Imaging Proliferation in Lung Tumors with PET: 18F-FLT Versus 18F-FDG

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Recently, the thymidine analog 3'-deoxy-3'-18F-fluorothymidine (FLT) was suggested for imaging tumoral proliferation. In this prospective study, we examined whether 18F-FLT better determines proliferative activity in newly diagnosed lung nodules than does 18F-FDG. **Methods:** Twenty-six patients with pulmonary nodules on chest CT were examined with PET and the tracers 18F-FDG and 18F-FLT. Tumoral uptake was determined by calculation of standardized uptake value (SUV). Within 2 wk, patients underwent resective surgery or had core biopsy. Proliferative activity was estimated by counting nuclei stained with the Ki-67–specific monoclonal antibody MIB-1 per total number of nuclei in representative tissue specimens. The correlation between the percentage of proliferating cells and the SUVs for 18F-FLT and 18F-FDG was determined using linear regression analysis. **Results:** Eighteen patients had malignant tumors (13 with non–small cell lung cancer [NSCLC], 1 with small cell lung cancer, and 4 with pulmonary metastases from extrapulmonary tumors); 8 had benign lesions. In all visible lesions, mean 18F-FDG uptake was 4.1 (median, 4.4; SD, 3.0; range, 1.0–10.6), and mean 18F-FLT uptake was 1.8 (median, 1.2; SD, 2.0; range, 0.8–6.4). Statistical analysis revealed a significantly higher uptake of 18F-FDG than of 18F-FLT (Mann–Whitney U test, P < 0.05). 18F-FDG SUV correlated better with proliferation index (P < 0.0001; r = 0.92) than did 18F-FLT SUV (P < 0.001; r = 0.59). With the exception of 1 carcinoma in situ, all malignant tumors showed increased 18F-FDG PET uptake. 18F-FLT PET was false-negative in the carcinoma in situ, in another NSCLC with a low proliferation index, and in a patient with lung metastases from colorectal cancer. Increased 18F-FLT uptake was related exclusively to malignant tumors. By contrast, 18F-FDG PET was false-positive in 4 of 8 patients with benign lesions. **Conclusion:** 18F-FLT uptake correlates better with proliferation of lung tumors than does uptake of 18F-FDG and might be more useful as a selective biomarker for tumor proliferation. **Key Words:** 18F-FLT; 18F-FDG; Ki-67; proliferation; lung cancer

**P**ET using the glucose analog 18F-FDG enables noninvasive tissue characterization based on metabolic differences between benign and malignant tumors. Several studies have found 18F-FDG PET to have a high sensitivity for staging lung cancer (1–3). However, 18F-FDG uptake is not tumor specific, and false-positive findings can occur in inflammatory lesions (4). Therefore, many efforts have been made to develop more selective tracers. In contrast to 18F-FDG uptake values, proliferative activity as measured by Ki-67 immunostaining has been shown to be a specific sign of malignant tumors (5). Furthermore, immunohistochemical studies using various biomarkers for proliferation showed significantly decreased survival in patients with highly proliferating tumors (6). In clinical studies, 18F-FDG uptake correlated with proliferative activity (7,8) and survival in non–small cell lung cancer (NSCLC) (9,10).

11C-Thymidine was the first radiotracer for noninvasive imaging of tumor proliferation (11). The short half-life of 11C and rapid metabolism of 11C-thymidine in vivo make the radiotracer less suitable for routine use. Hence, the thymidine analog 3'-deoxy-3'-18F-fluorothymidine (FLT) was recently introduced as a stable proliferation marker with a suitable nuclide half-life (12). 18F-FLT is phosphorylated to 3'-fluorothymidine monophosphate by thymidine kinase 1 and reflects thymidine kinase 1 activity in A549 lung cancer cells (13). In a first clinical study, our group demonstrated proliferation-dependent 18F-FLT uptake in NSCLC (14).

We devised a prospective study to evaluate whether PET with the novel tracer 18F-FLT better determines tumoral proliferation and better differentiates benign from malignant lung tumors than does PET with 18F-FDG.

**MATERIALS AND METHODS**

**Patients**

This prospective study included 26 patients (17 men, 9 women) with a mean age of 62 ± 9.9 y (range, 37–77 y; Table 1). PET with both tracers, 18F-FDG and 18F-FLT, was planned for 30 consecutive patients. Four patients had to be excluded from the study because only 18F-FDG or 18F-FLT PET was performed. Patients were selected when pulmonary nodules on CT scans strongly
TABLE 1
Patient Characteristics, Tumoral Tracer Uptake, and Proliferation Fraction (Ki-67 Index)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Histopathology finding</th>
<th>TNM</th>
<th>SUV of 18F-FLT</th>
<th>SUV of 18F-FDG</th>
<th>Ki-67 index (%)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>57</td>
<td>F</td>
<td>Non-small cell lung cancer</td>
<td>T1 N1 M0</td>
<td>5.6</td>
<td>6.3</td>
<td>11.3</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>M</td>
<td>Non-small cell lung cancer</td>
<td>T2 N1 M0</td>
<td>4.0</td>
<td>5.6</td>
<td>10.6</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>F</td>
<td>Non-small cell lung cancer</td>
<td>T2 N1 M0</td>
<td>4.0</td>
<td>5.3</td>
<td>10.6</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>F</td>
<td>Non-small cell lung cancer</td>
<td>T2 N1 M0</td>
<td>2.9</td>
<td>4.6</td>
<td>4.13</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>M</td>
<td>Non-small cell lung cancer</td>
<td>T2b N0 M0</td>
<td>3.1</td>
<td>5.7</td>
<td>6.5</td>
</tr>
<tr>
<td>6</td>
<td>53</td>
<td>F</td>
<td>Non-small cell lung cancer</td>
<td>T2 N1 M0</td>
<td>2.9</td>
<td>5.7</td>
<td>6.5</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>M</td>
<td>Non-small cell lung cancer</td>
<td>T3 N0 M0</td>
<td>4.9</td>
<td>6.8</td>
<td>5.1</td>
</tr>
<tr>
<td>8</td>
<td>76</td>
<td>M</td>
<td>Non-small cell lung cancer</td>
<td>T4 N2 M0</td>
<td>3.1</td>
<td>5.2</td>
<td>7.9</td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>M</td>
<td>Non-small cell lung cancer</td>
<td>T2 N2 MX</td>
<td>3.9</td>
<td>5.6</td>
<td>6.8</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>F</td>
<td>Non-small cell lung cancer</td>
<td>TX N0 M0</td>
<td>2.3</td>
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<td>T1 N1 M0</td>
<td>1.1</td>
<td>1.3</td>
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<tr>
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<td>Neg</td>
<td>Neg</td>
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<td>15</td>
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</tr>
<tr>
<td>16</td>
<td>51</td>
<td>M</td>
<td>Met from renal cell carcinoma</td>
<td>rTX N0 M1</td>
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<td>3.4</td>
<td>6.7</td>
</tr>
<tr>
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<td>65</td>
<td>M</td>
<td>Met from renal cell carcinoma</td>
<td>rTX N0 M1</td>
<td>1.3</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>18</td>
<td>37</td>
<td>F</td>
<td>Met from osteosarcoma</td>
<td>TX N0 M1</td>
<td>0.8</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>19</td>
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<td>M</td>
<td>Bronchiolitis</td>
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<td>Neg</td>
<td>6.9</td>
</tr>
<tr>
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<td>76</td>
<td>M</td>
<td>2 × 3 cm nodule, benign lesion indicated by clinical course</td>
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<td>Neg</td>
<td>3.0</td>
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<tr>
<td>21</td>
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<td>F</td>
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<td>Neg</td>
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</tr>
<tr>
<td>22</td>
<td>59</td>
<td>M</td>
<td>Bronchiolitis</td>
<td>—</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>23</td>
<td>69</td>
<td>M</td>
<td>Bronchiolitis</td>
<td>—</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>24</td>
<td>67</td>
<td>M</td>
<td>1 × 2 cm nodule, benign lesion indicated by clinical course</td>
<td>—</td>
<td>Neg</td>
<td>Neg</td>
<td>2.2</td>
</tr>
<tr>
<td>25</td>
<td>55</td>
<td>F</td>
<td>Fibroma</td>
<td>—</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>26</td>
<td>56</td>
<td>M</td>
<td>Chondroma</td>
<td>—</td>
<td>Neg</td>
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</tr>
</tbody>
</table>

Neg = negative; met = metastasis.

suggested a malignant tumor. Sixteen patients underwent resective surgery up to 14 d after 18F-FLT and 18F-FDG PET. In the other 10 patients, core-biopsy specimens were used for histopathologic evaluation. All patients gave written consent to participate in this study, which was approved by the local ethical committee.

Eighteen patients had malignant tumors. Histopathologic examination revealed NSCLC in 13 patients; small cell lung cancer in 1 patient; and pulmonary metastases from colorectal cancer, renal cell carcinoma, or osteosarcoma in 4 patients. Eight patients had benign tumors (1 case of bronchopulmonary chondroma; 3 of bronchiolitis; 1 of tuberculosis; 1 of focal fibrosis; and 2 of undefined tumors, for which malignancy was excluded by the clinical course).

Immunostaining and Morphometric Analysis

The detailed protocol for immunostaining was published elsewhere (5). Briefly, formalin-fixed and paraffin-embedded sections (5 µm) of resected specimens and biopsy samples were dewaxed, rehydrated, and microwaved in 0.01 mol/L citrate buffer for 30 min. For immunostaining, the monoclonal murine antibody MIB-1 (Dianova), specific for human nuclear antigen Ki-67, was used in a 1:500 dilution. Sections were lightly counterstained with hematoxylin. As a positive control for proliferating cells, sections of human lymph node tissue were used. The primary antibody was omitted on sections used as negative controls. Histopathologic slides were examined by a pathologist who was unaware of the patients’ clinical data.

An area with high cellularity was chosen for the evaluation of MIB-1 immunostaining. All epithelial cells with nuclear staining of any intensity were defined as positive. Proliferative activity was described as the percentage of MIB-1–stained nuclei per total number of nuclei in the sample. With light microscopy, 600 nuclei per slide and 3 slides per case were evaluated for Ki-67 expression to minimize tissue-sampling error. Representative images of each slide were transferred to the computer frame by a video camera using the computer-assisted imaging system OPTIMAS 6.2 (Media Cybernetics, Inc.).

18F-FLT Synthesis and PET Imaging

In accord with the method of Machulla et al. (15), benzoyl-protected anhydrothymidine was used for 18F-FLT synthesis. Radiosynthesis was performed in a PET tracer synthesizer from nuclear interface. After nuclophilic introduction of 18F-fluoride accompanied by an anhydro-ring opening, the benzylated intermediate was cleaved using 1% NaOH solution. 18F-FLT was purified via preparative high-performance liquid chromatography.
Corresponding 18F-FDG PET and CT scans show high 18F-FDG uptake and ribs represent proliferating bone marrow. (B and C) Corresponding 18F-FDG PET and CT scans show high 18F-FDG uptake in tumor margin and primary lung tumor. (D) On Ki-67 immunohistochemistry, Ki-67-positive nuclei (brown) demonstrate high proliferation rate of 54%, and hematoxylin background staining reveals Ki-67-negative nuclei (blue).

18F-FLT and 18F-FDG PET examinations were performed on consecutive days within 2 wk before resective surgery or core biopsy. PET was performed using a high-resolution full-ring scanner (ECAT EXACT or ECAT HR+; Siemens/CTI), which produces 47 or 63 contiguous slices per bed position. Axial field of view is 15.5 cm per bed position. Five bed positions were measured for each patient, covering a total field of view of 77.5 cm. The emission scan included the thorax and abdomen for all patients. Patients fasted for at least 6 h before undergoing PET. Static emission scans were obtained 45 min after injection of 265–370 MBq of 18F-FLT (mean, 334 MBq) or 345–550 MBq of 18F-FDG (mean, 391 MBq). The acquisition time was 10 min per bed position. Four-minute transmission scans with a 68Ge/68Ga ring source were obtained for attenuation correction after tracer application. Images were reconstructed using an iterative reconstruction algorithm described by Schmidlin (16).

All images were evaluated by 2 experienced nuclear medicine physicians. For calculation of standardized uptake value (SUV), circular regions of interest were drawn containing the area with focally increased pulmonary 18F-FLT and 18F-FDG uptake (lesional diameter at spiral CT, 4–48 mm).

Data Analysis
Data are presented as mean, median, range, and SD. The amount of Ki-67-positive cells and the SUVs for 18F-FDG and 18F-FLT were compared using linear regression analysis. Differences were considered statistically significant at \( P < 0.05 \). 18F-FDG and 18F-FLT uptakes were compared using the Mann–Whitney U test.

RESULTS

18F-FDG PET
All malignant lesions except 1 carcinoma in situ (NSCLC, patient 13) showed focally increased and easily detectable 18F-FDG uptake (Table 1). The mean 18F-FDG SUV in all visible lesions was 4.1 (median, 4.4; SD, 3.0; range, 1.0–10.6). The mean maximum 18F-FDG uptake was 6.9 (median, 7.0; SD, 5.8; range, 1.4–22.7).

The mean 18F-FDG SUV in the 13 patients with NSCLC was 5.6 (median, 5.5; SD, 2.6; range, 1.0–10.6; Fig. 1), and the mean maximum 18F-FDG SUV was 9.7 (median, 10.1; SD, 5.5; range, 1.4–22.7). Four of the 8 patients with benign lesions presented with focal 18F-FDG uptake. The reviewers visually interpreted 2 of 8 nodules as malignant. Histopathologic examination revealed unifocal tuberculoma in one patient (patient 21; mean 18F-FDG SUV, 1.1; maximum 18F-FDG SUV, 1.8; Fig. 2) and focal bronchiolitis in another patient (patient 19; mean 18F-FDG SUV, 6.9; maximum 18F-FDG SUV, 10.3). Inflammatory lesions were suspected in the other 2 patients. Tissue sampling was not performed because clinical follow-up at 3 mo indicated benign lesions (a 1 × 2 cm nodule disappeared on CT performed at the 3-mo follow-up examination, and a 2 × 3 cm nodule decreased to 1 × 1 cm). Mean 18F-FDG SUVs in these lesions were 2.2 and 3.0, respectively, and maximum 18F-FDG SUVs were 2.9 and 4.3, respectively.

18F-FLT PET
The mean 18F-FLT SUV in all visible lesions was 1.8 (median, 1.2; SD, 2.0; range, 0.8–6.4; Table 1), and the mean maximum 18F-FLT SUV was 2.7 (median, 1.6; SD, 3.1; range, 1.3–10.4). Mean 18F-FLT SUV in NSCLC was 3.2 (median, 3.1; SD, 2.0; range, 0.8–6.4), and the mean maximum 18F-FLT SUV was 4.7 (median, 5.2; SD, 3.1; range, 1.0–10.4). Increased 18F-FLT uptake within a nodule was identified in 11 of 13 patients with histologically confirmed NSCLC (Fig. 1). Patient 6, with highly differentiated...
NSCLC and a low proliferation fraction, and patient 13, with a carcinoma in situ, had no visible 18F-FLT uptake.

In pulmonary metastases, the mean 18F-FLT SUV was 1.1 (median, 1.3; SD, 0.8; range, 0.8–2.1), and the mean maximum 18F-FLT SUV was 1.6 (median, 1.9; SD, 1.3; range, 1.0–3.4). In the 1 patient with pulmonary metastases from colorectal cancer (patient 15), the metastases showed no 18F-FLT uptake (Fig. 3). Another patient, with small cell lung cancer (patient 14), showed weak but easily detectable 18F-FLT uptake (mean 18F-FLT SUV, 1.7). No benign tumors showed focal 18F-FLT uptake. Hence, SUV was not determined for these tumors.

In all pulmonary lesions, mean and maximum 18F-FLT uptake was lower than the respective 18F-FDG uptake. Mean 18F-FLT SUV was significantly lower than the respective 18F-FDG SUV (Mann–Whitney U test, P < 0.05). The mean maximum SUVs of 18F-FDG were also significantly higher (P < 0.0001).

**Ki-67 Immunohistochemistry**

Regional lymph nodes serving as a positive control showed an intense nuclear staining with Ki-67 antibody. In control sections, for which the primary antibody was omitted, no positive nuclear staining was visible.

All malignant tissue specimens contained Ki-67–positive cells. Stained nuclei belonged mainly to epithelial cells, and a very small portion belonged to inflammatory cells. Ki-67 positivity ranged from 1% to 70% of sampled epithelial nucleus profiles (median, 35%). The mean fraction of Ki-67–positive nuclei was 33% (SD, 6.5%). In 6 cases, more than 40% of nuclei showed immunoreactivity for Ki-67 antigen. In NSCLC, the mean proliferation fraction was 37.8% (median, 40%; SD, 19.1%; range, 10%–70%). In pulmonary metastases, the mean proliferative fraction was lower (11.5%; median, 11%; SD, 9%; range, 1%–23%).

Ki-67–positive cells were present in only 1 specimen with benign disease (patient 21, with tuberculoma; Ki-67 index, 5%). Seven benign tissue specimens showed no immunoreactivity to Ki-67 antigen. The range for Ki-67–positive cells was 0%–5%. Ki-67–positive nuclei belonged mainly to inflammatory cells rather than to epithelial cells. The mean of Ki-67–positive cells in benign lesions was 1% (SD, 1.4).

In all lung tumors, linear regression analysis indicated a highly significant correlation between 18F-FLT SUV and
Ki-67 index ($P < 0.0001; r = 0.92$; Fig. 4). Between Ki-67 and $^{18}$F-FDG SUV, statistical analysis also revealed a significant correlation ($P < 0.001; r = 0.44$) but a weak correlation coefficient ($r = 0.59$).

**DISCUSSION**

This is the first clinical study comparing the correlation between $^{18}$F-FDG uptake and proliferation rate and the correlation between $^{18}$F-FLT and proliferation rate for unclear lung lesions. Compared with conventional imaging modalities, $^{18}$F-FDG PET has been reported to offer the highest sensitivity for staging lung cancer (17,18). In agreement with these findings, $^{18}$F-FLT uptake was increased in all malignant tumors except 1 carcinoma in situ (in patient 13) in our series.

Despite high sensitivity, false-positive findings can occur with $^{18}$F-FDG PET, especially in inflammatory lesions (4). Concordantly, focal $^{18}$F-FDG uptake was present in 4 of our study patients with inflammatory or other benign lesions (1 case of bronchiolitis, 1 of tuberculosis, and 2 of undefined benign lung tumors). The relatively high number of false-positive findings in the present series is related to patient selection. Other studies with more patients found speciﬁcities averaging 78% for $^{18}$F-FDG PET in detecting lung cancer (3). Recently, unspeciﬁc $^{18}$F-FLT uptake has been reported in inflammatory cells such as macrophages (19). Furthermore, many other factors have been reported to influence $^{18}$F-FDG uptake, such as upregulation of glucose transporter 1 receptors (20,21), number of viable tumor cells (22), microvessel density, or hexokinase expression (23). In pancreatic cancer, we previously demonstrated that proliferation was a speciﬁc enzyme responsible for intracellular trapping of $^{18}$F-FLT (18). However, the detailed uptake mechanism is still unknown, and the inﬂuence of other factors, such as expression of nucleoside transporters, remains to be determined.

For patients with pulmonary nodules, our data show a highly signiﬁcant correlation between tumoral $^{18}$F-FLT uptake and proliferative activity as indicated by Ki-67 immunostaining. The correlation coefﬁcient was 0.92 ($r^2 = 0.85$). In contrast to the lower correlation coefﬁcient observed for $^{18}$F-FDG, 85% of tracer uptake can be explained by proliferative activity. In agreement with this ﬁnding, no $^{18}$F-FLT uptake was visible in nonproliferating tumors. $^{18}$F-FLT PET may therefore be used for the differentiation of benign from malignant lung tumors.

However, 2 patients with NSCLC (1 case of carcinoma in situ and 1 of large cell carcinoma with low proliferative activity), and another patient with pulmonary metastases from colorectal cancer with a proliferation rate of 12%, showed no $^{18}$F-FLT uptake but clear uptake of $^{18}$F-FDG. Compared with $^{18}$F-FDG, $^{18}$F-FLT seems less sensitive for staging disease in patients with malignant lung tumors. Further studies with larger patient populations are needed to determine the diagnostic accuracy of $^{18}$F-FLT PET in detecting malignant tumors.

Several studies have reported that $^{18}$F-FDG PET can be used to assess therapeutic response in various tumors (29–33). A first in vitro study demonstrated that $^{18}$F-FLT uptake in esophageal cancer cells was modiﬁed early after incubation with various cytotoxic drugs (34). Hence, $^{18}$F-FLT may be an alternative for therapeutic monitoring. However, for evaluation of $^{18}$F-FLT as a marker for therapy response, large clinical trials are needed.

**CONCLUSION**

$^{18}$F-FLT correlates signiﬁcantly better with the proliferative activity of lung tumors than does $^{18}$F-FDG. $^{18}$F-FLT may therefore be the superior PET tracer for assessment of therapy response and outcome. Because of 3 false-negative ﬁndings in our preliminary study, $^{18}$F-FLT PET may be less adequate than $^{18}$F-FDG for primary staging in patients with known lung cancer but may be more accurate for differentiation of unclear lung lesions.

**REFERENCES**


