Technology and Health Care 23 (2015) S311–S318
DOI 10.3233/THC-150967
IOS Press

Is the Glut expression related to FDG uptake in PET/CT of non-small cell lung cancer patients?

Woo Hee Choi[a], Ie Ryung Yoo[b,*], Joo Hyun O[b], Tae Jung Kim[c], Kyo Young Lee[d] and Young Kyoon Kim[e]
[a]*Department of Radiology, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea*
[b]*Department of Radiology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea*
[c]*Department of Hospital Pathology, Yeouido St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea*
[d]*Department of Hospital Pathology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea*
[e]*Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea*

Abstract. Though ^{18}F-FDG PET/CT scans are widely used in non-small cell lung cancer (NSCLC), the mechanism of FDG uptake by lung cancer cells has not yet been fully elucidated. This study evaluated the relationship between FDG uptake and the expression of glucose transporters in NSCLC. Sixty-four NSCLC patients who underwent both preoperative ^{18}F-FDG PET/CT scanning and thoracotomy were included. The maximum standardized uptake value (SUVmax) of the primary lung cancer was compared to the immunohistochemistry results for Glut expression and tumor size. In all the NSCLC cases, degree of FDG uptake significantly correlated with both Glut-1 and Glut-3 expression. When stratified by the histology, squamous cell carcinomas showed higher mean SUVmax, Glut-1 expression intensity, and percentage of area positive for Glut-1 expression than adenocarcinomas. Glut-1 and Glut-3 expressions correlated with SUVmax in adenocarcinomas, but there was no significant correlation in squamous cell carcinomas. No significant correlation was observed between tumor size and FDG uptake or Glut expression. These results show that Glut expression was significantly correlated with SUVmax in NSCLC, especially in adenocarcinomas, and that neither FDG uptake nor the expression of Glut was associated with tumor size.

Keywords: Glut-1, Glut-3, glucose transporter, FDG, non-small cell lung cancer

1. Introduction

Enhanced glucose consumption has been reported in cancer cells [1]. Because of this phenomenon, the staging and restaging of various cancers, including lung cancer, can be performed using PET with

*Corresponding author: Ie Ryung Yoo, Department of Radiology, Seoul St. Mary's Hospital, Seocho-gu, Banpo-dong 505, Seoul 137-701, Korea. Tel.: +82 2 2258 1552; Fax: +82 2 2258 1575; E-mail: iryoo@catholic.ac.kr.

^{18}F-FDG, an analog of glucose [2]. In general, changes in the glucose transporter (Glut), hexokinase activity, and glucose-6-phosphatase activity are thought to be associated with enhanced FDG uptake in cancer cells [3,4]. However, the mechanism of FDG uptake within lung cancer has not yet been exactly explained.

Studies in non-small cell lung cancer (NSCLC) have shown an increase in Glut-1 and Glut-3 mRNAs and proteins [5,6]. Several researchers have examined the relationship between Glut-1 or Glut-3 and degree of FDG uptake, but these results were contradictory [6–10].

This study aimed to investigate the relationship between the expression of Gluts (Glut-1 and Glut-3) and FDG uptake in NSCLC.

2. Materials and methods

2.1. Patients

Sixty-four NSCLC patients who performed preoperative ^{18}F-FDG PET/CT scans from 2003 to 2007 and subsequent thoracotomy were enrolled. Fifty-seven patients had undergone an operation with curative intent, and seven patients were referred for diagnosis or accurate staging. All patients had an operation within 4 weeks following the PET/CT scan. Patients who received chemotherapy or radiotherapy before the PET/CT scan were excluded. The histological type and tumor size were recorded for each primary tumor. The tumor size was evaluated from surgically resected specimens.

The ethical committee of our institution approved this study.

2.2. ^{18}F-FDG PET/CT

After 6 h fasting, 370–555 MBq FDG was injected intravenously. Blood glucose levels before the FDG injection did not exceed 130 mg/dL in all patients. All PET/CT scans were performed using a hybrid PET/CT scanner (Biograph DUO; Siemens, Knoxville, TN). Scans were performed an hour after FDG injection. The CT scan was acquired in the craniocaudal direction from the orbitomeatal line through the upper thigh at 130 kV and 30 mAs, immediately followed by PET imaging. The axial spatial resolution was 6.5 mm.

The maximum standardized uptake values (SUVmax) were computed by normalizing the measured tumor radioactivity to the injected doses and body weights of the patients.

2.3. Immunohistochemical staining

The 5-μm thick paraffin sections were dewaxed in xylene and ethanol. After antigen retrieval, the slides were incubated with 3% hydrogen peroxide in methanol solution for 10 min. The primary antibodies were diluted in Dako Antibody Diluent (Dako, Carpinteria, CA, USA) and incubated at room temperature. The immunoreaction was developed with diaminobenzidine (DAB; Dako), after which all slides were counterstaining with hematoxylin.

The staining intensity for Glut-1 and Glut-3 was categorized as none (0), weak (1), moderate (2), or intense (3). The immunoreactivity score was computed as the staining intensity grade multiplied by percentage of positive cells.

Table 1
Tumor characteristics

Histologic subtype	n	Size (cm)	SUVmax
Squamous cell carcinoma	26	3.6 (1.2∼7.0)	9.4 (1.7∼15.4)
Adenocarcinoma	35	3.8 (1.0∼11.0)	6.1 (1.2∼23.8)
Large cell carcinoma	2	5.0, 7.0	8.1, 10.1
Pleomoprhic carcinoma	1	3.8	12.9

2.4. Statistical analysis

To compare expression of Glut-1 and Glut-3 in each patient, a Wilcoxon signed rank test was used. An independent t-test and Mann–Whitney U test were used to compare the two different histology groups. Spearman's rho correlations were applied to analyze the correlation between SUVmax, Glut-1 and Glut-3 expression, and tumor size.

3. Results

3.1. Tumor characteristics

Thirty-five out of 64 patients (54.7%) were diagnosed with adenocarcinomas, and 26 patients (40.6%) were diagnosed with squamous cell carcinoma (SCC). The tumor characteristics are summarized in Table 1.

3.2. Expression of Glut

Fifty-three out of 64 tumors (83%) had positive immunostaining for Glut-1 and 61 tumors (97%) were positive for Glut-3. Three cases did not stained for both Glut-1 and Glut-3, and these were all adenocarcinoma cases with SUVmax ranging from 2.1 to 2.8. No significant differences were noted for the staining intensity of Glut-1 and Glut-3 in individual patients ($p = 0.109$). However, the percent of tumors expressing Glut-3 was significantly higher than those expressing Glut-1 ($p = 0.014$).

NSCLC patients were stratified by their histological diagnosis (adenocarcinoma or SCC). Compared to adenocarcinoma, the expression of Glut-1 was significantly higher in SCC than in adenocarcinoma for staining intensity and the percentage of positive cells ($p < 0.001$). No significant difference was found for the Glut-3 expression between SCC and adenocarcinoma ($p = 0.300$ for intensity and $p = 0.358$ for percentage of the area with positive staining).

There was modest but statistically significant association between Glut-1 and Glut-3 for both grade of intensity ($r = 0.374$, $p = 0.002$) and the percentage of area with positive staining ($r = 0.311$, $p = 0.012$) when all NSCLC cases were included. Glut-1 correlated with Glut-3 for both intensity and the percentage of positive area in adenocarcinoma, but not in SCC (Table 2).

3.3. FDG uptake

The mean SUVmax of the primary tumor was 7.6 ± 4.4 (range, 1.2–23.8). Two large cell carcinomas and one pleomorphic carcinoma case showed intense FDG uptake and had an SUVmax above 8.0. SCCs had a significantly higher mean SUVmax than adenocarcinoma (9.4 ± 4.1 vs. 6.1 ± 4.2, $p = 0.003$) (Fig. 1).

Table 2
Correlation between expression of Glut-1 and Glult-3 in NSCLC patients of all histology types, adenocarcinoma, and squamous cell carcinoma

	All NSCLC ($n = 64$)		Squamous cell carcinoma ($n = 35$)		Adenocarcinoma ($n = 26$)	
	r	p value	r	p value	r	p value
Intensity	0.374	0.002	0.044	0.830	0.534	0.001
% of positive cell	0.311	0.012	−0.135	0.511	0.502	0.002

r = correlation coefficient.

Table 3
Correlation between SUVmax and Glut expression, and tumor size in NSCLS patients of all histology types, adenocarcinoma, and squamous cell carcinoma

	All NSCLC ($n = 64$)		Squamous cell carcinoma ($n = 35$)		Adenocarcinoma ($n = 26$)	
	r	p value	r	p value	r	p value
Glut-1 intensity	0.594*	< 0.001	0.307	0.127	0.632*	< 0.001
Glut-1 percent	0.506*	< 0.001	0.163	0.427	0.433*	0.009
Glut-1 score	0.533*	< 0.001	0.215	0.291	0.512*	0.002
Glut-3 intensity	0.408*	0.001	0.128	0.533	0.591*	< 0.001
Glut-3 percent	0.267*	0.033	−0.122	0.554	0.528*	0.001
Glut-3 score	0.342*	0.006	0.015	0.941	0.614*	< 0.001
Summed score	0.536*	< 0.001	0.220	0.280	0.635*	< 0.001
Tumor size	0.128	0.312	0.247	0.223	0.086	0.623

r = correlation coefficient.

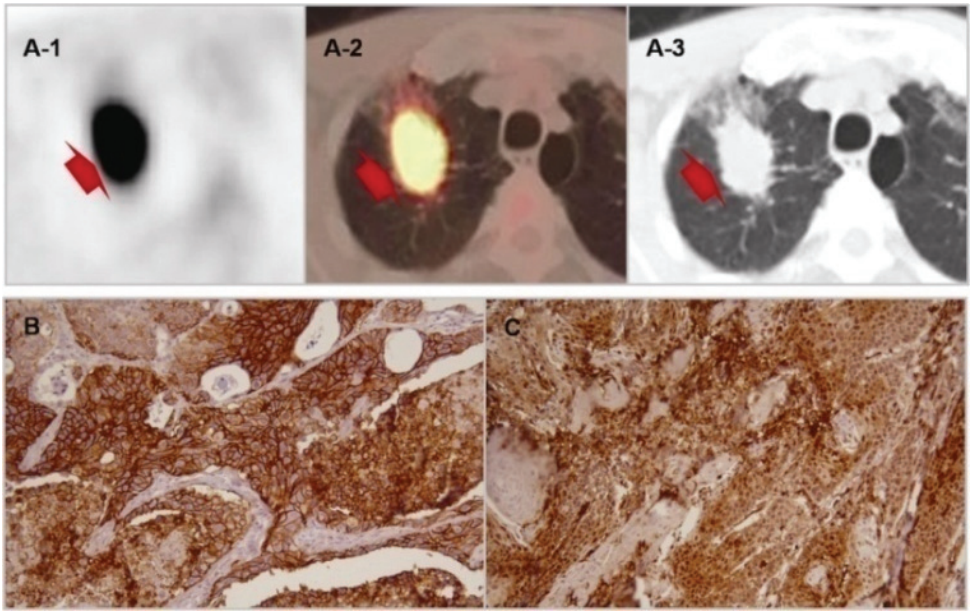

Fig. 1. PET/CT images (A) demonstrate high FDG uptake in right upper lobe mass, which is confirmed as squamous cell carcinoma by right upper lobe lobectomy. Immunohistochemical staining illustrates overexpression of Glut-1 (B, X100) and Glut-3 (C, X100).

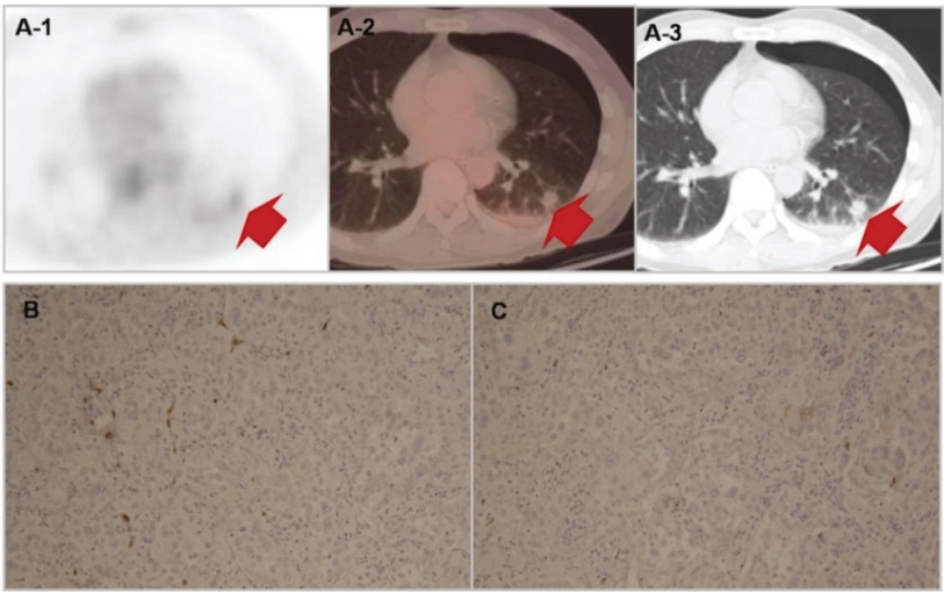

Fig. 2. On the PET (A-1) and fusion views (A-2), faint focal FDG uptake is noted in left lower lobe (SUVmax: 1.8). CT image (A-3) shows about 1.2 cm sized nodule with spiculate margin. This lesion is confirmed as adenocarcinoma (stage IA). Among the cancer cells, 2% were Glut-1 positive (B, X100), and 10% were Glut-3 positive (C, X100). Staging intensity was zero for both Glut-1 and Glut-3.

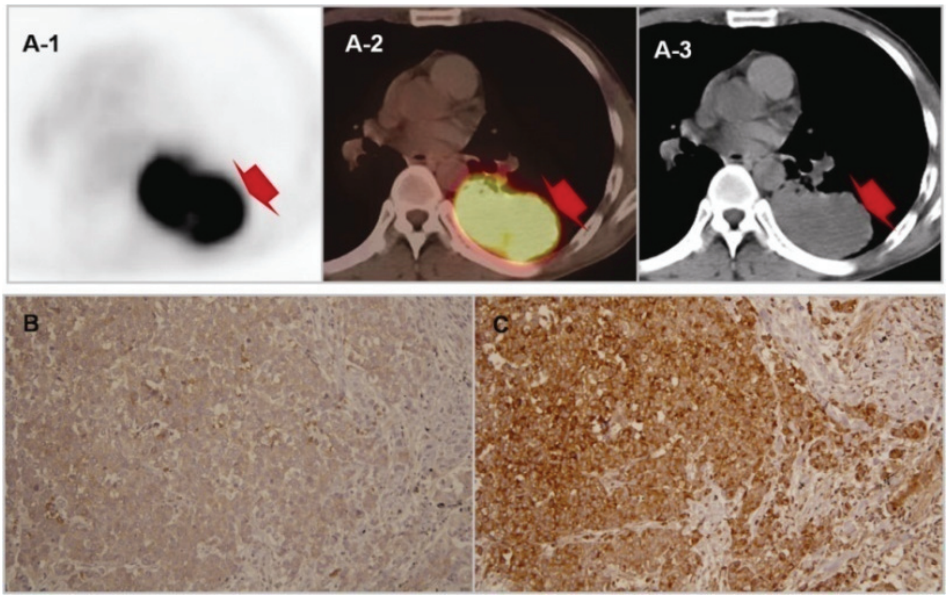

Fig. 3. PET (A-1) and fusion (A-2) images demonstrate intense localized FDG uptake in left lower lobe (SUVmax: 9.1). In CT image (A-3), 7.2 cm sized lobulated mass is seen. By operation, this case is confirmed as adenocarcinoma (stage IIIB). In this patient, 80% of cancer cells were positive for Glut-1 and staging intensity was 1 (B, original magnification X100). 90% of cancer cells were positive for Gout-3 and staining intensity was 3 (C, original magnification X100).

3.4. FDG uptake and expression of Glut

The degree of FDG uptake was significantly correlated with both staining intensity and the percentage of positive area for Glut-1, as well as Glut-3 (Figs 2 and 3). Table 3 shows correlation coefficients of SUVmax and Glut expression with their respective p values.

3.5. Relationship among FDG uptake, expression of Glut and tumor size

The SUVmax of the primary tumors were not significantly correlated with tumor size. No significant correlation was observed between expression of Glut and tumor size (Table 3).

4. Discussion

Malignant cells show accelerated glucose utilization, which is thought to be mediated by an enhanced glucose uptake rate, increased hexokinase enzyme activity, and underexpression of glucose-6-phosphatase [1,3,4]. The glucose uptake into the cancer cell is mediated by glucose transporters. Among the 14 isoforms of the mammalian glucose transporter family, Glut-1 and Glut-3 overexpression have been reported in NSCLC [5,6].

In our study, the percentage of Glut-3 expressing tumors was higher than for Glut-1. There were 11 tumors negative for Glut-1, and only three tumors were negative for Glut-3 expression. All but one that examined the expression of Glut in NSCLC found Glut-1 expression to be significantly greater than that of other Gluts [5,8,11–13], but the percentages of positively stained areas varied widely. Suzawa et al. [6] showed a higher percentage of expression for Glut-3 than for Glut-1, but there was a strong correlation in the expression between Glut-1 and Glut-3. Discrepancies in the results of the previous studies may be explained by differences in the antibodies and detection systems, and the different characteristics of each study population. Immunohistochemistry is not an absolutely quantitative method, and staining intensity can easily differ because of slight variations in the methods used at each institution.

This study demonstrated that SUVmax correlated with expression of both Glut-1 and Glut-3. Data from other studies comparing FDG uptake and the expression of Glut-1 have demonstrated conflicting results. Higashi et al. [7] reported that FDG uptake was significantly correlated with the Glut-1 protein levels in NSCLC, while Brown et al. [11] and Marom et al. [8] did not find a significant correlation between the degree of FDG uptake and Glut-1 expression.

FDG uptake and expression of Gluts differed based on the histology of the tumor. Higher SUVmax and Glut-1 expression were observed in SCC than in adenocarcinomas in this study, supporting the results reported by Brown et al. and Eschmann et al. [11,14]. While positive correlation was seen in adenocarcinomas, FDG uptake had no statistically significant correlation with Glut-1 expression in SCC. This implies that Glut-1 is not the only one of the important factors influencing glucose accumulation, and other factors such as hexokinase enzyme activity, glucose-6-phosphatase expression, cell proliferation rates, and tumor cell density may play a more important role in the FDG uptake mechanism for SCC.

In contrast to the study reported by Suzawa et al. [6], which suggested that SUVmax with partial volume colrrection correlated better with size than with Glut expression, the degree of Glut expression and FDG uptake were not significantly correlated with tumor size. Though the partial volume correction for SUVmax was not applied, only two cases had a tumor size (1.0 and 1.2 cm) smaller than twice the axial spatial resolution of our PET/CT. Therefore, the partial volume effect is not expected to significantly

affect the result. Further research will be needed to determine the relationship among the FDG uptake, expression of Glut, and tumor size.

This study has some limitations. First, both membranous and intracellular Glut expression were scored. Gluts are glycoproteins that deliver glucose into cells through the cell membrane. Therefore, only Gluts positioned in the cell surface can contribute to FDG entry into cells. There was no differentiation made between membranous Glut expression and biologically inactive intracellular Glut expression; consequently, Glut expression may have been overestimated.

Second, Glut expression in inflammatory cells was not considered. Some studies reported that human leukocytes express Glut-1 or Glut-3 [11,15]. In an *in-vitro* experiment, activation of lymphocytes or monocytes affects dramatically Glut-1 and Glut-3 expression [15]. Accumulation of FDG at site of infection or in inflammatory diseases is often noted [16]. In cases with concomitant inflammation in the area surrounding the lung cancer, Glut expression by inflammatory cells might have affected the result.

Last, this study investigated the expression of only Gluts. The expression of hexokinase, well known to be an important enzyme in regulating glucose metabolism in cancer cells, was not measured. A study has suggested that Glut-1 is the rate-limiting step in FDG accumulation within malignant cells, including primary lung cancer [17]. However, Mamede et al. [18] proposed that hexokinase also plays an important role together with Glut in cases of NSCLC. The findings of this study implied that other factors besides Glut may play a key role in the mechanism for FDG accumulation, at least in SCC. Further evaluation is needed that takes into consideration the other steps in glucose metabolism that could contribute to FDG uptake.

References

[1] G. Weber, Enzymology of cancer cells (second of two parts), New England Journal of Medicine, The (1977), 541-51.

[2] J. Oh, Y. Chung, Y. Kim, H. Jeon, S. Kim, Y. Park, and S. Chung, Value of F-18 FDG PET/CT in detection and prognostication of isolated extra-axillary lymph node recurrences in postoperative breast cancer, Bio-Medical Materials and Engineering (2014), 1173-84.

[3] N. Katz, E. Eigenbrodt, I. Ruschenburg, and G. Fischer, Decrease in glucokinase and glucose-6-phosphatase and increase in hexokinase in putative preneoplastic lesions of rat liver, Journal of Cancer Research (1987), 430-6.

[4] H. Imura, T. Manabe, K. Inoue, Y. Yamada, N. Inagaki, H. Yano, G. Koh, H. Fukumoto, Y. Seino, and T. Yamamoto, Over-expression of facilitative glucose transporter genes in human cancer, Biochemical and Biophysical Research Communications (1990), 223-30.

[5] S. Koide, H. Inoue, and J. Ogawa, Glucose-transporter-type-I-gene amplification correlates with sialyl-Lewis-X synthesis and proliferation in lung cancer, International Journal of Cancer (1997), 189-92.

[6] N. Suzawa, M. Ito, S. Qiao, K. Uchida, M. Takao, T. Yamada, K. Takeda, and S. Murashima, Assessment of factors influencing FDG uptake in non-small cell lung cancer on PET/CT by investigating histological differences in expression of glucose transporters 1 and 3 and tumour size, Lung Cancer (2011), 191-8.

[7] K. Higashi, Y. Ueda, A. Sakurai, X.M. Wang, L. Xu, M. Murakami, H. Seki, M. Oguchi, S. Taki, Y. Nambu, H. Tonami, S. Katsuda, and I. Yamamoto, Correlation of Glut-1 glucose transporter expression with, Eur J Nucl Med (2000), 1778-85.

[8] E.M. Marom, T.A. Aloia, M.B. Moore, M. Hara, J.E. Herndon, 2nd, D.H. Harpole, Jr., P.C. Goodman, and E.F. Patz, Jr., Correlation of FDG-PET imaging with Glut-1 and Glut-3 expression in early-stage non-small cell lung cancer, Lung Cancer (2001), 99-107.

[9] L.F. de Geus-Oei, J.H. van Krieken, R.P. Aliredjo, P.F. Krabbe, C. Frielink, A.F. Verhagen, O.C. Boerman, and W.J. Oyen, Biological correlates of FDG uptake in non-small cell lung cancer, Lung Cancer (2007), 79-87.

[10] M.D. Taylor, P.W. Smith, W.K. Brix, M.R. Wick, N. Theodosakis, B.R. Swenson, B.D. Kozower, C.L. Lau, and D.R. Jones, Fluorodeoxyglucose positron emission tomography and tumor marker expression in non-small cell lung cancer, Journal of thoracic and cardiovascular surgery (2009), 43-8.

[11] R.S. Brown, J.Y. Leung, P.V. Kison, K.R. Zasadny, A. Flint, and R.L. Wahl, Glucose transporters and FDG uptake in untreated primary human non-small cell lung cancer, J Nucl Med (1999), 556-65.

[12] M. Younes, R.W. Brown, M. Stephenson, M. Gondo, and P.T. Cagle, Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival, Cancer (1997), 1046-51.

[13] A. van Baardwijk, C. Dooms, R.J. van Suylen, E. Verbeken, M. Hochstenbag, C. Dehing-Oberije, D. Rupa, S. Pastorekova, S. Stroobants, U. Buell, P. Lambin, J. Vansteenkiste, and D. De Ruysscher, The maximum uptake of (18)F-deoxyglucose on positron emission tomography scan correlates with survival, hypoxia inducible factor-1alpha and GLUT-1 in non-small cell lung cancer, Eur J Cancer (2007), 1392-8.

[14] S.M. Eschmann, G. Friedel, F. Paulsen, M. Reimold, T. Hehr, W. Budach, H.J. Langen, and R. Bares, 18F-FDG PET for assessment of therapy response and preoperative re-evaluation after neoadjuvant radio-chemotherapy in stage III non-small cell lung cancer, Eur J Nucl Med Mol Imaging (2007), 463-71.

[15] Y. Fu, L. Maianu, B. Melbert, and W.T. Garvey, Facilitative glucose transporter gene expression in human lymphocytes, monocytes, and macrophages: a role for GLUT isoforms 1, 3, and 5 in the immune response and foam cell formation, Blood Cells, Molecules, & Diseases (2004), 182-90.

[16] J. Paquet, F. Maskali, S. Poussier, J. Scala Bertola, A. Pinzano, L. Grossin, P. Netter, P. Olivier, and P. Gillet, Evaluation of a rat knee mono-arthritis using microPET, Bio-Medical Materials and Engineering (2010), 195-202.

[17] H. Sasaki, M. Shitara, K. Yokota, Y. Hikosaka, S. Moriyama, M. Yano, and Y. Fujii, Overexpression of GLUT1 correlates with Kras mutations in lung carcinomas, Molecular Medicine Reports (2012), 599-602.

[18] M. Mamede, T. Higashi, M. Kitaichi, K. Ishizu, T. Ishimori, Y. Nakamoto, K. Yanagihara, M. Li, F. Tanaka, H. Wada, T. Manabe, and T. Saga, [18F]FDG uptake and PCNA, Glut-1, and Hexokinase-II expressions in cancers and inflammatory lesions of the lung, Neoplasia (2005), 369-79.