSI gastric cancers and characterize the associations between MSI status, PD-L1 expression, and clinicopathologic features in Vietnamese patients. We hypothesized that MSI and PD-L1 expression would correlate with specific pathologic characteristics. This is the first study to extensively evaluate MSI, PD-L1, and related prognostic factors in a Vietnamese gastric cancer cohort. The results of this study will delineate the landscape of MSI and PD-L1 in this population and may guide prognostication and immunotherapy treatment decisions.

2. Materials and methods

2.1. Study population

This retrospective, descriptive cohort study analyzed clinicopathologic features and biomarker status in 87 patients with gastric cancer who underwent gastrectomy with lymph node dissection at Military Hospital 103 from January 2020 to March 2023. Inclusion criteria: patients with primary gastric adenocarcinoma confirmed by histopathological examination after surgery; complete medical records including administrative, clinical, and necessary lab data; available tissue samples of sufficient quality for histopathological and immunohistochemistry analysis. Exclusion criteria: metastatic tumors to the stomach from other primary sites; incomplete medical records; insufficient tissue samples for analysis.

The study was approved by the Institutional Review Board of Military Hospital 103 (approval number 150/CNChT-HĐĐĐ). The study also adhered to the ethical guidelines established by the National Research Committee and the 1964 Helsinki Declaration. As this was a retrospective study using archived tissue samples, the requirement for informed consent was waived. Patient confidentiality was maintained by data anonymization and coding.

2.2. Study variables

Clinical data including age (<60 years, ≥60 years), sex, clinical stage at diagnosis (according to AJCC 8th edition)[11], tumor location, gross appearance (based on Bormann classification)[12], and tumor size (<5cm, ≥5cm) were extracted from medical records. H&E staining of tumor specimens and nodal were retrieved from pathology archives and reviewed by gastrointestinal pathologists to confirm the histopathologic diagnosis and assess additional features, including depth of invasion (pT1-4)[11], Lauren classification[13], WHO classification 2019[14], differentiation status (well, moderate, poor)[15], presence of tumor necrosis, lymph node metastasis status, lymphovascular invasion, perineural invasion, and level of tumor-infiltrating lymphocyte.

2.3. Hematoxylin and eosin and immunohistochemistry staining

Formalin-fixed, paraffin-embedded tissue samples were obtained from surgical resection specimens. Sections were cut at 4µm thickness and stained with hematoxylin and eosin (H&E). Additional sections were used for immunohistochemistry (IHC) staining using an automated immunostainer (Leica ST5010 Auto Stainer XL, Leica Biosystems). IHC was performed using monoclonal antibodies against PD-L1 (clone 73-10, Code PA0832, rabbit anti-human monoclonal primary antibody, Leica, UK) and Mismatch Repair (MMR) proteins including MLH1 (clone ES05, Code NCL-L-MLH1), PMS2 (clone M0R4G, Code NCL-L-PMS2), MSH2 (clone 79H11, Code NCL-L-MSH2-612), MSH6 (clone PU29, Code NCL-L-MSH6) with all were

mouse anti-human monoclonal primary antibodies, Leica, UK. Positive and negative control tissues were included in each IHC run.

2.4. Evaluation of tumor-infiltrating lymphocytes and IHC staining

Tumor-infiltrating lymphocytes (TILs) were evaluated using the standardized methodology of the International TILs Working Group[16]. TILs were categorized as low (0–50% stromal infiltration) or high (51%–100% stromal infiltration).

PD-L1 expression was determined by tumor proportion score (TPS), defined as the number of PD-L1 positive tumor cells divided by the total number of viable tumor cells and multiplied by 100 to calculate a percentage, following the previous study method[17]. Samples with TPS < 1% were considered negative, while those with TPS ≥ 1% were considered positive. The Combined Positive Score (CPS) incorporates PD-L1 staining on tumor and immune cells. CPS was calculated as CPS = (Number of PD-L1 positive tumor cells + Number of PD-L1 positive immune cells + Number of PD-L1 positive macrophages)/Total number of viable tumor cells × 100, as previously reported method[18]. A CPS ≥1 was considered positive, while a CPS < 1 was considered negative. By incorporating both tumor cell and immune cell staining, the CPS quantitatively measures overall PD-L1 expression within the tumor microenvironment.

To determine MMR status, we followed the guidelines of the previous study[19,20]. MMR protein expression was scored as positive (retained) when ≥10% of tumor cells showed nuclear staining or negative (loss) when <10% of tumor cells were stained. Samples with loss of ≥1 MMR proteins were considered MSI-high, while samples with intact MMR expression were MSS/MSI-low.

2.5. Data collection and statistical

Analysis data was collected from medical records using standardized collection forms and managed anonymously with coded identification numbers. Categorical data were reported as frequency and percentage. Univariate logistic regression analyses by Pearson's chi-square test or Fisher's exact test were used to compare categorical variables. Multivariate logistic regression analyses were performed to identify predictors of microsatellite instability and PD-L1 expression. Variables with $p < 0.05$ in univariate analysis were entered into multivariate models. Results were reported as odds ratios with 95% confidence intervals. *P* values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 22.0 (IBM SPSS Inc., New York, NY, USA).

3. Results

3.1. Patient demographics and tumor characteristics

Our study examined the demographics and clinicopathologic characteristics of 87 patients diagnosed with gastric cancer (**Table 1**).

Table 1. Clinicopathologic features of gastric cancer.

Characteristics		n (%)
Age		
	≤60	37 (42.5)
	>60	50 (57.5)
Sex		
	Female	27 (31.0)
	Male	60 (69.0)

Table 1. (*Continued*).

Characteristics		n (%)
Clinical staging		
	I	5 (5.8)
	II	29 (33.3)
	III	37 (42.5)
	IV	16 (18.4)
Tumor location		
	Fundus-body	14 (16.1)
	Antrum	49 (56.3)
	Lesser curvature	24 (27.6)
Gross appearance		
	Polypoid	13 (14.9)
	Fungating	15 (17.2)
	Ulcerative	40 (46.0)
	Infiltrative	19 (21.9)
Tumor size		
	<5 cm	50 (57.5)
	≥5 cm	37 (42.5)
Depth of tumor invasion		
	pT1	4 (4.6)
	pT2	39 (44.8)
	pT3	31 (35.6)
	pT4	13 (15.0)
Lauren classification		
	Intestinal type	38 (43.7)
	Diffuse type	33 (37.9)
	Mixed type	16 (18.4)
WHO classification		
	Tubular adenocarcinoma	43 (49.4)
	Mucinous carcinoma	11 (12.6)
	Poorly cohesive carcinoma	18 (20.7)
	Undifferentiated carcinoma	15 (17.3)
Tumor differentiation status		
	Well	13 (14.9)
	Moderately	35 (40.2)
	Poorly	39 (44.9)
Tumor necrosis		
	Positive	16 (18.4)
	Negative	71 (81.6)
Lymph node metastasis		
	Positive	50 (57.5)
	Negative	37 (42.5)
Lymphatic invasion		
	Positive	6 (6.9)
	Negative	81 (93.1)
Perineural invasion		
	Positive	16 (18.4)
	Negative	71 (81.6)

Table 1. (*Continued*).

Characteristics		n (%)
Tumor-infiltrating lymphocyte		
	Low	56 (64.4)
	High	31 (35.6)
Tumor proportion score		
	<1%	59 (67.8)
	≥1%	28 (32.2)
Combined positive score		
	<1%	47 (54.0)
	≥1%	40 (46.0)
Microsatellite instability		
	MSS/MSI-L	75 (86.2)
	MSI-H	12 (13.8)

MSS: Microsatellite stable; MSI-L: Low-level microsatellite instability; MSI-H: High-level microsatellite instability; n: number; WHO: World Health Organization.

We found that the majority were male (69.0%) and over 60 (57.5%). Regarding clinical stage, 5.8% were stage I, 33.3% were stage II, 42.5% were stage III, and 18.4% were stage IV. The most common tumor location was the antrum (56.3%), followed by the lesser curvature (27.6%) and fundus/body (16.1%). The most frequent gross appearance was ulcerative (46.0%), followed by fungating (17.2%), infiltrative (21.9%), and polypoid (14.9%). Over half of the tumors were <5 cm (57.5%). The depth of invasion was pT2 in 44.8%, pT3 in 35.6%, pT4 in 15.0%, and pT1 in 4.6%.

According to the Lauren classification histologic subtypes, the intestinal type was most frequent (43.7%), followed by diffuse (37.9%) and mixed (18.4%). The most common WHO classification was tubular adenocarcinoma (49.4%), followed by poorly cohesive carcinoma (20.7%), undifferentiated carcinoma (17.3%), and mucinous carcinoma (12.6%). Tumor differentiation was poor in 44.9%, moderate in 40.2%, and well in 14.9% of cases. Tumor necrosis was present in 18.4% and absent in 81.6%. Lymph node metastases were present in 57.5% and absent in 42.5%. Lymphatic and perineural invasion rates were 6.9% and 18.4%, respectively. Tumor-infiltrating lymphocytes were high at 35.6% and low at 64.4%. The tumor proportion score was ≥1% in 32.2% and <1% in 67.82%. The combined positive score was ≥1% in 46.0% (**Figure 1**) and <1% in 54.0% of patients. Microsatellite instability status was MSS/MSI-L in 86.2% and MSI-H in 13.8% (**Figure 2**).

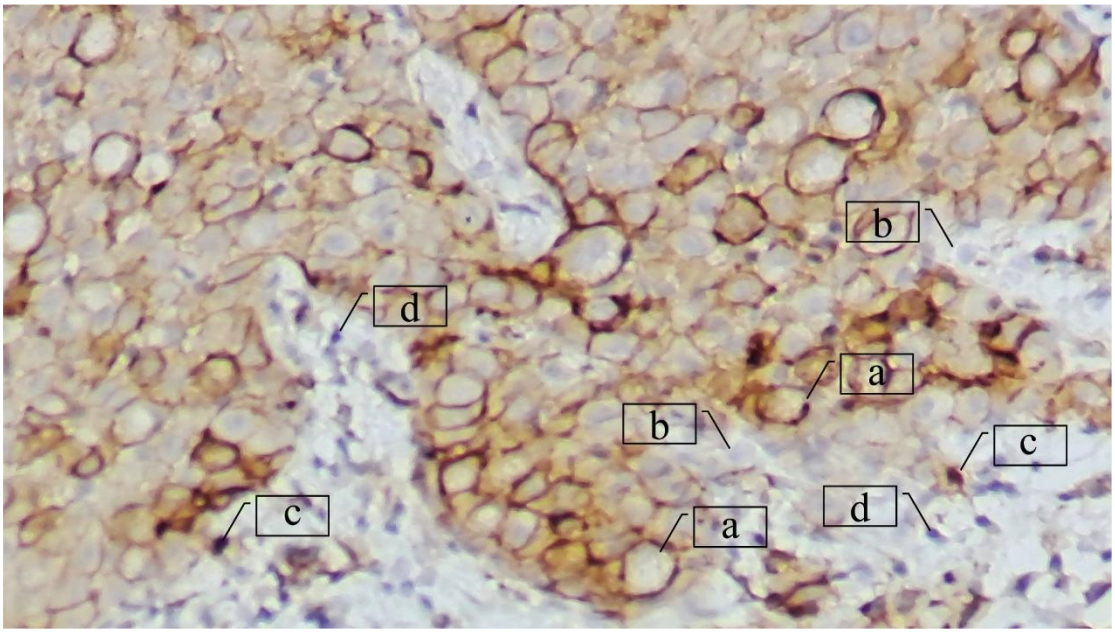

Figure 1. PD-L1 expression in tumor cells and immune cells from tumor specimens. Tumor specimens were stained for PD-L1 using immunohistochemistry at 400× magnification. The staining was used to calculate the Tumor Proportion Score (TPS) and Combined Positive Score (CPS). The figure shows representative images of (a) PD-L1 positive expression tumor cells, (b) PD-L1 negative expression tumor cells, (c) PD-L1 positive expression immune cells, and (d) PD-L1 negative expression immune cells.

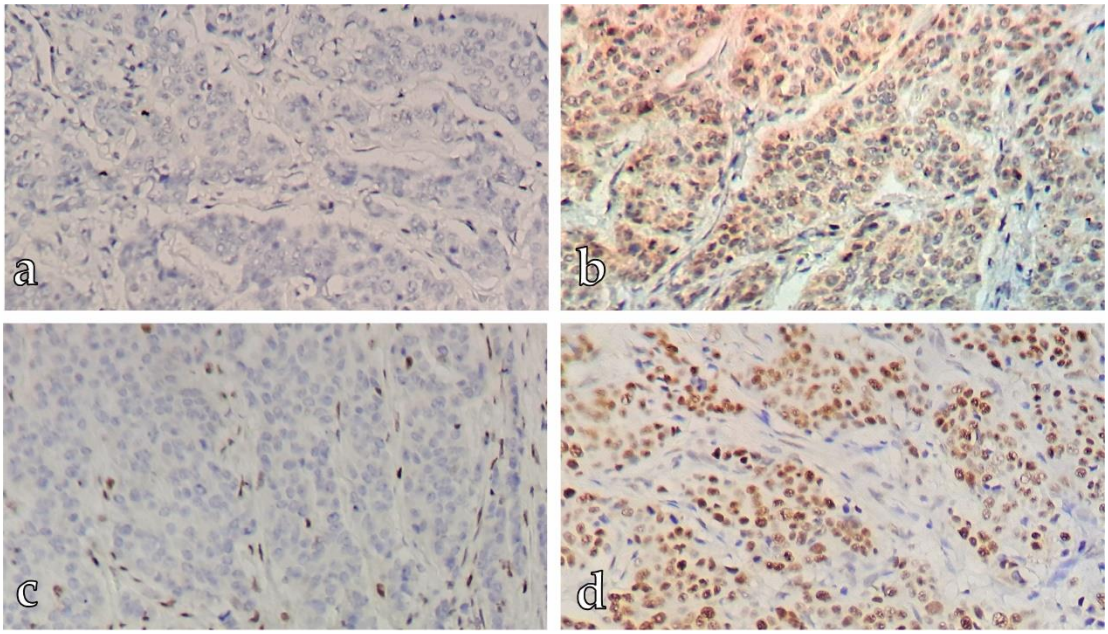

Figure 2. Representative immunohistochemistry of Mismatch Repair proteins in gastric cancer tissue. Formalin-fixed, paraffin-embedded gastric cancer tissue sections were stained by immunohistochemistry for the mismatch repair proteins MLH1, PMS2, MSH2, and MSH6. Staining was visualized at 400× magnification. Representative images show loss of nuclear protein expression for MLH1 (a) and PMS2 (c) but retained expression for MSH2 (b) and MSH6 (d). This staining pattern is indicative of high microsatellite instability.

3.2. Association between MSI status and clinicopathologic factors

We examined the phenomenon of microsatellite instability (MSI) in relation to various clinicopathologic characteristics in gastric cancer patients (**Table 2**). MSI-H was significantly associated with depth of tumor invasion ($p = 0.023$), Lauren classification ($p = 0.042$), tumor differentiation status ($p = 0.024$), tumor necrosis ($p = 0.001$), tumor-infiltrating lymphocytes ($p = 0.023$), tumor proportion score ($p = 0.016$), and combined positive score ($p = 0.03$). Specifically, we found MSI-H was more prevalent in tumors invading the subserosa or deeper (pT3-pT4) of the intestinal Lauren type, moderately differentiated, with tumor necrosis, high tumor-

infiltrating lymphocytes, tumor proportion score ≥1%, and combined positive score ≥1%. We observed no significant associations between MSI-H and age, sex, clinical staging, tumor location, gross appearance, tumor size, lymph node metastasis, lymphatic invasion, perineural invasion, or WHO classification.

In summary, the major clinicopathologic characteristics associated with MSI-H were the depth of invasion, histologic subtype, differentiation, necrosis, and immune markers. At the same time, we saw no associations for demographic, gross, or metastatic factors.

Table 2. Associations of clinicopathologic features and microsatellite instability in gastric cancer.

Characteristics		**Microsatellite instability, n (%)**		***p****
		MSS/MSI-L	**MSI-H**	
Age				
	≤60	34 (39.1)	3 (3.5)	0.223[#]
	>60	41 (47.1)	9 (10.3)	
Sex				
	Female	21 (24.1)	6 (6.9)	0.126
	Male	54 (62.1)	6 (6.9)	
Clinical staging				
	I	5 (5.7)	0 (0)	0.274
	II	27 (31.0)	2 (2.3)	
	III	29 (33.3)	8 (9.2)	
	IV	14 (16.1)	2 (2.3)	
Tumor location				
	Fundus-body	10 (11.5)	4 (4.6)	0.195
	Antrum	43 (49.4)	6 (6.9)	
	Lesser curvature	22 (25.3)	2 (2.3)	
Gross appearance				
	Polypoid	10 (11.5)	3 (3.4)	0.541
	Fungating	13 (14.9)	2 (2.3)	
	Ulcerative	34 (39.1)	6 (6.9)	
	Infiltrative	18 (20.7)	1 (1.1)	
Tumor size				
	<5 cm	43 (49.4)	7 (8.0)	0.948
	≥5 cm	32 (36.8)	5 (5.7)	
Depth of tumor invasion				
	pT1	4 (4.6)	0 (0)	0.023
	pT2	37 (42.5)	2 (2.3)	
	pT3	22 (25.3)	9 (10.3)	
	pT4	12 (13.8)	1 (1.1)	
Lauren classification				
	Intestinal type	29 (33.3)	9 (10.3)	0.042
	Diffuse type	32 (36.8)	1 (1.1)	
	Mixed type	14 (16.1)	2 (2.3)	
WHO classification				
	Tubular adenocarcinoma	35 (40.2)	8 (9.2)	0.273
	Mucinous carcinoma	9 (10.3)	2 (2.3)	
	Poorly cohesive carcinoma	18 (20.7)	0 (0)	
	Undifferentiated carcinoma	13 (14.9)	2 (2.3)	

Table 2. (*Continued*).

Characteristics		Microsatellite instability, n (%)		*p**
		MSS/MSI-L	MSI-H	
Tumor differentiation status				
	Well	13 (14.9)	0 (0)	0.024
	Moderately	26 (29.9)	9 (10.3)	
	Poorly	36 (41.4)	3 (3.4)	
Tumor necrosis				
	Positive	66 (75.9)	5 (5.7)	0.001#
	Negative	9 (10.3)	7 (8.0)	
Lymph node metastasis				
	Positive	43 (49.4)	7 (8.0)	0.948
	Negative	32 (36.8)	5 (5.7)	
Lymphatic invasion				
	Positive	4 (4.6)	2 (2.3)	0.191#
	Negative	71 (81.6)	10 (11.5)	
Perineural invasion				
	Positive	16 (18.4)	0 (0)	0.112#
	Negative	59 (67.8)	12 (13.8)	
Tumor-infiltrating lymphocyte				
	Low	52 (59.8)	4 (4.6)	0.023#
	High	23 (26.4)	8 (9.2)	
Tumor proportion score				
	<1%	55 (63.2)	4 (4.6)	0.016#
	≥1%	20 (23.0)	8 (9.2)	
Combined positive score				
	< 1%	44 (50.6)	3 (54.0)	0.03
	≥ 1%	31 (35.6)	9 (10.3)	

MSS: Microsatellite stable; MSI-L: Low-level microsatellite instability; MSI-H: High-level microsatellite instability; n: number; WHO: World Health Organization; *p* values were determined using the (*) Chi-square test and (#) Fisher's exact test.

3.3. Relationship between PD-L1 expression and clinicopathologic features

In our analysis of PD-L1 expression in gastric cancer, we found several significant associations between clinicopathologic characteristics and PD-L1 expression as measured by Tumor Proportion Score (TPS) and Combined Positive Score (CPS) (**Table 3**).

Overall, 28 (32.2%) of the tumors were classified as PD-L1 positive with a TPS ≥ 1%, while 40 (46.0%) had a CPS ≥ 1% (**Table 1**). PD-L1 expression by TPS was significantly associated with lymphatic invasion (p = 0.005). No other clinicopathologic factors demonstrated a statistically significant correlation with TPS. Using CPS, PD-L1 expression was significantly associated with Lauren classification (p = 0.033). No other characteristics showed a significant relationship with CPS.

Our key findings were that lymphatic invasion correlated with increased TPS, while intestinal type Lauren classification was associated with higher CPS.

3.4. Potential risk factors for microsatellite instability and PD-L1 expression

We conducted multivariate logistic regression analyses to identify potential risk factors for microsatellite instability (**Table 4**) and PD-L1 expression (**Table 5**).

Tumor depth of invasion was significantly associated with microsatellite instability. Notably, pT3 stages showed a substantial difference with OR of 7.227 (95% CI, 1.785-29.249; p = 0.006). Regarding Lauren

classification, intestinal type tumors displayed a higher OR of 4.758 (95% CI, 1.189-19.044; p = 0.027), indicating a stronger association with microsatellite instability. Tumor differentiation status also demonstrated a significant association, with moderately differentiated tumors having an OR of 5.653 (95% CI, 1.407-22.710; p = 0.015). Additionally, the presence of tumor necrosis OR of 10.267 (95% CI, 2.682-39.304; p = 0.001) and high tumor-infiltrating lymphocytes OR of 4.522 (95% CI, 1.236-16.537; p = 0.023) were identified as risk factors. Tumors with tumor proportion scores ≥1% had 5.5 times higher odds of microsatellite instability (95% CI, 1.492-20.278; p = 0.01). Similar results were seen with combined positive scores ≥1%, which had 4.258 times higher odds of microsatellite instability (95% CI, 1.066-17.012; p = 0.01) (**Table 4**).

For PD-L1 expression, we found lymphatic invasion significantly increased the OR to 12.608 (95% CI, 1.396-113.871; p = 0.024). Additionally, the Lauren classification again displayed a significant association, particularly the mixed type with an OR of 3.186 (95% CI, 1.001-10.146; p = 0.024).

In summary, our key findings were that pT3 invasion, intestinal type by Lauren classification, moderately differentiated tumors, presence of necrosis, high tumor-infiltrating lymphocytes, and tumors with TPS ≥ 1% and CPS ≥ 1% were potential predictive factors for microsatellite instability. Lymphatic invasion and mixed type by Lauren classification were most indicative of PD-L1 expression in gastric cancer.

Table 3. Associations of clinicopathologic features and PD-L1 expression in gastric cancer.

Characteristics		**Tumor proportion score, n (%)**		***p****	**Combined positive score, n (%)**		***p****
		<1%	≥1%		<1%	≥1%	
Age							
	≤60	26 (29.9)	11 (12.6)	0.673	19 (21.8)	18 (20.7)	0.667
	>60	33 (37.9)	17 (19.5)		28 (32.2)	22 (25.3)	
Sex							
	Female	18 (20.7)	9 (10.4)	0.878	16 (18.4)	11 (12.6)	0.643
	Male	41 (47.1)	19 (21.8)		31 (35.6)	29 (33.4)	
Clinical staging							
	I	5 (5.7)	0 (0)	0.342	3 (3.4)	2 (2.3)	0.873
	II	18 (20.7)	11 (12.6)		15 (17.2)	14 (16.1)	
	III	24 (27.6)	13 (14.9)		19 (21.8)	18 (20.7)	
	IV	12 (13.8)	4 (4.6)		10 (11.5)	6 (6.9)	
Tumor location							
	Fundus-body	8 (9.2)	6 (6.9)	0.218	6 (6.9)	8 (9.2)	0.639
	Antrum	37 (42.5)	12 (13.8)		28 (32.2)	21 (24.1)	
	Lesser curvature	14 (16.1)	10 (11.5)		13 (14.9)	11 (12.6)	
Gross appearance							
	Polypoid	7 (8.0)	6 (6.9)	0.432	4 (4.6)	9 (10.3)	0.340
	Fungating	9 (10.3)	6 (6.9)		9 (10.3)	6 (6.9)	
	Ulcerative	28 (32.2)	12 (13.8)		23 (26.4)	17 (19.5)	
	Infiltrative	15 (17.2)	4 (4.6)		11 (12.6)	8 (9.2)	
Tumor size							
	<5 cm	33 (37.9)	17 (19.5)	0.673	23 (26.4)	27 (31.0)	0.081
	≥5 cm	26 (29.9)	11 (12.6)		24 (27.6)	13 (15.0)	
Depth of tumor invasion							
	pT1	4 (4.6)	0 (0)	0.076	2 (2.3)	2 (2.3)	0.350
	pT2	29 (33.3)	10 (11.5)		19 (21.8)	20 (23.0)	
	pT3	16 (18.4)	15 (17.2)		16 (18.4)	15 (17.2)	
	pT4	10 (11.5)	3 (3.4)		10 (11.5)	3 (3.4)	

Table 3. (*Continued*).

Characteristics		**Tumor proportion score, n (%)**		***p****	**Combined positive score, n (%)**		***p****
		<1%	≥1%		<1%	≥1%	
Lauren classification							
	Intestinal type	27 (31.0)	11 (12.6)	0.544	19 (21.8)	19 (21.8)	0.033
	Diffuse type	23 (26.4)	10 (11.5)		23 (26.4)	10 (11.5)	
	Mixed type	9 (10.3)	7 (8.0)		5 (5.7)	11 (12.6)	
WHO classification							
	Tubular adenocarcinoma	30 (34.5)	13 (14.9)	0.489	22 (25.3)	21 (24.1)	0.945
	Mucinous carcinoma	7 (8.0)	4 (4.6)		6 (6.9)	5 (5.7)	
	Poorly cohesive carcinoma	14 (16.1)	4 (4.6)		10 (11.5)	8 (9.2)	
	Undifferentiated carcinoma	8 (9.2)	7 (8.0)		9 (10.3)	6 (6.9)	
Tumor differentiation status							
	Well	10 (11.5)	3 (3.4)	0.475	7 (8.0)	6 (6.9)	0.395
	Moderately	23 (26.4)	12 (13.8)		16 (18.4)	19 (21.8)	
	Poorly	26 (29.9)	13 (14.9)		24 (27.6)	15 (17.2)	
Tumor necrosis							
	Positive	11 (12.6)	5 (5.7)	0.929	10 (11.5)	6 (6.9)	0.451
	Negative	48 (55.2)	23 (26.4)		37 (42.5)	34 (39.1)	
Lymph node metastasis							
	Positive	36 (41.4)	14 (16.1)	0.332	29 (33.3)	21 (24.1)	0.387
	Negative	23 (24.6)	14 (16.1)		18 (20.7)	19 (21.8)	
Lymphatic invasion							
	Positive	1 (1.1)	5 (5.7)	0.005[#]	1 (1.1)	5 (5.7)	0.057
	Negative	58 (66.7)	23 (24.6)		46 (52.9)	35 (40.2)	
Perineural invasion							
	Positive	12 (13.8)	4 (4.6)	0.568[#]	9 (10.3)	7 (8.0)	0.843
	Negative	47 (54.0)	24 (27.6)		38 (43.7)	33 (37.9)	
Tumor-infiltrating lymphocyte							
	Low	36 (41.4)	20 (23.0)	0.343	17 (19.5)	14 (16.1)	0.910
	High	23 (26.4)	8 (9.2)		30 (34.5)	26 (29.9)	

n: number; WHO: World Health Organization; *p* values were determined using the (*) Chi-square test and (#) Fisher's exact test.

Table 4. Multivariate logistic regression analyses of the potential risk factors for microsatellite instability in gastric cancer.

Variables		Odds ratio	95% confidence interval	*p*
Depth of tumor invasion				
	pT1	1	-	-
	pT2	0.205	0.042-1.001	0.05
	pT3	7.227	1.785-29.249	0.006
	pT4	0.477	0.056-4.049	0.498
Lauren classification				
	Intestinal type	4.758	1.189-19.044	0.027
	Diffuse type	0.122	0.015-0.995	0.049
	Mixed type	0.871	0.171-4.427	0.868
Tumor differentiation status				
	Well	1	-	-
	Moderately	5.653	1.407-22.710	0.015
	Poorly	0.361	0.091-1.439	0.149
Tumor necrosis				
	Positive	10.267	2.682-39.304	0.001
	Negative	0.097	0.025-0.373	0.001
Tumor-infiltrating lymphocyte				
	Low	0.221	0.060-0.809	0.023
	High	4.522	1.236-16.537	0.023
Tumor proportion score				
	<1%	0.182	0.049-0.670	0.01
	≥1%	5.5	1.492-20.278	0.01
Combined positive score				
	< 1%	0.235	0.059-0.938	0.04
	≥ 1%	4.258	1.066-17.012	0.04

(-): omitted.

Table 5. Multivariate logistic regression analyses of the potential risk factors for PD-L1 expression in gastric cancer.

Variables		Odds ratio	95% confidence interval	*p*
Tumor proportion score				
Lymphatic invasion				
	Positive	12.608	1.396-113.871	0.024
	Negative	0.079	0.009-0.716	0.024
Combined positive score				
Lauren classification				
	Intestinal type	1.333	0.569-3.123	0.508
	Diffuse type	0.348	0.139-0.869	0.024
	Mixed type	3.186	1.001-10.146	0.050

4. Discussion

4.1. Clinicopathologic profile of gastric cancer in a regional population

Our study examined the demographics and clinicopathologic characteristics of 87 patients diagnosed with gastric cancer. We found that the majority of patients were male and over 60 years old with stage II–IV disease. The higher incidence of gastric cancer among older males aligns with epidemiologic data showing sex and age associations for this malignancy[21,22]. The preponderance of advanced stage III/IV disease mirrors prior reports and underscores the challenge of detecting gastric cancer early. However, the relative distribution across stages was slightly different than in some previous studies[23,24]. The advanced clinical stage likely reflects aggressive

tumor biology and ineffective early screening. The stage distribution provides insights into opportunities for improving early diagnosis.

The most frequent tumor location was the antrum (56.3%), followed by the lesser curvature (27.6%) and fundus/body (16.1%). Our finding of predominantly antral tumors is consistent with the established literature[25,26]. Some Western series have reported higher rates of proximal gastric cancers[27], contrasting with the lower incidence in our fundus/body cohort. The antral predilection implies a possible role of H. pylori infection, which characteristically causes antral-predominant gastritis. The rarity of cardia tumors argues against rising gastroesophageal junction cancers underlying the increase in gastric cancer in our population. These location patterns suggest geographically distinct gastric cancer subgroups that may benefit from targeted screening and prevention strategies.

The most frequent gross appearance was ulcerative (46.0%), followed by fungating (17.2%), infiltrative (21.9%), and polypoid (14.9%). Prior studies have reported ulcerative morphology as the most prevalent[28]. The high ulcerative rate implies an association with H. pylori-associated chronic gastritis. Over half of the tumors in our study were <5 cm (57.5%). However, the tumor size distribution differed somewhat from prior studies[29,30]. Tumor size correlates with depth of invasion and risk of nodal metastasis[30,31]. Smaller tumors may represent earlier-stage disease with better prognosis.

The depth of invasion was pT2 in 44.8%, pT3 in 35.6%, pT4 in 15.0%, and pT1 in 4.6% of cases. The predominance of pT2/pT3 tumors aligns with published data[32]. The high frequency of pT2 tumors reflects earlier-stage detection, though many patients still presented with locally advanced pT3/pT4 disease. The rarity of pT1 tumors indicates a need for improved endoscopic diagnosis. Earlier detection of pT1 cancers would increase eligibility for endoscopic resection.

Regarding histologic classification, the intestinal subtype was most common based on the Lauren system, while tubular adenocarcinoma was the predominant WHO subtype. Poorly differentiated tumors were frequent. The predominance of intestinal-type Lauren classification and tubular adenocarcinoma on WHO criteria agrees with the literature[33–36], but other studies have reported a higher prevalence of the diffuse type[37]. The high frequency of poorly differentiated tumors differed slightly from some reports[38]. The histologic subtypes suggest gastric cancer in our population may be somewhat more aggressive or advanced at diagnosis compared to other series based on the extent of poor differentiation. Tailoring treatment based on histologic classification could improve outcomes for certain subtypes. Understanding risk factors for undifferentiated tumors may elucidate prevention strategies.

Other pathologic features included frequent lymph node metastases, lower lymphatic/perineural invasion rates, and high tumor-infiltrating lymphocytes. Most tumors lacked MSI-H status. The pathologic characteristics were generally typical for gastric cancer. However, some findings, such as the proportion of MSI-H cases, differed slightly from other reports[39,40]. Subtle variations compared to prior studies may reflect distinctions in our regional population. However, the overall pathologic profile indicates a biologically aggressive disease. Understanding prognostic pathologic markers could enhance risk stratification and guide management. Tailoring adjuvant therapy based on features like MSI status may improve outcomes.

Overall, our findings confirm and extend the literature on gastric cancer patterns while identifying opportunities for screening, diagnosis, and tailored treatments. Further studies should clarify regional variations.

4.2. MSI-H as a biomarker defines a subset of gastric cancers with unique features

Our key findings demonstrate that microsatellite instability-high (MSI-H) significantly correlated with the depth of tumor invasion, Lauren classification, tumor differentiation, tumor necrosis, and immune markers

such as tumor-infiltrating lymphocytes, tumor proportion score, and combined positive score. We did not observe associations for demographic, gross, or metastatic factors.

These results align with and extend prior studies showing MSI-H was associated with Lauren subtypes[41–43], tumor necrosis[44], and immune cell infiltration in gastric cancer[45–47]. In our study, tumors with MSI-H were mainly in the pT2 stage. However, in other studies, MSI-H is related to pT3-pT4 tumors[48,49]. Thus, our findings suggest MSI-H predominated at the early invasion stage compared to others. Some studies have found that patients with MSI-H were associated with nodal metastasis[33,49,50], and that MSI-H links to older age and distal tumor location[44], which we did not observe in our sample. We did not find evidence directly linking MSI-H with tumor proportion score, even though MSI-H serves as a biomarker to match patients to PD-1 inhibitors[51]. However, in previous publications, MSI-H was associated with the combined positive score in gastric cancer[52–54]. The enrichment of MSI-H in early-stage type tumors suggests it may represent an alternate carcinogenic pathway in a subset of gastric cancers. The association with necrosis, lymphocytic reaction, and PD-L1 expression indicates MSI-H tumors provoke an active immune response.

Our findings imply that MSI-H identifies a distinct biomarker-defined subgroup of gastric cancer. Defining MSI-H gastric cancers could enable more personalized prognosis and treatment, as these patients may experience improved outcomes and differ in their response to chemotherapy and immunotherapy. Our results add to the characterization of MSI-H as an important biomarker in gastric cancer, possibly delineating a unique subtype. Further research on the prognostic and predictive roles of MSI-H is warranted to facilitate precision oncology approaches. However, additional studies with larger sample sizes are needed to confirm our findings and clarify the mechanisms linking MSI-H to gastric cancer characteristics.

4.3. PD-L1 expression in gastric cancer: Associations with lymphatic invasion and Lauren classification

Our study discovered that PD-L1 expression, as measured by TPS, was significantly associated with lymphatic invasion status. In contrast, PD-L1 expression, as measured by CPS, was significantly associated with Lauren classification. No other clinicopathologic factors showed significant relationships.

Our finding of an association between PD-L1 expression and lymphatic invasion aligns with prior studies showing higher PD-L1 expression in gastric cancers with lymphatic metastasis[55,56]. However, the link between Lauren classification and increased CPS has yet to be widely reported. One previous study found higher PD-L1 expression in intestinal compared to diffuse type gastric cancer[57], supporting our results.

The correlation between lymphatic invasion and elevated TPS suggests that PD-L1 upregulation may facilitate lymphatic spread in gastric cancer. The association between morphology and increased CPS indicates that PD-L1 expression differs based on Lauren classification, which may relate to distinct biological backgrounds between subtypes[58,59]. Our results confirm and extend prior knowledge about relationships between PD-L1 and clinicopathologic factors in gastric cancer.

These findings contribute to our understanding of PD-L1 expression patterns in gastric cancer. The links between PD-L1 and aggressive features like lymphatic invasion and morphology could help select patients likely to benefit from PD-1/PD-L1 inhibitor immunotherapy. Stratifying patients based on PD-L1 expression and clinicopathologic characteristics may improve outcomes.

Our results add evidence linking PD-L1 to more aggressive disease and support stratification by PD-L1 expression and clinicopathologic features. Further research with larger patient cohorts should investigate if these biomarkers can predict response to PD-1/PD-L1 blockade. Our findings suggest promise for personalized immunotherapy approaches in gastric cancer.

4.4. Clinicopathological predictors of microsatellite instability and PD-L1 expression in gastric cancer

In the present study, we conducted multivariate logistic regression analyses to uncover potential risk factors for microsatellite instability (MSI) and PD-L1 expression in gastric cancer. Our principal findings suggest that patients demonstrating pT3 invasion, Lauren classification-intestinal type, moderately differentiated tumors, presence of necrosis, high tumor-infiltrating lymphocytes, and tumors with TPS ≥ 1% and CPS ≥ 1% may exhibit an elevated risk for MSI. Likewise, patients presenting with lymphatic invasion and mixed type by Lauren classification potentially face a higher risk for PD-L1 expression.

Our results are in agreement with and extend upon several previous studies. Consistent with our findings, one research effort found an association between MSI and the intestinal Lauren histological type, reflected in an OR of 2.23 (95% CI, 1.94-2.57; $p < 0.001$)[60], implying the intestinal type of gastric cancer as a potential risk factor for MSI, this suggests that the intestinal type of gastric cancer may be a risk factor for MSI. Another study demonstrated a hazard ratio (HR) of 2.052 (95% CI, 1.211-3.477; $p = 0.008$) for PD-L1 expression of CPS ≥ 1 in the Lauren classification mixed vs. intestinal[61].

Interpreting these results, we propose that the significant association between pT3 invasion and MSI may imply a role of deeper tumor invasion in promoting genomic instability in gastric cancer. The correlation between intestinal type by Lauren classification and MSI could indicate distinct molecular underpinnings for this subtype of gastric cancer. The necrosis and high tumor-infiltrating lymphocytes may indicate an active immune response against the tumor, potentially contributing to MSI. Furthermore, the association between PD-L1 expression and lymphatic invasion suggests that upregulation of this immune checkpoint molecule may aid lymphatic spread in gastric cancer.

Our results could substantially affect clinical practice and the broader knowledge base. They provide fresh insights into potential risk factors for MSI and PD-L1 expression in gastric cancer and can help identify patients suitable for immunotherapy targeting these molecular pathways. Stratifying patients based on these biomarkers and clinicopathologic characteristics could enhance treatment outcomes.

4.5. Some limitations

Our study has limitations, including the small sample size from a single center. Our findings will require validation in larger, multicenter populations. We lacked detailed treatment data to correlate biomarkers with outcomes. Additionally, we did not assess PD1 expression, which would provide a more comprehensive evaluation of the tumor immune microenvironment along with PD-L1. Furthermore, due to the limited follow-up duration, we were unable to conduct survival analyses to examine associations between MSI status and clinical outcomes. Additional research should investigate the prognostic and predictive roles of MSI/PD-L1 and include analysis of both PD1 and PD-L1 expression to guide precision oncology approaches.

5. Conclusion

Our results delineate our population's clinicopathologic landscape of gastric cancer and identify opportunities to advance care through molecular characterization. We provide evidence that MSI-H marks a distinct subset of gastric cancer. PD-L1 expression is associated with aggressive parameters. Further research can clarify if biomarker-based stratification improves clinical outcomes. Our study underscores the promise of personalized medicine in this complex, heterogeneous disease.

Author contributions

Conceptualization, PVT and DTC; methodology, TND, VTT, NTC, and NTH; software, TDT; validation, DST, DTT, NMH, KVK and PXH; formal analysis, PVT and DTC; investigation, PVT and DTC; resources,

PVT; data curation, NVD and DTC; writing original draft preparation, PVT and DTC; writing review and editing, NVD and DTC; visualization, PVT; supervision, DTC; project administration, PVT. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* 2018; 68(6): 394–424. doi: 10.3322/caac.21492
2. Song Z, Wu Y, Yang J, et al. Progress in the treatment of advanced gastric cancer. *Tumour Biology* 2017; 39(7): 1010428317714626. doi: 10.1177/1010428317714626
3. Zepeda-Najar C, Xavier Palacios-Astudillo R, Chávez-Hernández JD, et al. Prognostic impact of microsatellite instability in gastric cancer. *Contemporary Oncology* 2021; 25(1): 68–71. doi: 10.5114/wo.2021.104939
4. Svenson N. Overcoming resistance in gastrointestinal cancers. Available online: https://www.targetedonc.com/view/overcoming-resistance-in-gastrointestinal-cancers (accessed on 25 August 2023).
5. Jin Y, Chen DL, Wang F, et al. The predicting role of circulating tumor DNA landscape in gastric cancer patients treated with immune checkpoint inhibitors. *Molecular Cancer* 2020; 19(1): 154. doi: 10.1186/s12943-020-01274-7
6. Eto S, Yoshikawa K, Nishi M, et al. Programmed cell death protein 1 expression is an independent prognostic factor in gastric cancer after curative resection. *Gastric Cancer* 2015; 19(2): 466–471. doi: 10.1007/s10120-015-0519-7
7. Sughayer MA, Dabbagh TZ, Battah AH. PD-L1 expression is a favorable prognostic marker in gastric carcinoma. *Applied Immunohistochemistry & Molecular Morphology* 2020; 28(10): 748–754. doi: 10.1097/pai.0000000000000834
8. Wu Y, Cao D, Qu L, et al. PD-1 and PD-L1 co-expression predicts favorable prognosis in gastric cancer. *Oncotarget* 2017; 8(38): 64066–64082. doi: 10.18632/oncotarget.19318
9. Yamashita K, Iwatsuki M, Harada K, et al. Prognostic impacts of the combined positive score and the tumor proportion score for programmed death ligand-1 expression by double immunohistochemical staining in patients with advanced gastric cancer. *Gastric Cancer* 2019; 23(1): 95–104. doi: 10.1007/s10120-019-00999-9
10. Pereira MA, Ramos MFKP, Dias AR, et al. Scoring systems for PD-L1 expression and their prognostic impact in patients with resectable gastric cancer. *Virchows Archiv* 2020; 478(6): 1039–1048. doi: 10.1007/s00428-020-02956-9
11. Brierley JD, Gospodarowicz MK, Wittekind C. Skin tumours. *TNM Online* 2017; 131–132. doi: 10.1002/9780471420194.tnmc26.pub3
12. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer* 2011; 14(2): 101–112. doi: 10.1007/s10120-011-0041-5
13. Laurén P. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. *Acta Pathologica Microbiologica Scandinavica* 1965; 64(1): 31–49. doi: 10.1111/apm.1965.64.1.31
14. Nagtegaal ID, Odze RD, Klimstra D, et al. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2019; 76(2): 182–188. doi: 10.1111/his.13975
15. Tumor grade. Available online: https://www.cancer.gov/about-cancer/diagnosis-staging/diagnosis/tumor-grade (accessed on 30 October 2023).
16. Fuchs TL, Sioson L, Sheen A, et al. Assessment of Tumor-infiltrating Lymphocytes Using International TILs Working Group (ITWG) System Is a Strong Predictor of Overall Survival in Colorectal Carcinoma: A Study of 1034 Patients. *The American Journal of Surgical Pathology*. 2020; 44(4):536–544.
17. Kang YK, Boku N, Satoh T, et al. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; 390(10111): 2461–2471. doi: 10.1016/S0140-6736(17)31827-5
18. Fuchs CS, Doi T, Jang RW, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: Phase 2 clinical KEYNOTE-059 trial. *JAMA Oncology* 2018; 4(5): e180013. doi: 10.1001/jamaoncol.2018.0013
19. Umar A. RESPONSE: Re: Revised bethesda guidelines for hereditary nonpolyposis colorectal cancer (lynch syndrome) and microsatellite instability. *JNCI Journal of the National Cancer Institute* 2004; 96(18): 1403–1404. doi: 10.1093/jnci/djh281
20. Bateman AC. DNA mismatch repair proteins: Scientific update and practical guide. *Journal of Clinical Pathology*

2021; 74(4): 264–268. doi: 10.1136/jclinpath-2020-207281
21. Lou L, Wang L, Zhang Y, et al. Sex difference in incidence of gastric cancer: An international comparative study based on the Global Burden of Disease Study 2017. *BMJ Open* 2020; 10(1): e033323. doi: 10.1136/bmjopen-2019-033323
22. Wong MCS, Huang J, Chan PSF, et al. Global incidence and mortality of gastric cancer, 1980–2018. *JAMA Netw Open* 2021; 4(7): e2118457. doi: 10.1001/jamanetworkopen.2021.18457
23. Wanebo HJ, Kennedy BJ, Chmiel J, et al. Cancer of the stomach. A patient care study by the American College of Surgeons. *Annals of Surgery* 1993; 218(5): 583–592. doi: 10.1097/00000658-199321850-00002
24. Zhong N, Yu Y, Chen J, et al. Clinicopathological characteristics, survival outcome and prognostic factors of very young gastric cancer. *Clinical and Experimental Medicine* 2022; 23(2): 437–445. doi: 10.1007/s10238-022-00822-3
25. Kim K, Cho Y, Sohn JH, et al. Clinicopathologic characteristics of early gastric cancer according to specific intragastric location. *BMC Gastroenterology* 2019; 19(1): 24. doi: 10.1186/s12876-019-0949-5
26. Tsurumaru D, Miyasaka M, Nishimuta Y, et al. Differentiation of early gastric cancer with ulceration and resectable advanced gastric cancer using multiphasic dynamic multidetector CT. *European Radiology* 2015; 26(5): 1330–1337. doi: 10.1007/s00330-015-3938-2
27. Crew KD, Neugut AI. Epidemiology of gastric cancer. *World Journal of Gastroenterology* 2006; 12(3): 354–362. doi: 10.3748/wjg.v12.i3.354
28. Lee YJ, Kim JH, Park JJ, et al. The implications of endoscopic ulcer in early gastric cancer: Can we predict clinical behaviors from endoscopy? *PLoS One* 2016; 11(10): e0164339. doi: 10.1371/journal.pone.0164339
29. Guo P, Li Y, Zhu Z, et al. Prognostic value of tumor size in gastric cancer: An analysis of 2,379 patients. *Tumour Biology* 2013; 34(2): 1027–1035. doi: 10.1007/s13277-012-0642-6
30. Chen Y, Jia Y, Peng Z, Wang G. The prognostic role of tumor size in stage T1 gastric cancer. *World Journal of Surgical Oncology* 2022; 20(1): 135. doi: 10.1186/s12957-022-02596-0
31. Ren MH, Qi XS, Chu YN, et al. Risk of lymph node metastasis and feasibility of endoscopic treatment in ulcerative early gastric cancer. *Annals of Surgical Oncology* 2020; 28(4): 2407–2417. doi: 10.1245/s10434-020-09153-7
32. Bilici A, Dane F, Seker M, et al. Is subdivision of pT2 tumors superior to lymph node metastasis for predicting survival of patients with gastric cancer? Review of 224 patients from four centers. *Digestive Diseases and Sciences* 2011; 56(11): 3226–3234. doi: 10.1007/s10620-011-1721-z
33. Kim DG, An JY, Kim H, et al. Clinical implications of microsatellite instability in early gastric cancer. *Journal of Gastric Cancer* 2019; 19(4): 427–437. doi: 10.5230/jgc.2019.19.e38
34. An JY, Kim H, Cheong J, et al. Microsatellite instability in sporadic gastric cancer: Its prognostic role and guidance for 5-FU based chemotherapy after R0 resection. *International Journal of Cancer* 2011; 131(2): 505–511. doi: 10.1002/ijc.26399
35. Hu B, Hajj NE, Sittler S, et al. Gastric cancer: Classification, histology and application of molecular pathology. *Journal of Gastrointestinal Oncology* 2012; 3(3): 251–261. doi: 10.3978/j.issn.2078-6891.2012.021
36. Berlth F. Pathohistological classification systems in gastric cancer: Diagnostic relevance and prognostic value. *World Journal of Gastroenterology* 2014; 20(19): 5679–5684. doi: 10.3748/wjg.v20.i19.5679
37. Assumpção PP, Barra WF, Ishak G, et al. The diffuse-type gastric cancer epidemiology enigma. *BMC Gastroenterology* 2020; 20(1): 223. doi: 10.1186/s12876-020-01354-4
38. Feng F, Liu J, Wang F, et al. Prognostic value of differentiation status in gastric cancer. *BMC Cancer* 2018; 18(1): 865. doi: 10.1186/s12885-018-4780-0
39. Guan WL, Ma Y, Cui YH, et al. The impact of mismatch repair status on prognosis of patients with gastric cancer: A multicenter analysis. *Frontiers in Oncology* 2021; 11: 712760. doi: 10.3389/fonc.2021.712760
40. Ott PA, Le DT, Kim JW, et al. Nivolumab (NIVO) in patients (pts) with advanced (adv) chemotherapy-refractory (CT-Rx) esophagogastric (EG) cancer according to microsatellite instability (MSI) status: checkmate 032. *Annals of Oncology* 2017; 28: v229–v230. doi: 10.1093/annonc/mdx369.058
41. Nakashima H, Honda M, Inoue H, et al. Microsatellite instability in multiple gastric cancers. *International Journal of Cancer* 1995; 64(4): 239–242. doi: 10.1002/ijc.2910640405
42. Falchetti M, Saieva C, Lupi R, et al. Gastric cancer with high-level microsatellite instability: Target gene mutations, clinicopathologic features, and long-term survival. *Human Pathology* 2008; 39(6): 925–932. doi: 10.1016/j.humpath.2007.10.024
43. Jahng J. Endoscopic and clinicopathologic characteristics of early gastric cancer with high microsatellite instability. *World Journal of Gastroenterology* 2012; 18(27): 3571. doi: 10.3748/wjg.v18.i27.3571
44. Kim JY, Shin NR, Kim A, et al. Microsatellite instability status in gastric cancer: A reappraisal of its clinical significance and relationship with mucin phenotypes. *Korean Journal of Pathology* 2013; 47(1): 28–35. doi: 10.4132/koreanjpathol.2013.47.1.28
45. Grogg KL, Lohse CM, Pankratz VS, et al. Lymphocyte-rich gastric cancer: Associations with epstein-barr virus, microsatellite instability, histology, and survival. *Modern Pathology* 2003; 16(7): 641–651. doi: 10.1097/01.mp.0000076980.73826.c0

46. Mansuri N, Birkman EM, Heuser VD, et al. Association of tumor-infiltrating T lymphocytes with intestinal-type gastric cancer molecular subtypes and outcome. *Virchows Arch* 2020; 478(4): 707–717. doi: 10.1007/s00428-020-02932-3
47. Choi E, Chang MS, Byeon SJ, et al. Prognostic perspectives of PD-L1 combined with tumor-infiltrating lymphocytes, Epstein-Barr virus, and microsatellite instability in gastric carcinomas. *Diagnostic Pathology* 2020; 15(1): 69. doi: 10.1186/s13000-020-00979-z
48. Polom K, Marrelli D, Roviello G, et al. PIK3CA mutation in gastric cancer and the role of microsatellite instability status in mutations of exons 9 and 20 of the PIK3CA gene. *Advances in Clinical and Experimental Medicine* 2018; 27(7): 963–969. doi: 10.17219/acem/70795
49. Quaas A, Biesma HD, Wagner AD, et al. Microsatellite instability and sex differences in resectable gastric cancer—A pooled analysis of three European cohorts. *European Journal of Cancer* 2022; 173: 95–104. doi: 10.1016/j.ejca.2022.06.025
50. Zhu L, Li Z, Wang Y, et al. Microsatellite instability and survival in gastric cancer: A systematic review and meta-analysis. *Molecular and Clinical Oncology* 2015; 3(3): 699–705. doi: 10.3892/mco.2015.506
51. Kim ST, Cristescu R, Bass AJ, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nature Medicine* 2018; 24(9): 1449–1458. doi: 10.1038/s41591-018-0101-z
52. Wang L, Zhang Q, Ni S, et al. Programmed death-ligand 1 expression in gastric cancer: Correlation with mismatch repair deficiency and HER2-negative status. *Cancer Medicine* 2018; 7(6): 2612–2620. doi: 10.1002/cam4.1502
53. Wang YL, Gong Y, Lv Z, et al. Expression of PD1/PDL1 in gastric cancer at different microsatellite status and its correlation with infiltrating immune cells in the tumor microenvironment. *Journal of Cancer* 2021; 12(6): 1698–1707. doi: 10.7150/jca.40500
54. Yoshida T, Ogura G, Tanabe M, et al. Clinicopathological features of PD-L1 protein expression, EBV positivity, and MSI status in patients with advanced gastric and esophagogastric junction adenocarcinoma in Japan. *Cancer Biology & Therapy* 2022; 23(1): 191–200. doi: 10.1080/15384047.2022.2038002
55. Haraguchi N, Arigami T, Uenosono Y, et al. Clinical significance of primary tumor score determined by tumor depth and size in patients with resectable gastric cancer. Oncotarget. 2018; 9(9): 8512–8520. doi: 10.18632/oncotarget.23953
56. Saito H, Kono Y, Murakami Y, et al. Highly activated PD-1/PD-L1 pathway in gastric cancer with PD-L1 expression. *Anticancer Research* 2018; 38(1): 107–112. doi: 10.21873/anticanres.12197
57. Kim DH, Bae GE, Suh KS, et al. Clinical significance of tumor and immune cell PD-L1 expression in gastric adenocarcinoma. *In Vivo* 2020; 34(6): 3171–3180. doi: 10.21873/invivo.12152
58. Tan IB, Ivanova T, Lim KH, et al. Intrinsic subtypes of gastric cancer, based on gene expression pattern, predict survival and respond differently to chemotherapy. *Gastroenterology* 2011; 141(2): 476–485.e11. doi: 10.1053/j.gastro.2011.04.042
59. Pereira MA, Ramos MFKP, Dias AR, Ribeiro R, Cardili L, Zilberstein B, et al. Scoring systems for PD-L1 expression and their prognostic impact in patients with resectable gastric cancer. Virchows Archiv. 2021;478(6):1039–1048.
60. Polom K, Marano L, Marrelli D, et al. Meta-analysis of microsatellite instability in relation to clinicopathological characteristics and overall survival in gastric cancer. *British Journal of Surgery* 2017; 105(3): 159–167. doi: 10.1002/bjs.10663
61. Chen X, Zhang H, Wang M, et al. Relationship between programmed death ligand 1 expression and other clinicopathological features in a large cohort of gastric cancer patients. *Frontiers in Immunology* 2022; 13: 783695. doi: 10.3389/fimmu.2022.783695