L-[3-^{18}F]-α-Methyltyrosine Uptake by Lymph Node Metastasis Is a Predictor of Complete Response to CRT in Esophageal Cancer

MAKOTO SOHDA[1], TATSUYA MIYAZAKI[1], HIROAKI HONJYO[1], KEIGO HARA[1], DAIGO OZAWA[1], SHIGEMASA SUZUKI[1], NARITAKA TANAKA[1], AKIHIKO SANO[1], MAKOTO SAKAI[1], TAKEHIKO YOKOBORI[1], MASANOBU NAKAJIMA[2], MINORU FUKUCHI1, HIROYUKI KATO[2], TETSUYA HIGUCHI[3], YOSHITO TSUSHIMA[3] and HIROYUKI KUWANO[1]

Departments of [1]General Surgical Science and [3]Diagnostic Radiology and Nuclear Medicine, Gunma University Graduate School of Medicine, Maebashi, Japan; [2]Department of Surgical Oncology, Dokkyo Medical University, Mibu, Japan

Abstract. *Background/Aim: The amino acid positron emission tomography (PET) tracer [^{18}F]-3-fluoro-alpha-methyltyrosine (^{18}F-FAMT) is known to be highly specific for malignancies. We evaluated the accumulation of ^{18}F-FDG or ^{18}F-FAMT in lymph nodes (LN) prior to definitive chemoradiotherapy (CRT) for esophageal cancer. Patients and Methods: We retrospectively reviewed 30 patients with esophageal squamous cell carcinoma. All patients received definitive CRT. The relationship between the accumulation of ^{18}F-FDG PET or ^{18}F-FAMT PET in LNs prior to CRT and clinical outcomes was assessed. Results: A correlation was observed between LNs in which most of ^{18}F-FAMT was accumulated and complete response (CR) rate, but was not for ^{18}F-FDG. Additionally, for ^{18}F-FAMT, the CR rate was significantly higher in the LN accumulated lesion ≤1 group than in the LN accumulated lesion >2 group. Discussion: To predict the outcome of definitive CRT in patients with esophageal cancer, it is important to evaluate the LN status.*

Esophageal cancer is a common malignant neoplasm. In spite of recent improvements in surgical techniques and adjuvant therapies, the prognosis of patients with advanced disease remains poor (1, 2). This is mainly because esophageal cancer commonly spreads aggressively through the lymphatic system. Zhang *et al*. proposed that the number of lymph node (LN) metastases could better reflect the prognosis of patients with esophageal cancer (3). They reported that the 5-year survival rate of patients with two or more metastatic LNs was 9.35%, which was significantly worse than that of patients with one LN or no metastatic LNs (5-year survival: 33.38%). Lin *et al*. also demonstrated that metastasis in 4 or more LNs was a significant independent prognostic factor in thoracic esophageal squamous cell carcinoma (4). Esophageal cancer generally presents as a locally advanced disease and requires multimodal therapy (5, 6). Chemoradiotherapy (CRT) is considered definitive when administered with curative intent for the treatment of locally advanced esophageal squamous cell carcinoma (SCC). Recent improvements in the clinical results of CRT have increased the options for treatment. However, an accurate diagnosis of tumor invasion and metastasis is crucial for decision-making regarding appropriate treatments. Furthermore, a more accurate prediction of treatment is important for avoiding unnecessary treatments in patients with esophageal cancer. ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG-PET) can accurately diagnose the tumor extent of esophageal cancer. ^{18}F-FDG-PET was also shown to be more sensitive, specific and accurate for detecting lymph node metastasis than computed tomography (CT), particularly in the neck and upper thoracic region (7). We also proposed that the standardized uptake value (SUV) of ^{18}F-FDG-PET prior to definitive CRT may be one of the most reliable predictors of the outcomes of patients with esophageal cancer in combination with tumor dimensions and classification (8). However, to the best of our knowledge, the relationship between the LN SUV on PET and responses to CRT has not yet been examined in patients with esophageal cancer.

Although the 6th edition of the UICC/AJCC TNM classification did not categorize LN metastasis according to

Correspondence to: Makoto Sohda, MD, Ph.D., Department of General Surgical Science, Gunma University Graduate School of Medicine, 3-39-22, Showa-machi, Maebashi Gunma, 371-8511, Japan. Tel: +81 272208224, Fax: +81 272208230, e-mail: msohda@med.gunma-u.ac.jp

Key Words: ^{18}F-FAMT, lymph node metastasis, CRT, esophageal cancer.

the number of metastatic LNs (9, 10), the 7th edition incorporated the number of metastatic LNs into its N-categories. We speculated that the LN SUV on PET may be an important predictor of the outcomes of patients with esophageal cancer. We previously investigated whether L-[3-^{18}F]-α-methyltyrosine (18F-FAMT) uptake prior to CRT was a predictor of complete responses to definitive CRT and reported correlations between clinical (complete) responses and the uptake of ^{18}F-FAMT by the primary tumor (11).

In the present study, we evaluated the predictive value of the accumulation of ^{18}F-FDG or ^{18}F-FAMT in LNs prior to definitive CRT for the CRs of patients with esophageal cancer to this treatment.

Materials and Methods

Patients. We evaluated 30 patients with esophageal SCC who were diagnosed as positive for lymph node metastasis by CT. All patients received definitive CRT at the Department of General Surgical Science, Graduate School of Medicine, Gunma University, Japan, between June 2008 and May 2012. All patients with histologically-confirmed primary esophageal SCC were eligible to be included in the present study. Table I shows the clinical characteristics of these patients. Clinical data from a consecutive series of patients were retrospectively reviewed. Patients were excluded from the study if they had any comorbid malignancies or heart disease. Patients were enrolled after providing their written informed consent, while none had received prior treatment. The tumor stage and disease grade were classified according to the 6th edition of the TNM classification of the International Union against Cancer (12). The tumor stage was conventionally determined by the following: CT of the neck, chest and abdomen; endoscopic ultrasound; endoscopy; esophagography; and FDG-PET/CT. Furthermore, none of the patients had diabetes and all blood sugar levels were <120 mg/dl when patients underwent PET scans.

Treatment and clinical outcomes. After the diagnostic procedures, all 30 patients underwent CRT without pre-treatments. All patients were treated with CRT because of the presence of one of the following: invasion to the surrounding tissue, distant organ metastasis, severe organ dysfunction or patient preference (refusal to undergo surgery). External radiotherapy was delivered by a two-field technique using a 10-15 MV photon beam at 2 Gy per fraction/day, 5 fractions/week, to a total of 60-66 Gy. Concurrent chemotherapy consisted of docetaxel (60 mg/m^2) and cisplatin (50 mg/m^2), which were administered intravenously over 1 h on days 1 and 29 and 5-fluorouracil (5-FU; 600 mg/m^2), which was administered as a continuous intravenous infusion on days 1-4 and 29-32.

All patients underwent CT scans of the neck, chest and abdomen with continuous scans with a 5-mm slice thickness being obtained from the neck to the bottom of the liver following an intravenous injection of contrast medium. Lymph nodes were considered positive for metastasis if the short axis was >1 cm. These positive lymph nodes were assigned a specific number to indicate localization according to the guidelines of the JSED (13). CT images were interpreted by two radiologists who were blinded to the results of the PET scans. Treatment evaluations were classified as follows: a complete response (CR: complete disappearance of all clinical evidence of existing lesions beyond 4 weeks) and non-complete response (non-CR: all states, except a complete response, such as a partial response, stable disease and progressive disease).

Table I. *Clinical Characteristics in 30 patients with esophageal squamous cell carcinoma in this study.*

Parameter	No.of cases
Gender	
Male/Female	28/2
Location	
Ce/Ut/Mt/Lt	5/7/16/2
Tumor type	
0/1/2/3/4	1/5/5/17/2
TNM clinical classification	
cT 1/2/3/4	0/2/6/22
cN N0/N1	0/30
cM M0/M1	27/3
cStage I/II/III/IV	0/1/4/25

Ce: Cervix, Ut: upper thoracic, Mt: middle thoracic, Lt: lower thoracic.

PET–CT studies. Both ^{18}F-FAMT and ^{18}F-FDG were produced at our cyclotron facility using the method developed by Tomiyoshi *et al*. (14) and a modified method based on that of Hamacher *et al*. (15). PET/CT studies were performed as described previously (16).

The Institutional Review Board approved the imaging protocols and all patients provided informed consent before partaking in this study. Two experienced nuclear medicine physicians qualitatively evaluated all PET images. Regional lymph nodes, which were evaluated using PET, were assigned specific numbers to indicate localization in accordance with the guidelines of the JSED. To compare CRs with the PET images obtained, we classified lymph node localization according to the anatomic region, *i.e*., cervical, upper thoracic, middle thoracic, lower thoracic and abdominal regions. The faint uptake of both ^{18}F-FAMT and ^{18}F-FDG was defined as a positive result, no visualized uptake was defined as a negative result and SUV was assigned as 0.

Statistical analysis. The relationship between the LN SUVs of ^{18}F-FDG or ^{18}F-FAMT and complete response (CR/non CR) were assessed by an analysis of variance (ANOVA). Probability values of $p<0.05$ indicated a significant difference.

Results

Relationship between the location of LNs in which most of ^{18}F-FDG or ^{18}F-FAMT was accumulated and CR to definitive CRT. We evaluated the relationship between the location of LNs in which the most accumulated ^{18}F-FDG or ^{18}F-FAMT accumulated and CR or non-CR to definitive CRT in patients with esophageal cancer. LNs in which the most accumulated ^{18}F-FDG were located in the cervical area (n=7), followed by the thoracic area (n=15), abdominal area (n=3) and then no accumulation (n=5), while those for ^{18}F-FAMT were 6, 14, 2 and 8, respectively. Figure 1 shows the relationship between

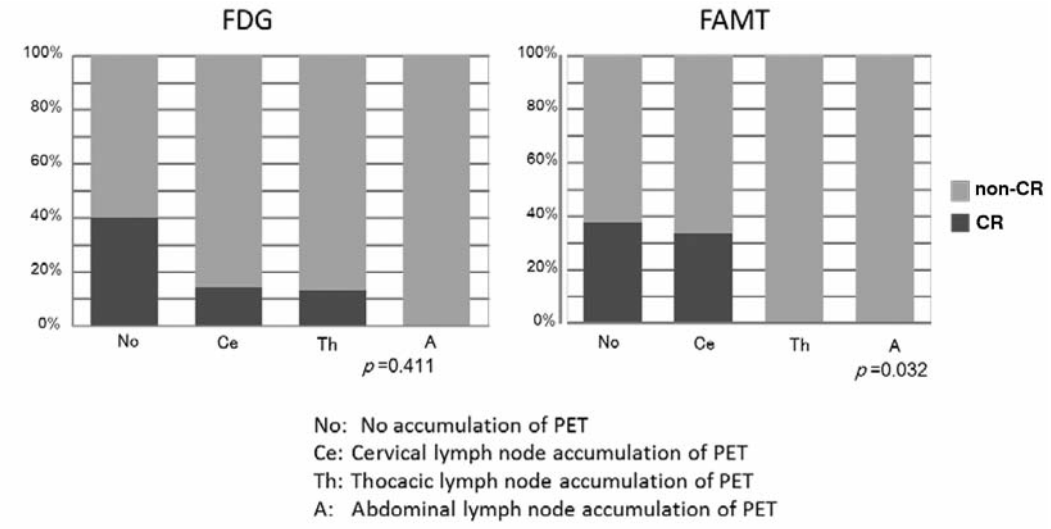

Figure 1. *Relationship between the location of LNs in which most of ^{18}F-FDG or ^{18}F-FAMT is accumulated and complete response (CR) to chemoradiotherapy (CRT). A correlation was observed between LNs in which most of ^{18}F-FAMT was accumulated and the CR rate (p=0.032), but was not for ^{18}F-FDG (p=0.411).*

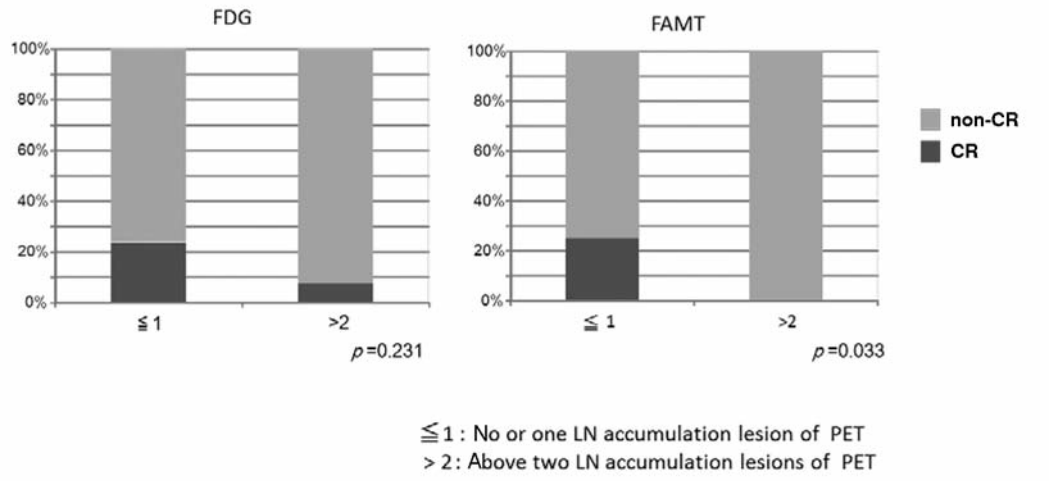

Figure 2. *Relationship between the number of LNs in which ^{18}F-FDG or ^{18}F-FAMT accumulated and complete response (CR) to chemoradiotherapy (CRT). A correlation was observed between the number of LNs in which most of ^{18}F-FAMT was accumulated and the CR rate (p=0.033) but was not for ^{18}F-FDG (p=0.231).*

the location of LNs in which the most ^{18}F-FDG or ^{18}F-FAMT was accumulated and CRs. The "no accumulation" LN group had a slightly higher CR rate to definitive CRT than the other lesion groups. A correlation was observed between LNs in which the most ^{18}F-FAMT was accumulated and the CR rate (p=0.032) but was not for ^{18}F-FDG (p=0.411). Thus, the patients with no ^{18}F-FAMT accumulated in the lymph nodes or ^{18}F-FAMT most markedly accumulated in the cervical lymph nodes showed a significantly higher possibility of achieving CR than those with ^{18}F-FAMT most markedly accumulated in the thoracic or abdominal lymph nodes. In other words, the patients with most markedly accumulated ^{18}F-FAMT in the thoracic or abdominal lymph nodes are unlikely to achieve CR. However, no significant difference was observed among additional partitioned lesions, including upper thoracic, middle thoracic and abdominal thoracic lesions (data not shown). Moreover, Figure 1 shows that the possibility of achieving CR to definitive CRT was reduced if the accumulation ^{18}F-FDG or ^{18}F-FAMT was observed in the abdominal region in patients with esophageal cancer.

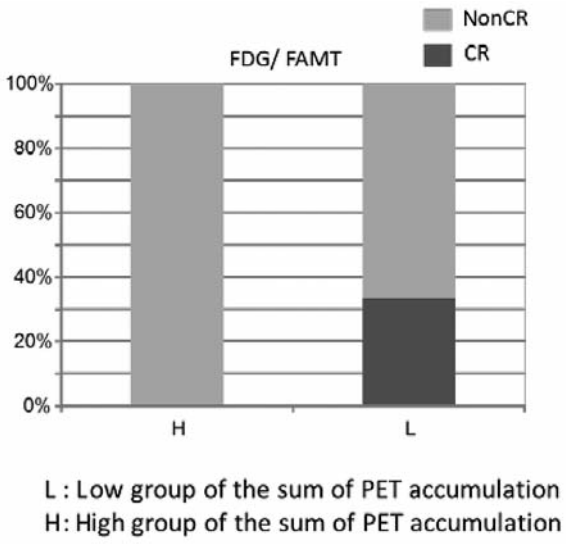

Figure 3. *Relationship between the sum of accumulated ^{18}F-FDG or ^{18}F-FAMT in LNs and complete response (CR) to chemoradiotherapy (CRT). The CR rate was significantly higher in the Low sum group than in the High sum group for both ^{18}F-FDG (p=0.005) and ^{18}F-FAMT (p=0.005).*

Relationship between the number of LNs in which ^{18}F-FDG or ^{18}F-FAMT was accumulated and CR to definitive CRT. We also evaluated the number of LNs in which ^{18}F-FDG or ^{18}F-FAMT was accumulated and CR to CRT. We divided the metastatic lymph node group into two groups based on the guidelines of the JSED (LN accumulation lesion ≤1 and >2). Figure 2 shows the relationship between the number of LNs in which ^{18}F-FDG or ^{18}F-FAMT accumulated and the CR rate to definitive CRT. No correlation was observed between the number of LNs in which ^{18}F-FDG accumulated and the CR rate (p=0.231). On the other hand, for ^{18}F-FAMT, the CR rate was significantly higher in the LN accumulation ≤1 group than in the LN accumulation >2 group (p=0.033). In the present study, for ^{18}F-FAMT, all CR cases belonged to the LN accumulation lesion ≤1 group. These results showed that the number of LNs in which ^{18}F-FAMT accumulated could be used as a predictor of the responses to CRT in patients with esophageal cancer.

Relationship between the sum of accumulated ^{18}F-FDG or ^{18}F-FAMT in LNs and CR to definitive CRT. We evaluated the relationship between the sum of the accumulation of ^{18}F-FDG or ^{18}F-FAMT in LNs and CR to definitive CRT. The median sums of the accumulation of ^{18}F-FDG and ^{18}F-FAMT in LNs were 11.4 (range=0-108.7) and 1.9 (range=0-18.9), respectively. We set the cut-off point at the median (high and low) in this study. The CR rate was significantly higher in the Low-sum group than in the High-sum group for both ^{18}F-FDG (p=0.005) and ^{18}F-FAMT (p=0.005) (Figure 3).

Discussion

The LN status is considered to be one of the most significant predictors of prognosis of patients with esophageal cancer (17). Stiles *et al*. concluded that the number of positive LNs was the best predictive marker of survival in patients undergoing neoadjuvant chemotherapy (18). Moreover, Mariette *et al*. reported that the mean number of dissected LNs and frequency of LN metastasis were significantly lower in patients who underwent preoperative CRT than in those who did not (19). However, the LN status of patients who undergo definitive CRT cannot be sufficiently evaluated because there are no pathological findings in the LNs. In the present study, we evaluated the predictive value of the accumulation of ^{18}F-FDG or ^{18}F-FAMT in LNs prior to definitive CRT for the CR of patients with esophageal cancer to this treatment from several angles. Our results demonstrated that the patients with most markedly accumulated ^{18}F-FAMT in the thoracic or abdominal lymph nodes are unlikely to achieve CR. In other words, if the LN with the highest accumulation of ^{18}F-FAMT was a cervical lesion, the CR rate was significantly higher than that of other lesions. These results demonstrated that radiation therapy can be easily performed with a sufficient radiation dose for the cervical region, including lymph node metastasis.

If ^{18}F-FAMT is accumulated in thoracic or abdominal lesions in esophageal cancer patients prior to definitive CRT, multimodality therapies including salvage surgery is considered necessary. Curative surgery should be selected as the initial treatment for esophageal cancer if the tumor is considered to be resectable. Knowledge of the location of LNs in which the accumulation of ^{18}F-FAMT is the highest prior to definitive CRT is important for deciding an appropriate treatment strategy for patients with esophageal cancer. Our results also showed that, for ^{18}F-FAMT, the CR rate was significantly higher in the LN accumulation lesion ≤1 group than in the LN accumulation lesion >2 group. However, patients in the LN accumulation lesion >2 group did not achieve CR. These results indicate that patients with >2 LNs that accumulated ^{18}F-FAMT should not choose definitive CRT for treatment of esophageal cancer. This is important for avoiding unnecessary treatments, which may cause adverse effects. The sum of the accumulation of ^{18}F-FDG or ^{18}F-FAMT in LNs correlated with responses to definitive CRT in patients with esophageal cancer. These results suggest that patients with advanced cancer, with many lymph node metastases, are unlikely to achieve CR.

In the present study, we evaluated the predictive value of the accumulation of ^{18}F-FDG or ^{18}F-FAMT in LNs prior to definitive CRT for the CR of patients with esophageal cancer to this treatment. Many previous studies using PET-reported correlations between the uptake of ^{18}F-FDG or ^{18}F-FAMT by the primary tumor and responses to CRT. However, PET

imaging studies have not examined the relationship between the LN status and response to CRT. Our LN uptake data for ^{18}F-FDG did not show the valuable result about response of definitive CRT compared with ^{18}F-FAMT PET. We previously reported that the ability of ^{18}F-FDG-PET to detect primary tumors was higher than that of ^{18}F-FAMT-PET (19). On the other hand, we also demonstrated that the specificity of ^{18}F-FAMT-PET for evaluating the LN status was significantly higher than that of ^{18}F-FDG-PET and CT (19). Diagnostic benefits differ between ^{18}F-FDG- and ^{18}F-FAMT-PET. ^{18}F-FAMT-PET is a more effective diagnostic modality than ^{18}F-FDG-PET in predicting the effects of definitive CRT based on their accumulation in the lymph nodes. To predict the responses of patients with esophageal cancer to definitive CRT, it is considered important to evaluate not only primary tumors but also the LN status, including the locations at which ^{18}F-FDG-PET and ^{18}F-FAMT-PET accumulate, as well as the number of LNs in which ^{18}F-FAMT-PET accumulates. However, an important limitation of the present study is that an insufficient number of patients were examined; further clinical research with a larger number of patients is required to confirm our results and demonstrate their reliability.

References

1 Daly JM, Karnell LH and Menck HR: National Cancer Data Base report on esophageal carcinoma. Cancer *78*: 1820-1828, 1996.

2 Wobst A, Audisio RA, Colleoni M and Geraghty JG: Oesophageal cancer treatment: studies, strategies and facts. Ann Oncol *9*: 951-962, 1998.

3 Zhang HL, Chen LQ, Liu RL, Shi YT, He M, Meng XL, Bai SX and Ping YM: The number of lymph node metastases influences survival and International Union Against Cancer tumor-node-metastasis classification for esophageal squamous cell carcinoma. Dis Esophagus *23*: 53-58, 2010.

4 Lin CS, Chang SC, Wei YH, Chou TY, Wu YC, Lin HC, Wang LS and Hsu WH: Prognostic variables in thoracic esophageal squamous cell carcinoma. Ann Thorac Surg 87: 1056-1065, 2009.

5 Burmeister BH, Smithers BM, Gebski V, Fitzgerald L, Simes RJ, Devitt P, Ackland S, Gotley DC, Joseph D, Millar J, North J, Walpole ET and Denham JW: Trans-Tasman Radiation Oncology Group; Australasian Gastro-Intestinal Trials Group. Surgery alone versus chemoradiotherapy followed by surgery for resectable cancer of the oesophagus: a randomised controlled phase III trial. Lancet Oncol *6*: 659-668, 2005.

6 Bedenne L, Michel P, Bouché O, Milan C, Mariette C, Conroy T, Pezet D, Roullet B, Seitz JF, Herr JP, Paillot B, Arveux P, Bonnetain F and Binquet C: Chemoradiation followed by surgery compared with chemoradiation alone in squamous cancer of the esophagus: FFCD 9102. J Clin Oncol *25*: 1160-1168, 2007.

7 Kato H, Miyazaki T, Nakajima M, Takita J, Kimura H, Faried A, Sohda M, Fukai Y, Masuda N, Fukuchi M, Manda R, Ojima H, Tsukada K, Kuwano H, Oriuchi N and Endo K: Comparison between whole-body positron emission tomography and bone scintigraphy in evaluating bony metastases of esophageal carcinomas. Anticancer Res *25*: 4439-4444, 2005.

8 Kato H, Fukuchi M, Miyazaki T, Nakajima M, Tanaka N, Inose T, Kimura H, Faried A, Saito K, Sohda M, Fukai Y, Masuda N, Manda R, Ojima H, Tsukada K, Oriuchi N, Endo K, Nonaka T, Shioya M, Ishikawa H, Sakurai H, Nakano T and Kuwano H: Prediction of response to definitive chemoradiotherapy in esophageal cancer using positron emission tomography. Anticancer Res *27*: 2627-2633, 2007.

9 Yamashita H, Nakagawa K, Tago M, Nakamura N, Shiraishi K and Ohtomo K: Salvage radiotherapy for postoperative loco-regional recurrence of esophageal cancer. Dis Esophagus *18*: 215-220, 2005.

10 Nakamura T, Ota M, Narumiya K, Sato T, Ohki T, Yamamoto M and Mitsuhashi N: Multimodal treatment for lymph node recurrence of esophageal carcinoma after curative resection. Ann Surg Oncol *15*: 2451-2457, 2008.

11 Sohda M, Honjyo H, Hara K, Ozawa D, Suzuki S, Tanaka N, Sano A, Sakai M, Yokobori T, Inose T, Miyazaki T, Ojima H, Higuchi T, Tsushima Y and Kuwano H: L-[3-18F]-α-methyltyrosine accumulation as a definitive chemoradiotherapy response predictor in patients with esophageal cancer. Anticancer Res *34*: 909-913, 2014.

12 Sobin LH and Wittekind C: TNM Classification of Malignant Tumors. 6th ed. New York: John Wiley & Sons. 2002.

13 Japanese Society for Esophageal Disease. (Guidelines for the clinical and pathological studies on carcinoma of the esophagus (10th edition)). Tokyo: Kanehara, 2008.

14 Tomiyoshi K, Amed K, Muhammad S, Higuchi T, Inoue T, Endo K and Yang D: Synthesis of isomers of ^{18}F-labelled amino acid radiopharmaceutical: position 2- and 3-L-^{18}F-alpha-methyltyrosine using a separation and purification system. Nucl Med Commun *18*: 169-175, 1997.

15 Hamacher K, Coenen HH and Stöcklin G: Efficient stereospecific synthesis of no-carrier-added 2-[^{18}F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med *27*: 235-238, 1986.

16 Sohda M, Kato H, Suzuki S, Tanaka N, Sano A, Sakai M, Inose T, Nakajima M, Miyazaki T, Fukuchi M, Oriuchi N, Endo K and Kuwano H: ^{18}F-FAMT-PET is useful for the diagnosis of lymph node metastasis in operable esophageal squamous cell carcinoma. Ann Surg Oncol *17*: 3181-3186, 2010.

17 Akutsu Y and Matsubara H: The significance of lymph node status as a prognostic factor for esophageal cancer. Surg Today *41*: 1190-1195, 2011.

18 Stiles BM, Christos P, Port JL, Lee PC, Paul S, Saunders J and Altorki NK: Predictors of survival in patients with persistent nodal metastases after preoperative chemotherapy for esophageal cancer. J Thorac Cardiovasc Surg *139*: 387-394, 2010.

19 Mariette C, Piessen G, Briez N and Triboulet JP: The number of metastatic lymph nodes and the ratio between metastatic and examined lymph nodes are independent prognostic factors in esophageal cancer regardless of neoadjuvant chemoradiation or lymphadenectomy extent. Ann Surg *247*: 365-371, 2008.

Received August 3, 2014
Revised September 11, 2014
Accepted September 18, 2014