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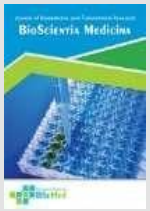

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Investigating the Landscape of Programmed Death-Ligand 1 (PD-L1) in Thymic Tumors: Implications for Histopathological Classification and Staging

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A B S T R A C T

Background: Thymic epithelial tumors (TETs) are uncommon malignancies originating in the mediastinum, characterized by considerable histopathological diversity and variable clinical trajectories. Programmed Death-Ligand 1 (PD-L1), an immune checkpoint protein, is implicated in mechanisms of tumor immune evasion. This study aimed to investigate the correlation between PD-L1 immunoexpression and distinct histopathological types, as well as the Masaoka-Koga stage, in TETs. **Methods:** This cross-sectional investigation analyzed 29 archival cases of TETs diagnosed between January 2019 and December 2024 at the Anatomical Pathology Laboratory of Dr. M. Djamil General Hospital Padang. Samples were procured via consecutive sampling from formalin-fixed paraffin-embedded (FFPE) tumor tissues. Histopathological classification was reassessed according to the WHO 2021 criteria. PD-L1 expression was evaluated immunohistochemically and quantified using the Tumor Proportion Score (TPS). Masaoka-Koga staging was determined from clinical records. Statistical analysis of correlations was performed using the Chi-square test. **Results:** PD-L1 immunoexpression was detected in the preponderance of cases. Low positive PD-L1 expression (TPS 1-49%) was observed in 82.8% of TETs, while high positive expression (TPS ≥50%) was noted in 10.3%. Thymic carcinoma constituted the most prevalent histopathological category (51.7%), and the majority of patients (91.7%) presented at an advanced Masaoka-Koga stage. Statistical analysis did not demonstrate a significant correlation between PD-L1 expression levels and histopathological type ($p=0.195$). Furthermore, no significant association was identified between PD-L1 expression and Masaoka-Koga stage ($p=0.800$). **Conclusion:** This study indicated that while PD-L1 is frequently expressed in TETs within this cohort, its expression level did not exhibit a significant correlation with specific histopathological subtypes or the Masaoka-Koga clinical stage. Further investigations incorporating larger sample sizes are warranted to delineate the precise role of PD-L1 within the complex biological spectrum of thymic neoplasms.

1. Introduction

Thymic epithelial tumors (TETs) constitute the most prevalent group of primary neoplasms arising in the anterior mediastinum; however, they are generally infrequent in the broader oncological landscape, with global incidence rates exhibiting some variation. These neoplasms, which encompass thymomas and thymic carcinomas, are characterized by a wide spectrum of histological appearances and diverse clinical behaviors. Thymomas typically demonstrate a more indolent clinical course, whereas thymic carcinomas are generally associated with greater biological

aggressiveness, manifesting a higher propensity for local tissue invasion and distant metastatic dissemination. The World Health Organization (WHO) classification system, most recently revised in 2021, provides a standardized framework for the histopathological categorization of TETs. This system delineates several distinct subtypes, including thymoma Type A, Type AB, Type B1, Type B2, and Type B3, alongside thymic carcinoma, each of which may possess implications for patient prognosis and therapeutic management.[1-5]

Within the Indonesian healthcare context, TETs represent a notable oncological challenge. These tumors frequently present with a range of histopathological features and are often diagnosed at advanced clinical stages, contributing to less favorable therapeutic outcomes. The management of advanced or recurrent TETs remains particularly problematic, given the restricted availability of effective systemic therapeutic modalities once the tumors are no longer amenable to complete surgical resection. This situation underscores a compelling need for an enhanced understanding of TET immunobiology and the identification of novel prognostic and predictive biomarkers to refine and guide treatment strategies.[6,7]

In recent years, the field of cancer therapeutics has been substantially advanced by the advent of immunotherapy, particularly through the development of immune checkpoint inhibitors (ICIs). programmed death-ligand 1 (PD-L1), a transmembrane protein, assumes a critical function in the process of adaptive immune resistance. When expressed on the surface of tumor cells, PD-L1 can engage its cognate receptor, programmed death-1 (PD-1), which is present on activated T lymphocytes. This interaction culminates in the inhibition of T-cell effector functions, thereby permitting cancer cells to circumvent immune-mediated destruction. Consequently, the PD-1/PD-L1 signaling axis has emerged as a pivotal therapeutic target for ICIs, with pharmacological agents designed to disrupt this interaction demonstrating notable efficacy in subsets of patients across a variety of cancer types.[8-10]

Preliminary investigations have suggested that PD-L1 is expressed in a considerable proportion of TETs, thereby raising the possibility that immunotherapy could represent a viable therapeutic option for these neoplasms. Nonetheless, the precise clinical significance of PD-L1 expression in TETs, particularly its association with specific histopathological subtypes and the extent of disease as defined by staging systems, continues to be an area of active research characterized by some degree of inconsistency in reported findings. Certain studies have posited a correlation between elevated PD-L1 expression levels and histologically more aggressive tumor types (Type B3 thymoma, thymic carcinoma) or advanced Masaoka-Koga stage. The Masaoka-Koga system is a widely adopted staging classification for TETs, predicated on the assessment of local tumor invasion and metastatic spread. Conversely, other studies have failed to identify a clear association or have presented discordant results. These discrepancies may be attributable to methodological variations, including the use of different anti-PD-L1 antibody clones, variations in immunohistochemical scoring methodologies, the application of diverse positivity cut-off values, and inherent differences in the characteristics of the patient cohorts under investigation.

The Masaoka-Koga staging system serves as a critical determinant of prognosis and influences the selection of therapeutic interventions for patients with TETs. An understanding of how PD-L1 expression patterns align with this established staging framework, as well as with the diverse histopathological entities defined by the latest WHO classification, could yield valuable insights into tumor biology. Furthermore, it could potentially refine patient stratification for immunotherapeutic interventions. For instance, the identification of specific TET subtypes or disease stages characterized by consistently high PD-L1 expression levels might facilitate the prioritization of patients who are more likely to derive clinical benefit from anti-PD-1/PD-L1 therapies.

Given the paucity of comprehensive data, particularly from Indonesian patient populations, and the extant controversies within the published literature, the present study was undertaken. This study critically examined the immunoexpression of programmed death-ligand 1 (PD-L1) in a cohort of Indonesian patients with thymic epithelial tumors, seeking to determine its correlative significance with the latest WHO histopathological classifications and Masaoka-Koga clinical staging to better understand its biological and potential clinical implications in this specific oncological setting.

2. Methods

This investigation utilized an observational, cross-sectional study design to assess the associations between programmed death-ligand 1 (PD-L1) expression, histopathological classification, and Masaoka-Koga staging in patients diagnosed with thymic epithelial tumors (TETs). The study population encompassed all confirmed cases of TETs, including thymoma subtypes (Type A, AB, B1, B2, B3) and thymic carcinoma, archived at the Anatomical Pathology Laboratory of Dr. M. Djamil General Hospital, Padang, Indonesia, over a period from January 2019 to December 2024. An initial cohort of 40 cases was identified. This research was conducted as a component of a broader, overarching study initiative.

Sample selection was performed using a consecutive sampling methodology. The criteria for inclusion in the study were as follows: a histopathologically confirmed diagnosis of a thymic epithelial tumor; availability of comprehensive medical records, including patient age, gender, primary tumor location, definitive histopathological type, and Masaoka-Koga stage; and accessibility of the corresponding Hematoxylin and Eosin (H&E) stained slides and formalin-fixed paraffin-embedded (FFPE) tissue blocks. Cases were excluded from the study if the archival paraffin blocks were either missing or exhibited damage, or if the quantity of tissue within the block was deemed insufficient for requisite further sectioning and subsequent immunohistochemical analysis.

Data were meticulously extracted from patient medical records, histopathology reports, and radiological (Computed Tomography - CT scan) expertise summaries. The variables under investigation comprised: Independent Variable: Level of PD-L1 expression. Dependent Variables: Histopathological type of TET and Masaoka-Koga stage. Ancillary Characteristics: Age at diagnosis, gender, smoking history (categorized using the Brinkman Index), anatomical location of the tumor, and the presence of comorbid myasthenia gravis.

Patient age was recorded in years and subsequently categorized into predefined intervals. Gender was documented as male or female. Smoking status was ascertained from medical records and classified as non-smoker, or as light, moderate, or heavy smoker based on the Brinkman Index (calculated as the product of the average number of cigarettes smoked per day and the duration of smoking in years). Tumor location was determined from CT scan reports. The presence or absence of myasthenia gravis as a comorbidity was noted from existing medical documentation.

All available H&E-stained slides from the selected cases underwent a thorough re-evaluation by two experienced anatomical pathologists (who also served as research supervisors). This review was conducted to confirm the initial diagnosis and to classify the TETs in accordance with the 2021 World Health Organization (WHO) Classification of Thoracic Tumours. The defined histopathological categories included thymoma Type A, Type AB, Type B1, Type B2, Type B3, and thymic carcinoma. In instances where existing H&E slides were either unavailable or deemed to be of suboptimal quality for definitive assessment, new sections were prepared from the FFPE blocks and subjected to standard H&E staining.

The clinical stage for each TET was assigned based on the Masaoka-Koga staging system. This system evaluates the extent of tumor invasion through the thymic capsule and into adjacent anatomical

structures, as well as the presence of pleural, pericardial, or distant metastatic disease. Staging information was primarily derived from radiological reports (CT scans) and comprehensive medical records. For analytical purposes, Masaoka-Koga stages were dichotomized into early stage (Stage I-II) and advanced stage (Stage III-IV).

Immunohistochemical staining for PD-L1 was performed on tissue sections 4-6 microns in thickness, obtained from the FFPE blocks. The glass slides utilized were pre-treated with a silane coating to enhance tissue adhesion. The IHC protocol involved sequential deparaffinization in xylene, followed by rehydration through a graded series of alcohols. Antigen retrieval was accomplished using Tris-EDTA buffer (pH 9.0) within a decloaking chamber maintained at 95-97°C for 30 minutes. Endogenous peroxidase activity was subsequently quenched by treatment with 0.1%-1% hydrogen peroxide. Non-specific antibody binding was minimized by the application of a normal blocking serum.

The primary antibody employed was the anti-PD-L1 monoclonal antibody, clone 28-8 pharmDx, utilized at a dilution of 1:300. Incubation with the primary antibody was carried out in a humidified chamber at 95°C for 30 minutes. A standard streptavidin-biotin complex (SBC) amplification method was used for signal detection. Visualization was achieved using DAB (3,3'-Diaminobenzidine) as the chromogen, followed by counterstaining with Mayer's hematoxylin. Sections of human placental tissue were processed in parallel and served as positive controls for PD-L1 staining.

PD-L1 expression was quantitatively assessed using the tumor proportion score (TPS). The TPS was defined as the percentage of viable tumor cells exhibiting discernible partial or complete membranous and/or cytoplasmic staining at any intensity. A minimum of 100 viable tumor cells was evaluated to determine the TPS for each case. Staining confined exclusively to the nucleus was considered non-specific and was not included in the scoring. The immunohistochemical slides were evaluated independently by two anatomical pathologists using a standard light microscope (Olympus BX51 equipped with a DP20 camera) at a magnification of 400x. Any discrepancies in scoring were resolved by consensus. ImageJ software was also employed as an ancillary tool to aid in the quantitative analysis of the proportion of positively stained cells. PD-L1 expression was stratified into three distinct levels based on the calculated TPS: No expression (Negative): TPS < 1%. Low expression: TPS 1-49%. High expression: TPS ≥ 50%

All collected data were systematically processed and subjected to statistical analysis using SPSS for Windows. Univariate analysis was performed to generate descriptive statistics, including frequencies and percentages, for patient demographic and clinical characteristics, histopathological subtypes, Masaoka-Koga stages, and PD-L1 expression levels. Bivariate analysis, employing the Chi-square test (or Fisher's exact test in instances where expected cell counts were low, typically <5), was conducted to ascertain the statistical significance of associations between PD-L1 expression levels and histopathological types, and between PD-L1 expression levels and Masaoka-Koga stage categories (early versus advanced). A p-value of less than 0.05 was predetermined as the threshold for statistical significance. The results of these analyses were presented in tabular formats and supplemented with narrative descriptions.

This research utilized archived biological specimens (H&E slides and paraffin blocks) and associated patient data. Strict measures were implemented to ensure patient confidentiality; all data were anonymized prior to analysis, and no personally identifiable information or patient photographs were included in any reports or publications. Given the retrospective nature of the study and the use of archived materials, the process of obtaining direct informed consent from individual patients was deemed impracticable, particularly as tracing all patients could prove difficult, and the research posed no direct physical risk or adverse effects to the subjects. The study protocol, including the provisions for patient

data handling and confidentiality, received formal ethical approval from the Medical Research Ethics Committee of Dr. M. Djamil General Hospital Padang.

3. Results

A total of 29 cases of thymic epithelial tumors (TETs) that met the predefined inclusion criteria were incorporated into the final analysis for this study. The demographic, clinical, and pathological characteristics of the 29 patients with TETs are comprehensively summarized in Table 1. The age of the patients at diagnosis ranged from 8 to 70 years, with a mean age calculated at 42 years. The demographic subgroup with the highest incidence was the 19-30 year age bracket, which included 9 patients (31.0%). A notable male predominance was observed, with males constituting 22 cases (75.9%) of the cohort. With respect to smoking history, a majority of the patients reported a history of smoking; specifically, 15 patients (51.7%) were categorized as moderate smokers according to the Brinkman Index (200-600). Myasthenia gravis was documented as a comorbid condition in 3 patients (10.3%). The anterior mediastinum was the most frequently identified primary tumor location, accounting for 19 cases (65.5%).

Following histopathological reassessment according to the WHO 2021 criteria, thymic carcinoma (predominantly the squamous cell carcinoma variant) was identified as the most common diagnosis, occurring in 15 patients (51.7%). Among the thymoma subtypes, Type B2 was the most prevalent (5 patients, 17.2%), followed by Type B1 and Type B3 (each comprising 3 patients, 10.3%), Type A (2 patients, 6.9%), and Type AB (1 patient, 3.4%). A substantial majority of the patients (27 cases, 91.7%) presented with advanced disease, corresponding to Masaoka-Koga stages III-IV. Analysis of PD-L1 expression revealed that 2 patients (6.9%) exhibited no detectable PD-L1 expression (TPS < 1%). Low-level PD-L1 expression (TPS 1-49%) was observed in 24 patients (82.8%), while 3 patients (10.3%) demonstrated high-level PD-L1 expression (TPS ≥ 50%).

The association between varying levels of PD-L1 expression and the distinct histopathological types of TETs was statistically evaluated using the Chi-square test. The distribution pattern of PD-L1 expression across these histopathological categories is presented in detail in Table 2. Negative PD-L1 expression (TPS < 1%) was identified in one case diagnosed as thymoma Type B2 and in one case of thymic carcinoma. Low-level PD-L1 expression (TPS 1-49%) was prevalent across multiple subtypes: it was observed in 2 cases of thymoma Type A, 2 cases of thymoma Type B1, 4 cases of thymoma Type B2, 3 cases of thymoma Type B3, and 13 cases of thymic carcinoma. High-level PD-L1 expression (TPS ≥ 50%) was detected in one case each of thymoma Type AB, thymoma Type B1, and thymic carcinoma.

The statistical analysis did not yield a significant association between the level of PD-L1 expression and the specific histopathological type of the thymic epithelial tumors ($p=0.195$). Representative photomicrographs illustrating the histopathological features of TETs and the patterns of PD-L1 immunoexpression are provided in Figures 1, 2, and 3.

The potential association between PD-L1 expression levels and the Masaoka-Koga clinical stage, dichotomized into early stage (I-II) versus advanced stage (III-IV), was subjected to statistical analysis. The pertinent findings are systematically presented in Table 3. A limited number of cases (2 patients, 8.3%) were diagnosed at an early Masaoka-Koga stage (I-II); both of these cases demonstrated low-level PD-L1 expression. The substantial remainder of the cohort (27 patients, 93.1%) presented with advanced-stage disease (III-IV). Within this advanced-stage subgroup, 2 patients exhibited no PD-L1 expression, 22 patients had low-level PD-L1 expression, and 3 patients displayed high-level PD-L1 expression. The application of the Chi-square test resulted in a p-value of 0.800. This outcome indicates the absence of a statistically significant relationship between the observed PD-L1 expression levels and the Masaoka-Koga clinical stage within this particular patient cohort.

Table 1. Clinicopathological and biomarker characteristics of patients with thymic epithelial tumors (N=29).

Characteristic	Category	Number of patients (n)	Percentage (%)
Age at diagnosis (Years)	0 – 18	1	3.4
	19 – 30	9	31.0
	31 – 40	2	6.9
	41 – 50	6	20.7
	51 – 60	5	17.2
	61 – 70	6	20.7
	> 70	0	0.0
Summary	Mean (Range)	42 (8 – 70)	-
Gender	Male	22	75.9
	Female	7	24.1
Smoking status	Non-smoker	9	31.0
	Brinkman Index: Light (0-199)	1	3.4
	Brinkman Index: Moderate (200-600)	15	51.7
	Brinkman Index: Heavy (≥600)	4	13.8
Myasthenia gravis	Yes	3	10.3
	No	26	89.7
Primary tumor location	Anterior Mediastinum	19	65.5
	Superior Mediastinum	1	3.4
	Superoanterior Mediastinum	6	20.7
	Anteromedial Mediastinum	3	10.3
Histopathological type (WHO 2021)	Thymoma Type A	2	6.9
	Thymoma Type AB	1	3.4
	Thymoma Type B1	3	10.3
	Thymoma Type B2	5	17.2
	Thymoma Type B3	3	10.3
	Thymic Carcinoma	15	51.7
Masaoka-Koga clinical stage	Early Stage (I – II)	2	8.3
	Advanced Stage (III – IV)	27	91.7
PD-L1 immunoexpression (TPS)	No Expression (TPS < 1%)	2	6.9
	Low Expression (TPS 1 – 49%)	24	82.8
	High Expression (TPS ≥ 50%)	3	10.3

Table 2. Distribution of histopathological types of thymic epithelial tumors according to programmed death-ligand 1 (PD-L1) immunoexpression levels (N=29).

PD-L1 immunoexpression level (TPS)	Thymoma Type A n (row %)	Thymoma Type AB n (row %)	Thymoma Type B1 n (row %)	Thymoma Type B2 n (row %)	Thymoma Type B3 n (row %)	Thymic Carcinoma n (row %)	Row Total N (100%)
Negative (TPS < 1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	1 (50.0%)	**2**
Low expression (TPS 1 – 49%)	2 (8.3%)	0 (0.0%)	2 (8.3%)	4 (16.7%)	3 (12.5%)	13 (54.2%)	**24**
High expression (TPS ≥ 50%)	0 (0.0%)	1 (33.3%)	1 (33.3%)	0 (0.0%)	0 (0.0%)	1 (33.3%)	**3**
Column total N (Overall % of Histotype in Cohort)	**2 (6.9%)**	**1 (3.4%)**	**3 (10.3%)**	**5 (17.2%)**	**3 (10.3%)**	**15 (51.7%)**	**29 (Grand Total 100%); p-value = 0.195**

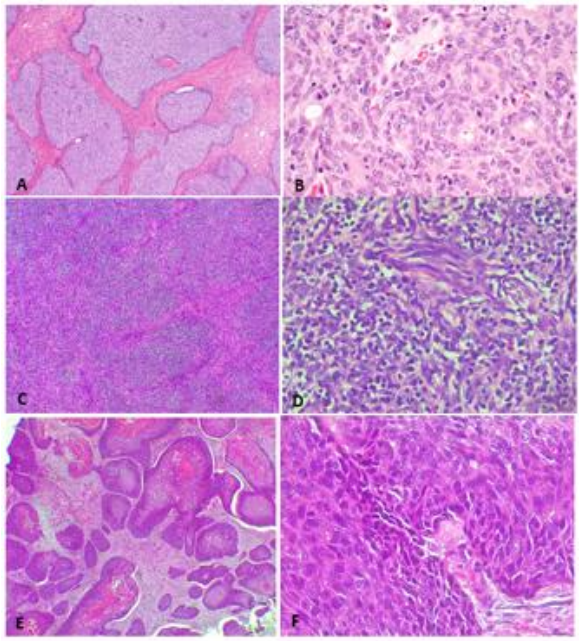

Figure 1. Microscopic features of thymoma type A, type AB, and thymic carcinoma: A. Visible dividing septa (H&E, 40x). B. Spindle cell morphology (arrow) (H&E, 400x). C. Thymoma type AB at low magnification (H&E, 40x). D. Mixed cell morphology demonstrating spindle cells (thick arrow) and round-oval/polygonal cells (thin arrow) (H&E, 400x). E. Thymic carcinoma exhibiting nesting patterns of tumor cells (H&E, 40x). F. Morphology of thymic carcinoma displaying intercellular bridges (arrow) (H&E, 400x).

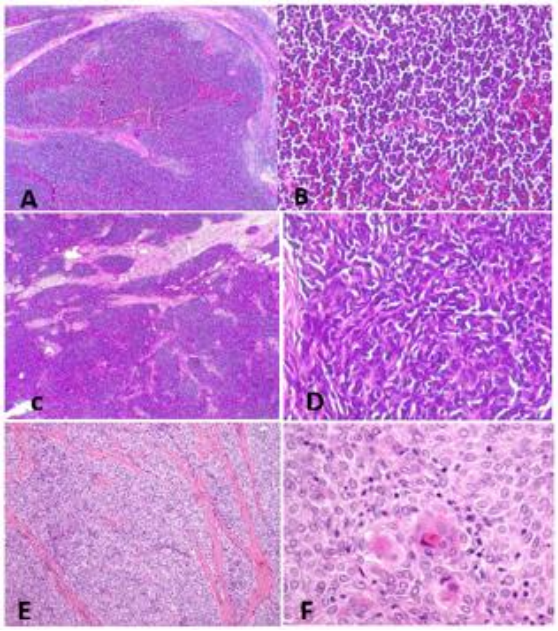

Figure 2: Microscopic appearances of thymoma type B subtypes. (A) Thymoma type B1 exhibiting a distinct lobular pattern separated by fibrous septa at low magnification (arrow) (H&E, 40x). (B) Higher magnification of thymoma type B1, illustrating the characteristic dense population of lymphocytes among which neoplastic epithelial cells are dispersed (H&E, 400x). (C) Thymoma type B2 shown at low magnification (H&E, 40x). (D) Thymoma type B2 at higher magnification, with clusters of neoplastic epithelial cells (thick arrow) admixed with numerous lymphocytes (thin arrow) (H&E, 400x). (E) Thymoma type B3 at low magnification, typically appearing more eosinophilic due to a higher proportion of epithelial cells (H&E, 40x). (F) Higher magnification of thymoma type B3, characterized by sheets of polygonal neoplastic epithelial cells with conspicuous nucleoli (thick arrow) and evidence of mitosis (thin arrow) (H&E, 400x).

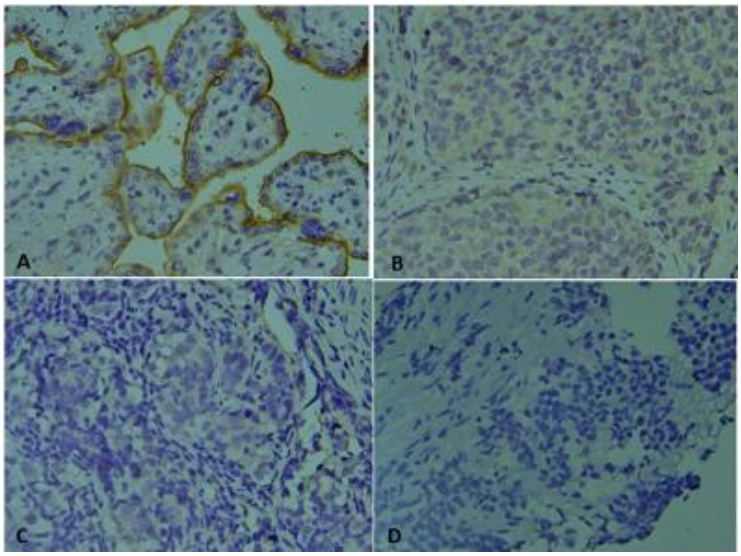

Figure 3. Representative images of programmed death-ligand 1 (PD-L1) immunoexpression. (A) Positive control (placental tissue) exhibiting distinct membranous and cytoplasmic PD-L1 staining (Immunohistochemistry [IHC], 400x). (B) High PD-L1 expression in tumor cells, evidenced by intense staining of the membrane and cytoplasm (IHC, 400x). (C) Low PD-L1 expression in tumor cells, indicated by faint to moderate membranous and cytoplasmic staining (IHC, 400x). (D) Absence of PD-L1 staining in tumor cells, representing a negative expression status (IHC, 400x).

Table 3. Correlation analysis between programmed Death-Ligand 1 (PD-L1) immunoexpression and Masaoka-Koga clinical stage in patients with thymic epithelial tumors (N=29)

PD-L1 immunoexpression level (TPS)	**Early stage (I – II) n (row %)**	**Advanced stage (III – IV) n (row %)**	**Row total N (100%)**
No expression (TPS < 1%)	0 (0.0%)	2 (100.0%)	**2**
Low expression (TPS 1 – 49%)	2 (8.3%)	22 (91.7%)	**24**
High expression (TPS ≥ 50%)	0 (0.0%)	3 (100.0%)	**3**
Column total N (Overall % of Stage in Cohort)	**2 (6.9%)**	**27 (93.1%)**	**29 (Grand Total 100%); p-value = 0.800**

4. Discussion

This investigation was undertaken to characterize the expression of programmed death-ligand 1 (PD-L1) within a cohort of 29 thymic epithelial tumors (TETs) from a single Indonesian institution, and to elucidate its potential correlations with histopathological classification (as per WHO 2021 criteria) and Masaoka-Koga staging. The results revealed that PD-L1 was commonly expressed in these neoplasms, with 82.8% of cases exhibiting low-level (Tumor Proportion Score [TPS] 1-49%) and 10.3% demonstrating high-level (TPS ≥50%) immunoexpression. However, in contrast to findings from some prior studies, the current analysis did not identify a statistically significant association between PD-L1 expression levels and specific histopathological subtypes or the Masaoka-Koga stage.[11,12]

The demographic and clinicopathological profile of our study cohort presented several noteworthy observations. The mean age at diagnosis was 42 years, with the 19-30 year age group representing the largest proportion (31%). This age distribution is somewhat

younger than the peak incidence typically documented in Western populations (often reported between 40-60 years), although variations have been noted in studies conducted among Asian populations. The observed male predominance (75.9%) in our cohort aligns with some reports in the literature, though a definitive gender predilection in TETs is not universally established. A high proportion of patients (69%) reported a history of smoking, with 51.7% classified as moderate smokers; this observation is congruent with emerging evidence implicating tobacco consumption as a potential etiological risk factor for thymoma. Myasthenia gravis was documented in 10.3% of cases, a prevalence lower than the 30-50% frequently cited in association with thymoma, particularly Type B thymomas. This lower incidence in our cohort may be influenced by the substantial representation of thymic carcinomas, which are less commonly associated with this autoimmune disorder.[13,14]

A particularly striking finding was the high prevalence of thymic carcinoma (51.7% of cases) and the predominance of advanced Masaoka-Koga stage (91.7%) at the time of diagnosis within our patient group. This contrasts with numerous reports from Western nations and some Asian countries, where thymomas typically constitute the majority of TETs, and a larger fraction of patients are diagnosed at an earlier stage of the disease. The observed preponderance of advanced-stage thymic carcinoma in our cohort could be multifactorial, potentially reflecting differences in patient referral pathways, variations in access to specialized healthcare services, levels of disease awareness within the population, or, conceivably, underlying biological or ethnic distinctions. This finding highlights a critical area for focused public health interventions and oncological care strategies within our region.[15,16]

The frequent detection of PD-L1 expression in our TET samples (93.1% exhibiting TPS ≥1%) is broadly consistent with the findings of previous studies, which have also reported PD-L1 positivity in a substantial percentage of TETs. The observed distribution of low (82.8%) versus high (10.3%) PD-L1 expression levels in our study underscores the inherent heterogeneity of PD-L1 expression profiles within TETs. However, our primary correlative analyses failed to demonstrate a statistically significant relationship between PD-L1 expression levels and the diverse histopathological subtypes of TETs (p=0.195). This outcome diverges from the conclusions of several other studies that have suggested an association between higher PD-L1 expression and histologically more aggressive tumor types, such as thymoma Type B2, B3, or thymic carcinoma, in comparison to Type A, AB, or B1 thymomas Previous study reported differential PD-L1 expression across subtypes, with Type B2 and B3 thymomas exhibiting elevated levels. The absence of such a correlation in the present study may be attributable to a confluence of factors. These include the relatively limited sample size within each histopathological subgroup, potential variations in the specific anti-PD-L1 antibody clones or immunohistochemical protocols utilized, inter-observer variability in interpretation, or genuine biological distinctions pertinent to our specific patient population. The notable predominance of thymic carcinoma within our cohort might also have influenced this result, potentially obscuring more subtle differences in PD-L1 expression among the various thymoma subtypes.[17,18]

Analogously, we did not identify a significant association between PD-L1 expression and Masaoka-Koga stage (p=0.800). This finding is in concordance with some previous reports, which also found no correlation between PD-L1 expression and Masaoka-Koga stage in their respective cohorts of thymoma and thymic carcinoma patients. However, this contrasts with other studies where high PD-L1 expression was linked to advanced Masaoka-Koga stage (III-IV) and, in some instances, to a less favorable prognosis. The fact that an overwhelming majority of our cases (91.7%) presented at an advanced stage may have constrained the statistical power to detect such a correlation. If most tumors within a cohort are already classified as advanced, variations in PD-L1 expression might not effectively segregate further by stage within this

predominantly advanced group.[19-20]

The absence of statistically significant correlations in our study does not inherently negate the potential biological relevance of PD-L1 in the pathobiology of TETs, nor does it preclude its utility as a biomarker in other contexts. PD-L1 expression within the tumor microenvironment is a complex phenomenon, subject to modulation by a variety of factors. These include intrinsic oncogenic signaling pathways activated within tumor cells (MAPK, PI3K/Akt pathways) and extrinsic stimuli, such as interferon-gamma (IFN-γ) produced by infiltrating immune effector cells. The regulation of PD-L1 expression can be either constitutive (intrinsic to the tumor cell) or adaptive (induced by microenvironmental cues), and its ultimate functional consequence is likely dependent upon the broader immune contexture of the tumor.

The present study is subject to several limitations that warrant acknowledgment. Firstly, its retrospective design and single-institution origin may introduce an element of selection bias, potentially limiting the generalizability of the findings. Secondly, the overall sample size of 29 cases, while satisfying the a priori calculation, remains relatively modest, particularly when cases are stratified by specific histopathological type and stage. This may have restricted the statistical power available to detect more subtle associations. The inherent challenge in precise microscopic assessment of capsular invasion for staging purposes, as alluded to in the source document, is a recognized complexity in TET pathology. Furthermore, while the anti-PD-L1 antibody clone 28-8 is FDA-approved for diagnostic use in certain malignancies, inter-observer variability in the interpretation of immunohistochemical staining, despite concerted efforts to standardize scoring, can persist. We did not evaluate PD-L1 expression on tumor-infiltrating immune cells, which can also possess prognostic and predictive significance. The high proportion of advanced-stage thymic carcinomas within our cohort might also render it distinct from other study populations with a different distribution of cases.

Notwithstanding these limitations, this study contributes valuable initial data regarding PD-L1 expression patterns in TETs within an Indonesian patient population, notably highlighting a high frequency of PD-L1 positivity. The observed lack of correlation with histotype and stage in this specific cohort suggests that if PD-L1 is to be employed as a predictive biomarker for immunotherapy in TETs, its interpretation may require a nuanced approach, possibly in conjunction with other biomarkers or more comprehensive immune profiling techniques. The predominance of advanced-stage disease at presentation also underscores an urgent imperative for the development and implementation of strategies aimed at improving early detection and enhancing access to specialized oncological care for patients with TETs in this region.

Future research endeavors should prioritize the assembly of larger, multi-center cohorts to validate these preliminary findings and to more definitively explore the molecular mechanisms that underpin PD-L1 expression in the various subtypes of TETs. Investigations into the broader tumor immune microenvironment, encompassing assessments of tumor-infiltrating lymphocytes (TILs) and other immune checkpoint molecules, would provide a more holistic understanding of the immune landscape in these tumors. Crucially, prospective clinical trials correlating PD-L1 expression with patient responses to immunotherapy in TETs are essential to establish its definitive predictive utility in this setting.

5. Conclusion

This study was conducted to assess the expression of programmed death-ligand 1 (PD-L1) in a series of 29 cases of thymic epithelial tumors (TETs) and to investigate its relationship with established histopathological classifications and Masaoka-Koga staging. The principal conclusions derived from this research are as follows: PD-L1 was found to be frequently expressed within this cohort of TETs. The majority of cases (82.8%) demonstrated low-level positive immunoexpression (Tumor Proportion Score

[TPS] 1-49%), while a smaller segment (10.3%) exhibited high-level expression (TPS ≥50%). The most commonly encountered histopathological diagnosis was thymic carcinoma (51.7% of cases). Furthermore, a significant preponderance of patients (91.7%) presented with advanced disease, as categorized by Masaoka-Koga stage (III-IV). Statistical analysis did not reveal a significant correlation between the observed levels of PD-L1 expression and the specific histopathological types of TETs (p=0.195) within this study group. Similarly, no statistically significant association was discerned between PD-L1 expression levels and the Masaoka-Koga clinical stage, when dichotomized into early versus advanced stages (p=0.800).

In summation, while PD-L1 is a commonly expressed protein in thymic epithelial tumors within this Indonesian patient cohort, its quantitative level of expression did not demonstrate a statistically significant independent association with either the histopathological classification or the Masaoka-Koga stage. These findings contribute to the ongoing scientific discourse concerning the role of PD-L1 in TETs and suggest that its utility as a standalone biomarker for stratification based on histology or stage may be limited, at least within this specific patient population. Further research, ideally involving larger and more demographically diverse cohorts, is imperative to fully elucidate the complex interplay between PD-L1 expression and the clinicopathological characteristics of thymic epithelial tumors, as well as to ascertain its potential implications for guiding immunotherapeutic interventions.

6. References

1. Abeygunawardhane S, Kanagasabapathy S, Jayawickrama M. Intrathyroid thymic carcinoma/carcinoma showing thymus-like elements (CASTLE): an extremely rare presentation of thyroid malignancy. J Diagn Pathol. 2022; 17(2): 9–13.
2. Dolan DP, Polhemus E, Lee DN, Mazzola E, Jaklitsch MT, Wee JO, et al. Hyperthermic intraoperative chemotherapy (HIOC) for Stage IVa thymic malignancy may improve 5-year disease-free survival. J Surg Oncol. 2023; 127(4): 734–40.
3. Bhansali PJ, Mishra GV, Parihar PH, Unadkat BS, Kabra R. International Thymic Malignancy Interest Group classification – the future of mediastinal mass evaluation using multidetector computed tomography. J Datta Meghe Inst Med Sci Univ. 2023; 18(2): 217–22.
4. Basse C, Botticella A, Molina T, Falcoz P-E, Oulkhouir Y, Kerjouan M, et al. 1742TiP RADIORYTHMIC: Phase III, opened, randomized study of post-operative radiotherapy (PORT) versus surveillance in stage IIb/III of Masaoka Koga thymoma after complete surgical resection. Ann Oncol. 2021; 32: S1203.
5. Bertocchi P, Meriggi F, Zambelli C, Zorzi F, Zaniboni A. Clear cell thymic carcinoma: a case report. Tumori. 2015; 101(2): e73–4.
6. Korst RJ, Fernando S, Catlin AC, Rutledge JR, Girard N, Huang J, et al. Positron emission tomography in thymic tumors: Analysis using a prospective research database. Ann Thorac Surg. 2017; 104(6): 1815–20.
7. Priola AM, Priola SM. Thymoma of the left thymic lobe with a contralateral small pleural implant successfully detected with diffusion-weighted MRI. Tumori. 2015; 101(1): e13–7.
8. Syed YA, Miller DH, Jiang L, Bagherpour A, Ko SJ. Durable response in a thymic neuroendocrine tumor: a case report and review of the literature. J Solid Tumors. 2017; 7(2):23.
9. Lesueur P, Chevalier F, Stefan D, Habrand J-L, Lerouge D, Gervais R. Review of the mechanisms involved in the abscopal effect and future directions with a focus on thymic carcinoma. Tumori. 2017; 103(3): 217–22.
10. Chen H-F, Wu L-X, Li X-F, Zhu Y-C, Pan W-W, Wang W-X, et al. PD-L1 expression level in

different thymoma stages and thymic carcinoma: a meta-analysis. Tumori. 2020; 106(4): 306–11.
11. Billè A, Bilkhu R, Benedetti G, Lucchese G. Improving outcomes of surgery in advanced infiltrative thymic tumours: the benefits of multidisciplinary approach. Tumori. 2022; 108(5): 477–85.
12. Cho S-H, Shim H-J, Hwang JE, Bae W-K, Chung IJ. The prognostic role of PD L1 expression according to MSI status in stage III colon cancer after curative resection. Ann Oncol. 2018; 29(Suppl_8): viii180.
13. Yamada S, Itai S, Kaneko MK, Kato Y. Detection of high PD-L1 expression in oral cancers by a novel monoclonal antibody L1Mab-4. Biochem Biophys Rep. 2018; 13: 123–8.
14. Stella GM, Bortolotto C, Antonacci F, Dore R, Coretti M, Antonio C, et al. Non-solid neoplastic nodules are associated with low PD L1 expression. In: Imaging. European Respiratory Society. 2019; PA4816.
15. Bortolotto C, Maglia C, Ciuffreda A, Coretti M, Catania R, Antonacci F, et al. The growth of non-solid neoplastic lung nodules is associated with low PD L1 expression, irrespective of sampling technique. J Transl Med. 2020; 18(1): 54.
16. Zhuang X, Li H, You Z, Cheng H, Guo G, Chen X, et al. PD - L1 (SP263) expression correlates with pathological aggressive parameters in prostate cancer. Ann Diagn Pathol. 2025; 78(152481): 152481.
17. Valavanis C, Stanc GM, Baltayiannis N. Classification, histopathology and molecular pathology of thymic epithelial tumors: a review. J BUON. 2021; 26(4): 1198–207.
18. Thomas-de-Montpréville V, Chalabreysse L, Hofman V, de-Muret A, Sizaret D, Dubois R, et al. Micronodular thymic epithelial tumors with lymphoid hyperplasia and mimicking lesions. Histol Histopathol. 2025; 40(1): 1–10.
19. Lattanzio R, La Sorda R, Facciolo F, Sioletic S, Lauriola L, Martucci R, et al. Thymic epithelial tumors express vascular endothelial growth factors and their receptors as potential targets of antiangiogenic therapy: a tissue micro array-based multicenter study. Lung Cancer. 2014; 85(2): 191–6.
20. Zhai Y, Chen D, Gao Y, Hui Z, Xue L, Zhou Z, et al. Role of modern neoadjuvant chemoradiotherapy in locally advanced thymic epithelial neoplasms. Tumori. 2021; 107(5): 407–15.