Table 1 Patient characteristics

Characteristic	LCNEC	Possible LCNEC	*p* value
Number of patients	10	24	–
Age			
Median (range)	69 (57–83)	67 (57–78)	0.29
Gender			
Male	10	20	–
Female	0	4	
Smoking status			
Ever	10	23	–
Never	0	1	
ECOG-PS			
0	1	4	0.17
1	9	16	
2	0	4	
Stage			
IIIA	0	1	<0.01
IIIB	0	1	
IV	4	22	
Recurrence after surgery	6[a]	0	

LCNEC large cell neuroendocrine carcinoma of the lung, *ECOG-PS* Eastern Cooperative Oncology Group performance status

[a] pStage IB (4), pStage IIIA (2)

pericardium biopsy ($n = 1$), and transanal colon biopsy ($n = 1$). Positive rates in immunohistochemical staining for NE markers were as follows: NCAM was 10 (100 %) in LCNEC and 22 (92 %) in possible LCNEC, chromogranin A was 5 (50 %) in LCNEC and 12 (50 %) in possible LCNEC, and synaptophysin was 7 (70 %) in LCNEC and 16 (67 %) in possible LCNEC.

Patient characteristics are shown in Table 1. Age was similar in the LCNEC and possible LCNEC groups ($p = 0.29$). All LCNEC patients were male and ever smokers. Among the 24 possible LCNEC patients, 83.3 % were male and only 1 patient was a never smoker. Four possible LCNEC patients had Eastern Cooperative Oncology Group (ECOG) performance status (PS) 2, but no statistically significant difference in PS was found between the 2 groups ($p = 0.17$). There was a difference in stage between the 2 groups ($p < 0.05$). Among the 10 LCNEC patients, 4 patients had distant metastasis (stage IV) and 6 patients had pulmonary recurrence after surgery. All possible LCNEC patients had stage III or IV disease.

The chemotherapy regimens used are shown in Table 2. Most patients were treated with SCLC-based regimens such as platinum plus irinotecan or platinum plus etoposide. Four LCNEC patients and 11 possible LCNEC patients were treated with cisplatin plus irinotecan. Two LCNEC patients and 5 possible LCNEC patients were treated with platinum plus etoposide.

Table 2 Chemotherapy regimens

	LCNEC ($n = 10$)	Possible LCNEC ($n = 24$)
Cisplatin plus irinotecan	4	11
Cisplatin plus etoposide	0	1
Carboplatin plus etoposide	2	4
Carboplatin plus paclitaxel	4	4
Others	0	4[a]

LCNEC large cell neuroendocrine carcinoma of the lung

[a] Cisplatin plus paclitaxel (1), cisplatin plus docetaxel (1), cisplatin plus vinorelbine (1), carboplatin plus S-1 (1)

Table 3 Clinical response to first-line chemotherapy

Response	LCNEC	Possible LCNEC	*p* value
CR	1	1	
PR	6	12	
SD	1	7	
PD	2	3	
NE	0	1	
Response rate (%)	70	54	0.39
95 % CI	40–90	35–72	

LCNEC large cell neuroendocrine carcinoma of the lung, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable, *CI* confidence interval

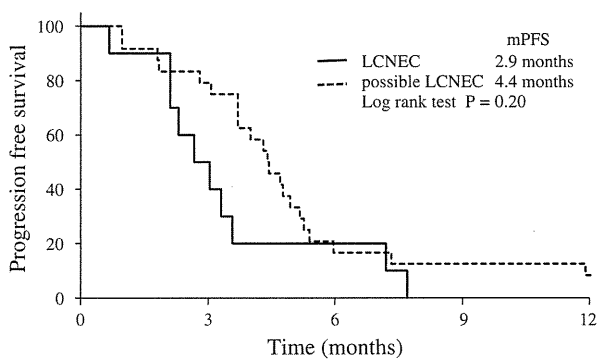

Fig. 1 Kaplan–Meier plot of progression-free survival (PFS) of patients with large cell neuroendocrine carcinomas (LCNEC) and possible LCNEC. The median PFS was 2.9 months in patients with LCNEC and 4.4 months in patients with possible LCNEC ($p = 0.20$)

The response rate was 70 % in LCNEC patients and 54 % in possible LCNEC patients (Table 3); and no statistically significant difference was found ($p = 0.39$).

The Kaplan–Meier curve for PFS is shown in Fig. 1. The median PFS was 2.9 months in the LCNEC group and 4.4 months in the possible LCNEC group ($p = 0.20$). The Kaplan–Meier curve for OS is shown in Fig. 2. The median

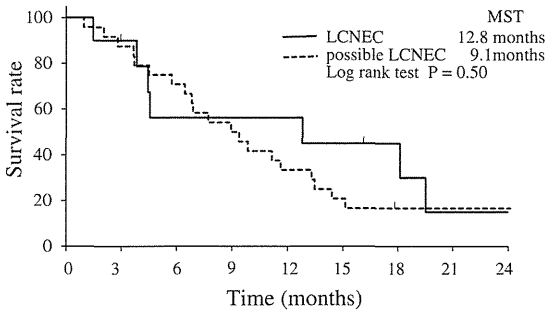

Fig. 2 Kaplan–Meier plot of overall survival of patients with large cell neuroendocrine carcinomas (LCNEC) and possible LCNEC. The median survival time (MST) was 12.8 months in patients with LCNEC and 9.1 months in patients with possible LCNEC ($p = 0.50$)

survival time (MST) was 12.8 months in the LCNEC group and 9.1 months in the possible LCNEC group ($p = 0.50$). In the present study, the median follow-up duration was 23.2 months.

Nine LCNEC patients and 15 possible LCNEC patients received second-line chemotherapy. Six LCNEC patients and 6 possible LCNEC patients were treated with amrubicin. Only 1 LCNEC patient who was treated with amrubicin showed a partial response.

Discussion

To the best of our knowledge, the present study is the first report comparing the efficacy of chemotherapy for LCNEC in patients diagnosed with LCNEC with that in patients diagnosed with possible LCNEC. In the present study, in the possible LCNEC group, the response rate was 54 % and the MST was 9.1 months. No statistically significant differences in the response rate and OS were found between the 2 groups.

Igawa et al. [7] evaluated 14 advanced possible LCNEC cases and showed that the response rate was 50 % and the MST was 10 months. In addition, Shimada et al. [8] analyzed 13 patients regarded as possible LCNEC with high-grade neuroendocrine carcinoma of the lung and reported that the response rate to first-line chemotherapy was 61 % and the MST was 12 months. These results were comparable to those of extensive disease (ED)-SCLC [7, 8] and to those in the possible LCNEC group in the present study. Resected LCNEC has been reported to be similar to SCLC in clinicopathological features and prognosis [5, 6].

Mazieres et al. [12] reported that 13 cases (72 %) of resected LCNEC relapsed with distant metastases, and 10 of these relapsed within 6 months. The 13 relapsed LCNEC cases were treated with platinum plus etoposide, and the response rate was 20 %. Other authors reported that the response rate of LCNEC was 50–59 % and the MST was 8–10.3 months, with most recurrences occurring after surgery [13, 14]. For LCNEC cases treated with cisplatin-based chemotherapy, the response rate was comparable to that of SCLC. Rossi et al. [15] reported that in 12 patients treated with platinum plus etoposide, the response rate was 50 % and the MST was 51 months, although 3 cases received radiotherapy in addition to chemotherapy. In previous studies, with 1 exception [12], the chemotherapeutic response of recurrent LCNEC was as good as that of SCLC [13–15]. In addition, Rossi et al. [15] reported that in another 15 patients treated with NSCLC-based regimens, the response rate was 0 % and the MST was 21 months. In the present study, an objective response was obtained in 4 of 6 LCNEC patients (66 %) who received platinum plus irinotecan or platinum plus etoposide, so-called SCLC-based regimens, and in 9 of 16 possible LCNEC patients (56 %) who received SCLC-based regimens. These results suggest that SCLC-based regimens might be effective for both LCNEC and possible LCNEC. In addition, the present study also indicated that treatment with paclitaxel-containing regimens might be effective for LCNEC and possible LCNEC. These anticancer drugs will be key to the treatment of LCNEC and possible LCNEC.

This study has several limitations. It was a retrospective study with an inherent potential for bias. Collection of clinical characteristics and treatment response data was retrospective and the follow-up interval for physical examinations was indefinite. The sample size was small. Therefore, future studies would benefit from investigating a much larger sample.

In conclusion, no statistically significant differences were found in the response rate, PFS, and OS between the LCNEC and possible LCNEC groups. These results suggest that possible LCNEC is similar to LCNEC in chemotherapeutic efficacy. In the future, a study of a larger series of LCNEC patients is mandatory to confirm the role of chemotherapeutic strategy.

Acknowledgments The authors thank Scientific Language for reviewing the English manuscript. No financial support was obtained for this study.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Travis WD, Brambilla E, Muller-Hermelink HK et al (2004) Pathology and genetics of tumours of the lung, pleura, thymus and heart. IARC Press, Lyon
2. Iyoda A, Hiroshima K, Toyozaki T et al (2001) Clinical characterization of pulmonary large cell neuroendocrine carcinoma

and large cell carcinoma with neuroendocrine. Morphology 91:1992–2000
3. Takei H, Asamura H, Maeshima A et al (2002) Large cell neuroendocrine carcinoma of the lung: a clinicopathologic study of eighty-seven cases. J Thorac Cardiovasc Surg 124:285–292
4. Paci M, Cavazza A, Annessi V et al (2004) Large cell neuroendocrine carcinoma of the lung: a 10-year clinicopathologic retrospective study. Ann Thorac Surg 77:1163–1167
5. Sun L, Sakurai S, Sano T et al (2009) High-grade neuroendocrine carcinoma of the lung: comparative clinicopathological study of large cell neuroendocrine carcinoma and small cell lung carcinoma. Pathol Int 59:522–529
6. Asamura H, Kameya T, Matsuno Y et al (2006) Neuroendocrine neoplasms of the lung: a prognostic spectrum. J Clin Oncol 24:70–76
7. Igawa S, Watanabe R, Ito I et al (2010) Comparison of chemotherapy for unresectable pulmonary high-grade non-small cell neuroendocrine carcinoma and small-cell lung cancer. Lung Cancer 68:438–445
8. Shimada Y, Niho S, Ishii G et al (2011) Clinical features of unresectable high grade lung neuroendocrine carcinoma diagnosed using biopsy specimens. Lung Cancer 75:368–373
9. Travis WD, Brambilla E, Noguchi M et al (2011) International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol 6:244–285
10. Eisenhauer EA, Therasse P, Bogaerts J et al (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228–247
11. Rami-Porta R, Ball D, Crowley J et al (2007) The IASLC Lung Cancer Staging Project: proposals for the revision of the T descriptors in the forthcoming (seventh) edition of the TNM classification for lung cancer. J Thorac Oncol 2:593–602
12. Mazieres J, Daste G, Molinier L et al (2002) Large cell neuroendocrine carcinoma of the lung: pathological study and clinical outcome of 18 resected cases. Lung Cancer 37:287–292
13. Yamazaki S, Sekine I, Matsuno Y et al (2005) Clinical responses of large cell neuroendocrine carcinoma of the lung to cisplatin-based chemotherapy. Lung Cancer 49:217–223
14. Fujiwara Y, Sekine I, Tsuta K et al (2007) Effect of platinum combined with irinotecan or paclitaxel against large cell neuroendocrine carcinoma of the lung. Jpn J Clin Oncol 37:482–486
15. Rossi G, Cavazza A, Marchioni A et al (2005) Role of chemotherapy and the receptor tyrosine kinases KIT, PDGFRalpha, PDGFRbeta, and Met in large-cell neuroendocrine carcinoma of the lung. J Clin Oncol 23:8774–8785

RESPIRATORY INVESTIGATION 50 (2012) 157–161

Contents lists available at SciVerse ScienceDirect

Respiratory Investigation

journal homepage: www.elsevier.com/locate/resinv

Original article

Feasibility of postoperative adjuvant chemotherapy of cisplatin plus vinorelbine for completely resected non-small-cell lung cancer: A retrospective study in Japan

Hirotsugu Kenmotsu[a,*], *Yasuhisa Ohde*[b], *Takehito Shukuya*[a], *Hirofumi Eida*[a], *Hiroaki Akamatsu*[a], *Akira Ono*[a], *Yukiko Nakamura*[a], *Asuka Tsuya*[a], *Kyoichi Kaira*[a], *Tateaki Naito*[a], *Haruyasu Murakami*[a], *Toshiaki Takahashi*[a], *Tomohiro Maniwa*[b], *Mitsuhiro Isaka*[b], *Masahiro Endo*[c], *Haruhiko Kondo*[b], *Nobuyuki Yamamoto*[a]

[a]*Division of Thoracic Oncology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho Sunto-gun, Shizuoka 411-8777, Japan*
[b]*Division of Thoracic Surgery, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho Sunto-gun, Shizuoka 411-8777, Japan*
[c]*Division of Diagnostic Radiology, Shizuoka Cancer Center Hospital, 1007 Shimonagakubo Nagaizumi-cho Sunto-gun, Shizuoka 411-8777, Japan*

ARTICLE INFO

Article history:
Received 12 June 2012
Received in revised form
15 September 2012
Accepted 19 September 2012
Available online 27 October 2012

Keywords:
Non-small-cell lung cancer
Adjuvant chemotherapy
Cisplatin
Vinorelbine
Feasibility

ABSTRACT

Background: The efficacy of postoperative adjuvant cisplatin (CDDP)-based chemotherapy, such as the combination of CDDP and vinorelbine (VNR), has been established for surgically resected non-small-cell lung cancer (NSCLC). However, the optimal treatment schedule and dosage for CDDP and VNR are unknown. We evaluated patient compliance with and the safety of adjuvant chemotherapy of CDDP at 80 mg/m^2 administered on day 1 plus VNR at 25 mg/m^2 administered on days 1 and 8, every 3 weeks.

Methods: Medical records of 100 surgically resected NSCLC patients, treated with a combination of CDDP and VNR at the Shizuoka Cancer Center between February 2006 and October 2011, were retrospectively reviewed.

Results: Eighty-three (83%) patients completed the planned 4 cycles of CDDP plus VNR and 59 (59%) received the planned doses. Sixty-eight percent of the patients experienced a decreased neutrophil count (grade 3/4 toxicity); 1%, a decreased platelet count; and 4%, febrile neutropenia. No treatment-related deaths were noted in this study. Univariate analysis of the factors influencing patient compliance with this adjuvant chemotherapy showed that neither patient characteristics nor surgical procedure was significantly associated.

Conclusions: Our results indicated that adjuvant chemotherapy with CDDP at 80 mg/m^2 administered on day 1 plus VNR at 25 mg/m^2 administered on days 1 and 8, every 3 weeks, was feasible for surgically resected NSCLC cases.

*Corresponding author. Tel.: +81 55 989 5222; fax: +81 55 989 5634.
E-mail address: h.kenmotsu@scchr.jp (H. Kenmotsu).

2212-5345/$ - see front matter © 2012 The Japanese Respiratory Society. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.resinv.2012.09.002

1. Introduction

Non-small-cell lung cancer (NSCLC) accounts for approximately 80–85% of lung cancer cases, and the 5-year survival rate of lung cancer resection patients is reported to be approximately 60%. The postoperative 5-year survival rate of Stage II–IIIA patients, in particular, is unsatisfactory at 30–60% [1]. The efficacy of postoperative adjuvant chemotherapy has been documented since 2004 [2,3]. According to a meta-analysis of 4584 patients enrolled in a recent large-scale comparative study for cisplatin (CDDP)-based postoperative chemotherapy (Lung Adjuvant Cisplatin Evaluation [LACE]), the hazard ratio (HR) against death in all patients was 0.89 (95% confidence interval [CI], 0.82–0.96), corresponding to an absolute survival benefit of 5.4% at 5 years. Subgroup analysis of the LACE study indicated that among the various drugs co-administered with CDDP, only vinorelbine (VNR) significantly prolonged survival ($p=0.005$). With regard to disease stage, postoperative chemotherapy significantly improved the survival time in Stages II and III, and an improvement trend was shown in Stage IB, whereas a deteriorating trend was found in Stage IA [4]. However, the treatment schedule and dosage for CDDP and VNR varied [2,3,5,6]. We have previously reported on the safety of and patient compliance with adjuvant chemotherapy with CDDP (80 mg/m^2 at day 1) and VNR (25 mg/m^2 at days 1 and 8) repeated every 3 weeks [7]. This schedule and dosage was found to be safe and effective for Japanese patients with advanced NSCLC [8]. The rate of completion of the planned 4 cycles was 92% in 25 resected NSCLC patients. However, this study involved only a small number of patients. Therefore, we retrospectively evaluated patient compliance with and the safety of adjuvant chemotherapy with CDDP at 80 mg/m^2 administered on day 1 plus VNR at 25 mg/m^2 administered on days 1 and 8, every 3 weeks, in surgically resected NSCLC patients.

2. Material and methods

The medical records of surgically resected NSCLC patients treated with adjuvant chemotherapy of CDDP plus VNR at the Shizuoka Cancer Center between February 2006 and October 2011 were retrospectively reviewed. As a rule at our institution, the inclusion of adjuvant chemotherapy patients in this study was based on the following criteria: (i) age less than 75 years; (ii) pathological Stage II–IIIA; and (iii) performance status (PS) 0 or 1. CDDP at 80 mg/m^2 was administered on day 1, and VNR at 25 mg/m^2 was administered on days 1 and 8. The combination of these drugs was repeated every 3 weeks, and each 3-week treatment schedule was counted as 1 cycle. We defined the completion of adjuvant chemotherapy as the administration of CDDP plus VNR on day 1 of 4 cycles. Treatment change, such as reducing, skipping, or delaying a dose, was based on the physician's decision. Chemotherapy-related toxicities were graded according to the National Cancer Institute Common Terminology Criteria version 4.0 (NCI-CTC v4.0). This study included the patients mentioned in our previous report [7]. Univariate analyses were performed to identify risk factors for not completing adjuvant chemotherapy of CDDP plus VNR. All categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. Clinical evaluation of relapse-free survival (RFS) after surgical resection was conducted using the Kaplan–Meier method to assess the time to relapse or death. All p values were reported as 2-sided, and values less than 0.05 were considered statistically significant. This study was approved by the Institutional Review Board (23-J69-23-1-3, October 24, 2011).

3. Results

3.1. *Patient characteristics*

One hundred NSCLC patients were treated with postoperative adjuvant chemotherapy of CDDP plus VNR. The characteristics of the patients are shown in Table 1. The median age was 63 years (range, 36–74 years) and 34% of the patients were female. Twenty percent of the patients had never smoked. Histologically, adenocarcinoma and squamous cell carcinoma were observed in 67% and 27% of patients, respectively. Eighty-seven percent of the patients had undergone a lobectomy, and 13% a pneumonectomy. Pathological Stages IIA, IIB, and IIIA were observed in 31%, 22%, and 47% of patients, respectively. The median time from surgical resection to the start of adjuvant chemotherapy was 45 days (range, 29–79 days).

Table 1 – Patient characteristics.

	Number of patients	(%)
Gender		
Male	66	(66)
Female	34	(34)
Age, years		
Median	63	
(Range)	(36–74)	
Smoking status		
Never-smoked	20	(20)
Prior or current smoker	80	(80)
Performance status (ECOG)		
0	62	(62)
1	38	(38)
Histology		
Adenocarcinoma	67	(67)
Squamous cell carcinoma	27	(27)
Others	6	(6)
Pathological stage		
IIA	31	(31)
IIB	22	(22)
IIIA	47	(47)
Surgical procedure		
Lobectomy	81	(81)
Lobectomy with chest wall resection	6	(6)
Pneumonectomy	13	(13)
Time from surgical resection to start of adjuvant chemotherapy, days		
Median	45	
(Range)	(29–79)	

($n=100$).

3.2. *Compliance with adjuvant chemotherapy and observed toxicities*

Of the 100 NSCLC patients treated with adjuvant chemotherapy, 83 (83%) completed the planned 4 cycles of CDDP plus VNR and 59 (59%) received the planned doses (Table 2). The reasons for discontinuation of chemotherapy were toxicity in 8 patients (8%) and patient refusal in 8 patients (8%). The median doses received were 320 mg/m^2 for CDDP and 200 mg/m^2 for VNR. In addition, the mean doses received were 283 mg/m^2 for CDDP and 173 mg/m^2 for VNR (Table 3). This study included only 12 patients who were 70 years or older, but 11 of these patients (92%) completed the planned 4 cycles of CDDP plus VNR.

The incidence of toxicity at grade 2 or worse is shown in Table 4. Sixty-eight percent of patients experienced a decreased neutrophil count (grade 3/4 toxicity); 34%, a decreased white blood cell count; 15%, anemia; and 1%, a decreased platelet count. Febrile neutropenia was reported in 4% of the patients. Although the use of oral antibiotics and granulocyte-colony stimulating factor (G-CSF) against afebrile neutropenia was based on the physician's decision, most patients were not treated with prophylactic oral antibiotics and G-CSF. Frequently observed non-hematological toxicities

Table 2 – Compliance with adjuvant chemotherapy of cisplatin and vinorelbine.

	Number of patients	(%)
Planned 4 cycles completed	83	(83)
Planned doses received		
(Cisplatin 320 mg/m^2 and vinorelbine 200 mg/m^2)	59	(59)
Discontinuation of adjuvant chemotherapy	17	(17)
Reason		
Toxicity	8	(8)
Patient refusal	8	(8)
Other	1	(1)

Table 3 – Dosage of cisplatin and vinorelbine received in adjuvant chemotherapy.

Cycle	1	2	3	4	Total
Cisplatin (*n*)	100	91	88	83	100
Dose (mg/m^2)					
Planned	80	80	80	80	320
Received (median)	80	80	80	80	320
(mean)	80	71	68	63	283
Vinorelbine (*n*)	100	91	88	83	100
Dose (mg/m^2)					
Planned	50	50	50	50	200
Received (median)	50	50	50	50	200
(mean)	48	44	42	40	173

Table 4 – Toxicities related to adjuvant chemotherapy of cisplatin and vinorelbine.

	Grade 2		Grade 3		Grade 4	
	No. of patients	(%)	No. of patients	(%)	No. of patients	(%)
Anemia	32	(32)	15	(15)	0	
Febrile neutropenia	–		4	(4)	0	
Constipation	16	(16)	1	(1)	0	
Nausea	38	(38)	8	(8)	0	
Vomiting	8	(8)	2	(2)	0	
Fatigue	20	(20)	1	(1)	0	
Infection	6	(6)	4	(4)	0	
ALT increase	0		2	(2)	0	
AST increase	1	(1)	2	(2)	0	
Blood bilirubin increase	3	(3)	0		0	
Creatinine increase	9	(9)	0		0	
Neutrophil count decrease	15	(15)	26	(26)	42	(42)
Platelet count decrease	1	(1)	0		1	(1)
WBC decrease	35	(35)	31	(31)	3	(3)
Anorexia	54	(54)	7	(7)		

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase and WBC: White blood cell.

Table 5 – Univariate analysis of factors influencing patient compliance to adjuvant chemotherapy of cisplatin and vinorelbine.

	Completion of 4 cycles		Discontinuation		*p*-value
	No. of patients	(%)	No. of patients	(%)	
Gender					0.305
Female	30	(36)	4	(24)	
Male	53	(64)	13	(76)	
Age, years					0.505
Median	64		63		
(Range)	(36–74)		(39–72)		
Smoking status					0.327
Never-smoker	18	(22)	2	(12)	
Previous or current smoker	65	(78)	15	(88)	
Performance status (ECOG)					0.403
0	53	(64)	9	(53)	
1	30	(36)	8	(47)	
Pathological stage					0.249
IIA	28	(34)	3	(18)	
IIB	16	(19)	6	(35)	
IIIA	39	(47)	8	(47)	
Surgical procedure					0.154
Lobectomy	69	(83)	12	(70)	
Lobectomy with chest wall resection	3	(4)	3	(18)	
Pneumonectomy	11	(13)	2	(12)	
Time from surgical resection to start of adjuvant chemotherapy, days					0.557
Median	45		44		
(Range)	(29–79)		(32–65)		

of grade 2 or worse included anorexia (61%) and nausea (46%). No treatment-related deaths were noted in this study.

The results of univariate analysis of the factors influencing adjuvant chemotherapy compliance are shown in Table 5. Patient characteristics were not significantly associated with compliance to adjuvant chemotherapy. In addition, surgical procedure and time from surgical resection to the start of adjuvant chemotherapy were not significantly associated with patient compliance. Lobectomy with chest wall resection tended to be associated with poor compliance to adjuvant chemotherapy.

3.3. *Relapse-free survival*

In this analysis, the median follow-up duration was 13.3 months. The Kaplan–Meier curve for relapse-free survival (RFS) from the time of surgical resection is shown in Fig. 1. The 1- and 2-year RFS rates were 75% and 62%, respectively.

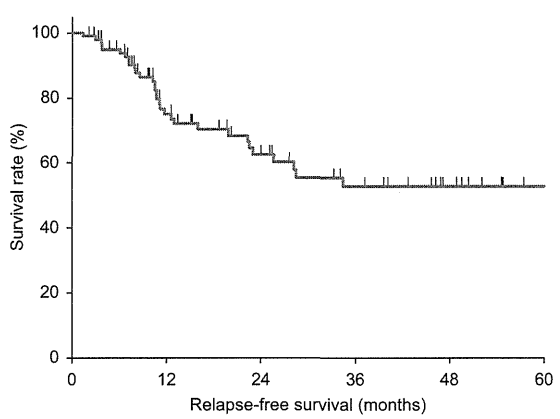

Fig. 1 – Relapse-free survival (RFS) curve for surgically resected NSCLC patients treated with adjuvant chemotherapy of cisplatin plus vinorelbine. Total number of patients = 100. The curve was plotted using Kaplan–Meier analysis. The 1- and 2-year RFS rates were 75% and 62%, respectively.

4. Discussion

This study demonstrated the feasibility of postoperative adjuvant chemotherapy of CDDP plus VNR (CDDP at 80 mg/m^2 was administered on day 1 and VNR at 25 mg/m^2 was administered on days 1 and 8, every 3 weeks) for surgically resected NSCLC patients. LACE meta-analysis and randomized trials have demonstrated the efficacy of CDDP-based adjuvant chemotherapy, in particular, CDDP plus VNR [3,4,6]. However, the treatment schedule and dosage were varied. In the trial conducted by the International Adjuvant Lung Cancer Trial Collaborative Group, CDDP at 80–120 mg/m^2 was administered every 3 or 4 weeks, and VNR at 30 mg/m^2 was administered weekly [2]. In the JBR.10 trial, CDDP at 50 mg/m^2 was administered on days 1 and 8 every 4 weeks, and VNR at 25 mg/m^2 was administered weekly [3]. In the Adjuvant Navelbine International Trialist Association (ANITA) study, CDDP at 100 mg/m^2 was administered every 4 weeks, and VNR at 30 mg/m^2 was administered weekly [6]. However, only 45% of the patients randomized to receive adjuvant chemotherapy completed the planned 4 cycles in the JBR.10 trial, and only 50% of the patients completed the cycles in the ANITA study. This

compliance with adjuvant chemotherapy of CDDP plus VNR is unsatisfactory, and the optimal treatment schedule and dosage for CDDP and VNR needs to be determined. The median dose received for CDDP was 320 mg/m^2, which is comparable to the results of the LACE meta-analysis (median dose of CDDP was 303 mg/m^2) [9]. The median dose of VNR is also comparable (this study, 200 mg/m^2 vs. LACE, 236 mg/m^2). Therefore, CDDP at 80 mg/m^2 on day 1 and VNR at 25 mg/m^2 on days 1 and 8 (every 3 weeks), as evaluated in this study, might be the optimal treatment schedule and dosage.

There have been few reports concerning factors influencing adjuvant chemotherapy compliance [10–12]. In the JBR.10 trial, the extent of the surgery and the patient's age and gender were reported to be related to compliance with adjuvant chemotherapy. In particular, patients who had undergone pneumonectomy were more likely to discontinue therapy because of toxicity than patients who had undergone less extensive resections [10]. In addition, a trend towards better compliance with adjuvant chemotherapy was reported in patients who underwent a thoracoscopic lobectomy than in those who underwent a thoracotomy [13]. On the other hand, some reports showed no effect of age and gender on compliance with adjuvant chemotherapy [12–14]. Our study did not identify any factors that influenced adjuvant chemotherapy compliance. This might have been because most of the patients in this study were younger than 75 years and showed good performance status.

A major limitation of this retrospective analysis was that the evaluation of toxicities, such as non-hematological toxicities, might be underestimated. The incidence of hematological toxicities was similar to that in previous reports [3,6,8]. Reducing, skipping, or delaying a dose in the planned chemotherapy was based on the physician's decision and might have been influenced by the physician's bias. However, we followed the chemotherapy regimen of CDDP at 80 mg/m^2 on day 1 and VNR at 25 mg/m^2 on days 1 and 8, every 3 weeks, and none of the patients received a modified dosage of CDDP or VNR from the first cycle of adjuvant chemotherapy. Although the RFS data was immature, the 2-year RFS rate was comparable to that in the trial conducted by the International Adjuvant Lung Cancer Trial Collaborative Group including Stage I patients (the 2-year disease-free survival rate was 61.0%) [2].

5. Conclusion

Our results indicated that adjuvant chemotherapy with CDDP at 80 mg/m^2 administered on day 1 plus VNR at 25 mg/m^2 administered on days 1 and 8, every 3 weeks, was feasible for surgically resected NSCLC patients. This treatment schedule and dosage for CDDP and VNR might be a good candidate for the reference arm of future phase III studies.

Role of funding source

This study was not supported by any funding.

Conflict of interest

The authors have no conflict of interest.

REFERENCES

[1] Asamura H, Goya T, Koshiishi Y, et al. A Japanese lung cancer registry study: prognosis of 13,010 resected lung cancers. J Thorac Oncol 2008;3:46–52.
[2] Arriagada R, Bergman B, Dunant A. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. N Engl J Med 2004;350:351–60.
[3] Winton T, Livingston R, Johnson D, et al. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 2005;352:2589–97.
[4] Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE collaborative group. J Clin Oncol 2008;26:3552–9.
[5] Waller D, Peake MD, Stephens RJ, et al. Chemotherapy for patients with non-small cell lung cancer: the surgical setting of the Big Lung Trial. Eur J Cardiothorac Surg 2004;26:173–82.
[6] Douillard JY, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. Lancet Oncol 2006;7:719–27.
[7] Shukuya T, Takahashi T, Tamiya A, et al. Evaluation of the safety and compliance of 3-week cycles of vinorelbine on days 1 and 8 and cisplatin on day 1 as adjuvant chemotherapy in Japanese patients with completely resected pathological stage IB–IIIA non-small cell lung cancer: a retrospective study. Jpn J Clin Oncol 2009;39:158–62.
[8] Ohe Y, Ohashi Y, Kubota K, et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-arm cooperative study in Japan. Ann Oncol 2007;18:317–23.
[9] Douillard JY, Tribodet H, Aubert D, et al. Adjuvant cisplatin and vinorelbine for completely resected non-small cell lung cancer: subgroup analysis of the lung adjuvant cisplatin evaluation. J Thorac Oncol 2010;5:220–8.
[10] Alam N, Shepherd FA, Winton T, et al. Compliance with postoperative adjuvant chemotherapy in non-small cell lung cancer. An analysis of National Cancer Institute of Canada and intergroup trial JBR.10 and a review of the literature. Lung Cancer 2005;47:385–94.
[11] Dediu M, Horvat T, Tarlea A, et al. Adjuvant chemotherapy for radically resected non-small cell lung cancer: a retrospective analysis of 311 consecutively treated patients. Lung Cancer 2005;47:93–101.
[12] Aljubran A, Leighl N, Pintilie M. Improved compliance with adjuvant vinorelbine and cisplatin in non-small cell lung cancer. Hematol Oncol Stem Cell Ther 2009;2:265–71.
[13] Lee JG, Cho BC, Bae MK, et al. Thoracoscopic lobectomy is associated with superior compliance with adjuvant chemotherapy in lung cancer. Ann Thorac Surg 2011;91:344–8.
[14] Keller SM, Vangel MG, Adak S, et al. The influence of gender on survival and tumor recurrence following adjuvant therapy of completely resected stages II and IIIa non-small cell lung cancer. Lung Cancer 2002;37:303–9.

doi:10.4149/neo_2012_033

^{18}F-FDG uptake on PET could be a predictive marker of Excision Repair Cross-Complementation Group 1 (ERCC1) expression in patients with thoracic neoplasms?

K. KAIRA[1,*], M. ENDO[2], T. SHUKUYA[1], H. KENMOTSU[1], T. NAITO[1], A. ONO[1], A. TSUYA[1], Y. NAKAMURA[1], T. TAKAHASHI[1], H. MURAKAMI[1], H. KONDO[3], T. NAKAJIMA[4], N. YAMAMOTO[1]

[1]Division of Thoracic Oncology; [2]Division of Diagnostic Radiology; [3]Division of Thoracic Surgery; [4]Division of Pathology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho, Sunto-gun, Shizuoka, 411-8777, Japan

**Correspondence: kkaira1970@yahoo.co.jp*

Received September 24, 2011 / Accepted November 10, 2011

The aim of this study is to examine the relationship between the expression level of excision repair cross-complementation group 1 (ERCC1) and of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) in various thoracic neoplasm.

Three hundreds-eight patients [non-small cell lung cancer (NSCLC)(n=56), malignant pleural mesothelioma (MPM)(n=21), pulmonary metastatic tumors (PMT)(n=148), thymic epithelial tumors (n=49) and pulmonary neuroendocrine tumor (n=34)] who underwent ^{18}F-FDG PET before treatment were included in this study. Tumors sections were stained by immunohistochemistry for ERCC1, glucose transporter 1(Glut1), vascular endothelial growth factor (VEGF) and microvessel density (MVD) by determinate by CD34.

The expression of ERCC1 in thoracic neoplasms had a positivity of 49% (152/308), and the positive rates of ERCC1 expression in NSCLC, PMT, thymic epithelial tumor, pulmonary neuroendocrine tumor and MPM were 52, 43, 53, 47 and 85%, respectively. The positivity of ERCC1 expression was significantly higher in MPM and SQC than in the other histological types. A statistically significant correlation between ERCC1 expression and ^{18}F-FDG uptake was observed in thymic epithelial tumors, especially thymoma. Moreover, ERCC1 expression was also closely associated with the expression of Glut1, VEGF and MVD.

Our results indicated that ^{18}F-FDG uptake may be an alternative biomarker for predicting ERCC1 expression in patients with thymoma.

Key words: ERCC1, ^{18}F-FDG PET, thoracic neoplasms, predictive, biomarker

Thoracic neoplasms include mainly the diseases such as primary lung cancer, malignant pleural mesothelioma (MPM), thymic epithelial tumors and pulmonary metastatic tumors (PMT). Although surgical resection is performed in patients with early stage, systemic chemotherapy represents the mainstay of thoracic malignancies with advanced or recurrent diseases. Especially, platinum-based regimens are the treatment of choice in patients with advanced or recurrent thoracic malignancies, but the response is usually recognized in approximately 30% only. Massive efforts have been carried out to identify biomarkers that might help clinicians to choose appropriate drugs, by identifying potentially sensitive subjects and spare adverse effects in patients who are unlikely to benefit from chemotherapy.

Nucleotide excision repair (NER) has been described to be involved in the repair of platinum-induced DNA damage [1]. Several researchers have investigated the prognostic and predictive significance of NER pathway biomarkers [2-4]. Excision repair cross-complementation group 1 (ERCC1) is involved in the NER system, and this protein is known to be associated with resistance to platinum-based chemotherapy [2-5]. Recently, the chemoresistance proteins such as thymidylate synthase (TS), ribonucletide reductase messenger 1 (RRM1), breast cancer gene 1 (BRCA1) and class III β-tubulin have been also described in patients with thoracic neoplasms [5,6]. However, it is sometime difficult that we can obtain an adequate specimen for immunohistochemical analysis in patients with advanced thoracic neoplasms eligible

for chemotherapy. Therefore, it remains unknown whether the immunohistochemical staining of these chemoresistance protein could be instrumental in predicting patient prognosis after chemotherapy in a clinical setting.

Recently, the usefulness of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) for the diagnosis of thoracic neoplasms has been investigated in some studies [7-12]. Previous studies demonstrated that the primary tumor standardized uptake value (SUV) measurement on ^{18}F-FDG PET has been described to be a useful marker for predicting outcome after treatment in patients with thoracic neoplasms [7-12]. The amount of ^{18}F-FDG uptake within tumor cells is determinate by the glucose metabolism, hypoxia and angiogenesis, but the uptake of ^{18}F-FDG is strongly associated with the expression of glucose transporter 1 (Glut1) [12]. Even if we cannot obtain adequate specimens from advanced thoracic malignancies, sufficiently clear image can be obtained by ^{18}F-FDG uptake within the primary tumor. Although it is unknown whether ^{18}F-FDG PET is useful as molecular imaging of the above chemoresistance proteins, it may be important as useful data for clinical practice to investigate whether the measurement by ^{18}F-FDG uptake could reflect the level of chemoresistance protein within tumor cells. Especially, since platinum agents such as cisplatin is a key drug for treatment of advanced thoracic malignancies, it may be meaningful that we know the level of ERCC1 protein before chemotherapy.

Based on these backgrounds, we examined the relationship between ^{18}F-FDG uptake on PET and ERCC1 expression in patients with various thoracic neoplasms. Moreover, ERCC1 expression was correlated with Glut1, vascular endothelial growth factor (VEGF) and microvessel density (MVD) determined by CD34.

Material and methods

Patients. Between April 2003 and May 2009, we analyzed 148 consecutive patients with PMT who underwent ^{18}F-FDG PET and lung resection for pulmonary metastasis from extrathoracic malignancies, 21 consecutive patients with MPM who underwent ^{18}F-FDG PET, 34 consecutive patients with pulmonary neuroendocrine (NE) tumors who underwent ^{18}F-FDG PET and curative resection, and 49 consecutive patients with thymic epithelial tumors who underwent ^{18}F-FDG PET at Shizuoka Cancer Center. In 148 patients with PMT [adenocarcinoma (AC) with 106, squamous cell carcinoma (SQC) with 15, sarcoma with 20 and other with 8], the primary site was colon in 80 patients, breast in 9, head and neck in 14, genital system in 12, esophagus in 3, gastrointestinal tract in 7, soft tissue and bone in 20 and other sites in 3. In 21patients with MPM, 11 underwent surgical resection, 6 patients surgical biopsy, and 4 patients only percutaneous needle-core biopsy. Disease stage was classified according to the TNM staging system proposed by the International Mesothelioma Interest Group (IMIG) [13]. Sixteen patients had a histology of epithelial type, two biphasic type, one sarcomatous type, and two unspecific type. Of the 21 patients, 8, 1, 5 and 7 had stage I, II, III and IV tumors, respectively. As the initial treatment, 11 patients underwent surgery, 5 systemic chemotherapy, 2 thoracic radiotherapy and 3 best supportive care alone. In 34 patients with pulmonary NE tumors, all underwent lobectomy for clinical stage I. All NE tumors had been diagnosed based on the definitions of the revised WHO classification of lung cancer [14.15]. The postoperative pathological stage was determined according to the Union Internationale Centre le Cancer (UICC) staging system. The pathological diagnoses were: typical carcinoid (n=5), atypical carcinoid (n=1), small-cell lung carcinoma (SCLC)(n=12) and large cell neuroendocrine carcinoma (LCNEC) (n=16). High-grade NE tumor was SCLC and LCNEC. Twenty-three patients had pathological stage I and 11 patients stage II. In 49 patients with thymic epithelial tumors, there was 38 patients with thymoma and 11 with thymic carcinoma. As the initial treatment, 38 patients were treated with surgery, 8 with chemotherapy and 3 patients by thoracic radiation. All thymic epithelial tumors had been diagnosed based on the WHO classification [20]. From all patients, 38 patients underwent surgical resection and 11 percutaneous needle-core biopsy.

NSCLC patients were consecutively assigned to the study between August 2003 and March 2004, and ^{18}F-FDG PET was performed as part of the preoperative work-up. These patients underwent surgical management, and the primary lesions were surgically resected. Finally, 56 patients with NSCLC (37 with AC, 12 with SQC and 7 with large cell carcinoma) were evaluated. These 56 patients had no metastatic pulmonary tumors that were due to primary malignancies outside the thorax. All surgical specimens were reviewed and classified according to the WHO classification by an experienced lung pathologist who was unaware of clinical or imaging findings [14.15]. Pathologic tumor-node-metastasis (TNM) stages were established using the International System for Staging Lung Cancer adopted by the American Joint Committee on Cancer and the Union Internationale Centre le Cancer. Of the total patients, 24, 17 and 15 had stage I, II and III tumors, respectively.

In total patients (n=308), 187 were male and 121 female. The age of the patients ranged from 19 to 84 years, and the median age was 65 years. None of the patients had insulin-dependent diabetes, and the serum glucose levels in all patients just before ^{18}F-FDG PET study was less than 120mg/dL. The study protocol was approved by the institutional review board.

Immunohistochemical staining. Immunohistochemical staining was performed according to the procedure described in the previous reports [6,11,12]. The following antibodies were used: a mouse monoclonal antibody against ERCC1 (ABI2356; Abcam, Tokyo, Japan; 1:200 dilution); a rabbit polyclonal against GLUT1 (AB15309, Abcam, Tokyo, Japan, 1:400 dilution); a monoclonal antibody against VEGF (1:200 dilution; Immuno-Biological Laboratories Co.,Ltd., Japan); a mouse monoclonal antibody against CD34 (1:800 dilution; Nichirei, Tokyo, Japan). Antibodies against TS, OPRT and DPD were kindly donated by Taiho (Tokyo, Japan).

ERCC1 was assessed semiquantitatively by estimating the percentage of tumor cells with positive nuclei and/or cytoplasmic staining of the whole slide, (0= no staining, 0.1=positive staining in 1-9% of the tumor cells, 0.5=positive staining in 10-49% of the tumor cells, 1=positive staining in > 50% of the tumor cells). The staining intensity was evaluated semiquantitatively representing the average intensity of the stained tumor cells (0=no staining, 1=weak staining, 2=moderate staining, 3=strong staining). The proportion and intensity scores were than multiplied to obtain a total score, which ranged from 0 to 3 (H-score).

The expression of Glut1 was considered positive if distinct membrane staining was present. For Glut1, a semi-quantitative scoring method was used: 1= <10%, 2=10-25%, 3=25-50%, 4=51-75% and 5=>75% of cells positive. The tumors in which stained tumor cells made up more than 25% were graded as positive.

The expression of VEGF was quantitatively assessed according to the percentage of immunoreactive cells in a total of 1000 neoplastic cells. The number of CD34-positive vessels was counted in four selected hot spots in a 0.26 mm^2 field area. MVD was defined as the mean count of microvessels per 0.26 mm^2 field area. Sections were assessed using a light microscope in a blinded fashion by at least two of the authors.

^{18}F-FDG PET imaging. Patients fasted for at least 4h before ^{18}F-FDG PET examination. Patients received an intravenous injection of 200-250MBq of ^{18}F-FDG and then rested for approximately 1h before undergoing imaging [11,12]. Image acquisition was performed using an Advance NXi PET scanner and Discovery PET-CT scanner (GE Medical Systems, Milwaukee, WI, USA). Two-dimensional emission scanning was performed from the groin to the top of the skull.☒PET/CT image was independently reviewed by two experienced physicians. Acquired data were reconstructed by iterative ordered subset expectation maximization. To evaluate ^{18}F-FDG accumulation, the tumor was first examined visually, and then the peak standardized uptake value (SUV) of the entire tumor was determined. SUV_{max} was defined as the peak SUV value on one pixel with the highest counts within the region of interest (ROI). The ROI, measuring 3 cm in diameter, was set at the mediastinum at the level of the aortic arch and the mean SUV of the mediastinum was calculated.

Statistical analysis. Probability values of <0.05 indicated a statistically significant difference. Fisher's exact test was used to examine the association of two categorical variables. Correlation of different variables was analyzed using the nonparametric Spearman's rank test. Statistical analysis was performed using JMP 8 (SAS, Institute Inc., Cary, NC, USA) for Windows.

Results

Immunohistochemical staining and SUV_{max} by ^{18}F-FDG uptake. Each protein revealed a profile pattern of the unique expression. The immunohistochemical staining of the biomarkers was evaluated in 308 thoracic tumor lesions. ERCC1 was expressed in 49% (152/308), with a median H-score of 0.1. A median value of 0.1 was used as the cutoff ERCC1 in the following analyses, and the ERCC1 in more than 0.1 was defined as positive expression. Glut1 was detected in tumor cells and localized predominantly on their plasma membrane. A positive rate of Glut1 expression was recognized in 68% (208/309). The staining pattern of VEGF was uniformly localized in the cytoplasm and/or membrane of neoplastic tissue. The median rate of VEGF positivity was 25% (range, 1-88%), and the value of 25% was chosen as a cutoff point. Positive expression was recognized in 47% of cases (145/308). The median number of CD34-positive vessels was 25 (4-68), and the value of cutoff point was 25. Positive expression of CD34 was seen in 49% of cases (153/308).

The SUV_{max} of the primary tumors in 308 patients ranged from 1.0 to 33.9 (median 5.2). A median value of 5.2 was used as the cutoff SUV in the following analyses, and the SUV_{max} in more than 5.2 was defined as positive expression. Positive expression of SUV_{max} was seen in 31% of cases (95/308). Figure 1 is representative imaging of ERCC1 expression and ^{18}F-FDG PET. Figure 2 shows the rate of positive expression of these different biomarkers according to disease types.

Relationship between ERCC1 expression and different variables. Table 1 shows a comparison of the different variables according to ERCC1 expression. A positive expression of ERCC1 was significantly correlated with thoracic primary site and the expression of Glut1, VEGF and CD34. In the analysis according to primary disease types, the positive rate (85%) of ERCC1 expression in MPM was significantly higher than the other diseases (NSCLC, PMT, NE tumor and thymic epithelial tumors). No statistically significant difference in the ERCC1 expression was recognized among NSCLC, PMT, NE tumor and thymic epithelial tumors. Positive rate of ERCC1 expression was then compared according to histological types. There were 144 patients with adenocarcinoma (AC), 26 squamous cell carcinoma (SQC), 28 high-grade NE tumors, 20 sarcomas, 38 thymomas, and 16 MPM with epithelial type. The positive rates of

Table 1. Different variables according to ERCC1 expression

Variables		ERCC1 (+) (n=152)	ERCC1 (-) (n=156)	*p*-value
Age	≤ 65 / > 65 yr	79 / 73	77 / 79	0.650
Sex	Male / Female	94 / 58	93 / 63	0.726
Primary site	Thoracic / Extrathoracic	88 / 64	72 / 84	**0.041**
SUVmax	Low / High	73 / 79	83 / 73	0.425
Glut1	Low / High	40 / 112	59 / 97	**0.031**
VEGF	Low / High	57 / 95	106 / 47	**0.037**
CD34	Low / High	58 / 94	97 / 59	**< 0.001**

Abbreviation: ERCC1, excision repair complementation group 1; SUVmax, maximal standardized uptake value; Glut1, glucose transporter 1; VEGF, vascular endothelial growth factor; Thoracic, Primary site is thoracic lesion; Extrathoracic, Primary site is extrathoracic lesion.

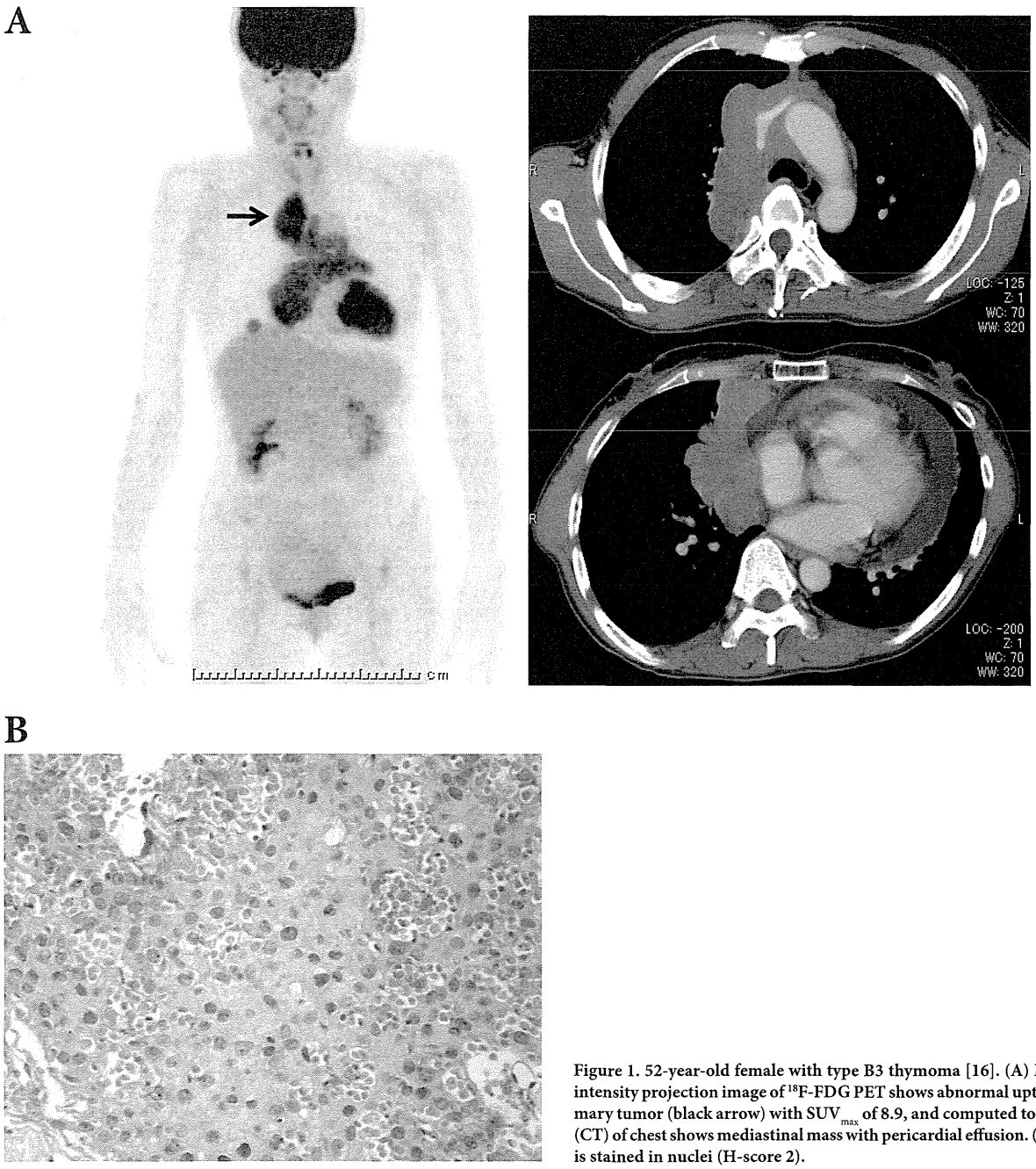

Figure 1. 52-year-old female with type B3 thymoma [16]. (A) Maximum intensity projection image of ^{18}F-FDG PET shows abnormal uptake in primary tumor (black arrow) with SUV_{max} of 8.9, and computed tomography (CT) of chest shows mediastinal mass with pericardial effusion. (B) ERCC1 is stained in nuclei (H-score 2).

AC, SQC, high-grade NE tumors, sarcoma, thymoma and MPM with epithelial type were 38% (55/144), 85% (22/26), 50% (14/28), 50% (10/20), 37% (15/38) and 88% (14/16), respectively. The positivity of ERCC1 expression was significantly lower in AC, high-grade NE tumor, sarcoma and thymoma than in SQC and MPM with epithelial type ($p<0.01$), demonstrating no significant difference ($p>0.99$). No statistically significant difference in the positivity of ERCC1 expression was recognized among AC, high-grade NE tumor, sarcoma and thymoma.

Correlation between ERCC1 expression and different variables. Table 2 shows the correlation between ERCC1 expression and different biomarkers according to various disease types. The expression of ERCC1 was significantly correlated with ^{18}F-FDG uptake, Glut1, VEGF and MVD. According to disease types, a statistically significant correlation

between ERCC1 expression and SUV_{max} by ^{18}F-FDG uptake was observed in patients with thymic epithelial tumors and pulmonary NE tumors. The analysis according to histological types demonstrated that ERCC1 expression in patients with thymoma was closely correlated with ^{18}F-FDG uptake, Glut1, VEGF and MVD (Table 3).

Discussion

This is the clinicopathological study evaluating the relationship between ^{18}F-FDG uptake on PET and ERCC1 expression in patients with various thoracic tumors. ERCC1 was expressed in 49% (152/308), and the positivity of ERCC1 expression in NSCLC, PMT, thymic epithelial tumors, NE tumors and MPM were 52, 43, 53, 47 and 85%, respectively. The analysis according to histology demonstrated that ERCC1 was highly expressed in patients with MPM and SQC. A statistically significant correlation between ERCC1 expression and SUV_{max} by ^{18}F-FDG uptake was recognized in thoracic neoplasms, especially thymoma. Our results suggest that ^{18}F-FDG uptake could be an alternative biomarker for predicting ERCC1 expression in patients with thymoma.

Complete surgical resection of the tumor offers the best chance for a favorable outcome in patients with thymic epithelial tumor. However, the extent of the disease at the time of presentation often precludes complete surgical resection. Therefore, platinum-based chemotherapy plays a very important role in the treatment of this disease. Recent study had documented that a positive ERCC1 expression was an independent factor for predict a poor outcome in thymic epithelial tumors [6]. Moreover, high ERCC1 expression has been also described to be related to resistance to platinum-based chemotherapy. *In vitro* data also supports that overexpression of ERCC1 was associated with resistance to platinum agent in thymic tumor cells [6]. Our study indicated that, out of these various thoracic neoplasms, the patients with thymoma had a significant correlation between ^{18}F-FDG uptake and ERCC1 expression. One preliminary study also described that ^{18}F-FDG PET is useful for monitoring response and prognosis after platinum-based chemotherapy in unresectable thymic epithelial tumors [16]. Although ^{18}F-FDG uptake is determined by glucose metabolism, hypoxia and angiogenesis, the expression level of ERCC1 within thymic tumor cells was also closely correlated with the expression of Glut1, VEGF and MVD. In this study, the expression level of ERCC1 was significantly correlated with not only ^{18}F-FDG uptake and Glut1 but also with VEGF and MVD. Especially, there was significant relationship between VEGF and ERCC1 expression in patients with different histological types except for MPM. MVD is closely related to the expression of ERCC1 in patients with PMT and thymoma. According to histology, ERCC1 expression yielded a positive correlation with angiogenesis in patients with AC. Our results suggest that ERCC1 expression may play a crucial role in the angiogenesis in patients with thymoma and AC of NSCLC or PMT. However, further study is warranted for confirming our results, because there is no

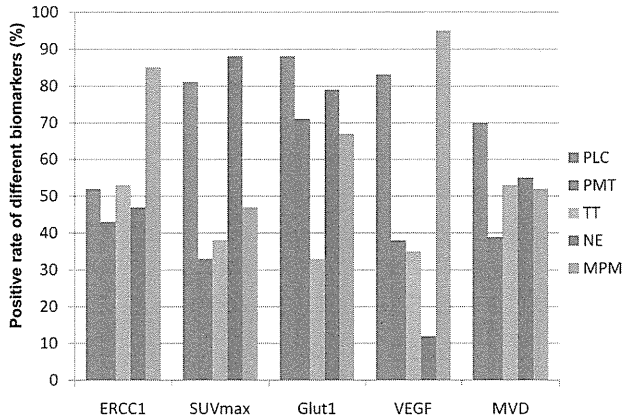

Figure 2. Positive rate according to different biomarkers (PLC, primary lung cancer; PMT, pulmonary metastatic tumor; TT, thymic epithelial tumor; NE, neuroendocrine tumor; MPM, malignant pleural mesothelioma). Positive rates of ERCC1 expression in PLC, PMT, TT, NE and MPM were 52, 43, 53, 47 and 85%, respectively. Those of SUV_{max}, Glut1, VEGF and CD34 in PLC, PMT, TT, NE and MPM were 81, 33, 38, 88 and 47%, respectively, 88, 71, 33, 79 and 67%, respectively, 83, 38, 35, 12 and 95%, respectively, and 70, 39, 53, 55 and 52%, respectively. (ERCC1, excision repair cross complementation group 1; SUV_{max}, maximal standardized uptake value; Glut1, glucose transporter 1; VEGF, vascular endothelial growth factor; MVD, microvessel density determinate by CD34).

Table 2. Correlation between ERCC1 expression and biomarkers according primary sites

	SUV_{max}	Glut1	VEGF	CD34
Total (n=308)				
Spearman γ 95% CI	0.154 0.039 – 0.264	0.268 0.158 – 0.372	0.432 0.334 – 0.521	0.329 0.222 – 0.428
***p*-value**	**0.006**	**<0.001**	**0.009**	**<0.001**
Primary lung cancer (n=56)				
Spearman γ	-0.054	0.315	0.375	0.101
95% CI	-0.320 – 0.219	0.049 – 0.540	0.116 – 0.586	-0.173 – 0.362
***p*-value**	0.688	**0.017**	**0.004**	0.457
Pulmonary metastatic tumors (n=148)				
Spearman γ	-0.038	0.123	0.250 0.087	0.312
95% CI	-0.203 – 0.128	-0.043 – 0.283	– 0.399	0.154 – 0.455
***p*-value**	0.641	0.136	**0.002**	**<0.001**
Thymic epithelial tumors (n=49)				
Spearman γ	0.723	0.713	0.840	0.626
95% CI	0.549 – 0.837	0.534 – 0.831	0.728 – 0.908	0.412 – 0.775
***p*-value**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Neuroendocrine tumors (n=34)				
Spearman γ	0.362	0.132	0.381	0.285
95% CI	0.017 – 0.631	-0.225 – 0.458	0.039 – 0.643	-0.068 – 0.576
***p*-value**	**0.035**	0.454	**0.025**	0.101
Malignant pleural mesothelioma (n=21)				
Spearman γ	0.231	0.285	0.168	-0.031
95% CI	-0.236 – 0.611	-0.181 – 0.646	-0.296 – 0.568	-0.468 – 0.416
***p*-value**	0.314	0.210	0.465	0.890

Abbreviation: ERCC1, excision repair complementation group 1; SUVmax, maximal standardized uptake value; Glut1, glucose transporter 1; VEGF, vascular endothelial growth factor; 95% CI, 95% confidential interval.

apparent rationale about the relationship between ERCC1 expression and the upregulation of these hypoxic markers.

Two recent studies demonstrated that no relationship was found between outcome and ERCC1 protein level in patients with MPM treated by platinum-based chemotherapy [9,17]. The results of these studies suggest that ERCC1 protein level may not be a predictor of responsiveness to platinum-based chemotherapy in patients with MPM. In our study, ERCC1 protein was highly expressed in patients with MPM, however, it remains unknown whether the expression level of ERCC1 could be correlated with the therapeutic resistance to platinum-based regimen. Therefore, it may not be instrumental to examine the relationship between ERCC1 expression and ^{18}F-FDG uptake within MPM tumor cells. Our results indicated that the pattern of ERCC1 expression varies according to the tumor subtype. Previous reports also described that a high expression of ERCC1 was observed in SQC as compared with AC [3,18], corresponding with our results. Olaussen et al concluded that NSCLC patients with complete resection and ERCC1-negative tumors appear to benefit from adjuvant cisplatin-based chemotherapy, whereas those with ERCC1-positive tumors do not [3]. Recent work suggests that ERCC1 expression is predictive in AC but not in other types of lung cancer [19]. In patients with SQC, the expression level of ERCC1 protein may not be also associated with resistance to platinum-based chemotherapy. Nowadays, the molecular techniques such as real-time quantitative polymerase chain reaction (qRT-PCR) and immunohistochemistry have been used in the measurement of ERCC1 expression. However, the expression profile of ERCC1 is different among the studies, and methods used in the studies also have a different technique. Therefore, it is necessary to apply standardized, optimized protocols and antibodies in order for immunohistochemistry to be validated as a reliable tool for therapeutic selection.

Table 3. Correlation between ERCC1 expression and different biomarkers according histological types

	SUV_{max}	Glut1	VEGF	CD34
Adenocarcinoma (n=144)				
Spearman γ	-0.033	0.154	0.307	0.340
95% CI	-0.200 – 0.135	-0.014 – 0.314	0.146 – 0.452	0.182 – 0.481
***p*-value**	0.691	0.065	**<0.001**	**<0.001**
Squamous cell carcinoma (n=26)				
Spearman γ	0.158	0.108	0.363	-0.029
95% CI	-0.255 – 0.522	-0.301 – 0.485	-0.040 – 0.664	-0.422 – 0.372
***p*-value**	0.441	0.596	0.068	0.884
High-grade neuroendocrine tumors (n=28)				
Spearman γ	0.224	-0.117	0.283	0.195
95% CI	-0.173 – 0.559	-0.479 – 0.278	-0.112 – 0.601	-0.203 – 0.538
***p*-value**	0.251	0.551	0.144	0.319
Sarcoma (n=20)				
Spearman γ	-0.013	0.068	0.121	0.041
95% CI	-0.453 – 0.432	-0.385 – 0.496	-0.339 – 0.535	-0.409 – 0.475
***p*-value**	0.955	0.766	0.600	0.859
Thymoma (n=38)				
Spearman γ	0.544	0.492	0.733	0.434
95% CI	0.262 – 0.740	0.196 – 0.706	0.533 – 0.855	0.123 – 0.667
***p*-value**	**<0.001**	**0.002**	**<0.001**	**0.006**
Thymic carcinoma (n=11)				
Spearman γ	-0.301	0.410	0.259	-0.179
95% CI	-0.771 – 0.382	-0.270 – 0.817	-0.421 – 0.752	-0.713 – 0.487
***p*-value**	0.367	0.210	0.441	0.597
Malignant pleural mesothelioma (epithelial type) (n=16)				
Spearman γ	0.177	0.271	0.037	-0.273
95% CI	-0.363 – 0.628	-0.274 – 0.684	-0.479 – 0.534	-0.686 – 0.272
***p*-value**	0.512	0.309	0.891	0.305

Abbreviation: ERCC1, excision repair complementation group 1; SUVmax, maximal standardized uptake value; Glut1, glucose transporter 1; VEGF, vascular endothelial growth factor; 95% CI, 95% confidential interval.

Our study is a retrospective analysis and includes heterogeneous groups with or without platinum-based treatment. Therefore, we cannot gain a useful information on the cisplatin sensitivity for our patients. This is one of the study limitations, and further study is warranted for evaluating the relationship between ERCC1 and ^{18}F-FDG uptake in patients treated by cisplatin.

In conclusion, the expression level of ERCC1 protein had a statistically significant correlation with SUV_{max} by ^{18}F-FDG uptake in thymic epithelial tumors (especially, thymoma). ERCC1 is highly expressed in MPM and SQC of thoracic neoplasms. Considering that ERCC1 is a possible marker for predicting chemoresistance to platinum-based chemotherapy, SUV_{max} by ^{18}F-FDG uptake in patients with thymoma may be an alterative marker for the expression of ERCC1. Further study is warranted for evaluating whether ^{18}F-FDG uptake could be a useful marker for predicting outcome after platinum-based treatment in patients with unresectable or recurrent thymoma.

Acknowledgements: This work was supported in part by Grant 21790793 (K. K) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and National Hospital Organization Policy Based Medical Services. We thank all staff of the Pathology Department of Shizuoka Cancer Center for their technical assistance in immunohistochemical analysis.

References

[1] VOLKER M, MONE MJ, KAMAKAR P, VAN HOFFEN A, SCHUL W. et al. Sequential assembly of the nucleotide excision repair factors in vivo. Mol Cell 2011; 8: 213-24. http://dx.doi.org/10.1016/S1097-2765(01)00281-7

[2] SIMON GR, SHARMA S, CANTOR A, SMITH P, BEPLER G. ERCC1 expression is a predictor of survival in resected patients with non-small cell lung cancer. Chest 2005; 127: 978-83. http://dx.doi.org/10.1378/chest.127.3.978

[3] OLAUSSEN KA, DUNANT A, FOURET P, BRAMBILLA E, ANDRE F. et al. DNA repair by ERCC1 in non-small- cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 2006; 355: 983-91. http://dx.doi.org/10.1056/NEJMoa060570

[4] ZHENG Z, CHEN T, LI X, HAURA E, SHARMA A, BEPLER G. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. N Engl J Med 2007; 356: 800-8. http://dx.doi.org/10.1056/NEJMoa065411

[5] KAIRA K, YAMAMOTO N. Prognostic and predictive factors in resected non-small cell lung cancer. Expert Opin Med Diagn 2010; 4: 1-9. http://dx.doi.org/10.1517/17530059.2010.506214

[6] KAIRA K, SERIZAWA M, KOH Y, MIURA S, KAIRA R. et al. Expression of Excision Repair Cross-Complementation Group 1, Breast Cancer Susceptibility 1, and β III-Tubulin in Thymic Epithelial Tumors. J Thorac Oncol. 20116: 606-13.

[7] SASAKI R, KOMAKI R, MACAPINLAC H, ERASMUS J, ALLEN P. et al. [18F] fluorodexyglucose uptake by positron emission tomography predicts outcome of non-small-cell lung cancer. J Clin Oncol 2005; 23: 1136-43. http://dx.doi.org/10.1200/JCO.2005.06.129

[8] HIGASHI K, UEDA Y, ARISAKA Y, SAKUMA T, NAMBU Y. et al. F-FDG uptake as a biologic prognostic factor for recurrence in patients with surgically resected non-small cell lung cancer. J Nucl Med 2002; 43: 39-45.

[9] BENARD F, STERMAN D, SMITH RJ, KAISER LR, ALBELDA SM. et al. Prognostic value of FDG PET imaging in malignant pleural mesothelioma. J Nucl Med 1999; 40: 1241-5.

[10] SONG YS, LEE WW, CHUNG JH, PARK SY, KIM YK. et al. Correlation between FDG uptake and glucose transporter type 1 expression in neuroendocrine tumors of the lung. Lung Cancer 2008; 61: 54-60. http://dx.doi.org/10.1016/j.lungcan.2007.11.012

[11] KAIRA K, OKUMURA T, OHDE Y, TAKAHASHI T, MURAKAMI H. et al. Correlation between 18F-FDG uptake on PET and molecular biology in metastatic pulmonary tumors. J Nucl Med 2011; 52: 705-11. http://dx.doi.org/10.2967/jnumed.111.087676

[12] KAIRA K, ENDO M, ABE M, NAKAGAWA K, OHDE Y. 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. http://dx.doi.org/10.1200/JCO.2009.27.4662

[13] RUSCH VW. The International Mesothelioma Interest Group. A proposed new international TNM staging system for malignant pleural mesothelioma. Chest 1995; 108; 1122-28. http://dx.doi.org/10.1378/chest.108.4.1122

[14] TRAVIS WD, RUSH W, FLIEDER DB. International Histological Classification of Tumors, Histological Typing of Lung and Pleural Tumors, 1999.

[15] ROSAI J, SOBIN LH. Histological typing of tumours of the thymus, International histological classification of tumours. 2nd ed. New York: Springer pp1-59, 1999. http://dx.doi.org/10.1007/978-3-642-60157-6_1

[16] KAIRA K, MURAKAMI H, MIURA S, KAIRA R, AKAMATSU H. et al. 18F-FDG uptake on help predict outcome and response after treatment in unresectable thymic epithelial tumors. Ann Nucl Med 2011;25:247-53. http://dx.doi.org/10.1007/s12149-010-0455-x

[17] ZUCALI PA, GIOVANNETTI E, DESTRO A, MENCOBONI M, CERESOLI GL. et al. Thymidylate synthase and excision repair cross-complementing group-1 as predictors of responsiveness in mesothelioma patients treated with pemetrexed/carboplatin. Clin Cancer Res 2011; 17: 2581-90. http://dx.doi.org/10.1158/1078-0432.CCR-10-2873

[18] JUN HJ, AHN MJ, KIM HS, YI SY, HAN J. et al. ERCC1 expression as a predictive marker of squamous cell carcinoma of the head and neck treated with cisplatin-based concurrent chemoradiation. Br J Cancer 2008; 99: 167-72. http://dx.doi.org/10.1038/sj.bjc.6604464

[19] VILMAR A, SANTONI-RUIGU E, SORENSEN JB. ERCC1, toxicity and quality of life in advanced NSCLC patients randomized in a large multicentre phase III trial. Eur J Cancer 2010; 46: 1554-62. http://dx.doi.org/10.1016/j.ejca.2010.02.045

European Journal of Radiology 81 (2012) 2423–2429

Contents lists available at SciVerse ScienceDirect

European Journal of Radiology

journal homepage: www.elsevier.com/locate/ejrad

^{18}F-FDG uptake on PET in primary mediastinal non-thymic neoplasm: A clinicopathological study

Kyoichi Kaira[a,*], Masato Abe[b], Kazuo Nakagawa[c], Yasuhisa Ohde[c], Takehiro Okumura[c], Toshiaki Takahashi[a], Haruyasu Murakami[a], Takehito Shukuya[a], Hirotsugu Kenmotsu[a], Tateaki Naito[a], Isamu Hayashi[b], Noboru Oriuchi[e], Masahiro Endo[d], Haruhiko Kondo[c], Takashi Nakajima[b], Nobuyuki Yamamoto[a]

[a] Division of Thoracic Oncology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan
[b] Division of Pathology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan
[c] Division of Thoracic Surgery, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan
[d] Division of Diagnostic Radiology, Shizuoka Cancer Center, 1007 Shimonagakubo Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan
[e] Department of Diagnostic Radiology and Nuclear medicine, Gunma University Graduate School of Medicine, Showa-machi, Maebashi 371-8511, Gunma, Japan

ARTICLE INFO

Article history:
Received 19 July 2011
Received in revised form
19 September 2011
Accepted 20 September 2011

Keywords:
^{18}F-FDG PET
Mediastinal neoplasm
Biological correlation
Glut1
HIF-1α
Glucose metabolism

ABSTRACT

Background: The usefulness of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) has been investigated in thymic epithelial tumors. However, little is known about PET imaging of ^{18}F-FDG in primary non-thymic mediastinal neoplasms. The aim of this study is to explore the clinicopathological significance of ^{18}F-FDG PET in primary mediastinal (non-thymic) neoplasms.
Methods: Twenty-one patients with mediastinal neoplasms who underwent ^{18}F-FDG PET before treatment were included in this study. Tumor sections were stained by immunohistochemistry for glucose transporter 1 (Glut1); glucose transporter 3 (Glut3); hypoxia-inducible factor-1 alpha (HIF-1α); hexokinase I; vascular endothelial growth factor (VEGF); microvessels (CD34); epidermal growth factor receptor (EGFR); Akt/mTOR signaling pathway (p-Akt and p-mTOR); cell cycle control (p53).
Results: Seventeen of 21 patients were imaged on PET system using ^{18}F-FDG, but 4 patients with a histology of cyst showed nothing abnormal in PET scans. The histology of the resected tumors was as follows: 6 schwannoma, 3 teratoma, 4 cyst, 3 sarcoma, 1 undifferentiated carcinoma, 1 seminoma, 1 mediastinal goiter, 1 ganglioneuroma, and 1 Hodgkin lymphoma. ^{18}F-FDG uptake was significantly correlated with Glut1, HIF-1α, EGFR, p-Akt and p-S6K. These biomarkers were highly expressed in schwannoma, teratoma and high grade malignancies, whereas all patients with cyst and ganglioneuroma had no positive expression of these biomarkers. High uptake of ^{18}F-FDG was significant associated with Glut1, VEGF, EGFR, p-Akt, p-S6K and tumor maximal size.
Conclusion: The amount of ^{18}F-FDG uptake in primary mediastinal non-thymic neoplasms is determined by the presence of glucose metabolism (Glut1), hypoxia (HIF-1α) and upstream components of HIF-1α (EGFR, p-Akt and p-S6K).

1. Introduction

Primary mediastinal tumors are rare diseases, representing approximately 3% of the tumors within the thorax [1,2]. They can originate from any organ or tissue but most commonly arise from thymic, neurogenic, lymphatic, germinal or mesenchymal tissues. Primary tumors of the mediastinum are a heterogenous group of tumors, ranging from relatively benign tumors to aggressive malignancies.

Recently, the usefulness of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) for the diagnosis of cancer has been investigated in many studies [3–5]. Determination of malignant lesions with ^{18}F-FDG PET is based on the glucose metabolism. The overexpression of glucose transporter 1 (Glut1) has been shown to be closely related to ^{18}F-FDG uptake in human cancer [3–5]. Glucose phosphorylation enzyme (Hexokinase) is also known to play an important subtype for glucose metabolism in cancer cells [4]. Glucose-6-phosphatase is decreasing by the increased concentrations of hexokinase, and the acceleration of glucose phosphorylation results in increased glucose consumption. Glut 1 is thought to be a possible intrinsic marker of hypoxia, and the expression of Glut 1 has been found to be regulated by hypoxia in a

* Corresponding author. Tel.: +81 55 989 5222; fax: +81 55 989 5634.
E-mail address: kkaira1970@yahoo.co.jp (K. Kaira).

0720-048X/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ejrad.2011.09.017

hypoxia-inducible factor (HIF)-1-dependent way [3,6,7]. Previous studies suggest that hypoxic conditions correspond to a higher ^{18}F-FDG uptake [3,6,7]. In addition, several researchers described the relationship between ^{18}F-FDG uptake and the expression of vascular endothelial growth factor (VEGF) or micro-vessel density (MVD) [8,9]. HIF-1α is considered to support tumor growth by the induction of angiogenesis via the expression of the VEGF and also by high and anaerobic metabolic mechanisms [10]. Recent preliminary report demonstrated that ^{18}F-FDG PET could be a valuable tool for assessing the effects of the mammalian target of rapamycin (mTOR) inhibition in lung cancer patients [11]. mTOR is a downstream component of the PI3K/AKT pathway involved in the regulation of cell proliferation, angiogenesis, and metabolism. However, there is no report about the relationship between ^{18}F-FDG uptake within tumor cells and PI3K/AKT/mTOR signaling pathway in human neoplasms. As many factors can influence the extent of ^{18}F-FDG uptake, the underlying mechanisms for ^{18}F-FDG accumulation are still a matter of debate in various human neoplasms.

Recently, we reported the biological correlation of ^{18}F-FDG uptake on PET in thymic epithelial tumors as primary mediastinal tumors [3]. Our report demonstrated that the amount of ^{18}F-FDG uptake in thymic epithelial tumors is determined by the presence of glucose metabolism (Glut1), hypoxia (HIF-1α), angiogenesis (VEGF and MVD) and cell cycle regulator (p53). However, there is still no data about the possible mechanisms for ^{18}F-FDG uptake in patients with primary mediastinal tumors excluding thymic epithelial tumors. Therefore, we conducted ^{18}F-FDG PET studies and immunohistochemical analyses in patients with primary mediastinal non-thymic tumors.

2. Materials and methods

2.1. Patients

Between November 2002 and August 2008, we analyzed 78 consecutive patients with primary mediastinal tumors who underwent ^{18}F-FDG PET and received a surgical resection at Shizuoka Cancer Center. The patients with 54 thymic epithelial tumors and 3 mediastinal metastases from extrathoracic malignancies were excluded from this study. Therefore, a total of 21 patients with mediastinal neoplasms were included in this study.

None of the patients had insulin-dependent diabetes, and the serum glucose levels in all patients just before ^{18}F-FDG PET infection were less than 120 mg/dL. The study protocol was approved by the institutional review board.

2.2. ^{18}F-FDG PET imaging

Patients fasted for at least 4 h before ^{18}F-FDG PET examination. Patients received an intravenous injection of 200–250 MBq of ^{18}F-FDG and then rested for approximately 1 h before undergoing imaging. Image acquisition was performed using an Advance NXi PET scanner and Discovery PET-CT scanner (GE Medical Systems, Milwaukee, WI, USA). Two-dimensional emission scanning was performed from the groin to the top of the skull. PET/CT image was independently reviewed by two experienced physicians. Acquired data were reconstructed by iterative ordered subset expectation maximization. To evaluate ^{18}F-FDG accumulation, the tumor was first examined visually, and then the peak standardized uptake value (SUV) of the entire tumor was determined. The region of interest (ROI), measuring 3 cm in diameter, was set at the mediastinum at the level of the aortic arch and the mean SUV of the mediastinum was calculated. Finally, the T/M ratio, which is the ratio of the peak SUV of the tumor to the mean SUV of the mediastinum, was determined for each patient.

2.3. Immunohistochemical staining

Immunohistochemical staining was performed according to the procedure described in the previous reports [3,12]. The following antibodies were used: a rabbit polyclonal antibody against GLUT1 (AB15309, Abcam, Tokyo, Japan, 1:200 dilution); a rabbit polyclonal antibody against GLUT3 (AB15311, Abcam, Tokyo, Japan, 1:100 dilution); a rabbit monoclonal antibody against hexokinase I (AB55144, Abcam, Tokyo, Japan, 1:200 dilution); a mouse monoclonal antibody against HIF-1α (NB100-123, Novus Biologicals, Inc., Littleton, 1:50 dilution); a murine monoclonal antibody against MIB-1 (Dako, Denmark, 1:40 dilution); a monoclonal antibody against VEGF (Immuno-Biological Laboratories Co.,Ltd., Japan, 1:300 dilution); a mouse monoclonal antibody against CD34 (Nichirei, Tokyo, Japan, 1:800 dilution); a mouse monoclonal antibody against p53 (D07; DAKO, 1:50 dilution); a mouse monoclonal antibody against EGFR (Novovastra laboratories Ltd., Newcastle, UK, 1:100 dilution); a rabbit polyclonal antibody against phosph-Akt (Abcam, Tokyo, Japan, 1:200 dilution); a rabbit monoclonal antibody against phosph-mTOR (Cell signaling, 80 dilution); a rabbit monoclonal antibody against phosph-S6K (Cell signaling, 100 dilution).

The expression of Glut1, Glut3 and EGFR was considered positive if distinct membrane staining was present. Five fields (400×) were analyzed to determine the frequency of the HIF-1α stained nuclei and hexokinase I stained cytoplasm. For Glut1, Glut3, EGFR, HIF-1α and hexokinase I, a semi-quantitative scoring method was used: 1 = <10%, 2 = 10–25%, 3 = 25–50%, 4 = 51–75% and 5 = >75% of cells positive. The tumors in which stained tumor cells made up more than 25% of the tumor were graded as positive.

The expression of VEGF was quantitatively assessed according to the percentage of immunoreactive cells in a total of 1000 neoplastic cells. The number of CD34-positive vessels was counted in four selected hot spots in a 400× field (0.26 mm^2 field area). Microvessel density (MVD) was defined as the mean count of microvessels per 0.26 mm^2 field area.

For p53, microscopic examination for the nuclear reaction product was performed and scored. According to previous report [12], p53 expression in more than 10% of tumor cells was defined as high expression.

p-AKT, p-mTOR and p-S6K were considered positive if membranous and/or cytoplasmic staining was present. For p-AKT, p-mTOR and p-S6K, a semi-quantitative scoring method was used: 1 = <10%, 2 = 10–25%, 3 = 25–50%, 4 = 51–75% and 5 = >75% of cells positive. The tumors in which stained tumor cells made up more than 25% of the tumor were graded as positive.

Sections were assessed using a light microscopic (E330-ADU1.2X, OLYMPUS, Japan) in a blinded fashion by at least two of the authors.

2.4. Statistical analysis

Probability values of <0.05 indicated a statistically significant difference. Fisher's exact test was used to examine the association of two categorical variables. Correlation of different variables was analyzed using the nonparametric Spearman's rank test. Statistical analysis was performed using JMP 8 (SAS, Institute Inc., Cary, NC, USA) for Windows.

3. Results

3.1. Patient characteristics

Patient's characteristics are listed in Table 1. The median age of the patients was 59 years (range, 12–82 years). Eleven patients

Table 1
Patient's characteristics.

No.	Age/sex	PS	Smoking history	Tumor size (mm)	T/M ratio	Histology
1	59F	0	Yes	27	1.87	Schwannoma
2	82M	0	Yes	63	(-)	Bronchogenic cyst
3	79M	1	Yes	139	(-)	Pericardial cyst
4	76M	0	Yes	30	(-)	Bronchogenic cyst
5	56M	0	Yes	43	4.25	Synovial sarcoma
6	17M	2	No	140	1.58	Mature teratoma
7	56F	0	No	21	(-)	Bronchogenic cyst
8	67M	0	Yes	70	9.28	Undifferentiated carcinoma
9	25M	0	Yes	12	2.52	Seminoma
10	80F	0	No	37	2.12	Mediastinal goiter
11	45F	0	No	28	3.38	Schwannoma
12	59M	0	Yes	42	1.64	Teratoma
13	72M	0	Yes	26	1.45	Schwannoma
14	45F	0	No	160	3.31	Liposarcoma
15	62F	0	No	20	1.74	Schwannoma
16	60F	0	No	46	3.12	Mature teratoma
17	12M	0	N0	105	1.05	Ganglioneuroma
18	33F	0	N0	54	1.75	Schwannoma
19	45M	0	Yes	17	1.59	Schwannoma
20	67F	0	No	65	2.58	Sarcoma
21	71F	1	Yes	45	6.48	Hodgkin lymphoma

Abbreviations: M, male; F, female; T/M ratio, the ratio of the peak SUV of the tumor to the mean SUV of the mediastinum; Tumor size, maximal tumor size.

were men and 10 were women. Performance status (PS) and smoking history were as follows: 18 patients with PS 0; 2 patients with PS 1; 1 patients with PS 2; 11 patients with smoker; 10 patients with never smoker. The maximal tumor size of resected pulmonary nodules ranged from 12 to 140 mm (median, 43 mm). The histology of the resected tumors was as follows: 6 schwannoma, 3 teratoma, 4 cyst, 3 sarcoma, 1 undifferentiated carcinoma, 1 seminoma, 1 mediastinal goiter, 1 ganglioneuroma, and 1 Hodgkin lymphoma.

3.2. ^{18}F-FDG PET findings

Of 21 patients, 17 patients were imaged on PET system using ^{18}F-FDG (Table 1). All of 4 patients with cyst showed nothing abnormal in PET scans. The mean value (mean and standard deviation) of T/M ratio was 3.06 ± 0.52 (range, 1.1–9.3). The maximal size of the primary tumor ($n = 17$) was not significantly correlated with ^{18}F-FDG uptake ($p = 0.278$).

The median value of T/M ratio was 2.51, therefore, a median value of 2.5 was used as the cutoff T/M ratio in the following analyses, and the T/M ratio in more than 2.5 was defined as high uptake.

3.3. Immunohistochemical analysis

Glut1, Glut3, Hexokinase I, HIF-1α, VEGF, CD34, EGFR, p-Akt, p-mTOR, p-S6K and p53 immunohistochemical staining were evaluated for the surgically resected 21mediastinal tumors (Figs. 1 and 2). Glut1 and Glut3 were detected in tumor cells and localized predominantly on their plasma membrane. A positive rate of Glut1 and Glut3 expression was recognized in 38.1% and 23.1%, respectively. A positive expression of HIF-1α was predominantly expressed in the cytoplasm with some nuclear staining, and was recognized in 47.6%. A positive expression of hexokinase I was expressed in the cytoplasm and/or membrane of neoplastic, and was recognized in 42.9%. The staining pattern of VEGF was uniformly localized in the cytoplasm and/or membrane of neoplastic. The median rate of VEGF positivity was 10% (range, 1–40%), and the value of 10% was chosen as a cutoff point. High expression was recognized in 38.1%. The median numbers of CD34 was 14 (1–45), and the value of 14 was chosen as a cutoff point. High expression of CD34 was seen in 33.3%. The positive rate of p-Akt, mTOR and p-S6K was recognized in 33.3%, 14.3% and 52.3%, respectively. The incidence of p53 positivity was 14.3%.

Next, we examined the expression of these immunohistochemical markers according to the histology of mediastinal tumors (Table 2). The positive expression of these biomarkers was observed in schwannoma, teratoma and other thoracic malignancies. But, all patients with cyst and ganglioneuroma had no positive expression of these biomarkers.

3.4. Relationship between ^{18}F-FDG uptake and different variables

The results of the statistical correlation between T/M ratio and different variables are listed in Table 3. The resected specimens of 17 patients with visible imaging on PET were correlated with the degree of ^{18}F-FDG uptake. Using Spearman rank correlation, the T/M ratio in mediastinal tumors was significantly correlated with the expression of Glut1, HIF-1α, EGFR, p-Akt and p-S6K (Fig. 3).

3.5. Comparison of ^{18}F-FDG uptake and different variables

The results of the statistical comparison between ^{18}F-FDG uptake and different variables are listed in Table 4. We analyzed 21 mediastinal neoplasms including 4 patients without visible imaging on PET. Glut1, VEGF, EGFR, p-Akt, p-S6K and tumor size yield a statistically significant positive correlation.

4. Discussion

This is the retrospective study to evaluate the biologic correlation of ^{18}F-FDG uptake and the expression of biomarkers such as Glut1, Gllut3, hexokinase I, HIF-1α, VEGF, CD34, EGFR, p-Akt, p-mTOR, p-S6K and p53 in primary mediastinal non-thymic neoplasms. Glucose transporter (Glut1), hypoxia (HIF-1α), EGFR and p-Akt were closely associated with ^{18}F-FDG uptake. These biomarkers were observed in schwannoma, teratoma and other thoracic malignancies. The significant factors of a high ^{18}F-FDG uptake were Glut1, VEGF, EGFR, p-Akt, p-S6K and tumor maximal size.

Of the 254 primary mediastinal masses reported, thymic neoplasms have been described to be most common [13]. We found that ^{18}F-FDG uptake in thymic neoplasms was closely associated with the expression of Glut1, HIF-1α, VEGF, CD34 and p53 [13]. However, there is still no data about the relationship between ^{18}F-FDG uptake and biomarkers in non-thymic mediastinal neoplasms. Since these diseases are a rare and hetergenous group, it may be

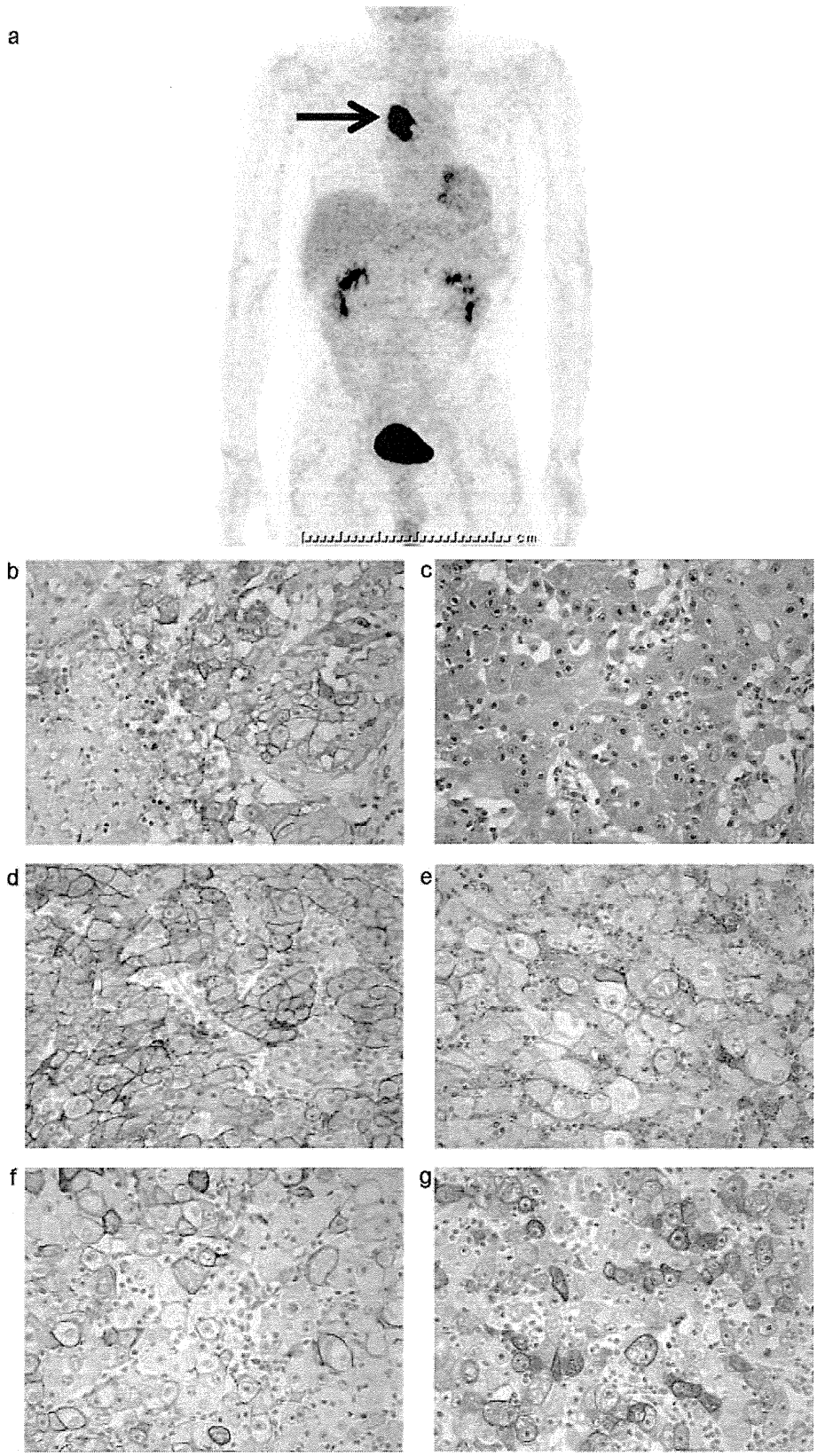

Fig. 1. PET imaging and immunohistochemical staining in a 62-year-old man with undifferentiated carcinoma in the mediastinum. PET showed increased ^{18}F-FDG accumulation in the mediastinal lesion (black arrow) (A). Immunohistochemical analysis showed positive staining of Glut1 (B), HIF-1α (C), EGFR (D), p-Akt (E), p-S6K (F) and p-mTOR (G).

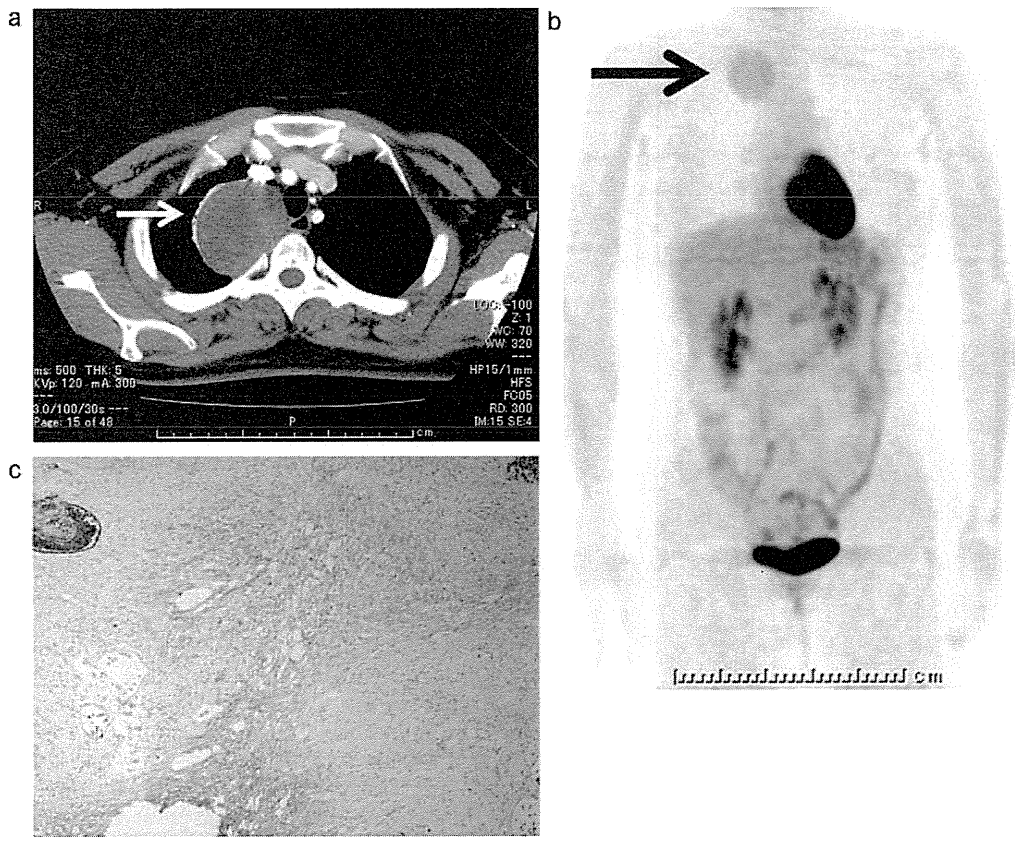

Fig. 2. PET imaging and immunohistochemical staining in a 59-year-old woman with schwannoma in the upper mediastinum. Chest computed tomography showed a mass in the upper mediastinum (A) and PET showed weak uptake of ^{18}F-FDG in the corresponding lesion (black arrow) (B). Immunohistochemical analysis of the resected mass showed positive staining of Glut1 (C).

difficult to investigate the immunohistochemical study of ^{18}F-FDG uptake on PET. Our results suggest that glucose metabolism and hypoxia play an important role on the uptake of ^{18}F-FDG in primary mediastinal non-thymic neoplasms, which is associated with the upstream component of Glut1/HIF-1α pathway such as Akt or EGFR. Since there is only our study assessing the expression of glucose metabolism and hypoxia including signal pathway in primary mediastinal neoplasms, further investigation is warranted.

Recently, ^{18}F-FDG PET analysis of schwannoma has been reported, and ^{18}F-FDG uptake showed a positive correlation with the tumor size and angiogenesis [14]. All of 26 patients with schwannoma showed negative Glut1, 4 patients (15%) positive Glut3, 15 patients (58%) positive Hexokinase, 9 patients (35%) positive VEGF and 9 patients (35%) positive MVD. However, there was no significant correlation between ^{18}F-FDG uptake and the expression of Glut1, Glut3 and Hexokinase. In our study, Glut1 expression was recognized in 3 (50%) of 6 patients with schwannoma, although our study includes a small sample size of schwannoma. Previous report also described that all tumors of schwannoma showed a positive study in ^{18}F-FDG PET, which was corresponding to our results. Therefore, the expression of Glut1, hexokinase, HIF-1α, and VEGF is considered to be necessary for ^{18}F-FDG accumulation within schwannoma cells. The discrepancy between our study and previous report [14] may be due to the differentiation of protocols and antibodies in order for immunohistochemistry.

In patients with bone and soft tissue sarcomas, Glut1 expression has been described to be closely associated with ^{18}F-FDG uptake [15]. Sarcoma patients have an enhanced glucose metabolism which is correlated with tumor grade. Moreover, the significant correlation between Glut1 expression and ^{18}F-FDG uptake has been also reported to be observed in patients with lymphoma [16]. But, several researchers described that intensity of lymphoma on ^{18}F-FDG PET is not significantly associated with the expression of hexokinase and Glut3 [16,17]. However, little is known about the relationship between ^{18}F-FDG uptake and these biomarkers in the metastatic sites secondary to sarcoma or lymphoma. In the present study, Glut1 and hexokinase were highly expressed in the histology of Hodgkin lymphoma and sarcoma. However, these diseases in our study are small sample size, and we could not investigate the correlation between ^{18}F-FDG uptake and Glut1 expression.

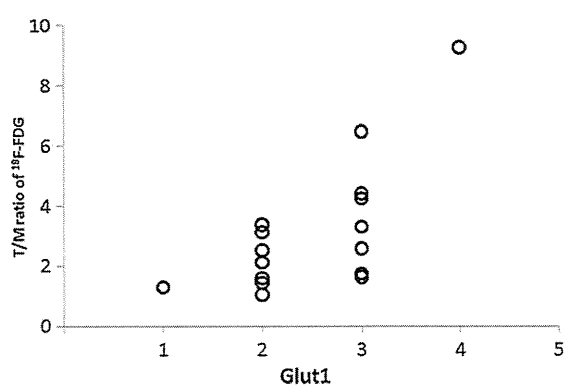

Fig. 3. Significant correlation was found between the T/M ratio of ^{18}F-FDG and scoring of Glut1 expression (Spearman's rank correlation coefficient $\gamma = 0.5965$, $p = 0.012$).