European Journal of Cardio-Thoracic Surgery 44 (2013) e105–e112
doi:10.1093/ejcts/ezt263 Advance Access publication 14 May 2013

ORIGINAL ARTICLE

^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography and the relationship between fluorodeoxyglucose uptake and the expression of hypoxia-inducible factor-1α, glucose transporter-1 and vascular endothelial growth factor in thymic epithelial tumours

Hiroaki Toba[a,*], Kazuya Kondo[b], Yohei Sadohara[a], Hideki Otsuka[c], Masami Morimoto[a], Koichiro Kajiura[a], Yasushi Nakagawa[a], Mitsuteru Yoshida[a], Yukikiyo Kawakami[a], Hiromitsu Takizawa[a], Koichiro Kenzaki[a], Shoji Sakiyama[a], Yoshimi Bando[d] and Akira Tangoku[a]

[a] Department of Thoracic and Endocrine Surgery and Oncology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan
[b] Department of Oncological Medical Services, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan
[c] Department of Radiology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan
[d] Department of Molecular and Environmental Pathology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

* Corresponding author. Department of Thoracic and Endocrine Surgery and Oncology, Institute of Health Biosciences, The University of Tokushima Graduate School, 18-15 Kuramoto-cho 3, Tokushima 770-8503, Japan. Tel: +81-88-6337143; fax: +8188-6337144; e-mail: toba1109@gmail.com (H. Toba).

Received 3 January 2013; received in revised form 6 March 2013; accepted 21 March 2013

Abstract

OBJECTIVES: The objective of this study was to evaluate the usefulness of ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) and the relationships among the expressions of hypoxia-inducible factor-1α (HIF-1α), glucose transporter-1 (Glut-1) and vascular endothelial growth factor (VEGF), histological type, other clinical factors and FDG uptake in thymic epithelial tumours.

METHODS: Thirty-three patients who underwent FDG-PET/CT before treatment were reviewed. All types of tumours were reclassified into three subgroups: low-risk thymomas (types A, AB and B1), high-risk thymomas (types B2 and B3) and thymic carcinomas. Tumour contour, pattern of FDG uptake, tumour size and maximum standardized uptake value (SUVmax) were obtained. Expressions of HIF-1α, Glut-1 and VEGF were analysed immunohistochemically, and these expressions were evaluated using grading scales.

RESULTS: FDG uptake was visually recognized in all (100%) tumours. A homogeneous pattern of FDG uptake was increasingly observed in the order of low-risk thymomas to high-risk thymomas to thymic carcinomas ($P = 0.016$). SUVmax for thymic carcinomas was significantly higher than that for thymomas ($P = 0.008$). With the optimal cut-off value of SUVmax of 5.6, the sensitivity, specificity and accuracy for diagnosing thymic carcinoma were 0.75, 0.80 and 0.79, respectively. Regarding the mean scoring of HIF-1α, Glut-1 and VEGF, increasing trends were observed in the order of low-risk thymomas to high-risk thymomas to thymic carcinomas. Tumour size revealed a significant correlation with SUVmax ($r = 0.60$, $P < 0.001$), and the expression of HIF-1α showed a moderate association, but the expression of Glut-1 showed no correlation with SUVmax. Regarding correlations between the expression of the three markers, there were moderate associations between HIF-1α and Glut-1, and HIF-1α and VEGF, and a significant correlation between Glut-1 and VEGF ($r = 0.60$, $P < 0.001$). In type B1 thymoma, HIF-1α and Glut-1 were partly expressed in non-neoplastic immature lymphocytes.

CONCLUSIONS: FDG-PET/CT should be performed in patients with tumours in the anterior mediastinum because the pattern of FDG uptake and SUVmax are useful in the differential diagnosis of thymic epithelial tumours. Furthermore, the expressions of HIF-1α, Glut-1 and VEGF might be associated with malignancy of thymic epithelial tumours. In contrast, FDG uptake might be dependent on tumour size rather than Glut-1 overexpression.

Keywords: FDG-PET/CT • Thymic epithelial tumour • SUVmax • WHO classification • HIF-1α • Glut-1 • VEGF

INTRODUCTION

Thymic epithelial tumours are uncommon neoplasms accounting for 0.2–1.5% of all malignancies, which are derived from the epithelial cells of the thymus [1]. The World Health Organization (WHO) proposed that thymic epithelial tumours consist of thymoma (type A, AB, B1, B2 and B3) and thymic carcinoma, including neuroendocrine epithelial tumours such as atypical

carcinoid and others [2]. In general, the WHO classification has been widely adopted for independent prognostic factors [3, 4]. Additionally, Masaoka *et al.* [5] presented a tentative TNM classification as a clinical staging system. Kondo [4] indicated that this classification is also an excellent prognostic predictor in large-scale studies.

^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) is an advanced imaging tool used for the diagnosis of various cancers. FDG-PET/CT can detect enhanced glycolysis of tumour cells [6], and FDG, like glucose, is known to enter tumour cells via glucose transporter-1 (Glut-1) [7]. In thymic epithelial tumours, several studies subsequently demonstrated the usefulness of FDG-PET. They investigated the correlations between FDG uptake and the WHO histological classification [8–11]. In recent years, the overexpression of biomarkers such as Glut-1 has been reported as an important factor to increase FDG uptake in non-small-cell lung cancer (NSCLC) [12, 13]. Only two studies have demonstrated that the same phenomenon was also recognized in thymic epithelial tumours [14, 15]. However, little is known about the pathogenesis and the mechanism of FDG uptake.

Hypoxia is a common phenomenon in solid tumours. In such conditions, hypoxia-inducible factor (HIF)-1 is activated. HIF-1α is the most ubiquitously expressed factor and regulates a large panel of target genes involved in metabolism, angiogenesis, invasion and metastasis; its targets include Glut-1 and vascular endothelial growth factor (VEGF) [16, 17]. In contrast, several studies have already suggested that the overexpression of HIF-1α and VEGF corresponds to higher FDG uptake in NSCLC [13, 18]. In thymic epithelial tumours, Kaira *et al.* [14] only indicated that FDG uptake is determined by the overexpression of HIF-1α, Glut-1 and VEGF. However, there are no reports about the relationship between these biomarkers and FDG uptake except for theirs; moreover, other factors might influence the extent of FDG uptake in thymic epithelial tumours.

In this study, we evaluated the clinical usefulness of FDG-PET/CT, and the relationships among the expression of HIF-1α, Glut-1, VEGF, histological type, other clinical factors and FDG uptake in thymic epithelial tumours.

PATIENTS AND METHODS

Patients

Between December 2005 and February 2011, 33 patients [15 males and 18 females, mean age: 60.2 (27–84) years] with a histological diagnosis of thymic epithelial tumour, who were treated at Tokushima University Hospital, were retrospectively reviewed. All patients underwent FDG-PET/CT before treatment. The protocol of this study was approved by the Institutional Review Board and informed consent was obtained from all patients before FDG-PET/CT.

Twenty-eight of the 33 patients were diagnosed by surgical excision, 4 by percutaneous core needle biopsy and 1 by transbronchial tumour biopsy for a bronchial lesion that a metastatic hilar lymph node had invaded directly. The stage was classified into stages I, II, III and IV according to Masaoka's classification [5] and determined by operative, pathological and radiological findings in a comprehensive manner. The histological types were classified into types A, AB, B1, B2, B3 and carcinoma according to the WHO classification [2], and all types of tumour were reclassified into three subgroups: low-risk thymomas (types A, AB and B1), high-risk thymomas (types B2 and B3) and thymic carcinomas (including atypical carcinoid).

Table 1 shows the characteristics of the patients. There were 15 low-risk thymomas, 10 high-risk thymomas and 8 thymic carcinomas. Thymic carcinomas were significantly larger than high-risk thymomas ($P = 0.037$).

As the initial treatment, 23 patients underwent surgery, 6 underwent chemotherapy and 4 underwent chemoradiotherapy.

FDG-PET/CT protocol and image interpretation

Patients were required to fast for 6 h and avoid strenuous work or exercise for 24 h before PET/CT. Sixty minutes after the intravenous injection of FDG (3.7 MBq/kg body weight), PET/CT was performed with an Aquiduo (Toshiba Medical Systems, Tokyo, Japan).

Table 1: Characteristics of patients

	Low risk	High risk	Carcinoma	*P*-value
No. of patients	15	10	8	
Sex				
Male	5	4	6	0.148
Female	10	6	2	
Age (years, range)	64.4 (32–84)	53.2 (27–73)	61.1 (51–72)	N.S.
Size (mm, range)	49.2 (20–103)	34.7* (17–74)	60.1* (18–115)	0.037*
WHO classification				
A/AB/B1	3 / 3 / 9			
B2/B3		7 / 3		
Carcinoma/atypical carcinoid			7 / 1	
Masaoka's classification				
I	8	2	0	0.004**
II	3	0	1	
III	4	2	2	
IVa	0	5	0	
IVb	0	1	5	

*High-risk vs carcinoma.
**I + II vs III + IV.

Patients were imaged from the skull base to the mid-thigh level. An emission scan was acquired immediately following the CT for 2 min per bed position in 3D mode. The images were usually reconstructed using ordered subset expectation maximization, selecting four iterations and 12 subsets, a 128 × 128 matrix, and post-smoothing with an 8-mm Gaussian filter. The reconstructed spatial resolution after smoothing was 9.2 mm.

Abnormal FDG uptake was defined as greater than background activity in surrounding normal tissue excluding physiological uptake sites. Regions of interest (ROI) were placed on visible uptake sites and standardized uptake values (SUV) were calculated as follows:

SUV = decay-corrected activity (kBq)/tissue volume (ml)/injected-FDG activity (kBq)/body weight (g). In this study, the maximum SUV (SUVmax) was used to minimize the partial volume effect and to ensure the reproducibility of measurements.

Image analysis

Using CT data, tumour size was measured in terms of the long axis on an axial image. Furthermore, tumour contour was also described as regular or irregular on the basis of a previously reported method [19]. Patterns of FDG uptake were described as heterogeneous or homogeneous. A heterogeneous pattern was defined when a tumour showed spotted or mottled FDG uptake, and a homogeneous pattern was defined when a tumour showed homogeneous uptake.

Immunohistochemical staining and analysis

The expressions of HIF-1α, Glut-1 and VEGF were studied in paraffin-embedded material cut into 4-μm sections. Immunohistochemical analyses were performed using the Envision system (ChemMate Envision kit; Dako, Glostrup, Denmark) for HIF-1α and VEGF, and using a biotin-free catalysed amplification system (CSAII, K1497; Dako North America, Carpinteria, CA, USA), for Glut-1. After de-paraffinization, endogenous peroxidase treatment was performed for 5 min with 0.3% hydrogen peroxidase in methanol solution. Enzymatic antigen retrieval was performed at pH 9.0, with heating for 10 min. Sections were then incubated overnight at 4°C with primary antibodies. The primary antibodies were as follows: anti-HIF-1α mouse monoclonal antibody (1 : 50 dilution; NB-100–123, Novus Biologicals, Littleton, CO, USA), anti-Glut-1 rabbit polyclonal antibody (RB-9052-R7, Ready-to-use, Neomarkers, Thermo Fisher Scientific, Fremont, CA, USA) and anti-VEGF rabbit polyclonal antibody (RB-222-R7, Ready-to-use, Neomarkers, Thermo Fisher Scientific). The secondary antibodies were as follows: dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins (ChemMate Envision kit, K5007; Dako, Glostrup, Denmark) for anti-HIF-1α and anti-VEGF, and anti-rabbit Ig ImmPRESS (1 : 500 dilution; Vector Laboratories, Burlingame, CA, USA) for anti-Glut-1. Sections were visualized using diaminobenzidine.

Each entire specimen was reviewed in five fields (×400) and the percentage of overexpressed tumour cells was graded. The expressions of HIF-1α and Glut-1 were considered positive if a distinct nucleus or membrane staining was present, respectively, and a 5-point grading scale was used: 1 = < 10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75% and 5 = >76% [13, 14]. The expression of VEGF was considered if a distinct cytoplasm staining was present, and a 4-point grading scale was used: 1 = <10%, 2 = 11–50%, 3 = 51–80% and 4 = >81% [20].

Statistical analysis

All values are expressed as mean ± standard deviation. A comparison of differences in SUVmax, staining scores and tumour size among the three classified WHO histological groups and the clinical stages was performed using Student's *t*-test for unpaired data. A comparison of differences in sex, the clinical stages, tumour contour and the patterns of FDG uptake among the WHO histological groups was performed using χ^2 test for unpaired data. The cut-off values of the SUVmax for predicting histological types were determined using a receiver operating characteristics (ROC) curve. Correlation of different variables was analysed using Spearman's rank correlation test. A value of $P < 0.05$ was considered to be statistically significant. Statistical analyses were performed using SPSS software (Version 11.0.1, SPSS).

RESULTS

FDG-PET/CT findings

FDG uptake within tumours was visually recognized in all 33 (100%) patients. Table 2 shows the tumour contour and patterns of FDG uptake within tumours. Regarding the tumour contour, there was no significant difference between the three groups. A homogeneous pattern of FDG uptake was observed in 1 (7%) of 15 low-risk thymomas, 3 (30%) of 10 high-risk thymomas and 5 (63%) of 8 thymic carcinomas. Regarding the pattern of FDG uptake, a homogeneous pattern was significantly more frequent in low-risk thymomas, high-risk thymomas and thymic carcinomas, in that order ($P = 0.016$).

SUVmax, WHO classification and Masaoka's classification

The mean SUVmax of all tumours was 4.2 ± 2.2 (range 0.7–8.3). According to the WHO classification, the mean SUVmax was 3.7 ± 2.0 in low-risk thymomas, 3.5 ± 2.1 in high-risk thymomas and 5.9 ± 1.9 in thymic carcinomas (Fig. 1A). SUVmax for thymic carcinomas was significantly higher than that for thymomas ($P = 0.008$). In contrast, there was no significant difference between low-risk and high-risk thymomas.

According to Masaoka's classification, the mean SUVmax was 2.4 ± 1.3 in stage I, 3.7 ± 1.8 in stage II, 5.1 ± 1.9 in stage III and 5.3 ± 2.2 in stage IV (Fig. 1B). The mean SUVmax values in stages III

Table 2: Tumour contour and the patterns of FDG uptake

	Low risk	High risk	Carcinoma	*P*-value
Regular	9	4	2	0.253
Irregular	6	6	6	
Homogeneous	1	3	5	0.016
Heterogeneous	14	7	3	

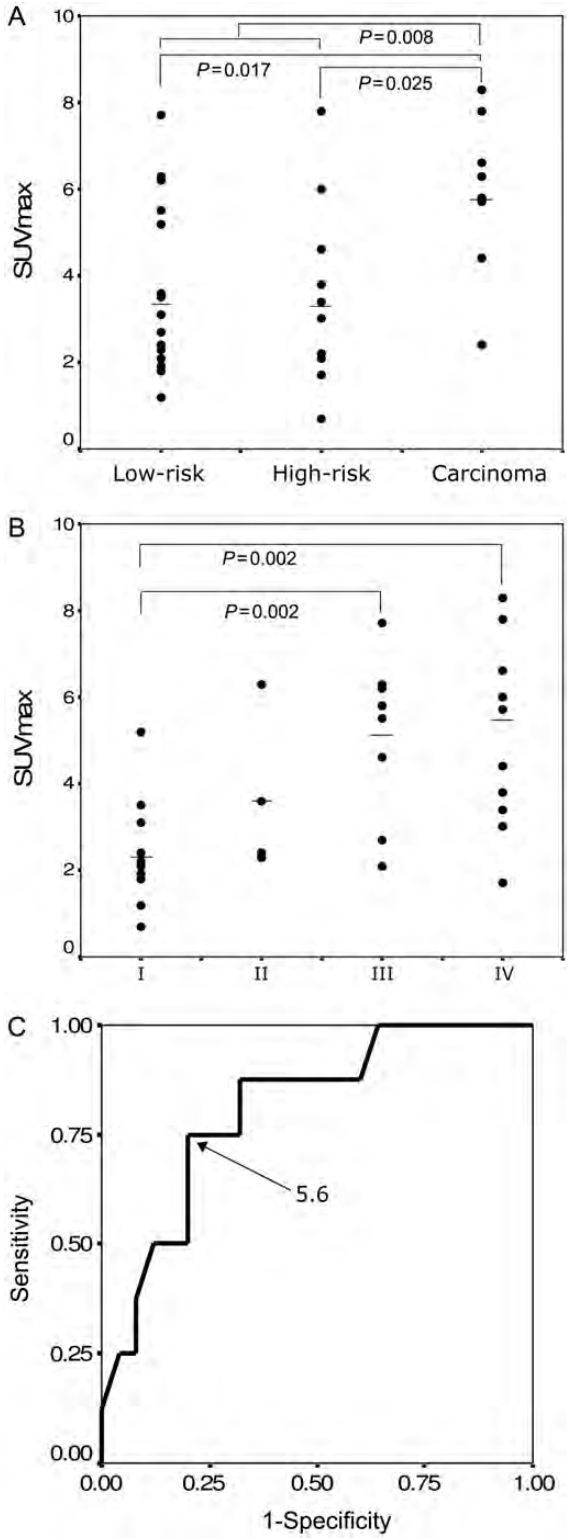

Figure 1: (**A**) SUVmax in subgroups of a simplified version of the WHO classification. (**B**) SUVmax in each of Masaoka's stages. (**C**) Receiver operating characteristic curve and SUVmax for diagnosing thymic carcinoma in FDG-PET/CT, showing the optimal cut-off value to be 5.6 (area under the curve, 0.81; 95% confidence interval 0.64–0.97).

and IV were significantly higher than that in stage I (both $P = 0.002$).

The ROC curve for the SUVmax for diagnosing thymic carcinoma revealed that the optimal cut-off value was 5.6 (Fig. 1C). Its sensitivity, specificity and accuracy were 0.75, 0.80 and 0.79, respectively.

Immunohistochemical analysis

Fig. 2 shows the different immunohistochemical staining patterns for HIF-1α, Glut-1 and VEGF, and the quantitative analysis of three markers according to the WHO classification. HIF-1α was expressed in the nucleus in tumour cells (Fig. 2A). The mean scoring of HIF-1α was 2.2 ± 1.0 in low-risk thymomas, 3.2 ± 1.1 in high-risk thymomas and 3.6 ± 1.2 in thymic carcinomas (Fig. 2B). The mean scoring in low-risk thymomas was significantly lower than that of high-risk thymomas and thymic carcinomas ($P = 0.037$, $P = 0.006$). Glut-1 was expressed in the cytoplasmic membrane of tumour cells (Fig. 2C). The mean scoring of Glut-1 was 3.0 ± 0.9 in low-risk thymomas, 4.1 ± 1.3 in high-risk thymomas and 4.4 ± 1.1 in thymic carcinomas (Fig. 2D). The mean scoring in low-risk thymomas was significantly lower than those of high-risk thymomas and thymic carcinomas ($P = 0.020$, $P = 0.004$). VEGF was expressed in the cytoplasm of tumour cells (Fig. 2E). The mean scoring of VEGF was 2.1 ± 0.8 in low-risk thymomas, 2.3 ± 0.7 in high-risk thymomas and 3.4 ± 0.5 in thymic carcinomas (Fig. 2F). The mean scoring in thymic carcinoma was significantly higher than those of low-risk thymomas and high-risk thymomas ($P < 0.001$, $P = 0.002$). Regarding the mean scoring of these three markers, an increasing trend was observed in the order of low-risk thymomas to high-risk thymomas to thymic carcinomas.

Relationship between SUVmax, immunohistochemical staining scores and tumour size

Table 3 shows the results of Spearman rho correlation coefficients between SUVmax, immunohistochemical staining scores and tumour size. Tumour size revealed a significant correlation with SUVmax ($r = 0.60$, $P < 0.001$) (Fig. 3). HIF-1α showed a moderate association with SUVmax ($r = 0.33$), although there was no significant difference ($P = 0.060$). Glut-1 showed no correlation with SUVmax ($r = -0.11$, $P = 0.544$).

Regarding correlation coefficients between the three markers, there were moderate associations between HIF-1α and Glut-1 ($r = 0.34$, $P = 0.053$), and HIF-1α and VEGF ($r = 0.34$, $P = 0.051$), although there were no significant differences. In addition, there was a significant correlation between Glut-1 and VEGF ($r = 0.60$, $P < 0.001$).

Type B1 thymoma

In type B1 thymoma, HIF-1α and Glut-1 were partly expressed in lymphocytes, but VEGF was not expressed (Fig. 4A–C). For 9 patients with type B1 thymoma, to evaluate whether the overexpression of Glut-1 in lymphocytes might affect the higher SUVmax rather than tumour size, we performed a comparison with other low-risk thymomas (types A and AB) in terms of the relationships between SUVmax and tumour size. The mean SUVmax of type B1 thymoma was significantly higher than that of type A and AB thymomas (4.7 ± 1.9 vs 2.2 ± 0.8, $P = 0.004$, Fig. 4D), although

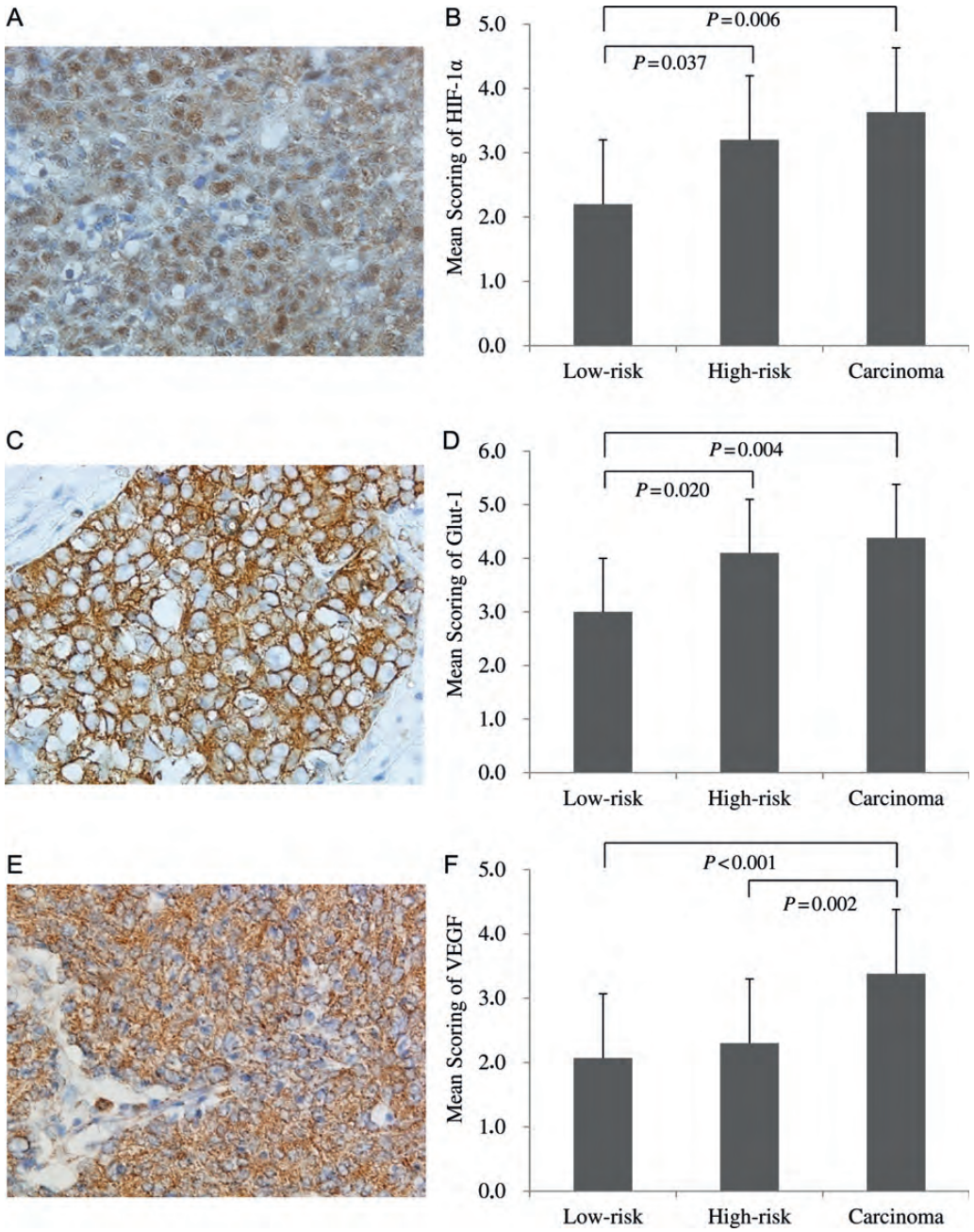

Figure 2: Immunohistochemical staining in the tumour cells. Strong nuclear staining (score 5) for HIF-1α (**A**), Strong membranous staining (score 5) for Glut-1 (**C**) and strong cytoplasmic staining (score 4) for VEGF (**E**). A–C: ×400 magnification. Mean scoring of HIF-1α (**B**), Glut-1 (**D**) and VEGF (**F**) in subgroups of a simplified version of the WHO classification. Data are presented as mean ± SD.

Table 3: Spearman rho correlation coefficients between SUVmax, immunohistochemical staining scores, tumour size and two-tailed *P*-values

	SUVmax	HIF-1α	Glut-1	VEGF
HIF-1α	0.33 (0.060)			
Glut-1	−0.11 (0.544)	0.34 (0.053)		
VEGF	0.15 (0.412)	0.34 (0.051)	0.60 (<0.001)	
Size	0.60 (<0.001)	0.22 (0.226)	−0.11 (0.539)	0.17 (0.344)

tumour size showed no significant correlation with SUVmax ($r = 0.58$, $P = 0.106$).

DISCUSSION

It is sometimes clinically difficult to determine whether a thymic epithelial tumour is a carcinoma or not, which is important because the therapeutic strategy for carcinoma is often different from that for thymoma. In this study, we could show the clinical usefulness of FDG-PET/CT in the differential diagnosis of thymic epithelial tumour. In particular, a homogeneous pattern of FDG

uptake and SUVmax could be useful indicators. First, a homogeneous pattern was increasingly observed in the order of low-risk thymomas to high-risk thymomas to thymic carcinomas, with a significant difference ($P = 0.016$). Sung *et al.* [8] also reported the same trend. Second, SUVmax for thymic carcinoma was significantly higher than that for thymomas ($P = 0.008$), which was compatible with the results of previous studies [8–11, 14]. The homogeneous pattern of FDG uptake and the high SUVmax of thymic carcinomas could be explained by the positive correlations between FDG uptake and the tumour growth rates or cell density [10]. Furthermore, in this study, we could propose an optimal cut-off value of SUVmax for diagnosing thymic carcinoma. With a cut-off value of 5.6, the sensitivity, specificity and accuracy were 0.75, 0.80 and 0.79, respectively, and the diagnostic capability was thought to be relatively high and feasible.

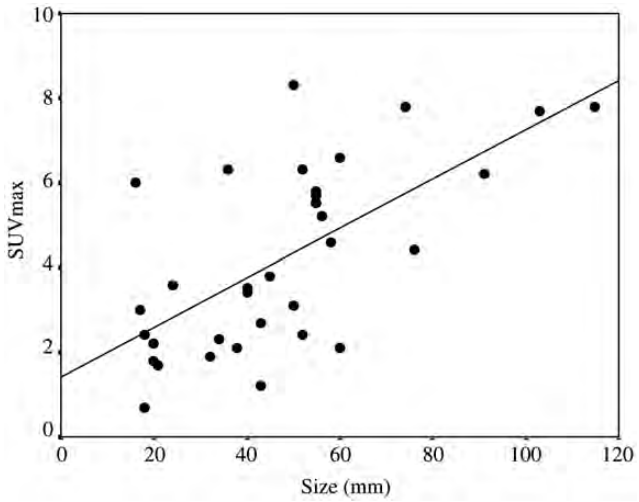

Figure 3: Correlation between tumour size and SUVmax. A significant correlation was shown between them.

However, in this study, there was no significant difference between SUVmax for low-risk and high-risk thymomas. Although this was compatible with the results of some previous studies [8, 10, 11], others indicated that a significant relationship was seen between FDG uptake and three histological subgroups [9, 14]. At present, this is still controversial. Regarding Masaoka's classification, SUVmax values in stages III and IV were significantly higher than that in stage I (both $P = 0.002$). This result was dependent on the fact that the number of patients with high-risk thymomas and thymic carcinomas in stages III and IV was larger than that in stage I. Our results suggested that it might be difficult to diagnose the histological type of thymoma and Masaoka's stage with only SUVmax although it is useful.

Hypoxia is a common phenomenon in solid tumours. It is known that HIF-1α is the most ubiquitously expressed factor related to hypoxia, and regulates a large panel of target genes such as Glut-1 and VEGF in such conditions [16, 17]. In addition, it has been determined that the overexpression of Glut-1 is associated with FDG uptake within tumours in NSCLC [12, 13]. Furthermore, angiogenesis, which is regulated by the overexpression of VEGF, is essential for tumour growth, and VEGF is an important prognostic factor in various cancers [21]. In this study, we performed immunohistochemical staining to evaluate the relationships between FDG uptake and WHO classification, and the expression of hypoxia-related markers, such as HIF-1α, Glut-1 and VEGF.

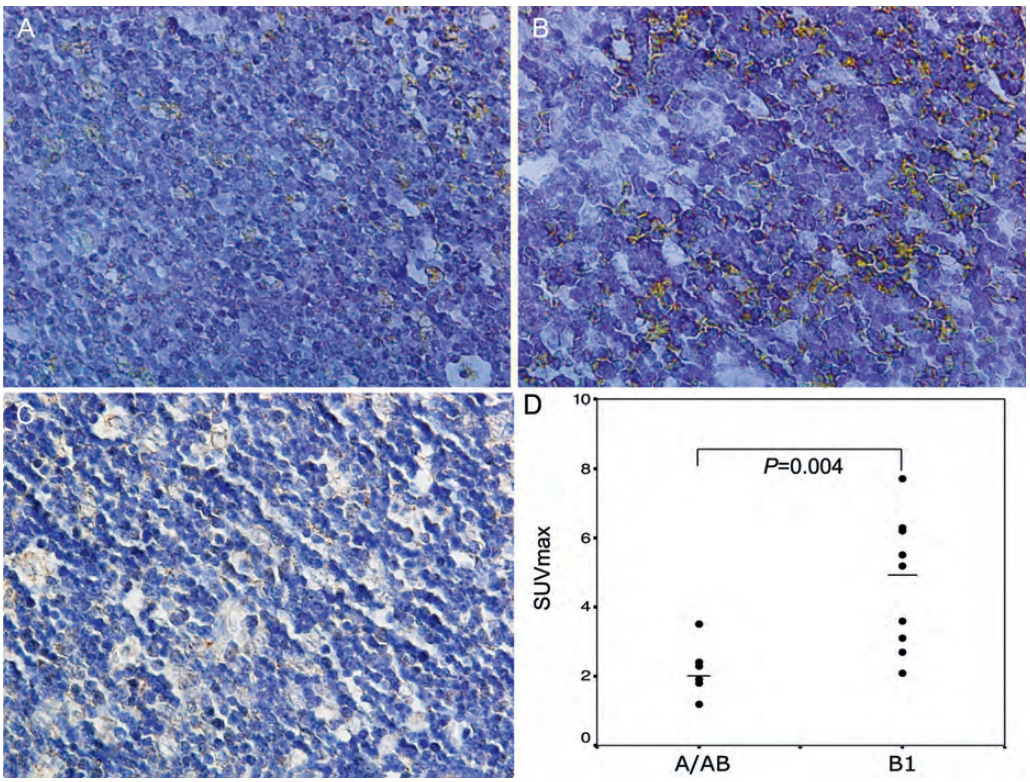

Figure 4: Immunohistochemical staining in the lymphocytes in type B1 thymoma. Positive nuclear staining for HIF-1α (**A**), positive membranous staining for Glut-1 (**B**) and negative staining for VEGF (**C**). A–C: ×400 magnification. (**D**) SUVmax in low-risk thymomas (type A and AB vs type B1).

First, regarding the relationship with FDG uptake, there was no correlation between SUVmax and the expression of Glut-1, although HIF-1α showed a moderate association ($P = 0.060$). Our results differ from those in a previous report [14]. Although researchers have investigated the relationship between FDG uptake and the expression of Glut-1 in NSCLC [12, 13, 22, 23], some studies reported a positive relationship [13, 22], but others reported a negative one [12, 23]. At present, this remains a controversial issue regarding thymic epithelial tumours.

In contrast, in this study, in type B1 thymoma, which is a lymphocyte-rich tumour, the overexpression of HIF-1α and Glut-1 was particularly recognized in non-neoplastic lymphocytes. As a result, SUVmax for type B1 thymoma might be relatively high compared with that for type A and AB thymomas ($P = 0.004$). Thymoma seems to maintain the ability of normal thymic epithelial cells to induce T-cell differentiation. This is indicated by the large number of non-neoplastic CD4+ CD8+ double-positive immature lymphocytes associated with thymoma, in particular type B1 [24]. Furthermore, some researchers demonstrated that increased Glut-1 expression and glucose metabolism were correlated with increased survival, differentiation and proliferation of activated T cells in the thymus [25]. We speculated that, in thymic epithelial tumours, in particular type B1 thymoma, FDG uptake might be affected by the expression of Glut-1 in not only tumour cells but also lymphocytes.

Second, regarding the relationship with the WHO classification, an increasing trend was observed in the order of low-risk thymomas to high-risk thymomas to thymic carcinomas in terms of the expression of each of the three markers. Furthermore, correlations among all of the three markers were basically recognized, although there was a significant correlation only between Glut-1 and VEGF ($P < 0.001$). Regarding the expressions of HIF-1α and Glut-1, the scoring of low-risk thymomas was significantly low compared with those of high-risk thymomas and thymic carcinomas. In addition, regarding the expression of VEGF, the scoring of thymoma was significantly low compared with that of thymic carcinoma. Our results were basically compatible with those of a previous report [14], and the expressions of HIF-1α, Glut-1 and VEGF could be useful biomarkers of tumour invasiveness in thymic epithelial tumours.

In this study, we found that only tumour size showed a very significant correlation with SUVmax ($r = 0.60$, $P < 0.001$). To our knowledge, this study is the first to show that tumour size is correlated with FDG uptake in thymic epithelial tumours. In NSCLC, Suzawa *et al.* [22] demonstrated that FDG uptake is associated with tumour size. Therefore, this might also be possible in thymic epithelial tumours or various other cancers.

Our study has certain limitations. Firstly, the number of patients was too small, and the study design was retrospective. However, considering the rarity of thymic epithelial tumours, a larger prospective and multicentre study is needed. Secondly, the immunohistochemical analyses using biopsy specimens were only included in 5 cases. However, this should be acceptable because chemotherapy was undergone as an initial treatment. Thirdly, the relationship among SUVmax, the expressions of the three markers and patient outcome was not investigated. However, longer follow-up periods are needed to investigate this in thymic epithelial tumours.

CONCLUSIONS

FDG-PET/CT should be performed in patients with tumours in the anterior mediastinum because the pattern of FDG uptake and SUVmax are useful in the differential diagnosis of thymic epithelial tumour. Furthermore, although the expressions of HIF-1α, Glut-1 and VEGF might be associated with malignancy of thymic epithelial tumours, further research on the clinical implication is needed. In contrast, FDG uptake might be dependent on tumour size rather than Glut-1 overexpression.

Conflict of interest: none declared.

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;105:546–51.
[2] Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC. Pathology and genetics of tumors of the lung, pleura, thymus and heart. In: Kleihues P, Sobin LH (eds). WHO Classification of Tumors, 2nd edn. Lyon: IARC Press, 2004, 145–97.
[3] Detterbeck FC. Clinical value of the WHO classification system of thymoma. Ann Thorac Surg 2006;81:2328–34.
[4] Kondo K. Tumor-node metastasis staging system for thymic epithelial tumors. J Thorac Oncol 2010;5:S352–6.
[5] Masaoka A, Monden Y, Nakahara K, Tanioka T. Follow-up study of thymomas with special reference to their clinical stages. Cancer 1981;48:2485–92.
[6] Rigo P, Paulus P, Kaschten BJ, Rigo P, Paulus P, Kaschten BJ *et al.* Oncological applications of positron emission tomography with fluorine-18 fluorodeoxyglucose. Eur J Nucl Med 1996;23:1641–74.
[7] Shankar LK, Sullivan DC. Functional imaging in lung cancer. J Clin Oncol 2005;23:3203–11.
[8] Sung YM, Lee KS, Kim BT, Choi JY, Shim YM, Yi CA. 18F-FDG PET/CT of thymic epithelial tumors: usefulness for distinguishing and staging tumor subgroups. J Nucl Med 2006;47:1628–34.
[9] Endo M, Nakagawa K, Ohde Y, Okumura T, Kondo H, Igawa S *et al.* Utility of 18FDG-PET for differentiating the grade of malignancy in thymic epithelial tumors. Lung Cancer 2008;61:350–5.
[10] Shibata H, Nomori H, Uno K, Sakaguchi K, Nakashima R, Iyama K *et al.* ^{18}F-fluorodeoxyglucose and ^{11}C-acetate positron emission tomography are useful modalities for diagnosing the histologic type of thymoma. Cancer 2009;115:2531–8.
[11] Fukumoto K, Taniguchi T, Ishikawa Y, Kawaguchi K, Fukui T, Kato K *et al.* The utility of [18F]-fluorodeoxyglucose positron emission tomography-computed tomography in thymic epithelial tumours. Eur J Cardiothorac Surg 2012;42:152–6.
[12] de Geus-Oei LF, van Kreiken JH, Aliredjo RP, Krabbe PF, Frielink C, Verhagen AF *et al.* Biological correlates of FDG uptake in non-small cell lung cancer. Lung Cancer 2007;55:79–87.
[13] van Baarwijk A, Dooms C, van Suylen RJ, Verbeken E, Hochstenbag M, Dehing-Oberije C *et al.* The maximum uptake of (18)F-deoxyglucose on positron emission tomography scan correlates with survival, hypoxia inducible factor-1alpha and GLUT-1 in non-small cell lung cancer. Eur J Cancer 2007;43:1392–8.
[14] Kaira K, Endo M, Abe M, Nakagawa K, Ohde Y, Okumura T *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;28:3746–53.
[15] Nakajo M, Kajiya Y, Tani A, Yoneda S, Shirahama H, Higashi M *et al.* ^{18}FDG PET for grading malignancy in thymic epithelial tumors: significant differences in ^{18}FDG uptake and expression of glucose transporter-1 and hexokinase II between low and high-risk tumors: preliminary study. Eur J Radiol 2012;81:146–51.
[16] Liao D, Johnson RS. Hypoxia: a key regulator of angiogenesis in cancer. Cancer Metastasis Rev 2007;26:281–90.
[17] Majmundar AJ, Wong WJ, Simon MC. Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 2010;40:294–309.
[18] Guo J, Higashi K, Ueda Y, Oguchi M, Takegami T, Toga H *et al.* Microvessel density: correlation with 18F-FDG uptake and prognostic impact in lung adenocarcinomas. J Nucl Med 2006;47:419–25.
[19] Tomiyama N, Johkoh T, Mihara N, Honda O, Kozuka T, Koyama M *et al.* Using the World Health Organization Classification of thymic epithelial neoplasms to describe CT findings. Am J Roentgenol 2002;179:881–6.

[20] Gou HF, Chen XC, Zhu J, Jiang M, Yang Y, Cao D *et al*. Expressions of COX-2 and VEGF-C in gastric cancer: correlations with lymphangiogenesis and prognostic implications. J Exp Clin Cancer Res 2011;30:14.
[21] Mineo TC, Ambrogi V, Baldi A, Rabitti C, Bollero P, Vincenzi B *et al*. Prognostic impact of VEGF, CD31, CD34, and CD105 expression and tumour vessel invasion after radical surgery for IB-IIA non-small cell lung cancer. J Clin Pathol 2004;57:591–7.
[22] Suzawa N, Ito M, Qiao S, Uchida K, Takao M, Yamada T *et al*. Assessment of factors influencing FDG uptake in non-small cell lung cancer on PET/CT by investigating histological differences in expression of glucose transporters 1 and 3 and tumour size. Lung Cancer 2011;72:191–8.
[23] Chung JK, Lee YJ, Kim SK, Jeong JM, Lee DS, Lee MC. Comparison of [18F] fluorodeoxyglucose uptake with glucose transporter-1 expression and proliferation rate in human glioma and non-small-cell lung cancer. Nucl Med Commun 2004;25:11–7.
[24] Kobayashi Y, Fujii Y, Yano M, Sasaki H, Yukiue H, Haneda H *et al*. Preoperative steroid pulse therapy for invasive thymoma: clinical experience and mechanism of action. Cancer 2006;106:1901–7.
[25] Swainson L, Kinet S, Manel N, Battini JL, Sitbon M, Taylor N. Glucose transporter 1 expression identifies a population of cycling CD4+ CD8+ human thymocytes with high CXCR4-induced chemotaxis. Proc Natl Acad Sci U S A 2005;102:12867–72.