Positron Emission Tomography Using Fluorine-18-Fluorodeoxyglucose in Malignant Lymphoma: A Comparison with Proliferative Activity

Junichi Okada, Kyosan Yoshikawa, Makiko Itami, Keiko Imaseki, Kimiichi Uno, Jun Itami, Junpei Kuyama, Atsuo Mikata, and Noboru Arimizu

Departments of Radiology and Pathology, University of Chiba School of Medicine, 1-8-1 Inohana, Chiba City, Chiba, Japan

To evaluate the relationship between PET using ^{18}F-fluorodeoxyglucose (FDG) and pathological findings and to compare indices obtained by FDG-PET, FDG-PET was performed in 23 patients with untreated malignant lymphoma. Three indices obtained by FDG-PET, tumor-to-normal contrast ratio (TCR), distribution absorption ratio (DAR), k1k2/(k2 + k3), correlated with proliferative activity which was pathologically estimated both by mitotic count and by proportion of cells in all phases of the cell cycle. The relationship did not significantly change according to which of the three indices was chosen. FDG-PET, which shows the proliferative activity of tumors, is considered to be a useful method for managing tumors.

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Positron emission tomography (PET) using ^{18}F-fluorodeoxyglucose (FDG) has been used to diagnose various kinds of malignant tumors. Good correlation between glucose metabolism measured by PET using FDG (FDG-PET) and grade of malignancy has been reported in brain, hepatic and musculoskeletal tumors (*1–4*). We compared pathological diagnosis, tumor size, ^{67}Ga scintigraphy, and clinical course in malignant lymphoma with FDG-PET. FDG-PET showed promise as a useful method for managing tumor and predicting prognosis (*5*). FDG-PET seems useful in accessing the biological behavior of malignant tumor. The relationship between glucose metabolism measured by FDG-PET and pathological findings, including proliferative activity, has not been studied closely. Minn et al. showed a clear correlation between the proliferative activity estimated by DNA flow cytometry and the uptake of FDG in thirteen patients with malignant head and neck tumors (*6*). However, the number of patients were low and the tumors consisted of several kinds of malignant tumors.

The number of mitoses counted in sections stained with hematoxylin and eosin has been considered one of indices of proliferative activity for a long time. However, mitotic counts are usually limited. Variations in cell cycle time and problems with the identification of mitotic figures can affect the counts (*7,8*). A monoclonal antibody, Ki-67, reacts with a nuclear antigen present in cells in G1, S, G2 and M phases of cell cycle but not G0 (*8,9*). The number of Ki-67 positive cells has been shown as a useful marker in determining a tumor proliferative index and has been helpful in assessing prognosis in malignant tumors including malignant lymphoma and breast cancer (*11–14*).

Some indices obtained by FDG-PET have been used to evaluate malignant tumors, but the question of which index is reasonable is not answered. In most studies, the accumulation of FDG in the tumor has been used as an index. However, using sequential (dynamic) scans and arterial blood sampling, an index more closely connected with glucose metabolism can be obtained.

We used three indices obtained by FDG-PET and compared them with proliferative activity estimated by mitotic counts and Ki-67 staining in biopsy specimens in patients with untreated malignant lymphoma in the head and neck region.

PATIENTS AND METHODS

We studied 23 patients, from 35 to 85 yr old, with untreated malignant lymphoma in the head and neck region (Table 1). Patients were diagnosed in Working Formulation for clinical usages and divided into low-, intermediate-, and high-grade malignancies (*15*) by fresh biopsies. After FDG-PET and the measurement of proliferative activity, the patients were treated by chemotherapy and radiotherapy, and observed at least 12 mo after treatment. In one patient with low-grade non-Hodgkin's lymphoma (NHL), disease progression was not observed within 12 mo after treatment. In 16 patients with intermediate-grade NHL and in one patient with Hodgkin's disease, complete clinical

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For reprints contact: Junichi Okada, MD, Department of Radiology, University of Chiba School of Medicine, 1-8-1 Inohana, Chiba City, Chiba, 280 Japan.

TABLE 1
Patient Characteristics

Case	Age/Sex	Diagnosis*	Grading	Staging (Ann Arbor)
1	56/M	NHL, small, lympho	Low	IV
2	59/F	NHL, follic, large	Intermediate	II
3	60/M	NHL, diffuse, small	Intermediate	IV
4	69/M	NHL, diffuse, small	Intermediate	I
5	54/M	NHL, diffuse, mixed	Intermediate	II
6	56/F	NHL, diffuse, mixed	Intermediate	I
7	77/F	NHL, diffuse, mixed	Intermediate	II
8	63/F	NHL, diffuse, mixed	Intermediate	II
9	51/M	NHL, diffuse, mixed	Intermediate	I
10	50/M	NHL, diffuse, large	Intermediate	II
11	43/F	NHL, diffuse, large	Intermediate	II
12	66/M	NHL, diffuse, large	Intermediate	II
13	85/M	NHL, diffuse, large	Intermediate	II
14	84/M	NHL, diffuse, large	Intermediate	III
15	42/M	NHL, diffuse, large	Intermediate	III
16	77/M	NHL, diffuse, large	Intermediate	II
17	84/M	NHL, diffuse, large	Intermediate	I
18	63/M	NHL, diffuse, large	Intermediate	I
19	78/M	NHL, diffuse, large	Intermediate	IV
20	60/M	NHL, diffuse, large	Intermediate	I
21	71/M	NHL, diffuse, large	Intermediate	I
22	49/M	NHL, immunoblastic	High	IV
23	35/M	Hodgkin		III

* Diagnosis classified by the Working Formulation.
Small lympho = small lymphocytic; follic large = follicular large cell; diffuse, small = diffuse small-cleaved cell; diffuse mixed = diffuse, mixed small and large cell; diffuse, large = diffuse large cell; and immunoblastic = diffuse, immunoblastic cell.

remission was obtained with therapy and no evidence of tumor recurrences or metastases was observed. In three patients with intermediate-grade NHL, complete remission was not obtained, and death occurred within 4 mo of treatment. One patient with high-grade NHL died as a result of disseminated intravascular coagulation during treatment. In another patient with high-grade NHL, remission was obtained but relapses followed outside the cervical region.

Mitotic Counts

In 22 patients, a section stained with hematoxylin and eosin was observed in a higher power field, ×400, and the number of mitoses was counted in ten fields chosen at random.

Ki-67 Immunoreactivity

Four-micrometer thick cryostat sections were mounted on slides pretreated with 5% bioden meschcement (Oken, Tokyo, Japan)/toluene. After being air-dried, the sections were fixed for 20 min in periodate-lysine paraformaldehyde (PLP) fixation (*16*) and washed twice for 4 min in phosphate-buffered saline (PBS), pH 7.2. Immunohistochemical stainings were performed according to the avidine-biotin-peroxidase complex (ABC) method (*17*). The sections were blocked with normal goat serum for 30 min at room temperature. After removal of excess normal serum, the tissue sections were incubated at room temperature with Ki-67 for 30 min. After being washed three times in PBS, the tissue sections were incubated with biotinylated antimouse immunoglobulin (Jackson Immunoresearch Laboratories, Inc.) as a second antibody for 30 min at room temperature. After washing, they were incubated with strept ABC solution for 30 min at room temperature. Finally, peroxidase activity was demonstrated using diaminobenzidine solution. The evaluation of the immunoreactivity of Ki-67 was expressed as a Ki-67 labeling index, a percentage of positively stained cells per total cells in the high power fields in 19 patients.

PET

In all patients, about 4 mCi (148 MBq) of FDG synthesized by CYPRIS and CUPID, a cyclotron system made by Sumitomo Heavy Industry, was injected intravenously in a fasting state. Following injection, a series of 2-min sequential scans were acquired for 60 min by a Shimazu-SET130W (HEADTOME III) PET scanner (*18*), whose spatial resolution was 1.04 cm FWHM. This machine can acquire three slices simultaneously with a slice thickness of 1.65 cm. Moreover, each patient was positioned to obtain PET images on a plane to which his tumor was shown at maximum size on x-ray CT. In 22 patients, arterial blood samples were also withdrawn to monitor the plasma radioactivity. Tumor-to-normal soft-tissue contrast ratios (TCR) were measured by setting a 10 × 10-mm square region of interest (ROI). A tumor ROI was set in the area where FDG activity was highest in the tumor. A normal soft-tissue ROI was set in soft tissue consisting mainly of paravertebral musculature around a vertebral bone.

Using the tumor activity of FDG, the distribution absorption ratio of FDG-to-tumor (DAR) (tumor activity concentration/(injected dose/body volume)) was calculated. We assumed that the specific gravity of tumor and body was 1, and DAR was derived from the following equation (*19,20*):

$$DAR = \text{tumor activity}/(\text{injected dose}/\text{body weight}).$$

Using the sequential scan, blood data and graphic method demonstrated by Patlak and Gjedde et al., k1k3/(k2 + k3) (a value given by kinetic rates of glucose) was calculated in 22 patients. The metabolic rate of glucose can be calculated by

$$Cpk1k3/(LC(k2 + k3)),$$

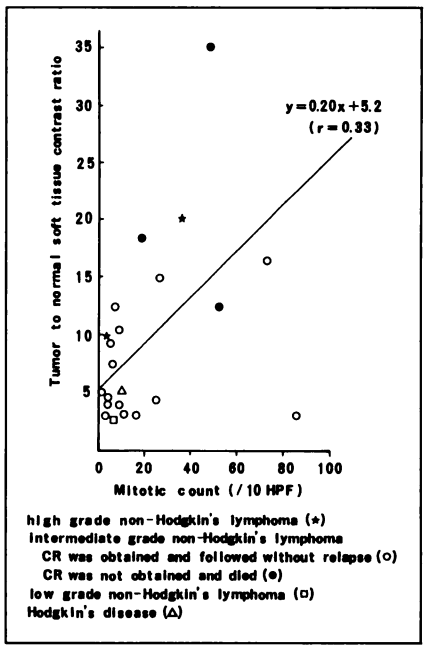

FIGURE 1. Distribution of TCR according to mitotic count.

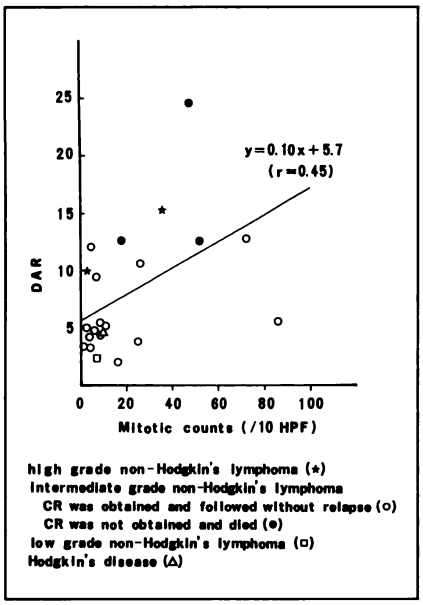

FIGURE 2. Distribution of DAR according to mitotic count.

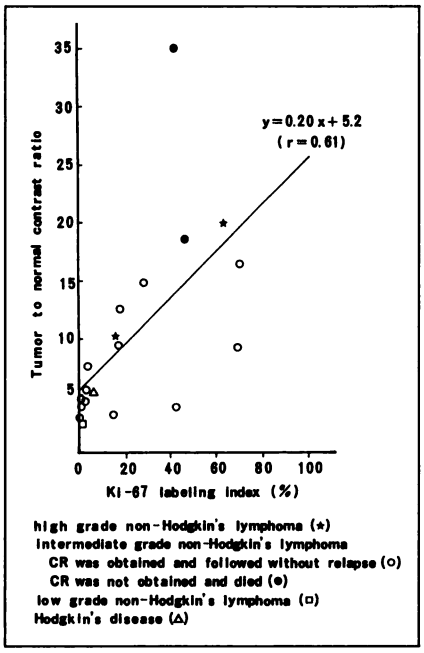

FIGURE 4. Distribution of TCR according to Ki-67 labeling index.

where Cp is blood sugar and LC is the lumped constant. We cannot know the lumped constant of each tumor and the metabolic rate of glucose cannot be measured exactly. However, k1k3/(k2 + k3) is thought to be an index closely connected with glucose metabolism (*5,21–23*).

Statistical Analysis

Scatter plots were analyzed by linear regression analysis. Student's t-test was used, and all p values are two-tailed.

RESULTS

The mean values ± standard deviations of mitotic count, Ki-67 labeling index, TCR, DAR and k1k3/(k2 + k3) were 20.9 ± 23.8, 23.4 ± 24.5, 9.49 ± 7.71, 7.93 ± 5.34 and 0.0437 ± 0.0325.

The relationships between mitotic count and TCR, DAR and k1k3/(k2 + k3) are shown in Figures 1, 2 and 3. Tumors with high mitotic count had a tendency to show high TCR, DAR and k1k3/(k2 + k3). The correlation coefficients (r) were 0.33, 0.45 and 0.45. One patient with low-grade NHL had a low mitotic count, TCR, DAR and k1k3/(k2 + k3). Five patients had poor prognoses. These were two high-grade NHL patients and three intermediate-grade NHL patients. In these patients, high mitotic count, TCR, DAR and k1k3/(k2 + k3) were shown. However, in some intermediate-grade NHL patients under good control, the values of mitotic count, TCR, DAR and k1k3/(k2 + k3) were not always lower than those of the poor prognostic patients.

The relationships between the Ki-67 labeling index and TCR, DAR and k1k3/(k2 + k3) are shown in Figures 4–6. The correlation coefficients were 0.61 ($p < 0.05$), 0.67

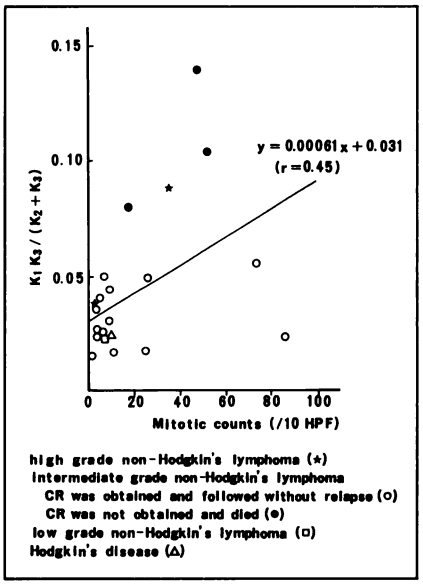

FIGURE 3. Distribution of k1k2/(k2 + k3) measured by Patlak plot according to mitotic count.

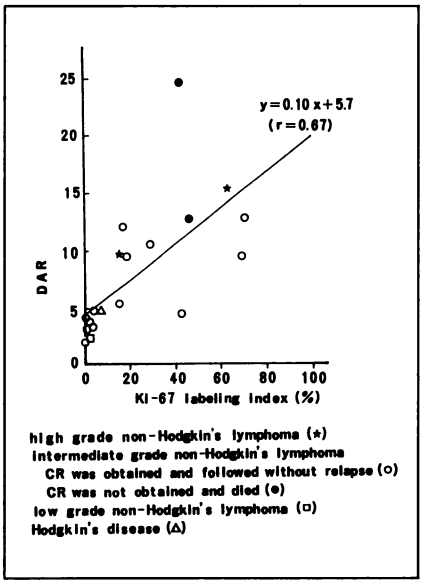

FIGURE 5. Distribution of DAR according to Ki-67 labeling index.

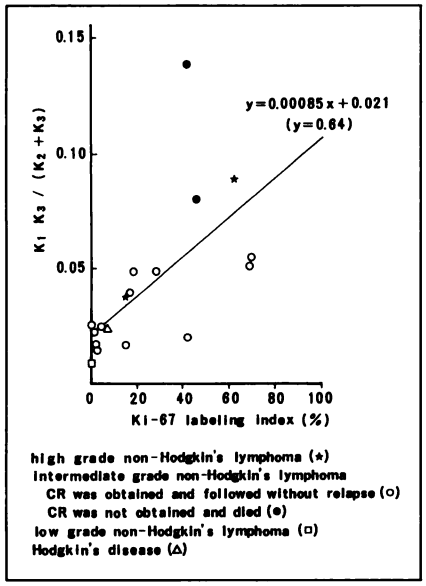

FIGURE 6. Distribution of k1k2/(k2 + k3) measured by Patlak plot according to Ki-67 labeling index.

($p < 0.01$) and 0.64 ($p < 0.01$), and significant relationships were observed. In the low-grade NHL patient, a low Ki-67 labeling index, TCR, DAR and k1k3/(k2 + k3) were shown. On the other hand, in the NHL patients with poor prognoses, the Ki-67 labeling index, TCR, DAR and k1k3/(k2 + k3) were high (Fig. 7). Among the three indices brought by FDG-PET, (namely TCR, DAR and k1k3/(k2 + k3)), significant changes were not observed in the plots.

DISCUSSION

The activity of glucose-6-phosphatase has been reported to be low in tumors and intracellular trapping of FDG has also been observed (*24,25*). Using the graphic method proposed by Patlak and Gjedde et al., we arrived at a value of k1k2/(k2 + k3), which correlated with the glucose utilization rate (*21,22*). However, this technique requires sequential arterial blood sampling. On the other hand, TCR and DAR can be measured only by placing an ROI on the PET image without sequential arterial blood sampling. In this study, three indices, TCR, DAR, k1k2/(k2 + k3), were obtained by FDG-PET and plotted versus mitotic counts and the Ki-67 labeling index. A significant change in the plot was not observed by choosing one of these three indices. The k1k2/(k2 + k3) did not provide information which could not also be derived from TCR or DAR. In consideration of the discomfort to patients caused by arterial blood sampling, it might not be necessary to add k1k2/(k2 + k3) into indices for evaluating tumors.

In our study, NHL with high mitotic count and high Ki-67 labeling index had a tendency to show an appreciable accumulation of FDG and high glucose metabolism. Especially in the comparison between FDG-PET and Ki-67 labeling index, the correlation coefficients were better than 0.6, and a good relationship was observed. In a tumor with high proliferative activity, metabolism of nuclear antigen and protein is increased, and glucose metabolism is also considered to be accelerated to supply sufficient energy for the proliferation. Our result was consistent with the biochemical mechanism.

Minn et al. studied the uptake of FDG and the proliferative activity estimated by DNA flow cytometry in 13 patients with several kinds of malignant head and neck tumors. A clear correlation between the proportion of the cells in S + G2/M phases of the cell cycle and the intensity of FDG accumulation was shown. The uptake of FDG also correlated with the percentage of S-phase cells (*6*). Flow cytometry is a method of measuring cell cycle kinetics. However, a single-cell suspension is required for the method and the equipment remains complicated and costly (*14,26–28*). Immunohistochemical labeling with the antibody Ki-67 also provides a sensitive and objective technique for estimation of the proliferative activity of malignant tumors including malignant lymphoma (*9–14*). We applied the mitotic counts and the Ki-67 technique in 23 patients with malignant lymphoma in the head and neck region. In our study, a good correlation between FDG uptake and proliferative activity was also observed. The k1k3/(k2 + k3) also correlated with the proliferative activity.

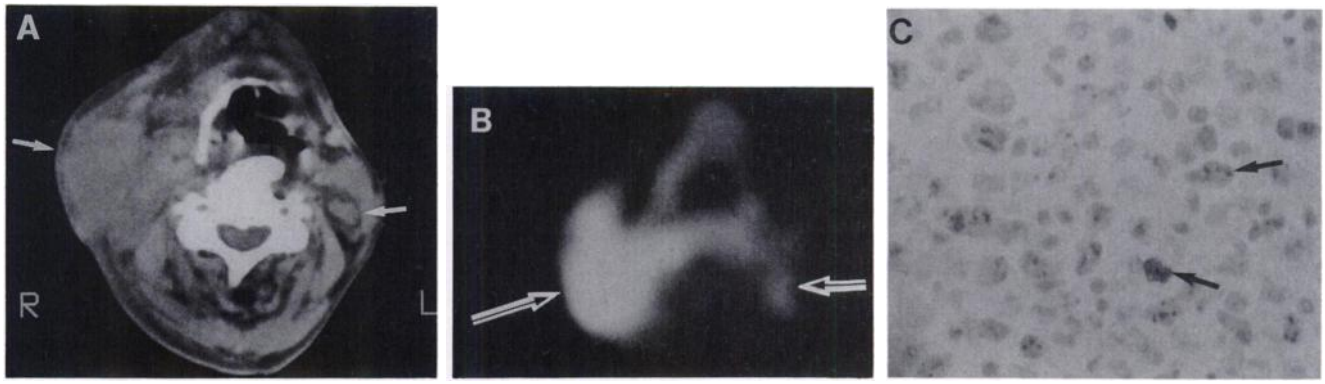

FIGURE 7. Case 13. Intermediate grade NHL in which complete remission was not obtained and the patient died. (A) X-ray CT shows tumors of the right and left jugular area (arrow). (B) PET shows high accumulation of FDG in the tumors (arrow). (C) Ki-67 immunostaining in the biopsy specimen showed a high percentage (45%) of positive cells whose nuclei were stained (arrow).

In malignant lymphoma, a strong correlation between a low Ki-67 labeling index and low-grade histology and between a high Ki-67 labeling index and high-grade histology was found, and close correlation between Ki-67 positivity and histological grading was reported. It also was reported in 99 patients with lymphoma that patients with a high Ki-67 labeling index had lower survival rates (*11*, *12*). We reported in a clinical study that high-grade NHL patients and intermediate-grade NHL patients with poor prognoses showed a high accumulation of FDG and increased glucose metabolism and that FDG-PET was helpful for grading and predicting prognosis in malignant lymphoma (*5*). The clinical usefulness of FDG-PET is supported by this pathological and PET study.

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