OBJECTIVE
Thyroid cancer is the most common endocrine neoplasm. About 10% develop invasive disease and 5% lymph node (LN) metastasis. An alternative immunoscore system to the tumor node metastasis (TNM) classification has been developed to predict the prognosis of solid tumors. The aim of this study is to investigate the potential roles of CD3+, CD8+ T lymphocyte infiltration and programmed death-ligand 1 (PD-L1) expression in immunoscore, the prognostic value of Immunoscore for Papillary thyroid cancer and its support for TNM classification.

METHODS
A total of 164 patients diagnosed with papillary thyroid cancer were included in our study. Immunoscore was evaluated by immunohistochemistry according to CD3+ and CD8+ T lymphocyte density at the tumor center and at the tumor margin. Immunoscore values were calculated. PD-L1 was evaluated by immunohistochemistry according to its density at the tumor center and at the tumor margin. The relationships between the parameters studied were evaluated statistically.

RESULTS
The presence of PD-L1 at the tumor centers in CD8+ T lymphocytes height was also significantly higher (p<0.001). There was no significant relationship between PD-L1 expression at the tumor center and LN metastasis and tumor size (p>0.999 and p=0.226, respectively). In the presence of PD-L1 expression at the tumor margin, LN metastasis (p<0.001) and tumor size (p=0.029) were higher. PD-L1 expression at the tumor margin was significantly associated with overall survival (p<0.001).

CONCLUSION
In particular, immunoscore value can be a good prognostic marker and its significant association with conditions associated with poor prognosis, such as PD-L1 expression, suggests that it may be a parameter that can be used and guided in stage scoring.

Keywords: Immunoscore; papillary thyroid cancer; programmed death-ligand 1; prognosis.

INTRODUCTION
Thyroid cancer is the most common endocrine malignancy, accounting for 3.4% of all cancers.[1] Its incidence has increased over the past three decades, as epidemiological data show.[2,3] Thyroid cancer includes differentiated thyroid carcinoma (DTC), poorly DTC, anaplastic thyroid carcinoma (ATC), and medullary thyroid carcinoma.[4] Subtypes of DTC are papillary thyroid carcinoma (PTC) and follicular thyroid car-
cinoma.[5] DTC is the most common type of thyroid cancer, accounting for 90% of all thyroid cancers. PTC accounts for 80% of this.[4] Although PTC has an overall good prognosis, 10% of patients develop invasive disease and distant metastases. In conclusion, it contributes to an increase in recurrence and mortality.[3,6] American Joint Committee on Cancer/International Association for Cancer Control (AJCC/UICC) staging is a classification system developed to define the extent of disease and estimate mortality based on a tumor node metastasis (TNM) scoring system.[7] It is the most commonly used staging system for thyroid cancer. [8] However, the clinical outcome can be very different among patients at the same stage.[9,10] The predictive accuracy of the traditional staging system assumes that disease progression is a tumor cell autonomous process and does not take into account the effects of the host immune response.[11] Recently, it has been reported that the risk of structural recurrence of patients with low-grade DTC may be overlooked by tumor classification in TNM, and the concept of the newly emerging molecular profile in prognosis is still lacking.[12] Evidence that tumor cells and immune cells have an important relationship in the tumor microenvironment is accepted worldwide.[13] The interaction between cancer and immune cells is an important determinant in cancer progression, and tumor infiltrating lymphocytes (TILs) are emerging as a powerful prognostic marker and therapeutic target in oncology.[14]

Contrary to the infiltration of cells responsible for chronic inflammation, it has been reported that the presence of a high number of T lymphocyte in cancers such as colon cancer, melanoma, lung cancer, pancreatic cancer, hepatocellular carcinoma, breast cancer is an indicator of good prognosis.[15] These findings highlight the relationship between endogenous antitumor immunity and cancer, and further highlight the potential role of patients immune system for the management of malignant disease. Indeed, there is a relationship between prognosis and immune cell infiltration in many types of cancer. This relationship validates the immune system's capacity to influence clinical outcome and predicts therapeutic responses.[16] Immune classification based on the degree of invasion of tumor tissue by immune cells (termed Immunoscore [IS]) has been found to increase the prognostic accuracy of tumor staging in colorectal cancer.[17] An international consortium has been initiated to validate and promote IS in routine clinical settings. The results of this international consortium resulted in the application of immunocorrection as a new component for cancer classification called TNM-Immune.[12] Studies in the field of thyroid cancer immunology support the importance of the immune system in thyroid cancer. The microenvironment of thyroid cancer is very important for prognosis and treatment.[9] However, its importance in PTC is not known enough.[18] The tumor microenvironment provides important prognostic and predictive information that has value in clinical management.[19] The discovery that intratumoral cytotoxic T lymphocytes (CTLs) have strong prognostic value provided the basis for the development of IS. IS evaluates the density of CD3+ and CD8+ T lymphocytes at the tumor center and at the tumor margin.[14] These parameters provide a scoring system ranging from IS 0 (10), which has low densities of both cell types in both regions, to IS 4 (14), which is characterized by high densities of both cell populations in both regions.[12] This test is a reproducible, quantitative, and standard immunologic test with high prognostic performance independent of all prognostic markers.[20] For colorectal cancer, the densities and type of CD8+ TILs were found to be correlated with tumor progression regardless of tumor stage in patients, making the IS an important prognostic factor.[21]

The immune checkpoint is of great importance in the recognition of CTLs by the tumor. Programmed death-ligand 1 (PD-L1) is expressed by B and T cells, monocytes, macrophages, and dendritic cells and plays an important role in the regulation of immune responses.[22] Overexpression of PD-L1 in various tumors and in vitro experimental models indicates compromised immune surveillance mechanism.[23] Tumor cells containing PD-L1 can induce T cell apoptosis, interleukin-10 production, and protect tumor cells from lysis by CTLs.[24] As a result, it leads to tumor cell resistance and immune escape of tumor cells, leading to a poor prognosis.[25] Blocking of immune checkpoints using monoclonal antibodies targeting the PD-L1 pathway has shown very promising results.[26] PD-L1 overexpression has been reported in a variety of human cancers, including head and neck, breast, ovarian, kidney, pancreatic, esophageal, non-small cell lung cancer, melanoma, and glioblastoma.[27] PD-L1 is associated with poor prognosis and increased resistance to anticancer treatments.[28] Therefore, the search for a predictive biomarker is imperative to identify patients likely to respond to anti-PD-L1 immunotherapy.

The relationship between IS and PD-L1 expression in PTC, clinical features and prognosis of PTC remain unclear. In this study, the relationship between IS and PD-L1 expression in PTC, clinical features, overall survival and prognostic value was investigated.
MATERIALS AND METHODS

A total of 164 patients who underwent total thyroidectomy in our institution between 2012 and 2021 and were diagnosed with PTC were included in the study. Tumor-registered selected blocks prepared from the surgical material were cut with a Leica RM2255 microtome at a thickness of 4 µm. IHC staining was done with the Dako Omnis automated IHC staining device. Immunohistochemically, CD 3 (Dako clone: polyclonal) and CD 8 (Dako clone: 1443) and PD-L1 (Dako clone: 22C3) staining were performed. Stained slides were individually scored from 0 to 2 under the light microscope (Zeiss Imager A1), and the scores both at the tumor center and tumor margin, and tumor center, and margin were summed to find IS values ranging from 0 to 4 (4). The data obtained were accepted as 0–2 points low and 3–4 points high.[29] In the evaluation of PD-L1 expression, cases with >1% tumor cell staining at the tumor center and at the tumor margin were considered positive, and cases without staining were considered negative[30] (Fig. 1a-d).

Statistical Analysis

All statistical analyses were performed using R version 3.6.0 (The R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org). Numerical variables such as age were expressed as mean ± standard deviation, and categorical variables were expressed as numbers (n) and percent (%). Yates continuity correction Chi-square and Fisher’s exact test were performed to determine whether there was a statistically significant relationship between CD3+ and CD8+ T lymphocytes, PD-L1 expression, and tumor size and lymph node (LN) involvement. In addition, significant relationships with phi (ϕ) coefficients as effect size and
odds ratio were expressed with 95% confidence interval over 2×2 cross tables. OS was defined as the time from diagnosis to date of death from any cause. The survival curves of CD3+, CD8+, and PD-L1 were estimated using the Kaplan–Meier method and Log-rank tests were used to compare the difference. Bipolar p<0.05 was considered statistically significant.

RESULTS

A total of 164 patients with PTC were included in our study. The mean age was 52.43±13.44 years and ranged from 20 to 79 years, and 126 (76.8%) of 164 patients were female and 38 (23.2%) were male. Of 164 patients, 49 (29.9%) were in the right lobe, 47 (28.7%) were in the left lobe, 13 (7.9%) were in the isthmus lobe, and 44 (26.8%) were multilobe. In addition, the location of 11 tumors (6.7%) could not be determined. Tumor size distribution was 40.5% for <1 cm and 59.5% for ≥1 cm. High CD3+ T lymphocyte infiltration was observed in 75 (45.7%) cases and high CD8+ T lymphocyte infiltration was observed in 70 (42.7%) cases. Twenty-two patients showed positive immunostaining for PD-L1 at the tumor center. Eleven patients showed positive immunostaining for PD-L1 at the tumor margin. Of 75 samples with high CD3+ T lymphocyte infiltration, 17 (22.7%) showed positive PD-L1 expression at the tumor center, and 5 (6.6%) of 89 samples with low CD3+ T lymphocyte infiltration showed positive PD-L1 expression at the tumor center. The rate of positive immunostaining for PD-L1 at the tumor center was approximately 5-fold higher in cases with high CD3+ T lymphocytes compared with low CD3+ T lymphocytes (22.7% vs. 5.6%, p=0.003). Of 75 samples with high CD3+ T lymphocyte infiltration, 51 (68%) showed high CD8+ T lymphocyte infiltration, and 19 (21.3%) of 89 samples with low CD3+ T lymphocyte infiltration showed high CD8+ T lymphocyte infiltration. The high infiltration rate of CD8+ T lymphocytes was approximately three-fold higher in cases with high CD3+ T lymphocytes compared with low CD3+ T lymphocytes (68% vs. 21.3%, p<0.001). Eighteen (25.7%) of 70 samples with high CD8+ T lymphocyte infiltration showed positive PD-L1 expression at the tumor center and 4 (4.3%) of 94 samples with low CD8+ T lymphocyte infiltration showed positive PD-L1 expression at the tumor center. The rate of positive immunostaining for PD-L1 expression at the tumor center was approximately 6-fold higher in cases with high CD8+ T lymphocytes compared with low CD8+ T lymphocytes (25.7% vs. 4.3%, p<0.001). There was no statistically significant correlation between CD3+ and CD8+ T lymphocyte levels and tumor size (p=0.330 and p=0.190, respectively). LN involvement was observed in 11 (14.9%) of 74 patients with high CD3+ T lymphocyte levels and 5 (5.6%) of 86 patients with low CD3+ T lymphocyte levels. The difference between these two ratios was not statistically significant (p=0.101). LN involvement was observed in 9 (13.2%) of 68 patients with high CD8+ T lymphocyte levels and 7 (7.6%) of 92 patients with low CD8+ T lymphocyte levels. The difference between these two ratios was not statistically significant (p=0.365) (Table 1).

LN involvement was observed in 2 (9.0%) of 22 patients with PD-L1 positive at the tumor center and 14 (10.1%) of 138 patients who were negative. LN involvement was lower in patients with PD-L1 expression at the tumor center. The difference between these two ratios was not statistically significant (p=0.999). Furthermore, there was no statistically significant correlation between PD-L1 at the tumor center and tumor size (p=0.226). LN involvement was observed in 11 (100%) of 11 patients with positive PD-L1 tumor margin. The difference between these two ratios was statistically significant (p<0.001) (Table 2).

Of the 164 PTC patients, 10 (7.2%) died during the follow-up period. Median follow-up was 1065 days (range: 78–3890 days). In Kaplan–Meier analysis, CD3+ and CD8+ T lymphocyte levels and PD-L1 expression at the tumor center did not show a significant association with OS (Log-rank χ²=0.921, p=0.337; Log-rank χ²=2.451, p=0.117; and Log-rank χ²=0.001, p=0.987; respectively) (Fig. 2a–c). No progression was observed in any of the 164 PTC patients. However, PD-L1 expression at the tumor margin was significantly associated with OS (Log-rank χ²=18.008, p<0.001; HR=10.41, 95% CI=2.75–39.36) (Fig. 2d).

DISCUSSION

Despite standard treatments, 5–10% of patients with PTC develop invasive and/or distant metastatic disease.[22] In addition, 1% of patients develop ATC. The prognosis of these patients is poor, survival is 3–5 months despite current treatment strategies.[31] Recurrent metastatic LN disease is common in patients with PTC.[32] The Joint International American Committee on Cancer/International Association for Cancer Control (AJCC/UICC) TNM staging system provides current guidelines for cancer classification.[7] However, the clinical outcome can be very different among patients at the same stage.[9] In addition, it has been
recently reported that the risk of structural recurrence in patients with low-grade PTC may be overlooked by tumor classification in TNM-8.[33] The recently emerged concept of molecular profile in determining prognosis is still incomplete.[12] New prognostic markers may guide a therapeutic approach. Developing an effective antitumor immune response reduces the likelihood of tumor recurrence and metastasis. Thus, it positively affects the outcome of the disease.[4] Gallon developed an IS system that shows useful prognostic value for colorectal cancer.[11] Similarly, it has been discussed that IS may be superior to TNM classification in terms of prognosis in other malignancies such as pancreatic, liver, and breast cancer, as was ob-

![Table 1 Comparisons of CD3+ and CD8+ T-lymphocyte, and PD-L1 expression, lymph node and tumor size](attachment:image1.png)

![Table 2 Comparisons of PD-L1 expression and lymph node metastasis and tumor size](attachment:image2.png)
served in colorectal cancer, and it was mentioned that a new cancer classification system might exist.[15,34,35]

Most studies have shown that high density of CD3+ and CD8+ T lymphocytes are associated with longer disease-free survival (DFS) and/or improved OS.[24] In PTC, CD8+ T lymphocytes were associated with lower tumor aggressiveness and a more favorable patient outcome.[21,36] Infiltration of CD8+ T cells was associated with increased DFS.[37] Positive expression of PD-L1 in PTC is associated with a higher risk of relapse and shortened DFS.[23,28] In another study, they found that PD-L1 expression was associated with lower survival in patients with thyroid cancer.[17] In our study, high CD3+ and CD8+ T lymphocytes infiltration may be associated with DFS. CD3+, CD8+ T lymphocytes, and PD-L1 expression at the tumor center did not show a significant association with OS (p=0.337, p=0.117, p=0.987, respectively). However, PD-L1 expression at the margin was significantly associated with OS (p<0.001).

In some studies, they did not find a significant relationship between CD8+ T lymphocytes infiltration and tumor size.[38] In our study, no statistically significant correlation was found between CD3+ and CD8+ T-lymphocyte levels and tumor size (p=0.330 and p=0.190, respectively). In PTCS, expression of PD-L1 was significantly associated with increased tumor size and multifocality.[39] In this study, they found a relationship between PD-L1 expiration and tumor size in advanced thyroid cancer.[17] In our study, no statistically significant correlation was found between PD-L1 expiration at the tumor center and tumor size (p=0.226). However, A significant correlation was found between PD-L1 expiration at the tumor margin and tumor size (p=0.029).

However, besides the relationship between PD-L1 expression and PTC, the clinical features and prognosis of PTC still remain unclear. In our study, the rate of positive immunostaining for PD-L1 expression at the tumor center was approximately six-fold higher in cases with elevated CD8+ T lymphocytes (p<0.001). The high infiltration rate of CD8+ T lymphocytes was approximately three-fold higher in subjects with high CD3+ T lymphocytes (p<0.001). These ratios show that CD8+ T lymphocytes are more valuable in determining the IS. It also supports that the IS is a strong prognostic marker. The IS can add additional power to the TNM classification, prognosis, and follow-up of patients. This information supports that CD3+ and CD8+ T lymphocytes are associated with progression and survival of thyroid cancer patients.

In our study, we examined whether there is a statistically significant relationship between CD3+ and CD8+ T lymphocytes, PD-L1 expression, and LN involvement. LN involvement was observed in 2 (9.5%) of 22 positive patients and 14 (10.1%) of 138 negative patients in the PD-L1 at the tumor center. The difference between these two ratios was not statistically significant (p>0.999). LN involvement was observed in 11 (100%) of 11 patients who were positive at the PD-L1 expression at the tumor margin. The difference between these two ratios is statistically significant (p<0.001). LN involvement was observed in 11 (14.9%) of 74 patients with high CD3+ T lymphocyte levels and 5 (5.8%) of 86 patients with low CD3+ T lymphocyte levels. The difference between these two ratios was not statistically significant (p=0.101). LN involvement was observed in 9 (13.2%) of 68 patients with high CD8+ T lymphocyte levels and 7 (7.6%) of 92 pa-
tients with low CD8+ T lymphocyte levels. The difference between these two ratios was not statistically significant (p=0.365). In our study, there was no statistically significant relationship between CD3+ and CD8+ T lymphocyte infiltrations, PD-L1 expression, and LN involvement. This shows us that LN metastasis develops independently of these parameters in early stage PTC. One study found that patients who were PD-L1 expression negative at the tumor center had a significantly longer survival than patients who were positive for PD-L1 at the tumor margin. [40] In our study, we found that LN involvement was less in patients with PD-L1 expression tumor center and LN involvement was higher in patients with PD-L1 expression at the tumor margin. This suggests that it is associated with shortened survival and shortened DFS. This information suggests that PD-L1 may be a biomarker for LN involvement in cases with PD-L1 expression at the tumor margin. As a biomarker, it can be used to monitor the survival and prognosis of cases.

Immune checkpoint inhibitors have made major contributions to the treatment of malignancies. [4,15,26] Our research may provide potential value for immunotherapy of PTC. At present, we think that effective biomarkers for predicting the prognosis of thyroid cancer are still lacking. Therefore, more studies should be conducted on IS, other factors in the microenvironment, and immune checkpoints in patients with PTC.

CONCLUSION

In our study, the correlation between IS levels, which is accepted as a prognostic biomarker in PTC, and PD-L1 levels, which acts as an immune checkpoint receptor ligand, among themselves and with other prognostic data was investigated. In particular, the IS value may be a good prognostic marker, and its significant association with conditions associated with poor prognosis, such as PD-L1 expression, suggests that it may be a parameter that can be used and guided in stage scoring. Similarly, considering the high cost of treatment protocols and the level of side effects, it was thought that it could be used in the selection of patients to be treated. In our study, no significant correlation was found between IS value and OS and tumor size. However, a significant correlation was found between the presence of PD-L1 expression at the tumor border and LN involvement and OS. Considering that these data are the parameters of the TNM system, the high IS value and PD-L1 expression at the tumor margin, which have a significant relationship with the prognosis, can be evaluated within the TNM staging system.

REFERENCES


