The feasibility of 18F-3'fluoro-3'-deoxy-L-thymidine PET (FLT PET) for detecting laryngeal cancer was investigated and compared with 18F-FDG PET. Methods: Eleven patients diagnosed with or strongly suspected of having recurrent laryngeal cancer and 10 patients with histologically proven primary laryngeal cancer underwent attenuation-corrected 18F-FLT PET imaging 60 min after injection of a median of 213 MBq (range, 175–400 MBq) 18F-FLT and attenuation-corrected 18F-FDG PET imaging 90 min after injection of a median of 340 MBq (range, 165–650 MBq) 18F-FDG. All patients were staged by endoscopy and CT according to the Union Internationale Contre la Cancer TNM staging system. All patients underwent biopsy of the laryngeal area after imaging. Lesions seen on 18F-FDG PET and 18F-FLT PET were compared with histopathologic results. Mean SUVs, maximum SUVs, and tumor-to-nontumor (TNT) ratios were calculated for 18F-FLT and 18F-FDG. Wilcoxon nonparametric testing was used for comparison of 18F-FLT with 18F-FDG PET. The Spearman correlation coefficient was used to correlate mean SUVs, maximum SUVs, and TNT ratios of 18F-FDG PET and 18F-FLT PET. Two-tailed P values < 0.05 were considered significant. Results: 18F-FDG PET and 18F-FLT PET detected laryngeal cancer correctly in 15 of 17 patients. One lesion judged as positive on 18F-FDG PET turned out to be normal tissue. Of 2 lesions judged as positive on 18F-FLT PET, 1 turned out to be inflammation and the other to be normal tissue. Maximum SUVs were 3.3 (range, 1.9–8.5) for 18F-FDG and 1.6 (range, 1.0–5.7) for 18F-FLT (P < 0.001). Mean SUVs were 2.7 (range, 1.5–6.5) for 18F-FDG and 1.2 (range, 0.8–3.8) for 18F-FLT (P < 0.001). TNT was 1.9 (range, 1.3–4.7) for 18F-FDG and 1.5 (range, 1.1–3.5) for 18F-FLT (P < 0.05). Conclusion: The numbers of laryngeal cancers detected with 18F-FLT PET and 18F-FDG PET were equal. In laryngeal cancer, the uptake of 18F-FDG is higher than that of 18F-FLT.

Key Words: 18F-FLT; 18F-FDG; recurrent laryngeal cancer; primary laryngeal cancer; PET

phorylated by the enzyme thymidine kinase 1 (TK₁), which leads to intracellular trapping (17). During DNA synthesis, TK₁ activity increases almost tenfold and is thus an accurate reflection of cellular proliferation (19). ¹⁸F-FLT uptake is probably related to TK₁ activity and, therefore, also related to proliferation. The aim of this study was to investigate the feasibility of ¹⁸F-FLT PET in comparison with ¹⁸F-FDG PET for detection of primary and recurrent laryngeal cancer. A second aim was to investigate possible differences in uptake of ¹⁸F-FLT and ¹⁸F-FDG in malignant and normal tissue.

MATERIALS AND METHODS

Patients

This prospective study included 21 consecutive patients: 11 with suspected recurrent laryngeal cancer and 10 with primary laryngeal cancer. Informed consent was obtained. Patients were enrolled from September 2001 through March 2003.

The patients with suspected recurrent laryngeal cancer had been treated initially with radiation therapy for laryngeal squamous cell carcinoma. Recurrent disease was suspected in these patients, because increased hoarseness, edema, pain in the larynx, or referred pain developed. All patients with suspected recurrent laryngeal cancer underwent diagnostic laryngoscopy for biopsy. The patients with primary laryngeal squamous cell carcinoma were candidates for radiotherapy. The disease of all patients had been clinically staged according to the Union Internationale Contre la Cancer TNM classification (20).

All patients underwent physical examination of the head and neck, chest radiography, endoscopic examination under general anesthesia, biopsies of suspected areas, and spiral CT of the neck. For inclusion, liver and kidney functions and hematologic parameters (hemoglobin, hematocrit, erythrocytes, thrombocytes, leucocytes, and white cell count) had to be within normal limits. Pregnant patients and patients with psychiatric disorders were excluded. All screened patients were included in the study. The medical ethics committee of the Groningen University Hospital approved the study protocol.

Tracer Synthesis

Synthesis of ¹⁸F-FLT was performed according to the method of Machulla et al. (21). ¹⁸F-FLT was produced by ¹⁸F fluorination of the 4,4'-dimethoxytrityl-protected anhydrothymidine, followed by a deprotection step. After purification by reversed-phase high-performance liquid chromatography, the product was made isotonic and passed through a 0.22-μm filter. ¹⁸F-FLT was produced with a radiochemical purity of >95% and specific activity of >10 TBq/mmol. The radiochemical yield was 7.5% ± 5.1% (at end of bombardment).

Synthesis of ¹⁸F-FDG was performed according to the method of Hamacher et al. (22) by an automated synthesis module. The radiochemical yield was 65.9% ± 7.1% (at end of bombardment).

PET Scanning

All studies were performed using an ECAT EXACT HR+ (Siemens/CTI Inc.). Before PET imaging, patients were instructed to fast for at least 6 h. They were also instructed to drink 1 L of water before imaging to stimulate ¹⁸F-FLT and ¹⁸F-FDG excretion from the renal calyces and subsequent voiding.

For injection of the radiopharmaceuticals, a venous cannula was inserted in each patient’s forearm. The interval between the ¹⁸F-FDG PET and ¹⁸F-FLT PET imaging was a maximum of 16 d. All patients were scanned in 4 positions from the nose down in emission-transmission-emission-transmission–emission mode, with 3 and 5 min per bed position for transmission and emission scanning, respectively. Twenty-one patients (20 men, 1 woman) with a median age of 65 y (range, 50–91 y) were included in this study (Table 1). Patients received a median of 340 MBq (range, 165–650 MBq) ¹⁸F-FDG and a median of 213 MBq (range, 175–400 MBq) ¹⁸F-FLT. Patients were scanned 60 min after ¹⁸F-FLT injection and 90 min after ¹⁸F-FDG injection. Previous experience with ¹⁸F-FLT indicated that tumor-to-nontumor (TNT) ratios were constant from 60 min after injection of the tracer. PET images were iteratively reconstructed (ordered-subset expectation maximization) (23).

Pathologic Evaluation

Biopsies of the laryngeal area and surgical specimens of total laryngectomies were examined on hematoxylin-and-eosin–stained sections. The pathologist was unaware of the results of the PET images. In patients in whom a total laryngectomy was performed, the maximum tumor diameter was obtained (Table 1). In the remaining patients only biopsies were performed, and these were used for histologic investigation.

Data Analysis

¹⁸F-FLT PET and ¹⁸F-FDG PET images were analyzed for uptake in malignant lesions and normal anatomic structures. A physician experienced with PET evaluated the images aware only of the location of the primary or suspected lesion and unaware of other clinical information.

¹⁸F-FLT PET and ¹⁸F-FDG PET images were first interpreted visually. The presence of a hypermetabolic lesion was judged as positive, and the absence of a hypermetabolic lesion was judged as negative. After visual interpretation, both the ¹⁸F-FLT PET and ¹⁸F-FDG PET images were analyzed randomly for hypo- and hypermetabolic lesions and sagittal sections. The slice with the highest uptake was selected for region-of-interest (ROI) analysis. After selecting the plane with the maximum SUV, an ROI was drawn manually. ROIs were placed at the 70% contour of the maximal SUV in the tumor when possible. In other cases, ROIs were drawn manually. The same ROI method was applied on the same slice in normal laryngeal tissue above or below the tumor site to calculate the background SUV. The mean SUV of the tumor was divided by the mean SUV of the background region to produce the TNT ratio. The TNT ratios and SUVs of ¹⁸F-FLT PET and ¹⁸F-FDG PET were compared. Images were displayed on a SUN workstation (Sun Microsystems). ROI calculation was performed using Clinical Applications Programming Package version 5 (CTI, Inc.).

Statistical Analysis

The results of the visually interpreted PET images were compared with the histologic data, which were used as standard. The Wilcoxon signed rank test was used to compare the mean SUVs, maximum SUVs, and TNT ratios of ¹⁸F-FDG PET and ¹⁸F-FLT PET. The Spearman correlation coefficient was used to correlate the mean SUVs, maximum SUVs, and TNTs of ¹⁸F-FDG PET and ¹⁸F-FLT PET. Two-tailed *P* values < 0.05 were considered significant.
RESULTS

Patients

Fifteen patients underwent biopsy of the laryngeal area after PET scans in the Groningen University Hospital. However, patients 7, 8, 11, 12, 15, and 20 underwent a biopsy in a referring hospital. These biopsies were performed at 7 mo and 11, 27, 42, and 21 d, respectively, before the PET scans.

Visualization of Laryngeal Cancer

In patients 2, 11, 14, and 19, no uptake of 18 F-FLT or 18 F-FDG could be observed, and, therefore, no SUV could be calculated. Histopathologic investigation showed no malignancy in patients 2 and 11. However, in patients 14 and 19 histopathologic investigation showed 2 recurrent laryngeal cancers. 18 F-FDG PET and 18 F-FLT PET detected 15 of 17 laryngeal cancers. One lesion (in patient 10) judged as positive on 18 F-FDG PET was found to be normal tissue. 18 F-FLT PET and 18 F-FDG PET missed 2 T1 tumors in patients 14 and 19. Two lesions (in patients 3 and 10) judged as positive on 18 F-FLT PET were found to be inflammation in one patient and normal tissue in the other. Figure 1 shows an 18 F-FDG image and an 18 F-FLT PET image of a patient with primary laryngeal cancer.

Comparison Between 18F-FDG and 18F-FLT Uptake

The uptake of 18 F-FDG was significantly higher than that of 18 F-FLT, whether expressed in mean SUV, maximum SUV, or TNT ratio (Table 2). The correlations between mean SUVs, maximum SUVs, and TNT ratios of 18 F-FDG and 18 F-FLT varied between 0.613 and 0.705 and were significant (P < 0.05).

DISCUSSION

This study was conducted on 21 patients and showed that both 18 F-FDG PET and 18 F-FLT PET could detect 15 of 17 laryngeal cancers. In 4 patients, lesions suggestive of laryngeal cancer turned out to be normal or inflammatory laryngeal tissue. 18 F-FDG PET detected 1 of these lesions as positive, and 18 F-FLT PET detected 2 of these lesions as positive. The uptake of 18 F-FDG (mean SUV of 2.7 [range,
1.5–6.5]) in laryngeal cancer was significantly higher than that of 18F-FLT (mean SUV of 1.2 [range, 0.8–3.8]).

Although few data are available, the limited 18F-FDG PET data on sensitivity in this study are in the same range as 18F-FDG PET data in the literature, where sensitivity for the detection of laryngeal cancer ranged from 80% to 97% (9,11,12,16,24,25) and specificity ranged from 61% to 82% (10,11,16). The specificity obtained in this study for 18F-FDG PET (100%, 3 of 3) is less reliable and not comparable with the literature because of the low number of false-positive and true-negative lesions.

False-positive results with 18F-FDG PET do occur, because 18F-FDG is also trapped in normal tissue. Secretion of these amino acids that image protein-related metabolites in tumor tissue (28). L-[1-11C]-tyrosine (TYR) also has been used to detect tumors and determine protein metabolism. In laryngeal and hypopharyngeal cancer, TYR was able to detect all primary tumors, with a correlation between protein synthesis rate and SUV (29–31). However, the disadvantage of this tracer is the low tumor uptake and accumulation in salivary glands, which could impair detection of metastatic lymph nodes in head and neck cancer (28,29). In 1998, Shields et al. (17) developed 18F-FLT, a pyrimidine analog that indirectly measures DNA synthesis through the DNA salvage pathway. Recent articles about 18F-FLT PET focused on the detection of colorectal and lung cancer and their metastases (32,33). A correlation was found between SUV and proliferation in single pulmonary nodules (32,34,35).

Despite the promising properties of 18F-FLT, 18F-FLT PET barely detected a recurrent laryngeal tumor (in patient 3) that displayed avid uptake of 18F-FDG. The tumor was large (1.8 mm), poorly differentiated, and surrounded with ulcers. This could explain the low 18F-FLT uptake and avid 18F-FDG uptake in the surrounding inflammation. 18F-FLT PET and 18F-FDG PET missed 2 T1 tumors that were detected on CT. The size of these tumors could be below the detection level of PET, and a partial-volume effect could be involved. In the literature, 18F-FDG PET has been reported to miss T1–T4 laryngeal tumors as well. However, no histologic explanation is given for these false-negative findings (11,24,25). Unfortunately, it is impossible to accurately quantify or assess the size of a primary laryngeal cancer. This is because diagnosis and extent of the tumor are obtained by a laryngoscopically guided biopsy and CT, because almost all T1–T3 tumors are treated with radiation therapy. The size of the biopsy obtained from the tumor is often too small to obtain reliable Ki-67 data for correlation with 18F-FLT uptake. In contrast, it is possible to obtain reliable Ki-67 data in patients with T4 tumors, who undergo total laryngectomy and are not palliatively treated with radiation therapy. In these patients, the tumor can be fully examined histologically. However, a T4 tumor often causes acute respiratory distress leading to urgent laryngectomy, making a PET scan unethical and impossible.

### TABLE 2

<table>
<thead>
<tr>
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<th>Max SUV* (range)</th>
<th>Mean SUV* (range)</th>
<th>TNT (range)</th>
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<tbody>
<tr>
<td>18F-FDG PET</td>
<td>3.3 (1.9–8.5)*</td>
<td>2.7 (1.5–6.5)†</td>
<td>1.9 (1.3–4.7)‡</td>
</tr>
<tr>
<td>18F-FLT PET</td>
<td>1.6 (1.0–5.7)</td>
<td>1.2 (0.8–3.8)</td>
<td>1.5 (1.1–3.5)</td>
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*SUV expressed as median (min–max).

†P < 0.001.

‡P < 0.05.
Malignant tissue displayed a significantly higher uptake of \(^{18}\)F-FDG than \(^{18}\)F-FLT. The mean \(^{18}\)F-FDG SUV reported in the literature for patients with primary or recurrent laryngeal cancer ranges from 4.6 to 10.7 \((9,12,13)\). These values are higher than the maximum \(^{18}\)F-FDG SUV of 3.3 \((\text{range}, 1.9–8.5)\) and mean \(^{18}\)F-FDG SUV 2.7 \((\text{range}, 1.5–6.5)\) in this study. No literature is available on \(^{18}\)F-FLT PET for detecting laryngeal cancer. However, the SUVs of \(^{18}\)F-FDG PET and \(^{18}\)F-FLT PET in patients with colorectal or lung cancer have been compared in the literature \((32,33)\). The mean \(^{18}\)F-FLT PET SUV in colorectal cancer was 4.2, compared with a mean \(^{18}\)F-FDG PET SUV of 8.7. The \(^{18}\)F-FLT SUV in lung cancer ranged from 1.2 to 3.1, and the \(^{18}\)F-FDG PET SUV ranged from 1.7 to 8.9. Although these articles discuss different types of malignancies, they also reported significantly lower uptake of \(^{18}\)F-FLT than \(^{18}\)F-FDG, just as was found for the detection of laryngeal cancer in this study \((32,33)\). The lower level of uptake probably raises the detection limit.

The correlation between \(^{18}\)F-FDG and \(^{18}\)F-FLT uptake was not strong. The SUV is the net result of several uptake processes. The availability of the tracer in the blood pool, nonspecific uptake, perfusion of the tumor, membrane transport, metabolic processes in which the tracer is involved, and (ir)reversible trapping of the tracer flow over the membrane are factors that can influence the SUV. It is our impression that both \(^{18}\)F-FLT and \(^{18}\)F-FDG are taken up by malignant cells but that the demand for glucose is larger than that for thymidine. The phosphorylation rate in vitro is about 30\% of the phosphorylation rate of serum thymidine by TK\(_1\), which could be an explanation for a low \(^{18}\)F-FLT uptake in the tumor \((36,37)\).

The number of patients in this study was too small to draw conclusions about the accuracy of \(^{18}\)F-FDG PET and \(^{18}\)F-FLT PET for detection of laryngeal cancer in general and for detection of primary or recurrent laryngeal cancer specifically. Larger groups of patients are needed with at least 2 observers to calculate the interobserver agreement to more accurately reflect clinical reality.

Future roles for \(^{18}\)F-FDG PET and \(^{18}\)F-FLT PET in laryngeal cancer are only partly clear. The role for \(^{18}\)F-FDG PET in detecting laryngeal cancer seems to be in differentiating between tumor and nontumor tissue in patients with suspected recurrent laryngeal cancer \((1.9–11,13)\). It is reasonable to delay biopsy on a negative \(^{18}\)F-FDG PET assessment and prevent unnecessary biopsies that could initiate or aggravate radionecrosis \((1.10,11)\). Imaging with PET lacks anatomic detail, but this could be overcome by the use of PET/CT. Too few data are available for the detection of primary laryngeal cancer untreated by radiation therapy \((9,24,25)\). Further research is needed to investigate whether \(^{18}\)F-FDG PET is an adequate tracer for the detection of primary laryngeal cancer. Because of the low uptake of \(^{18}\)F-FLT, \(^{18}\)F-FLT PET seems less adequate for the detection of laryngeal cancer \((11)\).

The numbers of laryngeal cancers detected with \(^{18}\)F-FLT PET and \(^{18}\)F-FDG PET were equal. The uptake of \(^{18}\)F-FDG in laryngeal cancer was higher than that of \(^{18}\)F-FLT.

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