Tumor Immunity is Related to 18F-FDG Uptake in Thymic Epithelial Tumor

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Abstract

Background

2-Deoxy-2-[fluorine-18]-fluoro-d-glucose (^{18}F-FDG) positron emission tomography (^{18}F-FDG-PET) is a convenient modality to assess metabolic activity within tumor cells. However, there is no consensus regarding the relationship between ^{18}F-FDG uptake and the immune environment in thymic epithelial tumors (TETs). We conducted a clinicopathological study to elucidate the relationship between ^{18}F-FDG uptake and programmed death ligands 1 and 2 (PD-L1/PD-L2) expression in patients with TETs.

Methods

A total of 108 patients with histologically confirmed TETs classified as thymomas or thymic carcinomas who underwent surgical resection or biopsy or needle biopsy and ^{18}F-FDG PET before any treatment between August 2007 and March 2020 were enrolled in this study. Tumor specimens underwent immunohistochemical staining for PD-L1, PD-L2, GLUT1, HIF-1α, VEGFR2, VEGF-C and β2 adrenergic receptor.

Results

High uptakes of SUV_{max}, SUV_{mean}, MTV and TLG were identified in 28 (25.9%), 61 (56.5%), 55 (50.9%) and 55 (50.9%) of 108 patients, respectively. High uptake of SUV_{max} significantly correlated with PS of 1-2, thymic carcinoma and advanced stage, and SUV_{max} on ^{18}F-FDG uptake displayed a close association with PD-L1 and PD-L2 expression, but not MTV and TLG. Multivariate analysis revealed that SUV_{max} was identified as an independent predictor for positive PD-L1 /PD-L2 expression. GLUT1 was clarified as a significant marker for the expression of PD-L1/PD-L2 from the biological viewpoint.

Conclusion

^{18}F-FDG accumulation was closely associated with the expression of PD-L1/PD-L2, which, in turn, was correlated with glucose metabolism and hypoxia. PD-L1/PD-L2 could affect glucose metabolism and hypoxia in thymic tumor cells.

1. Background

Thymic epithelial tumors (TETs), which are generally classified as thymomas and thymic carcinomas, are uncommon neoplasms presenting in less than 2.0% of all malignancies [1]. In particular, thymic carcinoma is a rare cancer with a dismal outcome and no available therapeutic agents for its advanced form. Thus, the identification of new targets that can serve as predictive and prognostic markers for the development of an optimal treatment plan are essential.

2-Deoxy-2-[fluorine-18]-fluoro-d-glucose (^{18}F-FDG) positron emission tomography (^{18}F-FDG-PET) is a convenient modality to assess the metabolic activity within tumor cells, although it shows some limitations such as false-positive findings [2]. As biological mechanisms, glucose metabolism, hypoxia, and angiogenesis are closely linked to the accumulation of ^{18}F-FDG within tumor cells. In particular, several studies have demonstrated that the expression levels of glucose transporter 1 (GLUT1) and hypoxia inducible factor-1α (HIF-1α) are correlated with ^{18}F-FDG uptake in thoracic tumors [3]. The ^{18}F-FDG uptake level can help predict the grade of malignancy in TETs, allowing staging of the extent of the disease, prognosis, and therapeutic sensitivity [3]. Programmed death ligand-1 (PD-L1) has been recently shown to be expressed in patients with TETs and is closely correlated with the grade of malignancy and survival [4 5]. Immune checkpoint inhibitors (ICIs) targeting programmed death-1 (PD-1) or PD-L1 have been identified as effective therapeutic agents for patients with various human cancers. In particular, PD-L1 expression within tumor cells is thought to be a predictor of response to and outcome of therapy in patients with advanced lung cancer who received anti-PD-1 antibody [6]. Therefore, ICIs could serve as a potential optimal treatment option for neoplasms with PD-L1 expression.

Several recent studies have shown that PD-L1 expression within tumor cells is closely related to ^{18}F-FDG uptake [7–10]. In patients with non-small cell lung cancer (NSCLC), PD-L1 expression is linked to ^{18}F-FDG uptake, GLUT1, and HIF-1α. Also, GLUT1 and HIF-1α have been described to be closely associated with angiogenesis such as vascular endothelial growth factor (VEGF)[2]. A recent investigation indicated that the increased expression of HIF-1α is associated with enhanced expression of PD-L1, and contributes to the activation of T-cell function and mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) signaling pathways [11]. Furthermore, HIF-1α directly binds to the hypoxia response element in the proximal promoter of PD-L1 and controls its expression under hypoxia [12]. Thus, the percentage of glucose metabolism determined by HIF-1α is suggestive of an immune reaction according to PD-L1 expression. However, little is known about the relationship between ^{18}F-FDG uptake and PD-L1 expression in patients with TETs. Moreover, anti-PD-1 antibody has been already known to provide an optimal blockade of PD-L1 and PD-L2, and some reports have shown that the expression of PD-L2 may be a potential progressive and prognostic marker in lung cancer [13 14]. Nevertheless, it remains unclear whether PD-L2 expression is associated with ^{18}F-FDG uptake and tumor aggressiveness in patients with

TETs. Although maximal standardized uptake value (SUV_{max}) has been generally used as a measurement of ^{18}F-FDG uptake, little is known about the correlation between PD-L1 expression and metabolic tumor volume (MTV) or total lesion glycolysis (TLG) on ^{18}F-FDG uptake. Thus, not only SUV_{max} but also MTV or TLG should be investigated for the association of PD-L1 expression with ^{18}F-FDG uptake.

To address this gap in the literature, we conducted a clinicopathological study to elucidate the relationship between ^{18}F-FDG uptake and PD-L1/PD-L2 expression in patients with TETs and correlated the findings with GLUT1 and HIF-1α expression.

2. Materials And Methods

2.1. Patients

A total of 118 patients with histologically confirmed TETs classified as thymomas or thymic carcinomas who underwent surgical resection or biopsy or needle biopsy and ^{18}F-FDG PET before any treatment at the Comprehensive Cancer Center, International Medical Center, Saitama University Hospital between August 2007 and March 2020. Of them, 10 patients were excluded because of inadequate tumor specimens and radiographic information, therefore, a total of 108 patients were enrolled in this study. Pathological diagnosis and tumor subtyping were performed according to the 2015 WHO histological classification of TETs and the TNM staging system[15]. Diagnoses were confirmed using light microscopy and immunohistochemistry. Surgically resected or biopsied primary tumors (n = 108) were included in this study in accordance with the institutional guidelines and the Helsinki Declaration. Ninety-four patients received surgical resection, and biopsy was performed in 14 patients. This study was approved by the institutional ethics committee of the International Medical Center, Saitama Medical University. The requirement for written informed consent was waived by the ethics committee of Saitama Medical University because of the retrospective nature of the study.

2.2. Immunohistochemical staining

For PD-L1 and PD-L2, immunohistochemical staining was performed according to previously described procedures [8 9]. Rabbit monoclonal antibodies against PD-L1 (clone 28 − 8; 1:100 dilution; Cell Signaling Technology, Danvers, MA, USA) and a mouse monoclonal antibody against PD-L2 (clone 366C.9E5; 1:100 dilution; Merck KGaA, Darmstadt, Germany) were used. Antigen retrieval was performed by autoclaving using Target Retrieval Solution (AR6, 10 × Universal HIER antigen retrieval reagent; Abcam, Tokyo, Japan), and the reaction was visualized using Signal Stain Boost IHC Detection Reagent. The expression of PD-L1 and PD-L2 was considered positive when membranous staining was observed. The following semiquantitative scoring method was used for PD-L1 and PD-L2: 1 = < 1%, 2 = 1%-24%, 3 = 25%-49%, and 4 = > 50% positively stained cells. Tumors with a score ≥ 2 were graded as showing positive expression.

The expressions of GLUT1 (1:100 dilution; Abcam, Tokyo, Japan), HIF-1α (1:100 dilution; Abcam, Tokyo, Japan), vascular endothelial growth factor receptor 2 (VEGFR2) (1:100 dilution; Abcam, Tokyo, Japan), VEGF-C (1:50 dilution; Immuno-Biological Laboratories Co.,Ltd., Gunma, Japan) and β2 adrenergic receptor (β2-AR) (1:100 dilution; Abcam, Tokyo, Japan) were scored according to the stained tumor areas as follows: 1 = ≤ 10% staining, 2 = 11% − 24% staining, 3 = 25–49% staining, and 4 = ≥ 50% staining. Low and high expression were defined by scores of 1–2 and 3–4, respectively, for GLUT1, HIF-1α and VEGFR2, and positive and negative expression were defined of 1 and 2–4, respectively, for VEGF-C and β2-AR.

Sections were evaluated using a light microscope in a blinded fashion by at least two authors. In case of discrepancies, both investigators evaluated the slides simultaneously until they reached a final consensus on the assessment. The investigators were blinded to the patient outcomes.

2.3. PET imaging and data analysis

Patients fasted for at least 6 h before PET imaging, which was performed using a PET/CT scanner (Biograph 6 or 16, Siemens Healthineers K.K., Japan) with a 585-mm field of view. Three-dimensional data acquisition was initiated 60 min after injecting 3.7 MBq/kg of FDG. We acquired eight bed positions (2-minute acquisition per bed position) according to the range of imaging. Attenuation-corrected transverse images obtained with ^{18}F-FDG were reconstructed with the ordered-subsets expectation-maximization algorithm, based on the point spread function into 168 × 168 matrices with a slice thickness of 2.00 mm.

For the semiquantitative analysis, functional images of the standardized uptake value (SUV) were produced using attenuation-corrected transaxial images with the injected dosage of ^{18}F-FDG, patient's body weight, and the cross-calibration factor between PET and the dose calibrator. The SUV was defined as follows:

SUV = radioactive concentration in the volume of interest (VOI) (MBq/g)/Injected dose (MBq)/Patient's body weight (g).

CT scanning for initial staging was performed with intravenous contrast medium, and the CT images were interpreted by board-certified radiologists. We used RAVAT software (Nihon Medi-physics Co. Ltd., Japan) on a Windows workstation to semi-automatically calculate the maximum of SUV (SUV_{max}) and MTV, TLG, defined as MTV multiplied by SUV_{mean}, of each lesion by using SUV thresholds obtained by the SUV in the liver VOI. Each threshold was defined as average of SUV (SUV_{mean}) plus 1.5 × S.D. of SUV in the liver. These SUV thresholds were the optimum values to generate a 3D volume of interest (VOI) in which the whole tumor mass is completely enclosed in all cases, with CT image as the reference. In case of the activity other than tumors, including myocardium, gastro-intestinal tracts, kidneys and urinary tracts, were eliminated by manually according to the diagnosis by the board-certified nuclear medicine physician.

2.4. Statistical analysis

Statistical analyses were performed using Student's *t-test* and the χ^2 test for continuous and categorical variables, respectively. A p value < 0.05 was considered statistically significant. Univariate and multivariate analyses of the relationship between PD-L1 expression and different variables were done by logistic regression analysis. Cut-off values for SUV_{max}, SUV_{mean}, MTV and TLG were determined by receiver operating characteristic (ROC) curve analyses. Correlations between SUVmax, MTV, and TLG on ^{18}F-FDG uptake were analyzed using Spearman's correlation coefficient test. All statistical analyses were performed using GraphPad Prism software (v.8.0; GraphPad Software, San Diego, CA, USA) and JMP 14.0 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

3.1. Patient characteristics and immunohistochemistry

A total of 118 patients (n_{males} = 54, $n_{females}$ = 54; median age = 64 years; age range = 34–85 years) were enrolled in the study. Patient characteristics are listed in Table 1. A total of 49 patients (45.3%) had a smoking history, and disease stages I, II, III, and IV were recorded in 37 (34.3%), 39 (36.1%), 14 (13.0%), and 18 (16.7%) patients, respectively.

Table 1

Patient's demographics according to ^{18}F-FDG uptake using different parameters

Variables	Total	SUV_{max}			SUV_{mean}			MTV			TLG		
	n = 108	High n = 28	Low n = 80	p-value	High n = 61	Low n = 47	p-value	High n = 55	Low n = 53	p-value	High n = 55	Low n = 53	p-value
Age (years) ≥ 69/<69	76/32	21/7	55/25	0.634	48/13	18/29	**< 0.001**	41/14	35/18	0.401	40/15	36/27	0.086
Gender Male / Female	54/54	19/9	35/45	**0.047**	33/28	21/26	0.437	32/23	22/31	0.085	31/24	23/30	0.248
PS (ECOG) 0 / 1–2	71/37	13/15	58/22	**0.019**	35/26	36/11	**0.042**	32/23	39/14	0.107	32/23	39/14	0.107
Smoking Yes / No	49/59	17/11	32/48	0.077	27/34	22/25	0.846	32/23	17/36	**0.007**	30/25	19/34	0.056
Histology Thymoma/Thymic ca.	81/27	8/20	73/7	**< 0.001**	38/23	43/4	**< 0.001**	32/23	49/4	**< 0.001**	32/23	49/4	**< 0.001**
Disease stage I-I / III-IV	75/33	6/22	69/11	**0.014**	37/24	38/9	**0.034**	28/25	47/6	**< 0.001**	27/28	48/5	**< 0.001**
PD-L1 Positive / Negative	58/50	21/7	37/43	**0.014**	38/23	20/27	0.052	34/21	24/29	0.122	33/22	25/28	0.246
PD-L2 Positive / Negative	61/47	22/6	39/41	**0.007**	35/26	26/21	0.847	34/21	27/26	0.331	35/20	26/27	0.174
GLUT1 High / Low	56/52	24/4	32/48	**< 0.001**	38/23	18/29	**0.019**	34/21	22/31	0.053	34/21	22/31	0.053
HIF-1α High / Low	32/76	14/14	18/62	**0.008**	21/40	11/36	0.288	19/36	13/40	0.295	18/37	14/39	0.531
VEGFR2 High / Low	57/51	15/13	42/38	> 0.999	28/33	29/18	0.122	29/26	28/25	> 0.999	30/25	27/26	0.847
VEGF-C Positive / Negative	59/49	9/19	50/30	**0.007**	30/31	29/18	0.243	26/29	33/20	0.127	25/30	34/19	0.056

Abbreviation: ^{18}F-FDG, 2-Deoxy-2-[fluorine-18]-fluoro-d-glucose; ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death ligand-1; PD-L2, programmed death ligand-2; GLUT1, glucose transporter 1; HIF-1α, hypoxia inducible factor-1; SUV_{max}, the maximum of standardized uptake value; SUV_{mean}, mean standardized uptake value; MTV, metabolic tumor volume; TLG, total lesion glycolysis; Thymic ca., thymic carcinoma; Glut1, glucose transporter 1; HIF-1α, hypoxia inducible factor-1α; VEGFR2, vascular endothelial growth factor receptor 2; VEGF-C, vascular endothelial growth factor-C; β2-AR, beta-2 adrenergic receptor.

Variables	Total	SUV_{max}			SUV_{mean}			MTV			TLG		
	n = 108	High n = 28	Low n = 80	p-value	High n = 61	Low n = 47	p-value	High n = 55	Low n = 53	p-value	High n = 55	Low n = 53	p-value
β2-AR Positive / Negative	35/73	16/12	19/61	**0.002**	26/35	9/38	**0.012**	21/34	14/39	0.221	19/36	16/37	0.683

Abbreviation: ^{18}F-FDG, 2-Deoxy-2-[fluorine-18]-fluoro-d-glucose; ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death ligand-1; PD-L2, programmed death ligand-2; GLUT1, glucose transporter 1; HIF-1α, hypoxia inducible factor-1; SUV_{max}, the maximum of standardized uptake value; SUV_{mean}, mean standardized uptake value; MTV, metabolic tumor volume; TLG, total lesion glycolysis; Thymic ca., thymic carcinoma; Glut1, glucose transporter 1; HIF-1α, hypoxia inducible factor-1α; VEGFR2, vascular endothelial growth factor receptor 2; VEGF-C, vascular endothelial growth factor-C; β2-AR, beta-2 adrenergic receptor.

The median FDG uptake values for SUV_{max}, SUV_{mean}, MTV and TLG, before operation or biopsy were 4.4, 3.3, 25.5 and 93.1, respectively. The optimal ^{18}F-FDG uptake cut-offs for SUV_{max}, SUV_{mean}, MTV and TLG as determined by ROC curve analysis, were 7.0 (sensitivity: 44.0%, specificity: 72.2%), 3.1 (sensitivity: 76.0%, specificity: 77.5%), 17.2 cm^3 (sensitivity: 66.0%, specificity: 82.8%), 56.7 gcm^3/mL (sensitivity: 68.0%, specificity: 80.8%), respectively (Fig. 1).

Representative ^{18}F-FDG PET images are shown in Fig. 2. The different variables according to ^{18}F-FDG uptake by SUV_{max}, SUV_{mean}, MTV and TLG were statistically compared (Table 1). High uptakes of SUV_{max}, SUV_{mean}, MTV and TLG were identified in 28 (25.9%), 61 (56.5%), 55 (50.9%) and 55 (50.9%) patients, respectively. High uptake of SUV_{max} and SUV_{mean} significantly correlated with PS of 1–2, thymic carcinoma, advanced stage, and high MTV and TLG were closely associated with histology and disease stage.

3.2. Immunohistochemical findings

Immunohistochemical examination was performed on the 108 primary sites of the TETs. Representative images for PD-L1, PD-L2, GLUT1, HIF-1α, VEGF-C and β2-AR are shown in Fig. 2. PD-L1 and PD-L2 immunostaining was localized predominantly in the plasma membrane of tumor cells. GLUT1 was stained on the cell membranes of tumor specimens; there was no evidence of normal tissue without red blood cells; and HIF-1α was stained in the nuclei. The PD-L1- and PD-L2-positive rates were 53.7% (58/108) and 56.5% (61/108), respectively, and the mean scores for PD-L1 and PD-L2 were 1.9 and 2.0, respectively. The percentages of scores of 1, 2, 3, and 4 for PD-L1 and PD-L2 were 46.3% (50/108), 26.0% (28/108), 15.7% (17/108), and 12.0% (13/108), respectively, and 43.5% (47/108), 26.9% (29/108), 13.9% (15/108), and 15.7% (17/108), respectively. The percentages of high expression and mean scores for GLUT1, HIF-1α, VEGFR2 were identified as 51.8% (56/108), 29.6% (32/108) and 52.7% (57/108), respectively, and 1.9, 1.5 and 1.9, respectively. The VEGF-C and β2-AR positive rates yielded 54.6% (59/108) and 32.4% (35/108), respectively, with the mean scores of 1.9 and 2.1, respectively. Using Spearman's correlation coefficient test, a statistically significant correlation was also observed between the expression of PD-L1 and PD-L2 ($\gamma = 0.27$, $p < 0.01$). Meanwhile, the expression of PD-L1 closely correlated with GLUT1 ($\gamma = 0.27$, $p < 0.01$), VEGFR2 ($\gamma = 0.31$, $p < 0.01$) and VEGF-C ($\gamma = -0.23$, $p = 0.02$), but not HIF-1α ($\gamma = 0.18$, $p = 0.08$) and β2-AR ($\gamma = 0.02$, $p = 0.86$), whereas, that of PD-L2 was significantly associated with GLUT1 ($\gamma = 0.54$, $p < 0.01$), HIF-1α ($\gamma = 0.42$, $p < 0.01$), VEGFR2 ($\gamma = 0.34$, $p < 0.01$) and VEGF-C ($\gamma = -0.26$, $p = 0.01$) and β2-AR ($\gamma = 0.32$, $p < 0.01$). Moreover, the comparison of scoring of different biomarkers according to PD-L1 and PD-L2 expression was performed. Positive expression of PD-L1 and PD-L2 was significantly linked to the increased expression of GLUT1, HIF-1α and VEGFR2. But, there was opposite relationship between PD-L1 and VEGF-C (Fig. 3A). The expression of PD-L1 and PD-L2 exhibited a significantly higher in patients with thymic carcinoma than in those with thymoma.

3.3. Comparison of different biomarkers related to ^{18}F-FDG uptake on PET

Table 2 also shows the comparison of various biomarkers according to ^{18}F-FDG uptake. High uptake of ^{18}F-FDG by SUV_{max} exhibited a significant association with positive or high expression of PD-L1, PD-L2, VEGFR2 and β2-AR, but, there was no relationship between MTV or TLG and different biomarkers. Moreover, the comparison of ^{18}F-FDG uptake according to the expression of PD-L1 and PD-L2 was listed in Fig. 2. SUV_{max} and SUV_{mean} on ^{18}F-FDG uptake displayed a close association with PD-L1 and PD-L2 expression, but not MTV and TLG.

Table 2
Univariate and multivariate analyses of relationship between PD-L1 /PD-L2 expression and different variables

Variables	PD-L1 expression			PD-L2 expression		
	Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
	p-value	OR (95% CI)	*p*-value	*p*-value	OR (95% CI)	*p*-value
Age (years)	0.442	1.435	0.423	0.092	2.358	0.078
≥ 69/<69		(0.591–3.487)			(0.905–6.141)	
Gender	> 0.999	1.082	0.868	**0.003**	2.616	**0.048**
Male/Female		(0.424–2.763)			(1.005–6.964)	
PS (ECOG)	0.091	1.574	0.311	0.388	1.108	0.826
0 / 1–2		(0.653–3.795)			(0.441–2.778)	
Smoking	0.610	1.537	0.367	0.610	1.001	0.998
Yes / No		(0.603–3.921)			(0.379–2.648)	
Disease stage	0.071	1.107	0.859	**0.006**	1.557	0.458
I-I / III-IV		(0.359–3.406)			(0.482–5.029)	
SUV_{max}	**0.007**	3.408	**0.048**	**0.004**	2.824	0.114
High/low		(1.101–11.499)			(0.778–10.251)	
SUV_{mean}	**0.041**			0.831		
High/low						
MTV	0.084			0.254		
High/low						
TLG	0.181			0.125		
High/low						

Abbreviation: ECOG PS, Eastern Cooperative Oncology Group performance status; PD-L1, programmed death ligand-1; PD-L2, programmed death ligand-2; SUV_{max}, the maximum of standardized uptake value; SUV_{mean}, mean standardized uptake value; MTV, metabolic tumor volume; TLG, total lesion glycolysis; OR, odds ratio; 95%CI, 95% confidence interval.

3.4. Univariate and multivariate analyses of association between PD-L1/PD-L2 expression and different variables

The relationship between PD-L1/PD-L2 expression and patient's demographics was examined by using logistic regression analysis (Table 2). By univariate analyses in the relationship between PD-L1 protein expression and other factors, SUV_{max} and SUV_{mean} were identified as significant predictors, and multivariate analysis revealed that SUV_{max} was identified as an independent predictor for positive PD-L1 expression. Likewise, the association between PD-L2 protein expression and other factors was also evaluated, and gender, disease stage and were SUV_{max} selected as significant predictors by univariate analysis. Multivariate analysis demonstrated that gender was an independent predictor for positive PD-L2 expression.

Next, we also investigated the relationship between PD-L1/PD-L2 expression and different biomarkers (Table 3). Univariate analysis in the PD-L1 expression demonstrated that GLUT1, HIF-1α, VEGFR2 and VEGF-C were significant factor for predicting its expression, and GLUT1 was selected as an independent predictor by multivariate analysis. Likewise, GLUT1, HIF-1α, VEGFR2, VEGF-C and β2-AR were identified as significant biomarkers for predicting positive PD-L2 expression, and multivariate analysis revealed that GLUT1 was an independent predictor .

Table 3
Univariate and multivariate analyses of relationship between PD-L1 /PD-L2 expression and different biomarkers

Variables	PD-L1 expression			PD-L2 expression		
	Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
	p-value	OR (95% CI)	*p*-value	*p*-value	OR (95% CI)	*p*-value
GLUT1	**< 0.001**	2.892	**0.049**	**< 0.001**	3.469	**0.017**
High / Low		(1.003–7.981)			(1.242–9.685)	
HIF-1α	**0.012**	1.691	0.329	**0.002**	1.987	0.217
High / Low		(0.588–4.856)			(0.667–5.917)	
VEGFR2	**0.004**	2.253	0.080	**0.023**	1.565	0.345
High / Low		(00907-5.597)			(0.615–3.97)	
VEGF-C	**0.009**	0.432	0.058	**0.036**	0.594	0.247
High / Low		(0.181–1.029)			(0.245–1.434)	
β2-AR	0.362	0.828	0.711	**0.027**	1.509	0.421
High / Low		(0.306–2.241)			(0.553–4.119)	

Abbreviation: PD-L1, programmed death ligand-1; PD-L2, programmed death ligand-2; GLUT1, glucose transporter 1; HIF-1α, hypoxia inducible factor-1α; VEGFR2, vascular endothelial growth factor receptor 2; VEGF-C, vascular endothelial growth factor-C; β2-AR, beta-2 adrenergic receptor; OR, odds ratio; 95%CI, 95% confidence interval.

4. Discussion

To our knowledge, this is the first study to evaluate the relationship between PD-L1/PD-L2 expression and ^{18}F-FDG uptake on PET in patients with TETs. We found that high expression of PD-L1 and PD-L2 was closely associated with high accumulation of ^{18}F-FDG; in particular, PD-L1/PD-L2 expression levels were significantly correlated with those of glucose metabolism and hypoxia. As angiogenetic markers, VEGFR2 and β2-AR was associated with the expression of PD-L1/PD-L2. Moreover, we confirmed that the expression of PD-L1 and PD-L2 was closely associated with not MTV or TLG but SUV_{max} on ^{18}F-FDG uptake, confirmed by multivariate analysis. Overall, PD-L1 and PD-L2 expressions indicated a strong correlation with glucose metabolism, as determined by GLUT1. Although SUV_{max} is closely correlated with MTV and TLG on ^{18}F-FDG uptake, the upregulation of PD-L1 and PD-L2 may play a crucial role in the pathogenesis of tumor glucose metabolism in patients with TETs. Further studies with an experimental approach using thymic tumor cell lines are warranted to elucidate the results of our study.

Several researchers have described that PD-L1 is frequently expressed in TETs, and a WHO classification is closely related to positive PD-L1 expression, but there was some discrepancy regarding the trend for worsened survival [4 16–19]. Padda *et al*. reported that high expression of PD-L1 could predict a significantly worse OS, which was correlated with more aggressive histology [16]. However, Yokoyama *et al*. described that low PD-L1 expression and a high number of PD-1-positive tumor infiltrative lymphocytes (TILs) were significant predictors of worse survival in patients with thymic carcinoma [18]. Considering the evidence from previous studies, it is debatable whether PD-L1 could absolutely predict a worse outcome for patients with TETs. As our study also indicated that the expression of PD-L1 was higher in thymic carcinoma than in thymoma, PD-L1 may highly express in human neoplasms with malignant phenotype.

PD-L1 is an important target for PD-1 blockade, whereas PD-L2, as another PD-1 ligand, may also play a crucial role in the inhibition of PD-1 in human neoplasms. A recent study demonstrated that PD-L2 was expressed in all tumor types. The prevalence of PD-L2 was significantly correlated with PD-L1, and PD-L2 status was also a significant predictor of PFS with pembrolizumab, independent of PD-L1 status [20]. Although there are some concerns about the clinicopathological significance of PD-L2 expression in human neoplasms, PD-L2 is frequently expressed in tumor cells and seems to play a crucial role in tumor growth and survival, adjusting the immune environment. A previous study reported that GLUT1 expression is associated with better clinical outcomes in advanced-stage classical Hodgkin's lymphoma and is significantly associated with PD-L1 and PD-L2 expression [21]. This study supports the hypothesis that GLUT1-related signaling pathways play an important role in the PD-L1 or PD-L2 pathway. Furthermore, a previous article reported that PD-L2-positive pheochromocytoma and paraganglioma were characterized by higher HIF-1α expression. That study reported the enrichment of transcripts involved in the hypoxic response in relation to PD-L2, but not PD-L1 expression [22]. When the researchers considered a broader subset of 200 genes involved in the hypoxic response, PD-L2 upregulation strikingly emerged as a stronger and more substantial determinant of tumor hypoxia than PD-L1, suggesting a potential mechanistic relationship between hypoxia and PD-L2-mediated antitumor immune control. Their data suggest that PD-L2 has a more predominant role than PD-L1 in shaping the immune-tolerogenic environment, given the highly significant association with key pathways involved in innate, adaptive immunity, and

inflammation in pheochromocytomas and paragangliomas. However, little is known about the clinicopathological significance of PD-L2 expression in patients with TETs. Recently, Rouquette *et al.* reported that the PD-L2 antibody stained no tumor epithelial cells in TETs [19]. Although we also performed PD-L2 staining using the same antibody, no staining was observed in our study, corresponding to their results [19]. Thus, different kinds of PD-L2 clones were explored, and we found the optimal PD-L2 clone for immunohistochemistry. Our investigation is the first study to demonstrate the expression of PD-L2 in TETs. In our study, PD-L2 was closely correlated with the expression of PD-L1 and was associated with histological grade, glucose metabolism, and hypoxia, but not survival.

The aim of our study was to focus on the role of PD-L1/PD-L2 expression as a mechanism underlying ^{18}F-FDG uptake in TETs. Previous investigations have supported the potential of PD-L1 as an alternative target of HIF-1α and suggested that the distribution of glucose metabolism determined by HIF-1α could reflect the immune response reflected by the expression of PD-L1 [11 12]. In addition, direct blockade of PD-L1 within cancer cells has been reported to diminish glycolysis by inhibiting the mTOR pathway and the expression of glycolysis enzymes [23]. Although the close relationship between ^{18}F-FDG uptake and PD-L1 expression was also supported by other evidence, assessments to elucidate its mechanism require further investigation. However, the relationship between ^{18}F-FDG uptake and PD-L2 expression has not yet been supported by experimental evidence. Takada *et al.* reported the radiological features of PD-L2 expression in 222 patients with lung adenocarcinoma [24]. In their study, the SUV_{max} for ^{18}F-FDG uptake was found to be significantly higher in PD-L2-positive than in PD-L2-negative cases [24]. This corresponds to the results of our study. It remains unknown why the expression level of PD-L2 is closely related to ^{18}F-FDG uptake. Our results suggest that the expression of PD-L2 is strongly associated with tumor glucose metabolism, hypoxia and angiogenesis as a mechanism of ^{18}F-FDG uptake. PD-L2 seemed to be more strongly correlated with glucose metabolism, hypoxia and angiogenesis, compared with PD-L1. Further investigation should be conducted to elucidate the relationship between PD-L2 and ^{18}F-FDG uptake from the perspective of basic science.

In the current study, SUV_{max} as assessment of ^{18}F-FDG uptake on PET was identified as a significant marker for predicting the expression of PD-L1/PD-L2. However, TLG or MTV are also significant indicators of ^{18}F-FDG uptake that can reflect tumor metabolic activity, and are calculated on the basis of SUV and are closely correlated with SUV_{max}. Our study is a first investigation to evaluate whether MTV or TLG could be correlated with the expression of PD-L1, thus, it remains unclear why SUV_{max} was chosen as a better marker for the prediction of PD-L1 expression than TLG or MTV. The relationship between metabolic tumor volume and PD-L1 expression may be obscure.

There are several limitations to our study. First, our study had a small sample size, which may have biased the results of our study. Since thymic cancer is a rare neoplasm, only limited numbers of samples were collected. Second, we tried to examine PD-L1 staining using clone 28 – 8; however, there are several kinds of PD-L1 clones. An additional investigation using other clones of PD-L1 may be needed to confirm the results of our study. Finally, the results of our study was not confirmed by experimental investigations. In the level of tumor cell lines, little is known about any data elucidating the association between PD-L1 expression and ^{18}F-FDG uptake. Further examination is needed to approach some basic mechanism.

Further studies are warranted to assess TLG and MTV in the context of the present study's findings.

In conclusion, the relevance and distribution of ^{18}F-FDG uptake on PET were significantly associated with the expression of PD-L1 and PD-L2 in patients with TETs. In particular, PD-L1 and PD-L2 exhibited a close relationship with upregulation of tumor glucose metabolism (GLUT1) and hypoxia (HIF-1α), which play essential roles in the mechanism of ^{18}F-FDG uptake within tumor cells. Further studies are needed to elucidate why PD-L1 and PD-L2 affect glucose metabolism and hypoxia in thymic tumor cells.

5. Declarations

Compliance with Ethical Standards:

Conflict of Interest:

Kyoichi Kaira has received research grants and a speaker honorarium from Ono Pharmaceutical Company, Boehringer Ingelheim, Chugai Pharmaceutical, Taiho Pharmaceutical, Eli Lilly Japan, and AstraZeneca. Atsuto Mouri has received a speaker honorarium from Eli Lilly, Taiho Pharmaceutical, Pfizer, Chugai Pharmaceutical, and AstraZeneca. Hiroshi Kagamu has received research grants and a speaker honorarium from Ono Pharmaceutical Company, Bristol-Myers Company, Boehringer Ingelheim, MSD, Daiichi Sankyo Company, Chugai Pharmaceutical, Taiho Pharmaceutical, Merck Biopharma Company, Eli Lilly Japan, and AstraZeneca. Kobayashi has received research grants and a speaker honorarium from Boehringer Ingelheim, AstraZeneca, and Bristol-Myers Company.

Ethical approval:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Abbreviations

CT Computed tomography

^{18}F-FDG 2-Deoxy-2-[fluorine-18]-fluoro-d-glucose

MAPK Mitogen-activated protein kinase

MTV Metabolic tumor volume

OS Overall survival

PET Positron emission tomography

PFS Progression-free survival

PS Performance status score

ROI Region of interest

SUV Standardized uptake value

TLG Total lesion glycolysis

GLUT1 Glucose transporter 1

HIF-1α Hypoxia inducible factor-1α

PD-1 Programmed death-1

PD-L1/PD-L2 Programmed death ligand-1/programmed death ligand-2

ICIs Immune checkpoint inhibitors

VEGFR2 Vascular endothelial growth factor receptor 2

VEGF-C Vascular endothelial growth factor C

β2-AR β2 adrenergic receptor

Declarations

Competing interests

Kyoichi Kaira has received research grants and a speaker honorarium from Ono Pharmaceutical Company, Boehringer Ingelheim, Chugai Pharmaceutical, Taiho Pharmaceutical, Eli Lilly Japan, and AstraZeneca. Atsuto Mouri has received a speaker honorarium from Eli Lilly, Taiho Pharmaceutical, Pfizer, Chugai Pharmaceutical, and AstraZeneca. Hiroshi Kagamu has received research grants and a speaker honorarium from Ono Pharmaceutical Company, Bristol-Myers Company, Boehringer Ingelheim, MSD, Daiichi Sankyo Company, Chugai Pharmaceutical, Taiho

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References

1. Engels EA, Pfeiffer RM. Malignant thymoma in the United States: demographic patterns in incidence and associations with subsequent malignancies. *Int J Cancer* 2003;105(4):546-51. doi: 10.1002/ijc.11099 [published Online First: 2003/04/25]
2. Kaira K, Oriuchi N, Otani Y, et al. Diagnostic usefulness of fluorine-18-alpha-methyltyrosine positron emission tomography in combination with 18F-fluorodeoxyglucose in sarcoidosis patients. *Chest* 2007;131(4):1019-27. doi: 10.1378/chest.06-2160 [published Online First: 2007/04/12]
3. Kaira K, Endo M, Abe M, et al. Biologic correlation of 2-[18F]-fluoro-2-deoxy-D-glucose uptake on positron emission tomography in thymic epithelial tumors. *J Clin Oncol* 2010;28(23):3746-53. doi: 10.1200/JCO.2009.27.4662 [published Online First: 2010/07/14]
4. Katsuya Y, Fujita Y, Horinouchi H, et al. Immunohistochemical status of PD-L1 in thymoma and thymic carcinoma. *Lung Cancer* 2015;88(2):154-9. doi: 10.1016/j.lungcan.2015.03.003 [published Online First: 2015/03/24]
5. Wei YF, Chu CY, Chang CC, et al. Different pattern of PD-L1, IDO, and FOXP3 Tregs expression with survival in thymoma and thymic carcinoma. *Lung Cancer* 2018;125:35-42. doi: 10.1016/j.lungcan.2018.09.002 [published Online First: 2018/11/16]
6. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* 2016;375(19):1823-33. doi: 10.1056/NEJMoa1606774 [published Online First: 2016/10/11]
7. Takada K, Toyokawa G, Okamoto T, et al. Metabolic characteristics of programmed cell death-ligand 1-expressing lung cancer on (18) F-fluorodeoxyglucose positron emission tomography/computed tomography. *Cancer Med* 2017;6(11):2552-61. doi: 10.1002/cam4.1215 [published Online First: 2017/10/06]
8. Kasahara N, Kaira K, Bao P, et al. Correlation of tumor-related immunity with 18F-FDG-PET in pulmonary squamous-cell carcinoma. *Lung Cancer* 2018;119:71-77. doi: 10.1016/j.lungcan.2018.03.001 [published Online First: 2018/04/17]
9. Kaira K, Shimizu K, Kitahara S, et al. 2-Deoxy-2-[fluorine-18] fluoro-d-glucose uptake on positron emission tomography is associated with programmed death ligand-1 expression in patients with pulmonary adenocarcinoma. *Eur J Cancer* 2018;101:181-90. doi: 10.1016/j.ejca.2018.06.022 [published Online First: 2018/08/05]
10. Zhang M, Wang D, Sun Q, et al. Prognostic significance of PD-L1 expression and (18)F-FDG PET/CT in surgical pulmonary squamous cell carcinoma. *Oncotarget* 2017;8(31):51630-40. doi: 10.18632/oncotarget.18257 [published Online First: 2017/09/09]
11. Noman MZ, Desantis G, Janji B, et al. PD-L1 is a novel direct target of HIF-1alpha, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *J Exp Med* 2014;211(5):781-90. doi: 10.1084/jem.20131916 [published Online First: 2014/04/30]
12. Chen R, Zhou X, Liu J, et al. Relationship between the expression of PD-1/PD-L1 and (18)F-FDG uptake in bladder cancer. *Eur J Nucl Med Mol Imaging* 2019;46(4):848-54. doi: 10.1007/s00259-018-4208-8 [published Online First: 2019/01/11]
13. Shinchi Y, Komohara Y, Yonemitsu K, et al. Accurate expression of PD-L1/L2 in lung adenocarcinoma cells: A retrospective study by double immunohistochemistry. *Cancer Sci* 2019;110(9):2711-21. doi: 10.1111/cas.14128 [published Online First: 2019/07/12]

14. Matsubara T, Takada K, Azuma K, et al. A Clinicopathological and Prognostic Analysis of PD-L2 Expression in Surgically Resected Primary Lung Squamous Cell Carcinoma. *Ann Surg Oncol* 2019;26(6):1925-33. doi: 10.1245/s10434-019-07257-3 [published Online First: 2019/03/01]
15. Travis W, Brambilla E, Burke A, et al. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. Lyon, France: International Agency for Research on Cancer 2015.
16. Padda SK, Riess JW, Schwartz EJ, et al. Diffuse high intensity PD-L1 staining in thymic epithelial tumors. *J Thorac Oncol* 2015;10(3):500-8. doi: 10.1097/JTO.0000000000000429 [published Online First: 2014/11/18]
17. Bagir EK, Acikalin A, Avci A, et al. PD-1 and PD-L1 expression in thymic epithelial tumours and non-neoplastic thymus. *J Clin Pathol* 2018;71(7):637-41. doi: 10.1136/jclinpath-2017-204788 [published Online First: 2018/02/14]
18. Yokoyama S, Miyoshi H, Nakashima K, et al. Prognostic Value of Programmed Death Ligand 1 and Programmed Death 1 Expression in Thymic Carcinoma. *Clin Cancer Res* 2016;22(18):4727-34. doi: 10.1158/1078-0432.CCR-16-0434 [published Online First: 2016/05/12]
19. Rouquette I, Taranchon-Clermont E, Gilhodes J, et al. Immune biomarkers in thymic epithelial tumors: expression patterns, prognostic value and comparison of diagnostic tests for PD-L1. *Biomark Res* 2019;7:28. doi: 10.1186/s40364-019-0177-8 [published Online First: 2019/12/13]
20. Yearley JH, Gibson C, Yu N, et al. PD-L2 Expression in Human Tumors: Relevance to Anti-PD-1 Therapy in Cancer. *Clin Cancer Res* 2017;23(12):3158-67. doi: 10.1158/1078-0432.CCR-16-1761 [published Online First: 2017/06/18]
21. Koh YW, Han JH, Park SY, et al. GLUT1 as a Prognostic Factor for Classical Hodgkin's Lymphoma: Correlation with PD-L1 and PD-L2 Expression. *J Pathol Transl Med* 2017;51(2):152-58. doi: 10.4132/jptm.2016.11.03 [published Online First: 2017/02/22]
22. Pinato DJ, Black JR, Trousil S, et al. Programmed cell death ligands expression in phaeochromocytomas and paragangliomas: Relationship with the hypoxic response, immune evasion and malignant behavior. *Oncoimmunology* 2017;6(11):e1358332. doi: 10.1080/2162402X.2017.1358332 [published Online First: 2017/11/18]
23. Chang CH, Qiu J, O'Sullivan D, et al. Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. *Cell* 2015;162(6):1229-41. doi: 10.1016/j.cell.2015.08.016 [published Online First: 2015/09/01]
24. Takada K, Toyokawa G, Azuma K, et al. Radiological Features of Programmed Cell Death-Ligand 2-positive Lung Adenocarcinoma: A Single-institution Retrospective Study. *In Vivo* 2018;32(6):1541-50. doi: 10.21873/invivo.11412 [published Online First: 2018/10/24]

Figures

Figure 1

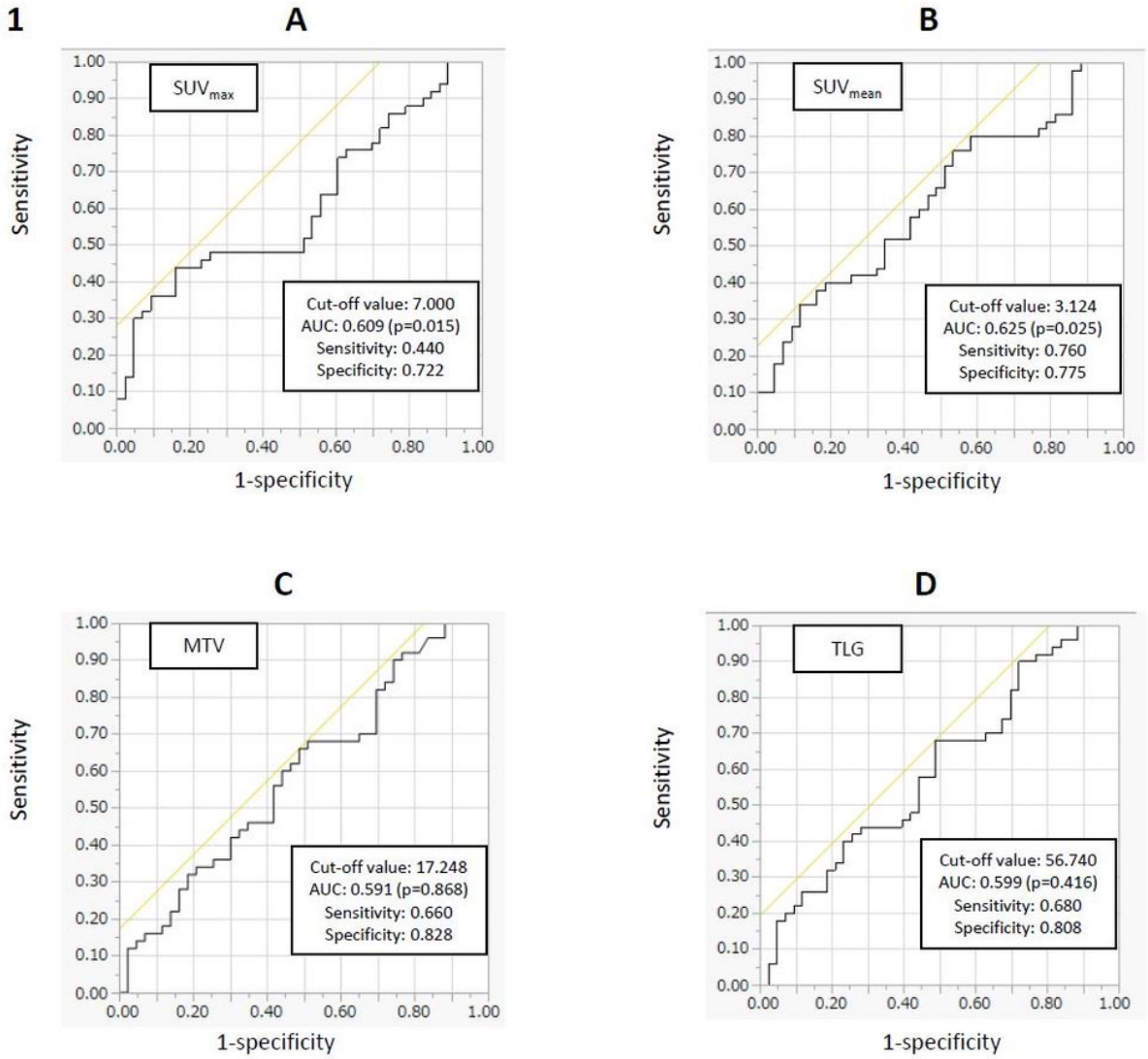

Figure 1

Cut-off values for SUVmax, SUVmean, MTV and TLG were determined by receiver operating characteristic (ROC) curve analyses. Optimal 18F-FDG uptake cut-offs for SUVmax, SUVmean, MTV and TLG as determined by ROC curve analysis, were 7.0 (sensitivity: 44.0%, specificity: 72.2%, p = 0.015), 3.1 (sensitivity: 76.0%, specificity: 77.5%, p = 0.025), 17.2 cm3 (sensitivity: 66.0%, specificity: 82.8%, p = 0.868), 56.7 gcm3/mL (sensitivity: 68.0%, specificity: 80.8%, p = 0.415), respectively

Figure 2A

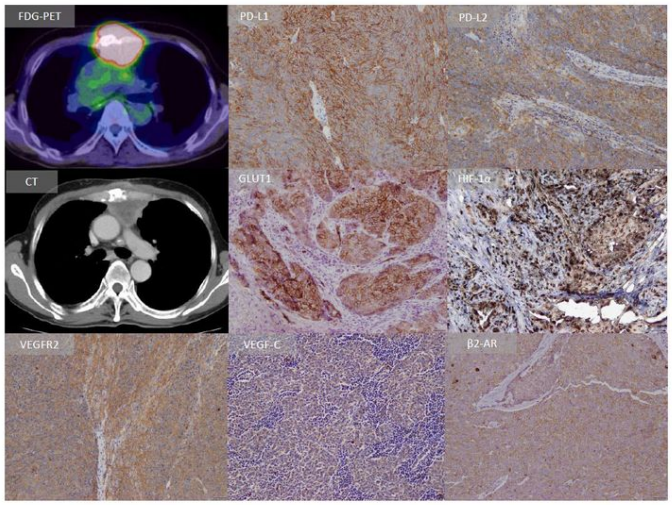

Figure 2B

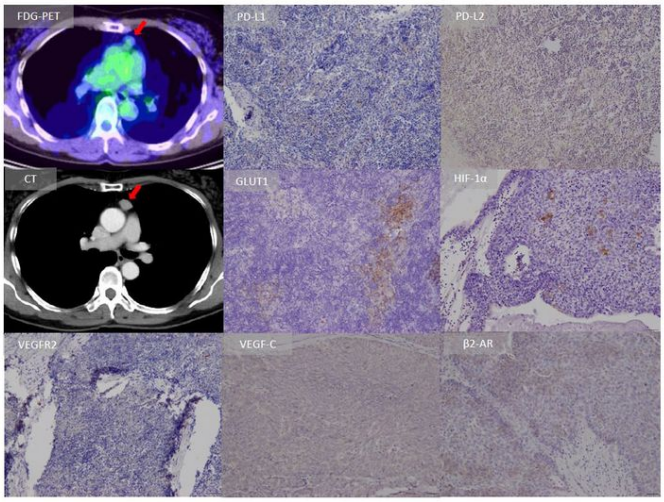

Figure 2

Representative 18F-FDG PET/CT images with high (A) and low (B) 18F-FDG accumulation and immunohistochemical staining showing PD-L1, PD-L2, GLUT1, HIF-1α, VEGFR2, VEGF-C and β2-AR expression. The maximum standardized uptake value was 19.0 for thymic cancer (A; thymic cancer), and the minimum standardized uptake value was 1.8 (B) for thymoma (red arrow). PD-L1 immunostaining was observed in the plasma membrane, and cases with scores of 4 (A; thymic cancer) and 1 (B; thymoma) are presented. PD-L2 immunostaining was also observed in the plasma membrane, and cases with scores of 4 (A; thymic cancer) and 1 (B; thymoma) are presented. GLUT1 immunostaining was observed in the cell membrane, and the cases with scores of 4 (A; thymic cancer) and 2 (B; thymoma) are shown. HIF-1α immunostaining was observed in the nuclei, and cases with scores of 4 (A; thymic cancer) and of 2 (B; thymoma) are presented. VEGFR2, VEGF-C and β2-AR immunostaining were observed in the cell membrane and cytoplasma, and cases with scores of 4 (A; thymic cancer) and 2 (B; thymoma) are shown. PD-L1, programmed death ligand-1; PD-L2, programmed death ligand-2; GLUT1, glucose transporter 1; HIF-1α, hypoxia inducible factor-1α; VEGFR2, vascular endothelial growth factor receptor 2; VEGF-C, vascular endothelial growth factor-C; β2-AR, β2 adrenergic receptor; 18F-FDG, 2-deoxy-2-[fluorine-18]-fluoro-D-glucose; PET, positron emission tomography.

Figure 3A

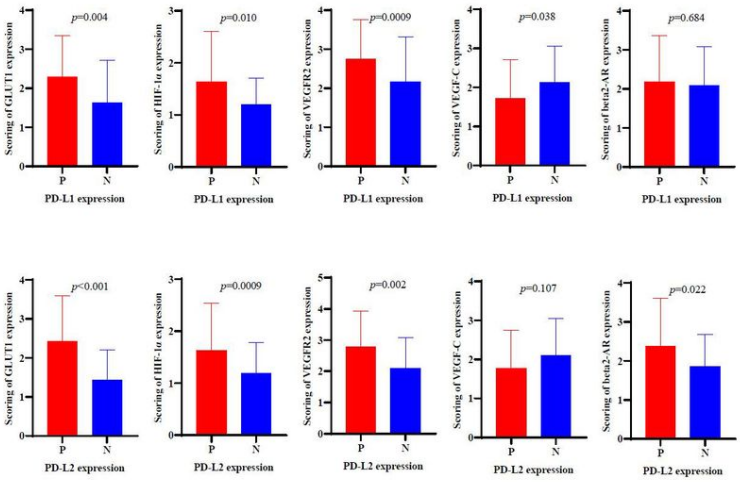

Figure 3B

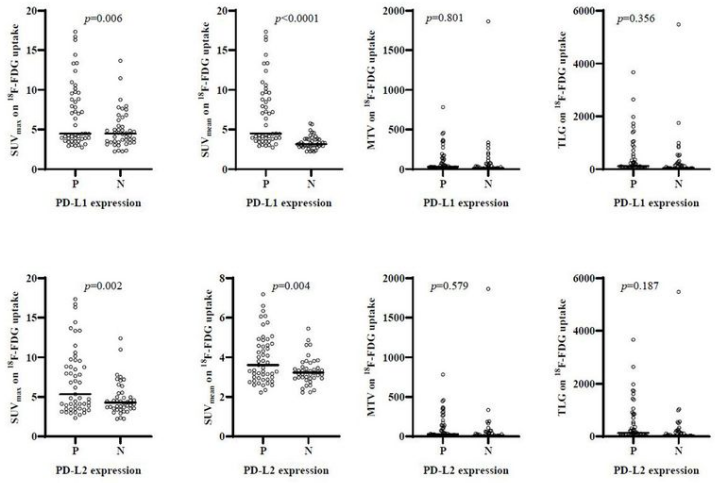

Figure 3

Comparison of GLUT1, HIF-1α, VEGFR2, VEGF-C and β2-AR expression scores according to PD-L1 and PD-L2 expression (A): Scoring of GLUT1 (p = 0.004), HIF-1α (p = 0.010) and VEGFR2 (p = 0.0009) were higher in patients with positive PD-L1 expression than in those with negative expression. GLUT1 score expression (p < 0.001), HIF-1α (p = 0.0009), VEGFR2 (p = 0.002) and β2-AR (p = 0.022) were higher in patients with positive PD-L2 expression than in those with negative expression. Comparison of SUVmax, SUVmean, MTV and TLG on 18F-FDG uptake according to PD-L1 and PD-L2 expression (B): SUVmax (p = 0.006 and p = 0.002) and SUVmean (p < 0.0001 and p = 0.004) on 18F-FDG uptake was higher in patients with positive PD-L1 and PD-L2 expression than in those with negative expression. No statistically significant differences in the MTV and TLG on 18F-FDG uptake were observed in patients with positive and negative PD-L1 and PD-L2 expression. PD-L1, programmed death ligand-1; PD-L2, programmed death ligand-2; GLUT1, glucose transporter 1; HIF-1α, hypoxia inducible factor-1α; VEGFR2, vascular endothelial growth factor receptor 2; VEGF-C, vascular endothelial growth factor-C; β2-AR, β2 adrenergic receptor; 18F-FDG, 2-deoxy-2-[fluorine-18]-fluoro-D-glucose; PET, positron emission tomography.