Correlation of glucose consumption and tumor cell density in astrocytomas

A stereotactic PET study

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To determine histological correlates of the variability of glucose consumption in astrocytomas, the authors performed positron emission tomography (PET) with 18F-2-fluoro-2-deoxy-D-glucose (FDG) and matched the PET scans three-dimensionally with computerized tomography scans obtained in a stereotactic frame before biopsy. Ten patients with astrocytomas of World Health Organization Grade 2 or 3 were studied; patients with glioblastomas, oligodendrogliomas, or oligoastrocytomas were excluded from the study to avoid any confounding effects of different cell types and necroses. In samples of pure tumor, glucose consumption correlated significantly with cell density, but not with nuclear polymorphism. It is concluded that tumor cell density is a major determinant of glucose consumption in astrocytomas. The use of PET with FDG may help to locate the highest cell density and thus improve the diagnostic yield of stereotactic biopsy.

KEY WORDS • astrocytoma • glucose metabolism • cell density • positron emission tomography

Positron emission tomography (PET) with 18F-2-fluoro-2-deoxy-D-glucose (FDG) is widely used to evaluate tumors in the brain and in other organs by measuring glucose consumption. This study may help to differentiate between recurrent tumor and necrosis, to detect early recurrence, and to guide selection of brain lesions for biopsy.10,17,18 Not all brain tumors show a high glucose metabolism, however, and histological correlates of glucose uptake have not yet been clearly identified. Although a significant correlation between histological tumor grade and FDG uptake has been reported in gliomas6-7 and non-Hodgkin's lymphomas10, a correlation was not confirmed for gliomas by Tyler, et al., and there was not a very close correlation (Kendall r = 0.45, p = 0.04) in our 1988 study.12 Increased nonoxidative glycolysis has often been seen in malignant tumors since the pioneering work of Warburg,13 but its relationship to proliferation in malignancy is complex and the mechanisms are not completely understood.14-31

Histological grading of gliomas includes assessment of several features: cell density, nuclear polymorphism, mitoses, vascularization, endothelial proliferation, and micro- and macrocereoses. To elucidate the relationship between FDG uptake and histological grading, we examined in a homogeneous group of untreated astrocytomas whether a more direct correlation to one of the histological features underlying grading may exist.

Clinical Material and Methods

Patient Population

Patients analyzed in the present study participated in a larger ongoing prospective study of suspected low-grade gliomas, which included PET with FDG in the preoperative diagnostic workup. Inclusion criteria for the current study were: 1) PET performed before surgery, irradiation, or cytostatic drug treatment; 2) subsequent computerized tomography (CT)-guided stereotactic biopsy; and 3) a diagnosis of astrocytoma, with the presence of pure tumor (no normal brain) in at least one stereotactic sample. Patients with oligoastrocytoma, evidence of necroses, or other features of glioblastoma were excluded from the study.

Ten patients (six men and four women), with a mean age of 46 ± 12 years, fulfilled the criteria and were
included in the study. Eight patients had astrocytomas of World Health Organization (WHO) Grade 2 and two had WHO Grade 3 astrocytomas. Tumor sizes, as estimated from the largest diameter of the abnormality on CT scans, ranged between 2 and 7 cm. Moderate contrast enhancement was observed in five tumors, including the two Grade 3 astrocytomas. All patients gave informed consent to the diagnostic procedures.

Positron Emission Tomography

Positron emission tomography was performed on a four-ring seven-slice scanner,* with 7.8-mm in-plane resolution and a slice thickness of approximately 11 mm. Scans used for calculation of the metabolic rate of glucose (MRGlut) were obtained starting 30 minutes after the intravenous bolus injection of approximately 185 MBq FDG. Two bed positions were used, yielding a set of 14 slightly overlapping slices with a center-to-center distance of 6.85 mm which covered nearly the entire brain. Multiple arterial blood samples were taken beginning at the time of FDG injection until the end of measurement and were used to calculate the MRGlut based on the Sokoloff model,' with adaption of \( K_i \) and \( k_3 \) to measured activity and a lumped constant of 0.418.

Stereotactic Biopsy

For stereotactic biopsy, the patient's head was placed in a modified CT-compatible Riechert-Mundinger stereotactic frame.' After administration of contrast medium† at a dose of 2.0 to 2.5 ml/kg body weight, a CT examination (2-mm slices, 2-mm table support)‡ with a stereotactic localizer' was performed. The CT data were transferred by magnetic tape into a computer, and a stereotactic coordinate system was established.26,28 Various biopsy tracks were simulated on the computer screen via three-dimensional treatment planning routines in horizontal, sagittal, coronal, and oblique section planes. The computer-generated parameters were transferred to the target simulator. After intracranial insertion of the cannula, serial biopsy specimens were obtained with a Backlund spiral needle' in 3- to 6-mm steps. The planned needle track allowed continuous sampling of specimens from peritumoral tissue and the lesion itself in order to obtain a representative histological diagnosis.

Histological Examination

Histological evaluation was based on tissue specimens fixed in formalin and embedded in paraffin. The evaluator (R.S.) was not aware of the PET results. Serial sections 4 μm thick were stained routinely. Cell density was counted from representative areas using an ocular grid measuring five fields at high-power magnification. In addition, the following features were evaluated: grade of malignancy according to the WHO classification system,31 gradation (low/medium/high) of nuclear polymorphism and vascularization, and presence of endothelial proliferation, mitoses, fibers, and micro- and macrocereoses. Only probes showing pure tumor without surrounding normal brain were used for comparison with PET studies.

Stereotactic Computerized Tomography

Stereotactic CT scans were stored digitally and transferred to a UNIX-based graphic workstation. An interactive program31 was used to display CT and PET scans simultaneously in 256 × 256 matrices with 1-mm pixels and to resample PET scans by bilinear interpolation in the same orientation and slice thickness as the stereotactic CT scans. To achieve registration, images were displayed in coronal cuts through the frontal and occipital lobes, sagittally and parasagittally through the basal ganglia, and in multiple transaxial levels. The brain contours were outlined on PET scans by appropriate filtering and then superimposed on CT scans. In addition, the correspondence of landmarks, such as the tentorium, the quadrigeminal cistern, and the anterior horns of the lateral ventricles, was checked. Coordinates of the stereotactic biopsies were located on the images, and regional MRGlut was recorded for each sample in a circular region of 5-mm diameter. If multiple biopsies with pure tumor were obtained from a patient, the average MRGlut of all samples was used for statistical calculations.

* PET scanner, Model PC-384, manufactured by Scanditronix, Uppsala, Sweden.
† Solutrast 300 obtained from Byk Gulden, Darmstadt, Germany.
‡ Somatom DR 2 manufactured by Siemens, Erlangen, Germany.

FIG. 1. Scattergram showing the relationship between the metabolic rate of glucose (MRGlu) and cell density in 19 stereotactic tumor samples (r = 0.74). Nuclear polymorphism was not significantly related to MRGlu. FOV = field of view.
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**Results**

**Cell Density**

There was a significant correlation in all 10 patients \((r = 0.71, p = 0.02)\) between tumor cell density and MRGlu. Without averaging samples from each patient, a similar correlation \((r = 0.74)\) was obtained for 19 specimens (Fig. 1). Nuclear polymorphism was classified as medium to high in five patients and as low in the other five patients. Neither cell density nor MRGlu differed significantly between these groups, although a tendency toward lower values in the group with medium or high nuclear polymorphism was noted (mean ± standard error of the mean: cell density 128 ± 30 cells/field of view (FOV) and MRGlu, 18.9 ± 5.6 μmol/100 gm/min in tumors with high nuclear polymorphism vs. cell density 187 ± 61 cells/FOV and MRGlu, 22.9 ± 10.9 μmol/100 gm/min in tumors with low nuclear polymorphism: Fig. 1). Findings from three illustrative cases are illustrated in Fig. 2.

**Other Histological Features**

The frequencies of the other histological parameters studied were less well balanced (Table 1). Vascularization was medium in seven tumors, low or medium in samples from two tumors, and low in one tumor. Endothelial proliferation was noted in one Grade 3 tumor, and mitoses were present in three tumors. The two Grade 3 tumors (Cases 9 and 10) exhibited mitoses and medium or high polymorphism, but did not show extreme values of cell density or MRGlu. Mitoses without other signs of anaplasia were also found in another tumor (Case 3) with low cellular density and low vascularility, which was therefore attributed to WHO Grade 2; a low MRGlu (15 μmol/100 gm/min) was also noted in this case. An overview of all patient findings is listed in Table 1.

**Discussion**

The present work identifies cell density as a main determinant of glucose metabolism in astrocytomas. The relationship between cell density and glucose metabolism has not been addressed in previous papers on FDG uptake and tumor proliferation. Malignant transformation often coincides with increased cell density, and most grading systems for gliomas explicitly take this feature into account as an indicator of higher grade. Cell density may therefore link glucose uptake and glioma grade.

Nuclear polymorphism was not related to the tumor MRGlu in our study. This finding differs from results in head and neck tumors and in lymphoma. More sophisticated methods such as flow cytometry and Ki-67 labeling were used in those studies to analyze nuclear anaplasia, probably increasing the ability to detect a correlation with MRGlu. In addition, tumor cell growth rates are much higher for head and neck tumors and lymphoma than for astrocytomas. Since there is no known direct biochemical link, it is not unreasonable that systems with different growth rates show a different relationship between nuclear polymorphism and MRGlu.

Other indicators of anaplasia, such as the presence of mitoses or endothelial proliferation, could not be analyzed separately in our small sample. Cell kinetics

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**TABLE 1**

Principal findings for 10 patients with astrocytomas*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>WHO Grade†</th>
<th>MRGlu (μmol/100 gm/min)</th>
<th>Cell Density (Cells/FOV)</th>
<th>Nuclear Polymorphism</th>
<th>Vascularization</th>
<th>Endothelial Proliferation</th>
<th>Mitoses</th>
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<tr>
<td>1</td>
<td>49, M</td>
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<td>16.7</td>
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<td>2</td>
<td>36, F</td>
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<td>low</td>
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<td>no</td>
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<td></td>
<td></td>
<td></td>
<td>17.3</td>
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<td>17.1</td>
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<tr>
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<td>3</td>
<td>23.8</td>
<td>177</td>
<td>high</td>
<td>medium</td>
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* MRGlu = metabolic rate of glucose; FOV = field of view.
† Grade according to the World Health Organization (WHO) grading system.
‡ Sample with blood imbibition, not used for analysis.
studies indicate that the fraction of S-phase cells is very low (less than 1%) in differentiated astrocytomas and only approximately 4% in anaplastic astrocytomas. Even in glioblastomas, this fraction is considerably lower (up to 20%) than in cerebral carcinoma metastases. Since most astrocytomas in our sample were classified as WHO Grade 2, no correlation between anaplasia and MR \( \text{Glu} \) could be anticipated.

In more malignant tumors, including most experimental brain tumors, necroses are frequent and may

![Image of tumor specimens and CT scans](image-url)
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confound the relationship between histological features and MR\textsubscript{Glu}. The uptake of FDG is usually high in viable tissue but close to zero in necroses.\textsuperscript{1,4} Since micronecroses cannot be resolved spatially on PET, they would reduce the average tumor MR\textsubscript{Glu} and possibly obscure the relationship with cell density. Tumors with necroses were therefore excluded from the present study.

Our analysis depended on the accuracy of the alignment of PET with stereotactic CT scans. Instead of using a rigid fixation device,\textsuperscript{15,20,25} which is rather impractical under usual clinical conditions, we relied on three-dimensional processing of the PET data. Resolution was limited more by the thickness of the original overlapping slices (approximately 11 mm) than by in-plane resolution (7.8 mm full width half-maximum height). The variability (standard deviation) of registration (2.4 mm measured at the center of the image) was determined in a reproducibility study with four independent users of the same program. This accuracy and resolution were sufficient with regard to the size of the tumors, but differentiation between adjacent tumor samples was probably incomplete and statistical evaluation was therefore based on averages.

In contrast to studies with labeled amino acids such as methionine,\textsuperscript{20} which may show higher uptake than normal brain even in low-grade gliomas, we did not attempt to delineate tumor borders. Injection of FDG would not be suited for such a task since FDG uptake is low (clearly less than in normal gray matter) in most low-grade gliomas, and the contrast to normal brain tissue is variable and location-dependent because of the large difference between gray and white matter MR\textsubscript{Glu}. This background variability also causes problems in samples from tumor border areas which may consist of a mixture of tumor and normal brain tissue. Such samples were therefore excluded from the present study. We also excluded patients with oligodendrogliomas or mixed tumors (oligoastrocytomas) because astrocytes and oligodendrocytes show very different MR\textsubscript{Glu} in cell culture,\textsuperscript{7} and oligodendroglialomas typically have a higher cell density than astrocytomas.

Our study lends further support to the diagnostic and prognostic use of PET with FDG in the evaluation of gliomas. Preliminary data indicate that FDG uptake (in relation to normal brain) correlates with survival time not only in high-grade gliomas\textsuperscript{22} but also in WHO Grade 2 gliomas.\textsuperscript{11} Our study also supports the use of PET with FDG to locate areas of high cell density in inhomogeneously enhanced tumors and thereby to improve the diagnostic yield of stereotactic biopsy.

References


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