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Original Article

The Expression of Programmed Death-Ligand 1 on Immune Cells Is Related to a Better Prognosis in Biliary Tract Cancer

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Background/Aims: Programmed death-ligand 1 (PD-L1) expression in tumor cells is associated with a poor biliary tract cancer (BTC) prognosis; tumor-infiltrating immune cells in the tumor microenvironment are associated with a better prognosis. The effect of PD-L1 expression on immune cells on survival is unclear. We investigated the relationship between PD-L1 expression in immune cells and BTC prognosis.

Methods: PD-L1 expression was evaluated using an anti-PD-L1 22C3 mouse monoclonal primary antibody, and its relationships with clinical characteristics and prognosis were analyzed using the Cox proportional hazard model to investigate the prognostic performance of PD-L1 in BTC.

Results: Among 144 analyzed cases, patients with positive PD-L1 expression in tumor cells and negative PD-L1 expression in immune cells showed poorer overall survival rates than those exhibiting other expressions (tumor cells: hazard ratio [HR]=1.023, p<0.001; immune cells: HR=0.983, p=0.021). PD-L1 expression in tumor cells was an independent predictor of poor overall survival (HR=1.024, p<0.001). In contrast, PD-L1 expression in immune cells was a predictive marker of good prognosis (HR=0.983, p=0.018).

Conclusions: PD-L1 expression in immune cells may be used as an independent factor to evaluate the prognosis of patients with BTC. **(Gut Liver, Published online December 13, 2022)**

Key Words: Biliary tract neoplasms; Prognosis; Survival

INTRODUCTION

Biliary tract cancer (BTC) is a well-known malignancy due to its high mortality rate and limited treatment. Only approximately 20% of patients are diagnosed at resectable stages. The 5-year overall survival (OS) rate is 16% to 30% depending on its origin, and the median survival duration is approximately 8 to 16 months.[1] Therefore, it is important to identify a robust prognostic biomarker for BTC.

Recently, breakthrough advances in treatment have been accomplished by the discovery of effective immune checkpoint inhibitor (ICI) therapy in various types of cancers.[2] Anti-programmed cell death protein 1/programmed death-ligand 1 (PD-L1) agents have been approved as standard treatments for solid tumors, including BTC.[3-5] Previous phase I/II trials, KEYNOTE-028 and KEYNOTE-158, showed modest pembrolizumab efficacy in patients with PD-L1-positive advanced BTC.[6]

PD-L1 expression has been reported in 8.5% to 83% of patients with BTC.[7-9] Dong *et al.*[10] showed an association between PD-L1 expression and poor prognosis in BTC. High PD-L1 expression was associated with poor OS in patients with BTC. In contrast, Sangkhamanon *et al.*[11] reported that PD-L1 expression did not seem to be associated with patient prognosis in BTC. However, most previous studies used surgical specimens for PD-L1 expression; therefore, their results were insufficient for generalization in most BTC patients. Furthermore, tumors are composed of immune as well as tumor cells. While the existence of tumor-infiltrating immune cells in the tumor microen-

vironment is associated with a better prognosis in other types of cancers, the importance of PD-L1 expression in immune cells is still unclear in BTC.[12,13]

Here, we aimed to investigate the significance of PD-L1 expression in tumor and immune cells in the prognosis of BTC patients to determine its possible role as a prognostic biomarker.

MATERIALS AND METHODS

1. Patient selection

We enrolled patients with pathologically confirmed BTC from January 1, 2017, to June 30, 2020, at Severance Hospital, South Korea. The recorded characteristics included age, sex, Eastern Cooperative Oncology Group performance score at the time of diagnosis, hypertension history, diabetes mellitus, liver cirrhosis, curative-intent operation history, chemotherapy type, laboratory results including levels of carbohydrate antigen 19-9, carcinoembryonic antigen, hepatitis B surface antigen, and histopathological results including cancer origin, pathological tumor-node-metastasis stage, tumor size, and the proportion of PD-L1 expression on tumor and immune cells. Tumor location was classified into the following three categories according to the National Comprehensive Cancer Network guidelines: intrahepatic, extrahepatic, and gallbladder. Tumors arising from the intrahepatic duct to the second-order branch of hepatic ducts are classified as intrahepatic BTC, and those from the perihilar area to distal common bile duct are classified as extrahepatic BTC. The tumor stages were divided according to American Joint Committee on Cancer eighth edition. OS was defined as the duration from the date of diagnosis until death from any cause. Recurrence-free survival (RFS) was defined as the duration from the operation day to time of proven recurrence of tumor via image or biopsy study. Progression-free survival (PFS) was defined as duration from start of the chemotherapy– either gemcitabine-based chemotherapy (PFS-G), or ICI (PFS-I)–to time of proven progression of tumor or death of the patient. This study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Boards of Severance Hospital (IRB number: 4-2021-1148). The informed consent was waived.

2. Definition of treatment modality

Patients received three treatment modalities, not necessarily mutually exclusive: surgical treatment, palliative gemcitabine-based chemotherapy, and ICI therapy. Patients with BTC of operable stage and performance status feasible for operation underwent surgical treatment for the first-line treatment. All patients who underwent operation received postoperative adjuvant gemcitabine-based chemotherapy. Patients with inoperable stage of BTC or those with performance status not feasible for operation or those who recurred after operation received gemcitabine-based chemotherapy as first-line treatment. Patients who recurred after operation or gemcitabine-based chemotherapy showed progression of disease received ICI therapy. Any therapy administered at least one cycle were all defined as the therapy received.

3. Immunohistochemical analysis for PD-L1

Tissue was acquired by tissue biopsy or surgical resection. Mission™ disposable biopsy forceps (catalog number, 1610MS; BD, Becton, NJ, USA) for percutaneous biopsy, EndoJaw™ disposable biopsy forceps (catalog number, FB-231K; Olympus, Tokyo, Japan) for endoscopic retrograde cholangiopancreatography biopsy, and EchoTip ProCore® HD Ultrasound Biopsy Needle (catalog number, G53585; Cook, Bloomington, IN, USA) or Acquire® 22-gauge FNB needle (Boston Scientific Co., Natick, MA, USA) for endoscopic ultrasound biopsy were used. PD-L1 expression was evaluated using an anti-PD-L1 22C3 (catalog number, SK006; Merck & Co., Inc., Kenilworth, NJ, USA) mouse monoclonal primary antibody (Dako, Santa Clara, CA, USA). Four-micrometer-thick sections of formalin-fixed, paraffin-embedded tissue blocks were immunostained with the EnVision FLEX visualization system on a Dako Autostainer Link 48 platform (Dako, Carpinteria, CA, USA) according to the manufacturer's instructions. The slides were then reviewed by two experienced pathologists (E.P. and Y.N.P.) in a blinded manner. Between the two pathologists, significantly different interobserver variation was not identified, and in few cases with subtle discrepancies, a consensus was reached between two pathologists. As the size of tumor tissues were different according to the specimen types, different number of high-power fields were evaluated between surgical and biopsy specimens; approximately 10 to 20 high-power fields for biopsy specimens and more than 30 high-power fields for surgical specimens. The tumor cells were considered PD-L1 positive if the viable tumor cells exhibited any perceptible membranous staining alone or membranous and cytoplasmic staining. For immune cells, any membranous or cytoplasmic PD-L1 staining mononuclear inflammatory cells (lymphocytes and macrophages) within tumor nests and/or immediately adjacent supporting stroma were counted for scoring. PD-L1 expression was measured using the tumor proportion score (TPS) and immune proportion score (IPS). TPS was defined as the percentage of viable

tumor cells showing partial or complete membrane PD-L1 staining at any intensity. IPS was defined as the number of immune cells with PD-L1 expression in the total tumor cells.[14]

4. Statistical analysis

Statistical analysis was performed using SPSS version 25 (IBM Corp., Armonk, NY, USA) and R programming language and R-Studio (v.1.4.1106; Affero General Public License, Boston, MA, USA). Categorical values were compared using the chi-square test. Differences were considered statistically significant at p-values less than 0.05. We used the Cox proportional hazards model analysis to determine the hazard of each patient and pathological factors on OS. Multivariate analysis was performed, including PD-L1 expression on the cells, clinicopathological characteristics including age, sex, types of chemotherapy administered, and cancer origin. Multicollinearity test was done for the covariates. Additional analysis for RFS, PFS-G, and PFS-I were also done with the same covariant. We determined the cutoff value for TPS and IPS that best differentiated the OS for each group of patients using the Contal and O'Quigley method.[15] This was accomplished using the R package survMisc (v. 0.5.5; R Foundation for Statistical Computing, Vienna, Austria). The log-rank test was used to graph the survival curve for the evaluated cutoff value for each score and to determine the statistical significance for each case.

RESULTS

1. Baseline characteristics

The baseline characteristics of the 144 patients are presented in Table 1. The median age of the patients was 63 years (range, 24 to 85 years). There were 78 patients diagnosed with stage IV disease and 52 patients who underwent curative-intent surgery. Out of 144 total patients, 28 patients received second-line treatment. ICIs were administered primarily according to the expression of PD-L1, regardless of cell type. All ICIs administered was pembrolizumab. One patient received ICI treatment even though there was no PD-L1 expression in neither of the cells. All other patients had to have at least one of the scores positive to receive the treatment. A total of 40 patients received ICI chemotherapy. Out of 40 patients who had ICI, 12 received the therapy as first line agent. Remaining 28 had gemcitabine-based chemotherapy as first line agent. Out of 94 cases where the tissue was acquired by biopsy, most of them were from liver with 57 cases. Biopsy site and average biopsy tissue count is shown in Supplementary Table 1.

In total, 74 patients had PD-L1 expression in their tumor cells, and 85 had it in their immune cells. The 104 out of 144 showed positive expression of PD-L1 in tumor cells or immune cells combined and remaining 40 showed no expression in the tissue at all.

The baseline characteristics of PD-L1 expression in tumor and immune cells are shown in Table 2. All baseline characteristics showed no significant difference within the

Table 1. Baseline Characteristics of Patients with Biliary Tract Cancer

Variable	Value (n=144)
Demographic variable	
Age, yr	63 (24–85)
Male sex	79 (54.9)
Liver cirrhosis	3 (2.1)
Hypertension	44 (30.6)
Diabetes mellitus	49 (34.0)
ECOG score	
0-1	136 (94.4)
2	7 (4.9)
3-4	1 (0.7)
Laboratory variable	
CA19-9, U/mL	122 (0.6–20,000)
CEA, ng/mL	2.87 (0.46–14,359)
Hepatitis B carrier	4 (2.8)
Tumor variable	
Cancer origin	
Intrahepatic duct	54 (37.5)
Gallbladder	38 (26.4)
Extrahepatic duct	52 (36.1)
Tumor stage*	
I	7 (4.9)
II	29 (20.1)
III	30 (20.8)
IV	78 (54.2)
Tumor size, cm	4 (0.3–16)
Surgery	52 (36.1)
Chemotherapy	
Gemcitabine-based chemotherapy	109 (75.7)
Immune checkpoint inhibitor	40 (27.8)
Tissue acquisition	
Surgery	50 (34.7)
Biopsy	94 (65.3)
Liver	57 (39.6)
Bile duct	15 (10.4)
Lymph node	7 (4.9)
Gallbladder	5 (3.5)
Other	10 (6.9)
PD-L1 expression	
Tumor proportion score (+)	74 (51.4)
Immune proportion score (+)	85 (59.0)

Data are presented as median (range) or number (%).
ECOG, Eastern Cooperative Oncology Group; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; PD-L1, programmed death-ligand 1.
*Tumor stage was defined according to the eighth edition of the American Joint Committee on Cancer staging system.

groups, except for those who had undergone surgical treatment and exhibited higher PD-L1 expression in immune cells. Expression of PD-L1 on tumor cells and immune cells were shown to be correlated to each other. Those with PD-L1 expression present in tumor cells, were also likely to have it in immune cells, and vice versa in negative cases (p<0.001). Most TPS and IPS of the patients were below 10 (Fig. 1). Median values of TPS and IPS were both 1, and mean were 12.2 and 7.0, respectively. Notable tissue samples with high PD-L1 expression in tumor and immune cells are shown in Fig. 2. TPS expression by tumor origin and tissue acquisition method are shown in Supplementary Table 2. The expression did not differ by tissue acquisition method, but there was significant difference of PD-L1 expression on tumor cells depending on tumor origin such that expression is in increasing intensity by extrahepatic origin, gallbladder origin, intrahepatic origin order.

2. PD-L1 expression and multivariate analysis

The correlation between OS and PD-L1 expression and covariance were evaluated using the Cox proportional hazard method, and the results are shown in Table 3. Univariate analysis showed that higher Eastern Cooperative Oncology Group score, carbohydrate antigen 19-9 greater or equal to 35, cancer from gallbladder origin, higher stage and higher TPS were associated with poor OS, while high IPS were associated with better OS. Multivariate analysis showed the same results as the univariate analysis in overall tendency and in significance. ICI therapy did not satisfy statistical significance in its association with OS, but it showed tendency of having better OS in both univariate and multivariate analysis. The multicollinearity test was performed for all covariates, and two variables, surgical resection and tumor stage, had variance inflation factor greater than 2 (Supplementary Table 3). When PD-L1 expression was evaluated against RFS, gemcitabine-based chemotherapy was associated with better RFS (hazard ratio [HR], 0.244; 95% confidence interval [CI], 0.115 to 0.514; p<0.001), and high TPS was associated with worse RFS (HR, 1.032; 95% CI, 1.011 to 1.053; p=0.003). When PD-L1 expression was evaluated against PFS-G, high TPS was associated with worse PFS-G (HR, 1.012; 95% CI, 1.005

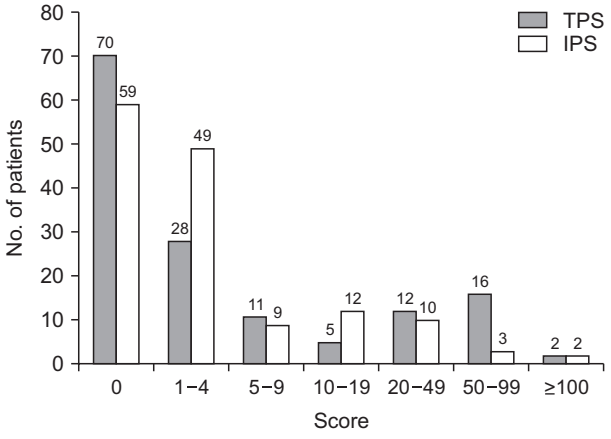

Fig. 1. Number of patients with programmed death-ligand 1 expression in tumor cells and tumor-infiltrating immune cells.
TPS, tumor proportion score; IPS, immune proportion score.

Table 2. Baseline Characteristics According to PD-L1 Expression

Characteristic	PD-L1 expression on tumor cell			PD-L1 expression on immune cell		
	Positive (n=74)	Negative (n=70)	p-value	Positive (n=85)	Negative (n=59)	p-value
Male sex	42 (56.8)	37 (52.9)	0.638	49 (57.6)	30 (50.8)	0.420
Age ≥65 yr	35 (47.3)	31 (44.3)	0.717	40 (47.1)	26 (44.1)	0.723
ECOG score ≥2	3 (4.1)	5 (7.1)	0.419	4 (4.7)	4 (6.8)	0.593
CA19-9 ≥35 U/mL	53 (71.6)	46 (65.7)	0.445	59 (69.4)	40 (67.8)	0.837
Cancer origin			0.216			0.624
Intrahepatic duct	28 (37.8)	24 (34.3)		28 (32.9)	24 (40.7)	
Gallbladder	23 (31.1)	15 (21.4)		24 (28.2)	14 (23.7)	
Extrahepatic duct	23 (31.1)	31 (44.3)		33 (38.8)	21 (35.6)	
Tumor size ≥4 cm	34 (45.9)	36 (51.4)	0.511	44 (51.8)	26 (44.1)	0.363
Tumor stage			0.066			0.551
I	1 (1.4)	6 (8.6)		4 (4.7)	3 (5.1)	
II	18 (24.3)	11 (15.7)		19 (22.4)	10 (16.9)	
III	12 (16.2)	18 (25.7)		20 (23.5)	10 (16.9)	
IV	43 (58.1)	35 (50.0)		42 (49.4)	36 (61.0)	
Surgery	25 (33.8)	27 (38.6)	0.550	36 (42.4)	16 (27.1)	0.061
Gemcitabine-based chemotherapy	54 (73.0)	55 (78.6)	0.434	64 (75.3)	45 (76.3)	0.893
Immune checkpoint inhibitor therapy	29 (39.2)	11 (15.7)	0.002	31 (36.5)	9 (15.3)	0.005

Data are presented as number (%).
PD-L1, programmed death-ligand 1; ECOG, Eastern Cooperative Oncology Group; CA19-9, carbohydrate antigen 19-9.

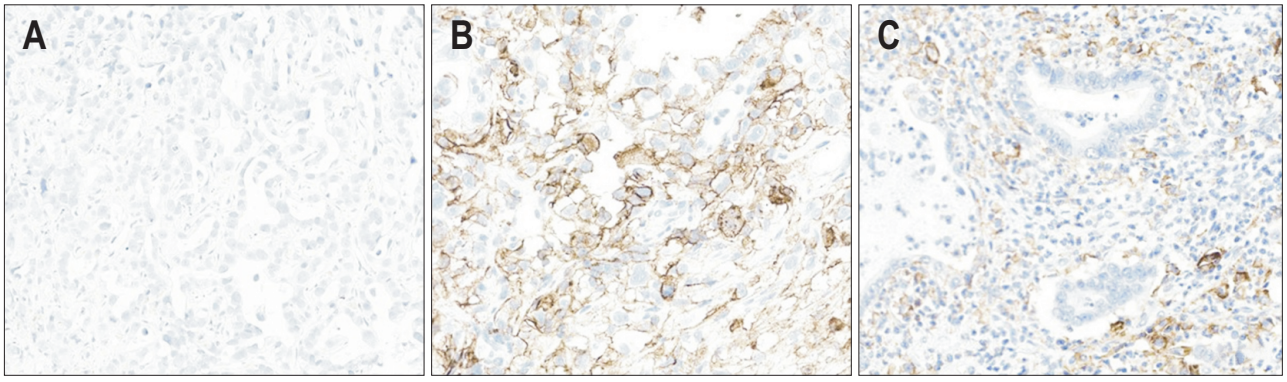

Fig. 2. Immunohistochemistry of PD-L1 expression in tumor and immune cells viewed under a light microscope (×200). (A) No expression of PD-L1 in tumor and immune cells (TPS 0, IPS 0). (B) High expression of PD-L1 in tumor cells (TPS 80%). (C) High expression of PD-L1 in immune cells (IPS 150).
PD-L1, programmed death-ligand 1, TPS, tumor proportion score; IPS, immune proportion score.

Table 3. Univariate and Multivariate Analysis for Overall Survival of Biliary Tract Cancer Patient

Overall survival	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Male sex	1.272 (0.839–1.928)	0.257		
Age ≥65 yr	1.267 (0.845–1.900)	0.251		
ECOG score	3.099 (1.442–6.657)	0.004	3.115 (1.463–6.630)	0.003
CA19-9 ≥35 U/mL	1.615 (1.031–2.530)	0.036	1.634 (1.049–2.546)	0.030
Cancer origin		0.005		0.011
Intrahepatic duct	1.000		1.000	
Gallbladder	2.636 (1.426–4.871)		2.436 (1.336–4.439)	
Extrahepatic duct	1.515 (0.824–2.784)		1.541 (0.849–2.796)	
Stage		0.002		0.001
I	1.000		1.000	
II	0.911 (0.259–3.205)		0.950 (0.273–3.305)	
III	1.804 (0.510–6.380)		1.830 (0.522–6.417)	
IV	2.996 (0.862–10.410)		3.013 (0.879–10.334)	
Gemcitabine-based chemotherapy	1.079 (0.661–1.762)	0.761		
Immune checkpoint inhibitor therapy	0.652 (0.413–1.028)	0.066	0.656 (0.417–1.031)	0.068
Tumor proportion score	1.023 (1.014–1.033)	<0.001	1.024 (1.015–1.034)	<0.001
Immune proportion score	0.983 (0.968–0.997)	0.021	0.983 (0.969–0.997)	0.018

HR, hazard ratio; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; CA19-9, carbohydrate antigen 19-9.

to 1.020; p=0.001). PFS-I was associated only with cancer origin (Supplementary Tables 4-6).

3. PD-L1 expression and survival plot

We used the Contal and O'Quigley method to find the cutoff values of TPS and IPS that emphasized survival difference from the maximum. Based on the statistics, the cutoff values for each score were TPS 20 and IPS 0. We performed a Kaplan-Meier survival analysis for each cutoff value (Fig. 3). A significant difference in OS between the groups separated by the selected cutoff values for each score was observed. For example, when patients were separated by a TPS with a cutoff value of 25, the two groups had maximal survival difference (median OS 17.6 months vs 6.2 months, p<0.001). For IPS, patients with no expression showed worse OS than those who did (median OS 10.5 months vs 18.8 months, p=0.018). For TPS, most of the other cutoff values resulted in significant survival differences between groups, while there was no other cutoff value for IPS that separated the groups such that they had significant survival differences other than 0. TPS and IPS with aforementioned cutoff values had shown to have same prognostic value as continuous variable when applied to Cox multivariate analysis compared with Table 3 and

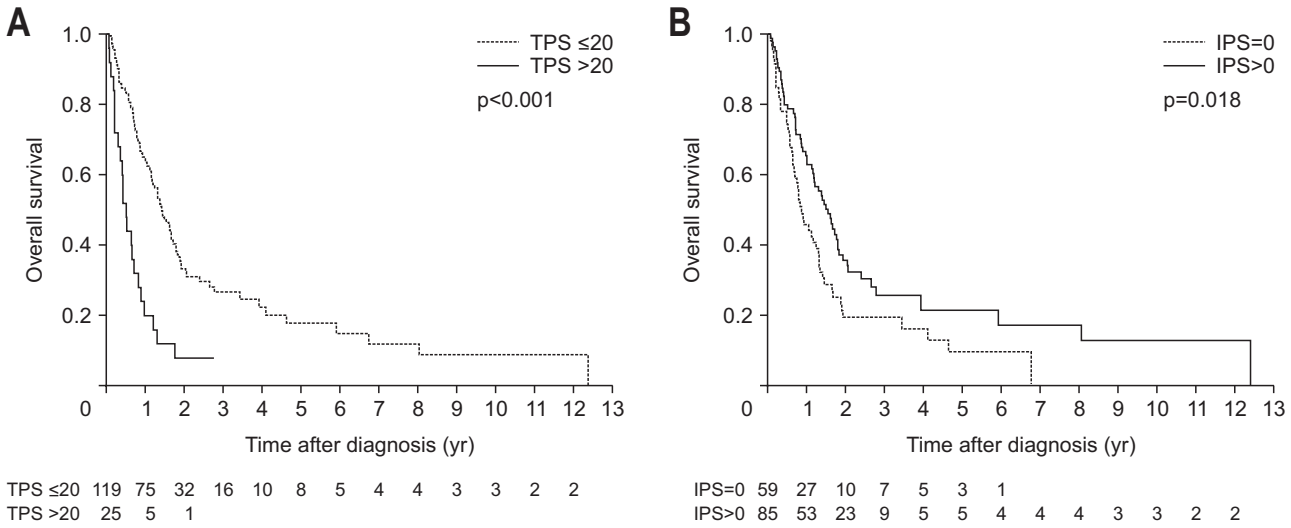

Fig. 3. Kaplan-Meier survival curve for each PD-L1 expression cutoff value. (A) High TPS is associated with a poor prognosis (median overall survival: 17.6 months vs 6.2 months, p<0.001). (B) High IPS is associated with a better prognosis (median overall survival: 10.5 months vs 18.8 months, p=0.018).
PD-L1, programmed death-ligand 1; TPS, tumor proportion score; IPS, immune proportion score.

Supplementary Table 7. TPS as continuous variable also showed significant correlation with worse OS (Supplementary Fig. 1).

DISCUSSION

In the present study, PD-L1 expression in immune cells was associated with a better prognosis in patients with BTC. It has been shown that the prognosis of BTC is affected not only by PD-L1 expression itself but also by the site of its expression in the tissue. Previously, PD-L1 expression in BTC has been investigated in several studies regarding its correlation with OS and tumor characteristics. Dong *et al.*[10] reported that PD-L1 expression in tumor tissues was associated with BTC prognosis (p<0.05). Previous meta-analyses revealed that PD-L1 expression was associated with patient outcomes.[7] In contrast, other studies did not show a correlation between PD-L1 expression in tumor tissues and OS.[11] These contrasting results were attributed to the mixture of tumor tissues in BTC. In particular, tumor tissues are composed of not only tumor cells but also immune cells. Therefore, we further analyzed PD-L1 expression in both the tumor and immune cells. In the present study, PD-L1 expression in tumor cells was associated with a poor BTC prognosis. The association of PD-L1 expression in tumor cells with patient outcome may be due to its role in the mechanism by which tumors acquire immune evasion against antitumor immunity, termed adaptive immune resistance.[16,17] This study was novel in such that we included patients who not only had surgical treatment but also underwent chemotherapy only.

In the present study, PD-L1 expression in tumor cells was associated with worse OS, and its expression on immune cells was associated with a better OS. It is known that PD-L1 expression in immune cells is a favorable prognostic factor in other types of cancer.[18-21] Its exact mechanism, however, is not yet clear. There may be some possible explanations for our results. For example, cytokines with antitumor activity, such as interferon-γ and tumor necrosis factor-α, are known to induce PD-L1 expression;[22] thus, a high IPS may reflect upregulated immunity against tumor cells. This can be seen from our results on PD-L1 expression in tumor or immune cells, where a clear correlation between the two was observed. For other treatment modalities, overall cohort did not show any significance on gemcitabine-based chemotherapy or ICI therapy, but when limited to specific treatment cohort, multivariate analysis showed significant favorable result toward gemcitabine-based chemotherapy in surgical treatment cohort. This may be due to diverse outcomes when evaluated as a mixed cohort.

In most previous studies, tissue specimens and their PD-L1 expression were obtained from surgical tissues.[8] However, 60% to 80% of patients diagnosed with BTC had unresectable BTC. Therefore, most diagnoses were made using percutaneous liver biopsy or bile duct biopsy via endoscopic retrograde cholangiopancreatography or endoscopic ultrasound. As a result, previous data are limited in that they do not reflect the entire spectrum of patients with BTC. In the present study, 65.3% of patients with BTC were diagnosed using biopsy tissues. Our study did

not show the statistical difference of PD-L1 expression of the tissue regardless of whether it was acquired by biopsy or operation. But PD-L1 expression by tumor origin was different which was similar finding in previous studies.[23] Also, Cox multivariate analysis showed that tumor origin and PD-L1 expression was not collinear and they both had independent significant effect on OS.

Cancers with high microsatellite instability, mismatch repair deficiency, and high tumor mutation burden have been reported as predictors of the effects of ICI and may benefit from programmed death-1 receptor blockers, such as pembrolizumab. The phase II KEYNOTE-158 study investigated the use of pembrolizumab in patients with advanced non-colorectal high microsatellite instability/mismatch repair deficiency tumors.[24,25] Analyses of a BTC subgroup revealed an objective response rate of 40.9% (95% CI, 20.7% to 63.6%). The median PFS and OS were 4.2 and 24.3 months, respectively.[26] Cells from mismatch repair deficient tumors express PD-L1 on their membranes;[27] therefore, programmed death-1 inhibitors can be considered second-line therapy for BTC with high PD-L1 expression.[28,29] TPS is the primary determinant of treatment decisions and predictors of prognosis.[23,30]

This study has several limitations. First, the study was retrospective in nature. To reduce selection bias, we reviewed the tissue slides again. Two pathologists independently reviewed the stained slides without any clinical information. Second, even if we checked the PD-L1 expression level in immune cells, it did not directly reflect the level of immune cell immunity. Cell counting, such as fluorescence-activated single-cell sorting, may be an ideal method for such a result. Third, our evaluation consists of mixed tissue acquisition method. While this study has a strength in that it included biopsy tissue, it also can be seen as limitation because there has not been previous study about spatial heterogeneity of PD-L1 expression or comparison of PD-L1 expression according to specimen types. In lung and ovarian cancers, some previous studies reported concordant PD-L1 results between biopsy or tissue microarray and resection specimens.[31,32] In our data, biopsy and surgical specimen showed different mean PD-L1 expression even if there was no statistical significance. Further investigation may be possible for confirmation in this area. Lastly, PD-L1 expression can be evaluated differently by the type of PD-L1 antibody. There are various clones of PD-L1 antibodies such as 22C3, 28-8, SP263, SP142, and 73-10, and the resulting score may differ when using different clones.[14] In the present study, we specifically used 22C3 anti-PD-L1 because the Food and Drug Administration has approved its use as an aid in identifying patients who may be appropriate for treatment with pembrolizumab (Keytruda®).[33]

In conclusion, PD-L1 expression is a valuable prognostic marker for BTC patients. PD-L1 expression in tumor-infiltrating immune cells may be used as an independent factor to evaluate favorable prognoses of patients with BTC.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Study concept and design: E.P., H.S.L. Data acquisition: S.C.K., S.B., Y.N.P., J.H.J., M.J.C., J.Y.P., S.W.P., S.Y.S., E.P., H.S.L. Data analysis and interpretation: S.C.K., Y.N.P., E.P., H.S.L. Drafting of the manuscript: S.C.K., E.P., H.S.L. Critical revision of the manuscript for important intellectual content: S.C.K., S.B., Y.N.P., J.H.P., S.J.K., J.H.J., M.J.C., J.Y.P., S.W.P., S.Y.S., E.P., H.S.L. Statistical analysis: S.C.K., H.S.L. study supervision: S.B., Y.N.P., E.P., H.S.L. Approval of final manuscript: all authors.

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SUPPLEMENTARY MATERIALS

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