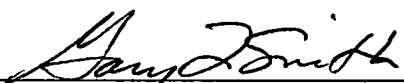


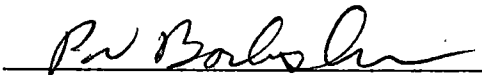
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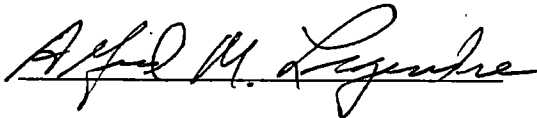
I am submitting herewith a dissertation written by Cary Lee Matwichuk entitled "Characterization of Fluorine-18-Fluorodeoxyglucose Accumulation in *Blastomyces dermatitidis*-Associated Inflammation." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Comparative and Experimental Medicine.


Gregory B. Daniel, Co-Major Professor

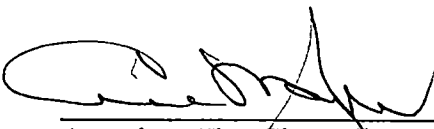

Gary T. Smith, Co-Major Professor

We have read this dissertation and recommend its acceptance:





Accepted for the Council:


Associate Vice Chancellor and Dean of the
Graduate School

**CHARACTERIZATION OF FLUORINE-18-FLUORODEOXYGLUCOSE
ACCUMULATION IN *BLASTOMYCES DERMATITIDIS*-ASSOCIATED
INFLAMMATION**

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Cary Lee Matwichuk
May 2001

DEDICATION

This thesis is dedicated to my mentors

Dr. Susan M. Taylor

and

Dr. Gregory B. Daniel

who exemplify the class of veterinarian, scientist, and person that I aspire to be.

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ABSTRACT

Whole-body positron emission tomography (PET) using the radiopharmaceutical fluorine-18-fluorodeoxyglucose (FDG) is widely used for tumor imaging in people. Lesions caused by fungal infections can have high accumulation of FDG and result in misdiagnosis for malignancy. Little information was available in the literature to draw firm conclusions regarding the uptake of FDG by fungal lesions. The purpose of this research was to determine the typical intensity of uptake of FDG by fungal lesions, to describe the distribution of FDG in fungal lesions, and to determine if fungal-associated inflammatory lesions could be detected using whole-body FDG-PET. A technique for creating lesions caused by the fungal agent *Blastomyces dermatitidis* in rats was developed. Accumulation of FDG in these experimentally created fungal lesions was compared with uptake by experimentally induced non-fungal inflammation (turpentine abscesses) and malignant tumors (lymphomas) in rats using the differential uptake ratio. Results showed that experimental *Blastomyces* lesions typically exhibit high intensity uptake of FDG. The intensity of uptake of FDG by the *Blastomyces* granulomas was higher than for the turpentine abscesses and as high as for malignant lymphomas. The uptake of FDG in the experimentally created *Blastomyces* lesions was associated with the region occupied by the granulomatous inflammatory reaction and the yeast organisms. Uptake of FDG was then evaluated in dogs with naturally occurring blastomycosis and compared with uptake in dogs with naturally occurring lymphoma using the standardized uptake value (SUV). The mean ($\pm$ sd) SUV for naturally occurring lesions of blastomycosis in dogs was 7.7 ± 2.0 , and was higher than the mean SUV for naturally

occurring lymphomas in dogs of 4.8 ± 1.8 . A cutoff SUV of 7.0 gave 100% specificity for blastomycosis. FDG-PET reliably detected lesions of *Blastomyces*-associated inflammation and may have a role in determining extent of disease and monitoring therapy in patients with blastomycosis. Because of the similarities of blastomycosis and lymphoma in dogs and people, similar results would be expected in human patients. Fungal granulomas should be considered a differential diagnosis for lesions with high intensity accumulation of FDG in areas where blastomycosis is endemic.

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CHAPTER 1

Introduction to the Research

Statement of the Problem

Whole-body positron emission tomography (PET) using the radiopharmaceutical fluorine-18-fluorodeoxyglucose (FDG) is an imaging technique widely used for oncologic evaluation in people.¹⁻⁷ The images acquired using PET are unique in that functional rather than structural factors define the images. In the case of FDG-PET, images are a reflection of tissue glucose metabolism.

The rationale for FDG-PET imaging in oncology is based on increased glucose metabolism by malignant cells. Biochemical changes characteristic of transformed cells include increased numbers of glucose transporters and increased activity of enzymes of glycolysis, resulting in increased glucose utilization by tumor cells.⁸⁻¹² A glucose analogue, FDG is initially processed by cells in the same manner as glucose. FDG thereby accumulates in any tissue in an amount proportional to the rate of glucose metabolism.^{8;10;13;14} Tumors are distinguished from normal tissues by their marked accumulation of FDG.^{8;10;13;14}

FDG-PET is highly sensitive for detecting malignancies.^{1-7;15-18} Accumulation of FDG is not specific for malignancy, however. Any tissue with increased glucose utilization will

exhibit increased uptake of FDG.¹⁹⁻²⁴ Inflammatory cells use increased amounts of glucose during chemotaxis and phagocytosis, so inflammatory lesions demonstrate increased uptake of FDG.^{21;24-27} In most cases, however, uptake of FDG by benign lesions is significantly less than for malignant lesions.^{1-7;15-18} Accumulation of FDG can be measured semi-quantitatively using the standardized uptake value (SUV). A cutoff SUV of 2.5-3.5 is used to distinguish benign from malignant disease; lesions having an SUV below this value are considered benign, and lesions with an SUV above this value are characterized as malignant.^{8;8;10;10} Benign lesions such as abscesses, fibrosis, and benign tumors typically have SUVs ranging from 1-3.^{1-7;15-18} Malignant lesions have SUVs ranging from 3-20.^{5;6;16}

Inflammatory lesions will occasionally show high intensity uptake of FDG and be misdiagnosed as malignancy.^{1;2;4-7;16;17;28-30} Many of the lesions with high intensity uptake of FDG are granulomatous inflammatory reactions including those caused by tuberculosis, sarcoidosis, and fungal infections.^{1;2;4-7;16;17;28-30} High intensity lesions misdiagnosed as malignancy in studies using FDG-PET for identifying malignant pulmonary nodules are commonly due to fungal infections.^{2;4-6;16;28;30} SUVs for fungal masses resulting in false positive diagnosis for malignancy may be as high or higher than for malignancies.^{2;4-6;16;28;30} However, not all fungal granulomas show high intensity uptake of FDG. Many fungal granulomas have SUVs below the cutoff for malignancy and are readily identified as benign.¹⁶ However, of lesions misdiagnosed for malignancy, many are due to fungal infections.

These observations are based on the small number of reported cases of uptake of FDG by fungal lesions. Because there are only a few reports of fungal disease imaged using FDG-PET in the literature, typically those involving cases that resulted in false positive diagnosis for malignancy, there is not enough information to draw firm conclusions regarding characteristics of uptake of FDG by fungal-associated inflammatory lesions. There are no reports in the literature describing a series of fungal lesions that provides a range of SUVs typical for fungal-associated inflammatory lesions, and no studies that compare a series of fungal lesions with other benign or malignant lesions. Thus the current literature prompts, but cannot conclusively answer, the following questions:

What is the typical intensity of uptake of FDG for fungal lesions?

How often does high intensity uptake of FDG by fungal lesions occur?

Can the traditional cutoff SUV for malignancy be used to reliably distinguish fungal infection from malignancy?

Can FDG-PET accurately detect fungal-associated inflammatory lesions?

Is there a role for FDG-PET in clinical management of patients with fungal infections?

The clinical implications of an incorrect diagnosis made using FDG-PET are obvious. Patients with fungal disease mistakenly suspected to be malignancy might undergo invasive and costly diagnostic tests that are not indicated. In addition, while these tests are being performed, appropriate therapy for the fungal infection is delayed. A study to definitively characterize uptake of FDG by fungal-associated inflammatory lesions would

be worthwhile to document whether fungal lesions can be consistently differentiated from malignancies. If fungal lesions are shown to have SUVs that are typically less than those for malignancies, fungal disease can be distinguished using the same criteria as for other benign diseases. Alternatively, if fungal lesions are shown to most often have SUVs higher than for malignancies, an additional cutoff SUV may allow distinction of fungal disease from malignancy. That is, lesions with an SUV above the cutoff would be identified as most likely fungal lesions, and lesions with an SUV below the cutoff would be identified as malignancy. The ability to differentiate between fungal lesions and malignancies would be particularly valuable in patient populations where both diseases may occur, for example, in patients with acquired immune deficiency syndrome, or in patients in a region where fungal disease is endemic. Additionally, if FDG-PET was shown to accurately detect sites of fungal infection, FDG-PET may provide a sensitive, non-invasive means by which to define the extent of disease and monitor response to therapy in patients with known fungal infection. Persistent foci of infection requiring prolonged anti-fungal therapy could be identified, minimizing the likelihood for relapse of disease.

Purpose of the Research

The purpose of this research is to test the following hypotheses:

Hypothesis 1. Inflammatory lesions caused by the fungal agent *Blastomyces dermatitidis* typically exhibit high intensity uptake of FDG.

Hypothesis 2. Uptake of FDG by the fungal organisms contributes significantly to the uptake of FDG by *Blastomyces* granulomas.

Hypothesis 3. Sites of fungal-associated inflammation in dogs with the naturally occurring fungal infection blastomycosis can be accurately detected using whole-body FDG-PET.

The specific objectives of the project designed to test these hypotheses are:

1. To define the mean and range of intensities of uptake of FDG for a series of fungal-associated inflammatory lesions caused by the fungal agent *Blastomyces dermatitidis*.
2. To describe the location/distribution of FDG uptake in *Blastomyces*-associated inflammatory lesions.
3. To compare the uptake of FDG by *Blastomyces* granulomas with uptake by non-fungal inflammatory lesions and malignant tumors in experimentally created lesions.
4. To determine if sites of fungal-associated inflammation in dogs with naturally occurring blastomycosis could be accurately detected with whole-body FDG-PET.
5. To determine the range of SUVs characteristic of naturally occurring fungal-associated inflammatory lesions due to blastomycosis.

6. To compare the SUVs of naturally occurring fungal-associated inflammatory lesions in dogs with blastomycosis with the SUVs of naturally occurring tumors in dogs with lymphoma.
7. To evaluate the role of yeast organisms in FDG uptake by *Blastomyces* granulomas.

We propose that the information gathered from the animal models in this study will be representative of characteristics of uptake of FDG by fungal-associated inflammatory lesions in human patients. This information could be used to assess the impact (if any) of uptake of FDG by fungal-associated inflammatory lesions on specificity of whole-body FDG-PET imaging for malignancy, and to define a possible role for FDG-PET in imaging fungal infections in human patients.

Overview of the Research

FDG uptake by fungal granulomas was first evaluated using experimentally created lesions in rats. Uptake of FDG by the fungal granulomas was compared with experimentally created non-fungal inflammatory lesions and malignant tumors. The fungal granulomas were also evaluated using macroautoradiography to determine the distribution of FDG within the fungal-associated lesions.

Subcutaneous injection with *Blastomyces dermatitidis* yeast organisms and malignant rat lymphoma cells were used to create fungal-associated inflammatory lesions and tumors,

respectively, for many reasons. Blastomycosis occurs in people in addition to many animal species, and has been reported as a cause of false positive diagnosis for malignancy using FDG-PET in people. Secondly, FDG-PET is commonly used for evaluation of human patients with lymphoma. Blastomycosis and lymphoma both affect lymph nodes, such that SUVs of lesions of the 2 diseases could be compared in the same tissue type. Finally, blastomycosis and lymphoma occur commonly in dogs, providing an accessible animal model to study naturally occurring disease. For the sake of consistency the same diseases were created experimentally in the rats. Turpentine injection, a widely used experimental method for creating non-infectious abscesses, was used to induce non-fungal inflammatory lesions.

This approach necessitated development of a technique to create localized, subcutaneous *Blastomyces* lesions in rats. A yeast suspension was prepared from a clinical isolate of *Blastomyces dermatitidis* obtained from a dog with systemic blastomycosis. The dose of yeast organisms and duration from inoculation to harvest of the nodules required to create optimal lesions were determined. We were successful in defining a technique to consistently produce localized subcutaneous nodules.

Localized *Blastomyces* granulomas and turpentine abscesses were created in opposite limbs in one group of rats to compare uptake of FDG by these two different inflammatory lesions. In a second group of rats, *Blastomyces* granulomas and lymphomas were created and uptake of FDG was compared between these two lesions. Uptake of FDG was measured by harvesting the lesions 1 hour after intravenous administration of FDG then

measuring the radioactivity of the tissues in a well counter. The amount of radioactivity was quantified using the differential uptake ratio (DUR). Tissue sections from the radioactive *Blastomyces* granulomas and the turpentine abscesses were also evaluated using macroautoradiography. The macroautoradiographs were used to determine the location of FDG within the *Blastomyces* nodules, and to compare the intensity of uptake of FDG between the different types of inflammatory reactions caused by *Blastomyces* and turpentine.

Results from this experiment indicated that high uptake of FDG was associated with the region occupied by the *Blastomyces* yeast organisms and the granulomatous inflammatory response of *Blastomyces* nodules. Uptake of FDG by *Blastomyces*-associated lesions was higher than for non-fungal inflammatory lesions (turpentine abscesses), and was as high as for experimentally induced lymphomas. A marked inflammatory response, not characteristic of naturally occurring lymphomas, was associated with the experimentally induced lymphomas, and may have contributed to the overall uptake of FDG by the lymphomas.

Next, FDG-PET scans were done in dogs with naturally occurring blastomycosis to determine if FDG-PET could accurately detect sites of *Blastomyces*-associated inflammation, and to define the range of SUVs observed for naturally occurring *Blastomyces* lesions. The SUVs of these *Blastomyces* lesions were compared with the SUVs of naturally occurring lymphomas in dogs. Results from this experiment showed that naturally occurring *Blastomyces* lesions typically exhibit high intensity uptake of

FDG, and that whole-body FDG-PET can accurately detect sites of *Blastomyces*-associated inflammation. The SUV for *Blastomyces*-associated inflammatory lesions was significantly higher than the SUV for lymphomas. Eight of 13 *Blastomyces* lesions had SUVs above 7.0, and none of the 17 lymphomas had SUVs above 7.0. Thus a cutoff SUV of 7.0 was 100% specific for *Blastomyces* lesions in this study. Because of the similarities in canine and human blastomycosis and lymphomas similar results would be predicted in human patients.

In summary, it was concluded from this research that *Blastomyces*-associated inflammatory lesions exhibit high intensity uptake of FDG due to the associated granulomatous inflammatory reaction. Uptake of FDG by the yeast organisms was suspected, but could not be proven in this study. Whole-body FDG-PET can reliably detect sites of fungal infections and has a useful role in defining the extent of fungal disease and monitoring response to therapy. The SUV of *Blastomyces* granulomas is higher than for lymphomas. In this study, a cutoff SUV of 7.0 was highly specific for *Blastomyces* infection.

CHAPTER 2

Literature Review

Positron Emission Tomography

Introduction

Traditional imaging methods such as radiology, ultrasound, computed tomography and magnetic resonance imaging provide information regarding tissue structure and anatomy.³¹ In contrast, functional rather than structural factors define the images obtained using positron emission tomography (PET).³¹ A radiopharmaceutical tagged with a positron-emitting isotope is administered to the patient and the PET detector maps the distribution of the radioactivity. The resulting image is a 3-dimensional map of radiopharmaceutical distribution (location and intensity) in the patient.³¹ Because accumulation of the radiopharmaceutical is dependent on cellular function, PET conveys information regarding physiologic and metabolic processes in the body.³¹ PET can be used to provide complementary information to anatomic images and vice versa.³¹

Radioactive Decay by Positron Emission

A radioactive isotope has an atomic nucleus composed of either too many protons or too many neutrons, rendering the isotope "unstable".³¹ In an attempt to reestablish the

balance of neutrons and protons and return to a stable state, the nucleus will undergo radioactive decay.³¹ PET cameras detect radioactivity produced by radioactive nuclei decaying by positron emission.

Positron emitting isotopes are created using cyclotrons, which bombard stable (non-radioactive) isotopes with protons.³¹ The resulting isotopes are “proton heavy”, that is, the nucleus contains too many protons.³¹ In essence, decay by positron emission entails transformation of a proton to a neutron and release of energy in the form of a positron.³¹ The resulting “daughter” isotope has a neutron:proton ratio that is more stable than the radioactive “parent” isotope.

The positron released from the radioactive nucleus during decay is a positively charged particle with the same mass as an electron.^{31;32} The positron is discharged from the nucleus, travels a very short distance, and collides with an orbital electron (from an atom in the matter in which it is traveling). The positron and electron undergo an “annihilation reaction”, which converts the mass of the positron and electron into an equivalent amount of energy in the form of gamma radiation.^{31;32} The mass (and therefore the energy) of an electron and a positron are the same. Therefore, the energy produced by the annihilation reaction is in the form of two gamma rays, each with energies of 0.511 MeV that are emitted at almost exactly 180° to each other.³¹ Detection of these two gamma rays traveling in opposite directions is the foundation of imaging with PET.

The PET Detector

PET cameras are designed to detect gamma rays of 0.511 MeV produced by positron emitting radioisotopes.³¹ PET cameras are composed of a ring of detectors that encircles the patient. Each detector is comprised of a bismuth germanate crystal coupled to a photocathode and a photomultiplier tube.^{31;33} The crystal translates the gamma radiation into a light flash, which is subsequently converted into an electrical signal by the photocathode.³³ The gamma rays from the annihilation reaction emitted at 180° to each other are registered by detectors on opposite sides of the patient.³¹ The detectors record “true” events, that is, events where the two gamma rays arrive simultaneously at detectors on the opposite side of the patient (“coincidence detection”).³¹ “Simultaneously” refers to gamma rays arriving within a preset coincidence window. That is, the gamma rays will not arrive at the detectors at *exactly* the same time, due to differences in scintillation time of the crystal and time delays in detector electronics. A typical coincidence window is set at 10-15 nanoseconds (ns); gamma rays arriving at opposite detectors within 10-15 ns of each other are considered as a “true” event.^{31;33}

Detection of the gamma rays by opposite detectors means that the annihilation reaction (indicating the location of the radiopharmaceutical) occurred somewhere along the line between the two detectors. This line is called the Line of Response (LOR).^{31 33} By placing detectors 360° around the patient, the location of radioactivity in the body can be mapped by creating multiple LORs.³¹ The emission scan is the image of radiopharmaceutical distribution generated by reconstruction of recorded events along all

of the LORs.³¹

Body tissues attenuate the gamma photons as they pass through the body.³¹ Attenuation must be accounted for to accurately quantify the concentration of radioactivity.^{31 33} To achieve this, a transmission scan is acquired with the patient in exactly the same position as for the emission scan.³¹ The source of radioactivity for the transmission scan is a ⁶⁸Ge ring or rod external to the patient, which produces a pair of annihilation photons.³¹ Attenuation of these annihilation photons by the body is measured. Emission data is corrected for attenuation by dividing it by the transmission data to produce the final image.³¹

Unique Features of PET

PET has some inimitable features that enable it to provide information not available using any other nuclear imaging modality. First, many molecules in the body are composed of organic elements including carbon, nitrogen, oxygen, and hydrogen. These “building blocks” have isotopes that decay by positron emission: carbon-11, nitrogen-13, oxygen-15, and fluorine-18 (which is similar to hydrogen and can be used to replace hydrogen atoms).^{31;33} Thus biologic processes can be measured by replacing stable molecules with radioactive ones. Individual steps within metabolic pathways can be measured using positron-emitting isotopes tagged to analogues of normal physiologic compounds.³³ Secondly, the high sensitivity of PET detectors facilitates use of very small amounts of radiotracer, which allows physiologic processes to be observed undisrupted in their

natural states. Finally, the count density for each pixel of attenuation corrected PET images is directly proportional to the number of positron emissions occurring, so that the image data precisely reflects the concentration of FDG that has accumulated in a specific region.^{31 33} Thus, the metabolic process under study with the positron emitting radiopharmaceutical can be quantified.

Fluorine-18-Fluorodeoxyglucose

Introduction

One of the most commonly used radiopharmaceuticals for PET imaging is fluorine-18-fluorodeoxyglucose (FDG), an analogue of glucose. FDG is an example of a radiopharmaceutical that is designed to permit observation and measurement of a single metabolic step in vivo. The FDG model is an ideal model for quantifying the rate of the hexokinase reaction of glycolysis.

Glycolysis

Glucose enters cells via a family of glucose-transporting proteins named GLUT1 to 6.³⁴ Once inside the cell glucose is converted to pyruvate via a series of enzymatic reactions called glycolysis (Figure 1).³⁴ Glycolysis is a fundamental pathway in the generation of cellular energy and functions both to degrade glucose to generate ATP and to provide

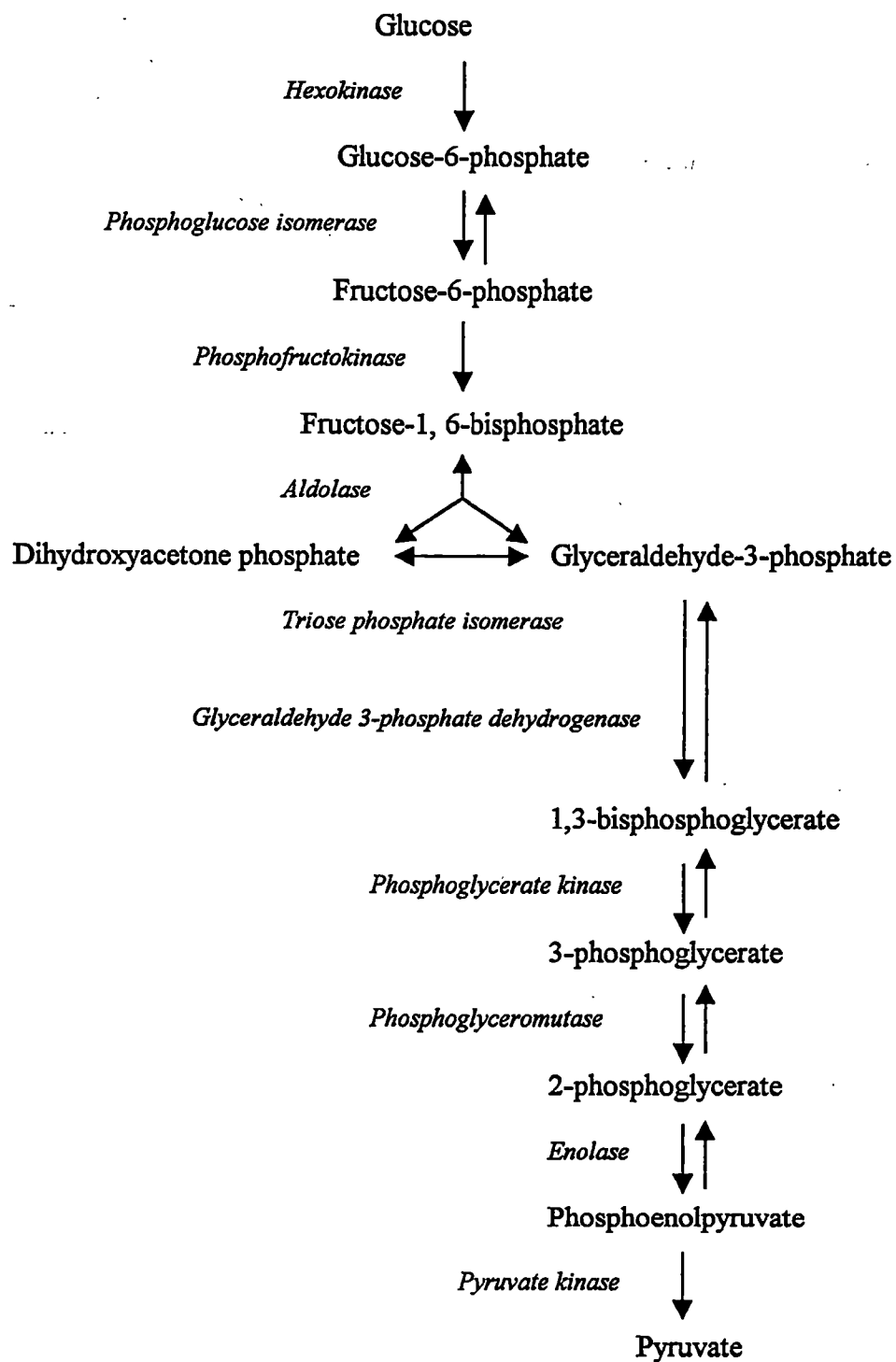


Figure 1. Enzymes of glycolysis (Embden-Meyerhof pathway).

synthetic building blocks.³⁴ The first step in glycolysis is phosphorylation of glucose to glucose-6-phosphate (G-6-P) by the enzyme hexokinase.³⁴ Binding of glucose to hexokinase induces a conformational change in hexokinase, an example of "induced fit" enzyme action.³⁴ The active site on the enzyme changes shape and becomes complementary to the substrate only after the substrate is bound. Thus hexokinase is active only once it is bound to glucose.³⁴ Phosphorylation of glucose by hexokinase is an irreversible reaction that traps glucose within the cell.³⁴ Hexokinase has such high affinity for glucose that its activity is saturated in the brain and (in the presence of insulin) liver and fat. Therefore, the rate of glycolysis is essentially equal to the maximum velocity of the hexokinase reaction.

The next step in glycolysis is isomerization of G-6-P to fructose-6-phosphate (F-6-P) by the enzyme glucose phosphate isomerase.³⁴ There are strict structural requirements for the enzyme substrate in order for this reaction to occur.³⁴

Deoxyglucose and Fluorodeoxyglucose

2-deoxyglucose (2-DG) is an analogue of glucose. 2-DG differs from glucose in that the hydroxyl group at carbon-2 (C-2) has been removed. 2-DG competes with glucose for transport into cells via the glucose transporters. Like glucose, 2-DG is a substrate for hexokinase, and is phosphorylated by hexokinase to 2-deoxyglucose-6-phosphate (2-DG-6-P).^{13;14} However, because of the structural difference between glucose and deoxyglucose, 2-DG-6-P is not a substrate for glucose phosphate isomerase.¹⁴ 2-DG-6-P

is also a poor substrate for glucose-6-phosphate dehydrogenase, the first enzyme in the pentose phosphate shunt. Thus, in the absence of significant glucose-6-phosphatase activity (which dephosphorylates glucose and 2-DG), 2-DG is phosphorylated by hexokinase and trapped within the cell, unable to undergo any further metabolism.

A radioactive compound tagged to 2-DG provides a means to measure accumulation of 2-DG within tissues.¹³ 2-flourine-2-deoxyglucose (FDG) is 2-DG with the hydrogen molecule at the C-2 position replaced by a fluorine-18 molecule. That is, glucose with the hydroxyl group at C-2 replaced by fluorine.¹⁴ The FDG model is an elegant example of a biochemical approach designed to measure a specific metabolic pathway, in this case, glycolysis. FDG retains the chemical character of 2-DG with no disruption of geometry. FDG remains an appropriate substrate for hexokinase but like 2-DG, is not a substrate for glucose phosphate isomerase.¹⁴ Therefore, phosphorylated FDG (FDG-6-P) cannot be further metabolized and all radioactivity will be in the form of either the original substrate, FDG, or the product of the hexokinase reaction, FDG-6-P.¹³ This principal of "biochemical trapping" of FDG-6-P thereby permits biochemical isolation of the hexokinase reaction.¹⁴ the radioactivity accumulating in a tissue over time is proportional to the rate of the hexokinase reaction in that tissue, which, at steady state, and in the absence of significant glucose-6- phosphatase activity, reflects the rate of glycolysis.

FDG-PET in Oncology

Introduction: Roles of PET in Oncology

Early uses of PET included imaging of brain and cardiac metabolism.^{8;35} Over the past 10 years, PET has been primarily used for evaluation and management of cancer patients.^{8;10} Recently, the following was said of PET's role in oncology: "The impact of PET imaging in cancer will be one of, if not the most important development in nuclear medicine in the next decade".⁸ FDG-PET is a uniquely sensitive imaging technique and has the following indications for use in cancer patients:

1. Tumor detection and characterization: FDG-PET is an exceptionally sensitive tool for detecting sites of malignancy and is unique in its ability to accurately differentiate malignant from benign lesions.^{8;10;36-40}
2. Staging malignant disease: FDG-PET is used as a sensitive, non-invasive method to assess regional lymph nodes for tumor involvement and to detect distant metastatic disease.^{8;10;37-46}
3. Determining prognosis: Higher uptake of FDG in malignancies has been reported to be a poor prognostic indicator in some tumors.^{8;36;38-40;47-52}
4. Assessing response to therapy: FDG-PET is more sensitive than traditional methods such as CT for detecting residual tumor following chemotherapy, because FDG-PET measures response to therapy by evaluating tumor

metabolism, an earlier indicator of treatment response than structural change.^{8;10;36;38-40;53-58}

5. Detecting recurrent disease and differentiating recurrent neoplasia from post-treatment scar tissue or necrosis: FDG-PET is effective for detecting recurrent neoplasia, and distinguishing recurrent tumor from benign changes such as fibrosis.^{8;10;36-40;59-63}

Glucose Transport and Metabolism in Tumor Cells

An identifying feature of malignant cells is aberrant energy metabolism.⁸ Transformation from a normal to a malignant cell was hypothesized by Warburg to be a 2-phase process: Phase 1 was an irreversible decrease in cellular respiration (due to many possible causes), and Phase 2 was the cell's attempt to compensate for loss of energy production via cellular respiration by increasing glucose fermentation.⁶⁴ While significantly decreased cellular respiration was subsequently proven not to occur in transformed cells, increased glycolysis is a well-documented characteristic of malignant cells.⁶⁵

In malignant cells, differences in gene expression manifest as changes in the end protein product: the enzymes. Weber reviewed the pattern of enzymatic changes linked to malignant transformation of cells.^{11;12} Malignant cells have a characteristic imbalance in "key" enzymes of biochemical pathways.^{11;12} Key enzymes are defined by characteristics relating to their biologic role, position in the metabolic pathway, and regulatory properties.^{11;12}

1. **Biologic role:** Key enzymes may regulate rate and direction of opposing metabolic pathways, be involved in surmounting thermodynamic barriers, catalyze 1-way reactions, or participate in the final common pathway of 2 or more metabolic pathways.
2. **Position in the metabolic pathway:** Key enzymes may occupy the first or last position in a reaction sequence, may be pathways in themselves, and are opposed by other 1-way enzymes.
3. **Regulatory properties:** Key enzymes characteristically have relatively low activity in the pathway, may be rate-limiting in the pathway, are the targets of feedback regulation, may exhibit allosteric properties, and may have an isozyme pattern.

The importance of key enzymes is that *the amount and activity of the key enzymes govern the rate and direction of opposing synthetic and catabolic biochemical pathways.*^{11;12}

In malignant cells, there is an imbalance in the ratios of opposing key enzymes of cellular glucose metabolism.^{11;12} The catabolic pathway of glucose is glycolysis. The opposing synthetic pathway is gluconeogenesis. There are 3 enzymes which meet the criteria of a key enzyme in glycolysis: hexokinase, phosphofructokinase, and pyruvate kinase (Figure 2), and 4 key enzymes in the pathway of gluconeogenesis: glucose-6-phosphatase, fructose 1,6-diphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase.^{11;12} Reprogramming of cancer cell gene expression results in a biochemical shift with predominance of glycolysis over gluconeogenesis due to increases in the

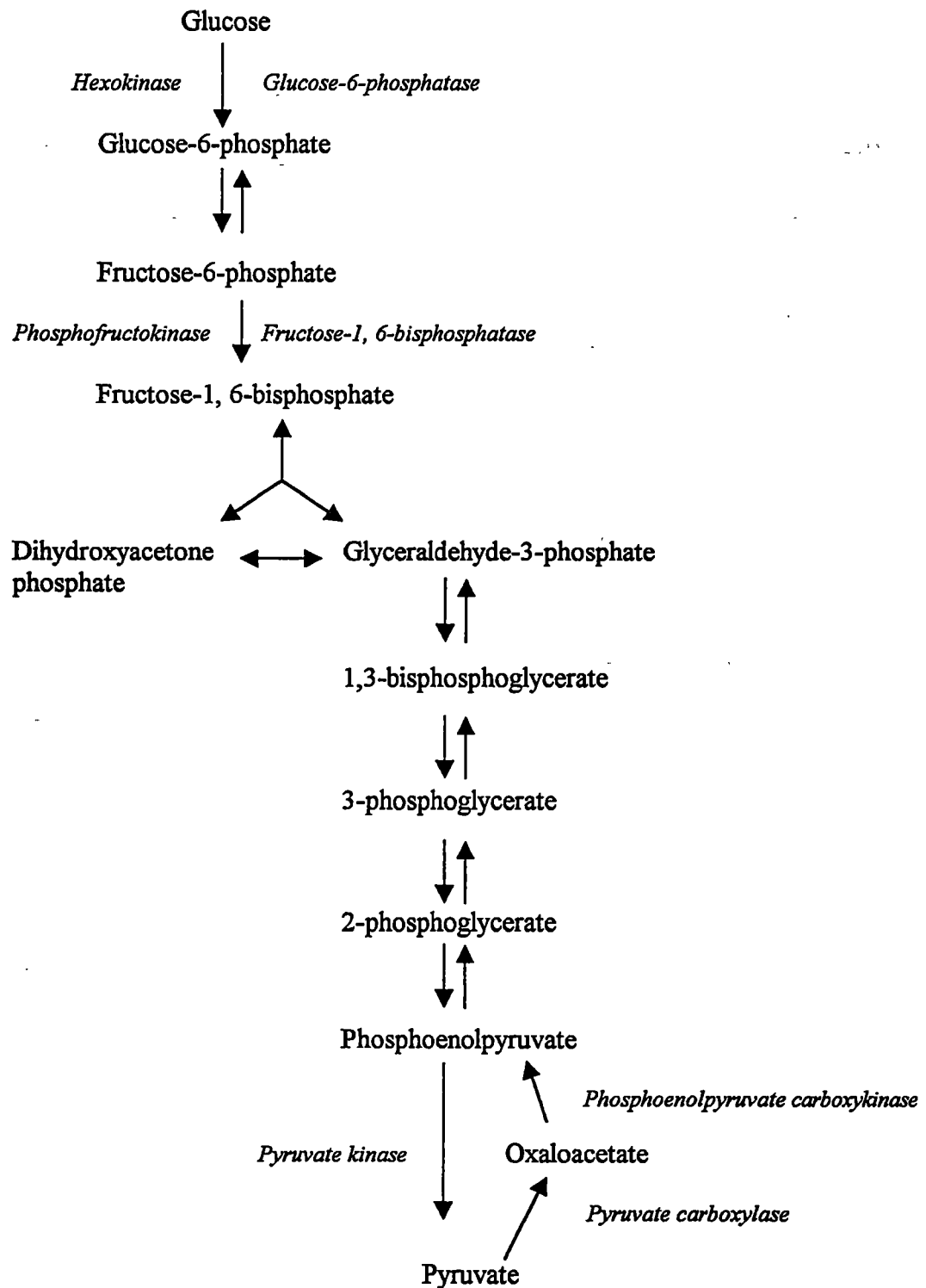


Figure 2. Key enzymes of glycolysis and opposing key enzymes of gluconeogenesis.

activity of key glycolytic enzymes with a concurrent decrease in the activity of key gluconeogenic enzymes.^{11;12} In addition, there is an "isozyme shift" in key enzymes: hexokinase with a low affinity for glucose is replaced by a predominance of a hexokinase isozyme with a high affinity for glucose, resulting in loss of responsiveness of cancer cell glycolysis to normal regulation.^{11;12}

Experimental evidence demonstrating these changes is shown in studies by Knox et al, Monahkov et al, and Weber and Cantero. Knox et al showed the presence of a marked increase in hexokinase concentration in 18 types of transplanted rat tumors.⁶⁶ The hexokinase concentration was correlated with measured growth rates and with loss of histologic differentiation. The increased hexokinase concentration was not simply a function of rate of growth; normal tissues (regenerating liver and fetal liver), grew as fast or faster than tumors and did not demonstrate the marked increase in hexokinase concentration. Monahkov et al assessed the physiochemical properties and isozyme composition of hexokinase in normal and malignant human tissues.⁶⁷ They found that:

1. Tumors have increased hexokinase activity relative to normal cells.
2. The isozyme spectrum of hexokinase differs in tumors from normal cells.
3. Tumor hexokinase isozymes demonstrate a 10x greater affinity for glucose than the corresponding isozyme from normal tissue.

Finally, Weber and Cantero demonstrated the absence of key enzymes of gluconeogenesis in a variety of transplanted and induced tumors in rats.⁶⁸

In addition to changes in glycolytic enzymes, over expression of glucose transporters is a characteristic marker of malignant transformation. Increased glucose transport in transformed cells is due to increased expression of genes encoding glucose transport proteins. Flier et al studied cultured rodent fibroblasts transfected with activated oncogenes.⁹ Cells transfected with the activated oncogenes *ras* and *src* (2 oncogenes that cause classical cell transformation) exhibited distinctly increased uptake of 2-DG. The increased uptake of 2-DG was paralleled by markedly increased amounts of glucose transport protein and glucose transporter mRNA.

Increased glucose transporters are present in many naturally occurring malignancies. GLUT 1 is the transporter most commonly over expressed. Increased amounts of GLUT 1 have been identified in non-small cell lung cancer, breast carcinoma, and pancreatic carcinoma.⁶⁹⁻⁷¹ Current studies are focusing on correlating increased numbers of glucose transporters with uptake of FDG and with prognosis.^{72;73}

To summarize, the rate of glucose consumption by tumor cells is amplified due to changes in amounts and isozyme composition of key enzymes of glycolysis, over expression of glucose transporter proteins, and a decrease in gluconeogenesis compared with normal cells. These biochemical changes result in increased uptake and sequestration of FDG by cancer cells due to increased influx of FDG, higher rate of phosphorylation of FDG, and decreased rates of dephosphorylation of FDG-6-P. These traits are exploited by using increased accumulation of FDG on FDG-PET images to detect malignancies.

Quantification of Uptake of FDG

Malignancies are detected on FDG-PET images by their high level of accumulation of FDG.^{8;10;39} Assessment of tissue glucose metabolism can be made from FDG-PET images at varying levels of complexity:^{74;75}

1. Qualitative visual inspection of static images to identify focal areas of increased FDG accumulation.
2. Semiquantitative analysis of images using the standardized uptake value or region of interest ratios (For example, lesion:background ratios).
3. Quantitative measurement of the local glucose metabolic rate using a static image with presupposed deductive estimates of the rate constants. Use of this method is limited because these rate constants are not universal. Required population estimates of these constants for a given tumor type are available for only a limited number of tumors of the central nervous system.
4. Quantitative (direct) measurement of the local glucose metabolic rate using dynamic imaging and simultaneous arterial blood sampling to determine the rate constants using compartmental modeling.

Direct measurement of the local glucose metabolic rate is the most sophisticated measure of glucose metabolism. The equation for calculating the local glucose metabolic rate is:⁷⁵

$$MR = \frac{C_p (k_1 * k_3)}{LC (k_2 + k_1)}$$

Where MR equals the estimate of the metabolic rate of glucose, C_p equals the plasma glucose concentration, $k_1 - k_3$ are the rate constants for FDG, and LC is the lumped constant.

The lumped constant is the ratio of net extraction of glucose to the net extraction of FDG (Fischman). It describes the differences in transport and phosphorylation characteristics of glucose and FDG⁷⁵⁻⁷⁷ and is comprised of 6 individual terms:

$$LC = (K_m/V_{max})_{gluc} * (V_{max}/K_m)_{FDG} * \lambda / \phi$$

Where K_m and V_{max} are the Michaelis-Menton parameters of affinity and maximum velocity of the hexokinase reaction, respectively, for glucose and FDG; λ is the ratio of the volumes of distribution for FDG and glucose; and ϕ is the measure of G-6-P that undergoes further metabolism.^{74;75}

These constants vary under different physiologic conditions *and for different tissues*.⁷⁴

While the direct measurement of glucose metabolic rate has the most sound theoretical basis for quantifying glucose metabolism,⁷⁸ its use for quantifying uptake of FDG by malignancies is limited because the lumped constant has only been determined for a limited number of CNS tumors.⁷⁵ Without knowledge of the lumped constant for a given tumor type, the direct local glucose metabolic rate cannot be determined.

Because the lumped constant is not known for most tumor types, and because the repeated arterial blood sampling necessary for measuring the absolute glucose metabolic rate is not practical for routine use in clinical patients, an alternative method for quantifying uptake of FDG by tumors was introduced. The standardized uptake value (SUV) is a simple, practical, semi-quantitative measure of FDG uptake (referred to as semi-quantitative because it is not a measure of the absolute glucose metabolic rate).^{8;10} Use of the SUV is based on the principle that it is correlated with the absolute metabolic rate of glucose.⁷⁸ Hamberg et al. reported a positive correlation of SUV with the metabolic rate of glucose at 60 minutes ($R^2 = 0.65$) in eight patients with lung cancer.⁷⁸ Lindholm et al. reported a correlation with an $R^2 = 0.75$ in 5 patients with head and neck tumors.⁷⁹

The SUV [also referred to as the differential uptake ratio (DUR) and the standard uptake ratio (SUR)] is a dimensionless measure of the concentration of radioactivity in a lesion corrected for the dose of FDG administered and the weight of the patient:^{8;10}

$$\text{SUV} = \frac{\text{Concentration of Tissue Radioactivity}}{\text{Injected Dose/Body Weight}}$$

Where the tissue radioactivity is calculated by multiplying the counts/pixel by a camera-specific calibration factor derived from a quality control phantom.

Computer software programs convert attenuation and decay corrected (to the time of injection) images into SUV images on a pixel-by-pixel basis. The SUV is then easily determined by drawing a region of interest (ROI) encompassing the desired area.

Intense controversy exists over the usefulness of the SUV.^{78;80;81} The correlation with the absolute glucose metabolic rate is only fair,⁷⁸ and there are many factors that can influence the SUV.^{78;81} Despite the recognized deficiencies of the SUV, this measurement is used routinely in the literature.

Factors other than actual lesion activity contributing to variability in the SUV include size and placement of the region of interest, scanner limitations, blood glucose concentrations, time of measurement, and patient body composition.⁸¹ These factors may alter the SUV by as much as 50% and are often not controlled or accounted for in any given study.⁸¹ A brief review of the influence of each of these factors is included below.

1. Size and placement of the ROI.^{81;82} The operator may choose to measure the mean SUV or the maximum SUV of a lesion. When measuring the mean SUV, the size and placement of the (ROI) can markedly affect the value. An ROI that extends beyond the borders of the lesion will falsely lower the SUV due to inclusion of low activity areas outside the lesion. By measuring a sphere phantom 2.7 times the full width half maximum (FWHM) of the detector, Kessler et al. showed that the mean SUV calculated using an ROI with a diameter equal to the object size resulted in a loss of 24% of activity compared with the SUV for the pixel with maximum activity.⁸³ Consequently, the maximum SUV is considered to be the most accurate representation of the activity in a lesion.⁸¹⁻⁸³

2. The partial volume effect.^{81;84} If a finite area of a tomographic image is occupied partly by a lesion with intense concentration of radioactivity and partly by adjacent normal tissue of low intensity, the activities of the lesion and adjacent tissue will be averaged for that portion of the image. This "partial volume effect" falsely lowers the amount of activity measured in the lesion due to averaging of the intense activity in the lesion with the lower activity of the neighboring tissue. In clinical patients this effect occurs for very small lesions bordered by normal tissue.

The amount by which the actual lesion activity is underestimated due to the partial volume effect is the recovery coefficient: the ratio of the measured activity in a lesion to the true activity.⁸¹ Objects measuring less than twice the resolution limit of the detector are especially susceptible to the partial volume effect. Hoffman et al showed that the recovery coefficient of a cylindrical phantom with a diameter equal to the FWHM of their camera was only 50%.⁸⁵ Kessler et al showed the recovery coefficient for a sphere diameter equal to the FWHM of their camera was only 31.6%.⁸³ Many factors, including resolution of the imaging system; lesion size; lesion geometry; and size, shape and placement of the ROI affect the recovery coefficient.⁸¹

Two approaches to account for the partial volume effect are used. One is to correct for the partial volume effect by applying a correction factor to the measured SUV that is based on known recovery coefficients of phantoms of different sizes and shapes.⁸¹ The accuracy and usefulness of this approach are hindered because knowledge of the *exact* size of the lesion is necessary, and a recovery coefficient that takes into account the

unique geometry of each lesion is required.⁸¹ Recovery coefficients are available only for specific shapes such as cylinders or spheres, resulting in inability to apply a correction factor based on a recovery coefficient that accurately represents the exact, diverse geometry of naturally occurring lesions.⁸¹

The second approach is to consider the SUV to be accurate only in lesions measuring greater than twice the resolution limit (FWHM) of the detector.⁸¹ For most scanners, this includes lesions measuring greater than 2 cm diameter.⁸¹ This is the recommended approach.⁸¹

3. Effect of time of measurement:⁸¹ The time from FDG administration to the time of measurement of the SUV markedly influences the SUV. The SUV is commonly measured 45-90 minutes after administration of FDG.^{78;81} This time point is valid in brain where plateau concentrations are achieved within one hour of administration of FDG.⁷⁸ However, FDG kinetics and glucose metabolism in tumors are not the same as in the brain and extrapolations from normal brain data may not be valid.⁷⁸ Little information regarding FDG kinetics of different tumors is available.⁷⁸ The rate of uptake and time to plateau for FDG likely differs for specific tumor types. Hamberg et al., in 8 patients with stage III non-small-cell lung cancer, showed that the (mean +/- sd) time to reach 95% of plateau was 298 +/- 42 minutes, and this value *changed* following therapy, to 154 +/- 31 minutes.⁷⁸ Measurement of the SUV during the rapid uptake phase prior to plateau of FDG accumulation results in variability of the SUV.^{74;81} In the 8 patients with non-small-cell lung cancer, Hamberg et al showed a difference between the DUR at 60

minutes and at plateau (5 hours) of $46 \pm 6\%$.⁷⁸ Even the time required to complete a scan on a single patient is enough to alter the SUV. Keyes reported an increase in the SUV of a lung tumor from 5.5 to 7.7 between 30 and 60 minutes post FDG administration.⁸¹ Weber showed an increase of the (mean $\pm$ sd) SUV from 4.5 ± 2.3 to 5.1 ± 2.3 between 40 and 60 minutes after FDG administration in 50 tumors of various types from 16 patients.⁸⁶

4. Effect of blood glucose concentration:⁸¹ Because FDG competes with glucose for entry into cells, a high blood glucose concentration will competitively inhibit uptake of FDG by cells, resulting in lower uptake of FDG and lower SUVs.^{8,87,88} Langen et al, in patients with bronchogenic carcinoma, showed that doubling of the plasma glucose concentration following glucose infusion resulted in decrease of the SUV from a mean of 5.07 to 2.84.⁸⁹ Lindholm et al, in five patients with head and neck squamous cell carcinoma, showed that SUVs ranged from 4.1 - 10.9 (mean 6.9) in the fasting state, and from 2.2 - 5.9 (mean 4.0) after doubling of blood glucose values following oral glucose loading one hour prior to FDG administration.⁷⁹

Because of the effect of hyperglycemia on the SUV, prior to FDG-PET scanning, fasting for a minimum of 4 hours,^{75,79} documenting normoglycemia⁸⁶, and establishing euglycemia in diabetic patients are recommended.

Some investigators advocate routine correction for blood glucose concentration.⁸¹

However, in euglycemic patients, Weber showed that correction for blood glucose

concentration made little difference in the SUV: without correction for serum glucose concentration the mean $\pm$ sd SUV was 5.05 $\pm$ 2.36, and with correction for serum glucose concentration the mean $\pm$ sd SUV was 4.83 $\pm$ 2.16.⁸⁶ These investigators also observed that correcting the SUV for blood glucose values did not improve reproducibility of SUV measurements compared with the conventional uncorrected SUV.⁸⁶ The authors also suggested that correction for blood glucose concentration may introduce additional error, given that glucose measurements obtained by reagent strips have a coefficient of variance of 5%.⁸⁶

5. Patient size/body composition:⁸¹ The conventional SUV represents the amount of FDG in a lesion, corrected for dose of FDG and body weight of the patient. Total body fat contributes substantially to body weight, but fat accumulates less FDG than other body tissues.⁸¹ Consequently, the SUV of non-fat tissues in obese patients may be overestimated compared with thin patients.⁹⁰⁻⁹² Some investigators have recommended correction for the SUV using body surface area or lean body mass instead of body weight.⁹⁰⁻⁹³ Other reports show no advantage to correcting the SUV using body surface area or lean body mass.³⁰

Distinguishing Malignant from Benign Disease

The importance of determining definitively whether a mass is malignant or benign is obvious as both treatment and prognosis differ. Accurately distinguishing patients with malignant disease from patients with benign disease allows patients to receive appropriate therapy as quickly as possible. The distinction between malignant and benign lesions is difficult with anatomic imaging modalities. These techniques provide detailed structural information about a lesion but do not reliably distinguish between malignant and benign masses. FDG-PET is an exceptionally sensitive tool for detecting sites of malignancy and is unique in its ability to accurately differentiate malignant from benign lesions.

FDG-PET is currently accepted as a reliable method to differentiate between malignant and benign pulmonary nodules.^{8;10;36;94-97} Evaluation of indeterminate pulmonary nodules was the first indication for FDG-PET use in oncology approved for reimbursement by the Health Care Finance Administration and Medicare (in 1998).^{8;36;37} It is estimated that 130,000-150,000 solitary pulmonary nodules (SPN) are detected annually in the United States.^{8;10;36;94-97} Pulmonary malignancy often presents as a focal nodule or pulmonary opacity on thoracic radiographs.⁹⁷ However, 40-50% of nodules resected at surgery turn out to be benign.^{94;96} Radiographs and CT are poor methods for distinguishing malignant from benign pulmonary opacities.⁹⁷ Definitive criteria for establishing a diagnosis of benign pulmonary disease on thoracic radiographs or CT include characteristic centralized calcification lesions, and/or stable nodule size after longer than 2 year follow

up.⁴ Characteristic calcification is observed infrequently and large numbers of pulmonary nodules remain indeterminate.^{4;8;97} Invasive methods for assessing indeterminate nodules such as transthoracic or transbronchial biopsies are risky and are associated with a high false negative rate of 15-50%.⁸

Many small studies have shown FDG-PET to be highly sensitive for detecting malignant pulmonary lesions.^{1;4-7;15;17;18;28;29} A historical review of these studies is outlined below and study results are summarized in Table 1. For all of these studies, the diagnosis of benign or malignant disease was made based on histopathology, cytology, or long-term (> 1 year) follow up.

In 1990, Kubota et al studied 22 prospective cases of indeterminate pulmonary masses (non-calcified on CT).²⁹ Using tumor:muscle (T:M) ratios of FDG uptake, the authors reported a sensitivity and specificity of 83% and 90% respectively for identifying solitary pulmonary nodules as malignant. The (mean +/- sd) T:M ratio for 12 malignant lesions of 4.4 +/- 2.2 was significantly greater than the T:M ratio of 1.5 +/- 0.3 for 10 benign lesions ($p < 0.001$). The authors concluded that FDG-PET may be useful for the differential diagnoses of lung tumors.

In 1993, Dewan et al studied 30 patients from 2 centers with indeterminate solitary pulmonary nodules on chest radiographs and CT.²⁸ The (mean +/- sd) DUR for 20 malignant lesions was 5.55 +/- 2.79 and was significantly greater than the DUR of 0.95 +/- 0.99 for 10 benign lesions ($p < 0.001$). Using visual inspection, the sensitivity and

Table 1. Summary of Literature Studies Evaluating FDG-PET for Detecting Malignant Solitary Pulmonary Nodules.

Author	Year	Reference	SUV (Malignant)	SUV (Benign)	P value	Sensitivity	Specificity
Lowe et al.	1998	16	6.9 +/- 3.9	1.7 +/- 1.0	< 0.001	92	90
Hubner et al.	1996	6	6.39 +/- 3.05	2.86 +/- 2.22	ND	100	73
Bury et al.	1996	1	ND	ND	ND	100	88
Gupta et al.	1996	4	6.28 +/- 3.247	1.15 +/- 0.96	yes	93	88
Knight et al.	1996	7	8.9 +/- 5	2.8 +/- 2.9	< 0.001	100	62.5
Hubner et al.	1995	5	6.4 +/- 3	2.8 +/- 2.2	< 0.001	100	67
Lowe et al.	1994	15	ND	ND	ND	97	89
Scott et al.	1994	18	6.4 +/- 0.56	1.14 +/- 0.26	< 0.0001	93.6	80
Patz et al.	1993	17	6.5 +/- 2.9	1.7 +/- 1.2	0.0001	89	100
Dewan et al.	1993	28	5.55 +/- 2.79	0.95 +/- 0.99	< 0.001	95	80
Kubota et al.	1990	29	ND	ND	ND	83	90

ND = Not done.

SUV = Standardized uptake value shown as mean +/- sd.

specificity for detecting malignant pulmonary nodules were 95% and 80% respectively. The authors concluded that FDG-PET, "a new, non-invasive diagnostic test", could accurately distinguish benign from malignant pulmonary nodules and could complement CT for evaluating patients with SPN.

In 1993, Patz et al assessed the ability of FDG-PET to distinguish malignant from benign pulmonary lesions in 51 patients with focal pulmonary abnormalities seen on chest radiographs.¹⁷ Thirty-three patients had malignant disease and 18 patients had benign disease. The SUR for the malignant lesions was 6.5 ± 2.9 and was significantly greater than the SUR of 1.7 ± 1.2 for the benign lesions ($p < 0.0001$). Using an SUR of < 2.5 to detect benign pulmonary lesions, the authors reported a sensitivity and specificity of 89% and 100% respectively for detecting benign pulmonary nodules. The authors concluded that FDG-PET was accurate for differentiating benign from malignant pulmonary nodules.

In 1994, Lowe et al compared semiquantitative analyses (SUR, mean and maximum activity ratios) and visual evaluation of FDG-PET studies for differentiating malignant from benign pulmonary lesions in 88 patients with indeterminate focal pulmonary nodules. Sixty-one lesions were malignant and 27 were benign.¹⁵ Using visual analysis, FDG-PET had a sensitivity of 97% and a specificity of 89% for detecting malignancy. Receiver operator characteristic curve analysis did not show significant differences between accuracy of semi-quantitative analyses and visual inspection for detecting

pulmonary malignancies. The authors concluded that each method was equally accurate for distinguishing malignant from benign pulmonary nodules.

Scott et al in 1994 retrospectively reviewed records from 62 patients with pulmonary masses that underwent FDG-PET scans.¹⁸ The investigators evaluating the FDG-PET scans were blinded to the patients' tissue diagnosis. Forty-seven patients had malignancies, and 15 patients had benign lesions. The (mean $\pm$ sd) DUR for malignancies was 6.4 ± 0.56 and was significantly greater than the DUR of 1.14 ± 0.26 of benign lesions ($p < 0.0001$). The authors determined that a cutoff DUR of 2.0 gave the greatest number of true positive and true negative results. Using this cutoff, the sensitivity and specificity of FDG-PET for identifying malignant lung tumors were 94% and 80% respectively. The authors concluded that FDG-PET was an accurate method for distinguishing malignant from benign lung masses.

In 1995, Hubner et al evaluated 23 patients (24 lesions) with unknown lung masses using FDG-PET.⁵ Nineteen masses were malignant and 5 were benign. Using visual inspection, FDG-PET had a sensitivity of 100% and a specificity of 67% for detecting malignant pulmonary masses. Hubner et al followed this study with a retrospective study in 1996, evaluating differentiation of malignant from benign lung lesions using semi-quantitative parameters of FDG-PET images.⁶ Twenty-six patients with benign pulmonary lesions were matched to gender, age, and blood glucose concentration with 26 patients with malignant lung tumors. Quantitation of FDG uptake in lesions was done using the SUV and Patlak analysis (accumulation rate of FDG). The mean (range) SUV

of malignant lesions was 6.39 (2.56 – 16) and was greater than the SUV of 2.86 (0.51-10.8) of benign lesions. Using visual inspection, sensitivity and specificity for detecting malignant lesions were 100% and 73% respectively. Using a cutoff SUV of 3.8 sensitivity and specificity were both 81%. Using a cutoff slope of the Patlak curve of 0.025 min^{-1} , sensitivity and specificity were both 85%. The authors concluded that FDG-PET image interpretation may be facilitated using parameters such as the SUV or Patlak analysis and that, with additional validation, these measures could have a significant impact on management of patients with indeterminate pulmonary masses.

Three additional studies evaluating the ability of FDG-PET to differentiate malignant and benign pulmonary lesions were published in 1996. Gupta et al prospectively evaluated 61 patients with radiographically indeterminate pulmonary masses.⁴ Forty-five patients had malignant lesions and 16 patients had benign lesions. The (mean +/- sd) DUR of the malignant lesions was 6.28 ± 3.247 , which was significantly higher than the DUR of the benign lesions of 1.15 ± 0.957 . Using visual analysis, the authors reported a sensitivity and specificity of 93% and 88% respectively for detecting malignancy in indeterminate solitary pulmonary nodules. Knight et al evaluated 48 patients with pulmonary lesions: 27 with no history of prior pulmonary malignancy and 21 with a history of prior pulmonary malignancy that had been surgically treated.⁷ The (mean +/- sd) SUR of all malignant lesions (n=29) was 8.9 ± 5.0 . The SUR of the benign lesions (n=16) was 2.8 ± 2.9 . The SUR of the malignant lesions was significantly greater than the SUR of the benign lesions ($p < 0.001$). Using a cutoff SUR of 2.5, the sensitivity and specificity for identifying malignancy were 100% and 63% respectively. The authors concluded that

FDG-PET could identify malignant lung tumors with high sensitivity in patients with or without a history of prior malignancy. Bury et al evaluated 50 patients with indeterminate SPNs on chest radiographs and CT scan prior to biopsy, aspirate, or thoracotomy.¹ Thirty-three lesions were malignant, and 17 lesions were benign. Using visual inspection, the sensitivity and specificity for identifying malignant pulmonary nodules were 100% and 88% respectively. The authors concluded that FDG-PET was a highly accurate technique for distinguishing malignant from benign pulmonary nodules.

A prospective multicenter trial evaluating the ability of FDG-PET to discriminate between benign and malignant pulmonary nodules was published in 1998.¹⁶ This study of a large number of patients from multiple centers provided definitive evidence for FDG-PET's ability to distinguish benign from malignant pulmonary nodules. Eighty-nine patients from 8 centers underwent FDG-PET for evaluation of newly diagnosed solitary pulmonary nodules. These patients had nodules indeterminate for malignancy on chest radiographs and CT. Findings on FDG-PET were compared with results of histopathologic examination of the nodules.

An SUV greater than 2.5 was considered positive for malignancy. The (mean +/- sd) SUV for 60 patients with pulmonary malignancies was 6.9 +/- 3.9, and the SUV for 29 patients with benign pulmonary nodules was 1.7 +/- 1.0. Sensitivity and specificity of FDG-PET for detecting malignant pulmonary nodules were 92% and 90% respectively. The authors concluded, supporting the results of the previous studies, that FDG-PET

could distinguish malignant from benign solitary pulmonary nodules with a high degree of accuracy.

Recently, FDG-PET has been evaluated as a means to distinguish malignant from benign disease of many organs, including lesions of the soft tissues, central nervous system, bone, breast, pleura, liver, adrenal gland, peripheral nerve sheath, and pancreas.⁹⁸⁻¹¹⁰ A summary of findings from the recent literature is shown in Tables 2 and 3. For example, pancreatic carcinomas typically appear as focal pancreatic masses on CT or ultrasound. Chronic pancreatitis can also appear as a focal mass, necessitating exploratory surgery to obtain a definitive diagnosis. Studies show that the SUV of pancreatic carcinoma is significantly higher than the SUV for pancreatitis.^{98;103-105;110} Most authors have concluded that FDG-PET is highly sensitive for identifying pancreatic carcinoma.^{98;103-105;110} As with SPN, specificity of focal pancreatic uptake of FDG for pancreatic carcinoma is less than sensitivity in the majority of studies. Shreve et al showed that focal, high intensity uptake of FDG in the pancreas could be misinterpreted as neoplasia.¹⁰⁶ SUVs of 3.1-11.2, covering the range of SUVs observed for pancreatic carcinoma, were measured on FDG-PET images from 12 patients with pancreatitis.¹⁰⁶

Table 2. Summary of Literature Studies Evaluating FDG-PET for Detecting Malignant Pancreatic Masses.

Author	Year	Reference	SUV (Malignant)	SUV (Benign)	P value	Sensitivity	Specificity
<i>Delbeke et al.</i>	1999	110	5.1 +/- 2.6	0.85 +/- 1.7	>0.0001	92-100	77-85
<i>Imdahl et al.</i>	1999	103	7.3 +/- 2.9	3.5 +/- 0.4	ND	96	100
<i>Zimny et al.</i>	1997	105	6.4 +/- 3.6	3.6 +/- 1.7	<0.001	85-89	53-84
<i>Ho et al.</i>	1996	104	4.1 +/- 0.7	2.2 +/- 0.2	0.003	100	67
<i>Stolfuss et al.</i>	1995	98	3.16 +/- 1.22	1.0 +/- 0.55	<0.001	93-95	90-93

ND = Not done.

SUV = Standardized uptake value shown as mean +/- sd.

Table 3. Summary of Literature Studies Evaluating FDG-PET for Detecting Malignancies in Various Tissues.

Author	Year	Reference	Tissue	SUV (Malignant)	SUV (Benign)	P value	Sensitivity	Specificity
<i>Ferner et al.</i>	2000	101	PN Sheath	5.4 +/- 2.4	1.54 +/- 0.7	0.002	ND	ND
<i>Delbeke et al.</i>	1998	102	Liver	7.8 +/- 4.5	2.0 +/- 1.7	<0.001	ND	ND
<i>Benard et al.</i>	1998	109	Pleura	4.9 +/- 2.9	1.4 +/- 0.6	<0.0001	91	100
<i>Avril et al.</i>	1996	108	Breast	3.3 +/- 1.8	1.4 +/- 0.5	<0.01	75	100
<i>Dehdashti et al.</i>	1996	99	Intraosseous	5.5 +/- 4.8	1.3 +/- 0.7	0.004	93	80
<i>Villringer et al.</i>	1995	100	CNS	3.2-13.2	1-3.9	<0.05	ND	ND

ND = Not done.

SUV = Standardized uptake value shown as mean +/- sd.

PN = Peripheral Nerve

Cancer vs Inflammation

Uptake of FDG by Inflammatory Lesions

FDG-PET is not 100% accurate for diagnosis of malignancy, and both false negative and false positive results occur. Because of the high sensitivity of FDG-PET for identifying malignancies, few false negative results are observed. False negative findings may be due to small lesion size (resulting from the partial volume effect and/or resolution limits of the camera) or hyperglycemia.⁸ Some bronchogenic carcinomas have little uptake of FDG resulting in a false negative diagnosis.^{8;97;111} FDG-PET is not specific for malignancy because any tissue with increased glucose metabolism will accumulate FDG.¹⁹⁻²⁴ Uptake of FDG by inflammatory cells occurs because inflammatory cells utilize increased amounts of glucose for chemotaxis, phagocytosis, and respiratory burst.²⁴⁻²⁷ Accumulation of FDG occurs in infectious and non-infectious inflammatory lesions, including CNS¹¹² and abdominal¹¹³ abscesses, pneumonia,^{113;114} pancreatitis,^{106;113} CNS toxoplasmosis,¹⁰⁰ mastitis,¹¹⁵ rheumatoid arthritis-associated lung disease,¹¹⁶ osteomyelitis,^{27;117} sarcoidosis,^{118;119} and tuberculosis.^{120;121} In most cases the intensity of uptake of FDG by inflammatory lesions is less than for malignancies, helping to distinguish malignant from benign lesions.^{3;4;7;16;17;28;122}

Some inflammatory lesions, however, have intense uptake of FDG and are mistaken for malignancy. This results in specificities of FDG-PET for identifying malignancy that are less than sensitivities in most studies. In evaluating SPNs using FDG-PET, benign

lesions misdiagnosed for malignancy are most often lesions of granulomatous inflammation, including lesions of tuberculosis, sarcoidosis, and fungal infections^{1;3-7;16-18;28-30;122}. High intensity uptake of FDG by granulomatous inflammatory lesions is likely due to avid uptake of FDG by macrophages. Kubota et al showed that macrophages accumulate 3-4 times as much FDG as tumor cells in a transplanted mammary carcinoma model.²⁰

Fungal Lesions Misdiagnosed for Malignancy

In the studies evaluating FDG-PET for identifying malignant SPNs, lesions misdiagnosed for malignancy due to intense uptake of FDG were commonly due to fungal infections. Seven of 12 studies investigating use of FDG-PET for imaging pulmonary nodules reported pulmonary masses due to fungal infection that resulted in misdiagnosis for malignancy.^{2;4-6;16;28;30} In 3 of these studies, the only false positive results were due to fungal infections.^{2;4;28} False positive results in these and the remaining studies included lesions caused by fungal infections as well as other granulomatous lesions.^{1;2;4-7;16;28-30} When the SUVs of the false positive fungal lesions were reported, SUVs ranged from just slightly above the cutoff SUV for malignancy to as high as for malignancies in the same study. Dewan et al reported 2 caseating *Histoplasma* granulomas with SUVs of 1.92 and 3.38, examples of fungal lesions with SUVs in the low range for malignancy.²⁸ Hubner et al reported a *Blastomyces* granuloma with an SUV of 10.8, well into the high range for malignancy.⁶ This SUV of 10.8 was greater than the (mean +/- sd) SUV of benign lesions of 2.86 +/- 2.22, and malignant lesions of 6.39 +/- 3.05 in the same study, and

exceeded the SUV of 20/23 malignancies in the study. The 3 malignancies with SUVs greater than the *Blastomyces* granuloma had SUVs of 10.96, 12.09, and 15.94.

Hubner et al also reported a *Blastomyces* granuloma misdiagnosed for malignancy with an SUV of 5.53.⁵ The SUV of 5.53 for the *Blastomyces* granuloma was in the moderate range for malignancy, as the SUV for malignancies in this study was 6.4 +/- 3.0.

In the multicenter trial investigating FDG-PET in pulmonary nodules, a *Histoplasma* granuloma with an SUV of 5.0 was the highest SUV of 3 false positive lesions (The other 2 lesions were granulomas with SUVs of 3.0 and 3.6 for which the etiology was not specified).¹⁶ The SUV for the *Histoplasma* granuloma was higher than the SUV of 20/60 of the malignancies in this study. The blood glucose of this patient was markedly elevated at 411 mg/dl, and the SUV of this lesion may have been even higher if the patient had been euglycemic.

High uptake of FDG has not been limited to fungal infections in the lung. Delbeke et al, in a study evaluating the ability of FDG-PET to distinguish malignant from benign hepatic lesions, reported 3 granulomatous inflammatory lesions misdiagnosed for malignancy.¹⁰² The etiology of only one of the granulomatous lesions was specified and was a yeast abscess with an SUV of 9.6. The SUV of the malignant lesions in these studies was 7.8 +/- 4.5. Individual SUVs were not reported for all lesions, but an SUV of 9.6 for this fungal granuloma would be expected to be greater than for many of the malignant lesions in the study.

Kuwabe et al reported an SUV of 3.7 for paranasal sinus Aspergillosis.¹²³ The authors reported that an SUV of 3.7 is commonly seen in head and neck tumors. In one study cited, the SUV of head and neck tumors ranged from 2 – 5.9, with 32/48 of these malignancies having SUVs < 3.7.¹²⁴ In another study, 8/22 head and neck malignancies had an SUV < 3.7.^{124;125} The authors concluded that Aspergillosis could be difficult to differentiate from head and neck malignancies.^{123;124}

These studies show that fungal lesions can have high intensity uptake of FDG and be misdiagnosed for malignancy with relative frequency. Of the inflammatory lesions misdiagnosed for malignancy, many are fungal-associated inflammatory lesions. Not all fungal granulomas show high intensity uptake of FDG, however. Many fungal granulomas have SUVs below the cutoff for malignancy and are readily identified as benign.¹⁶ However, these observations are based on limited numbers of fungal lesions. There is no series of fungal lesions reported to provide a range of SUVs typical for fungal-associated inflammatory lesions, and no studies published which compare a series of fungal lesions with other benign or malignant lesions. Further information is needed to draw firm conclusions regarding uptake of FDG by fungal-associated inflammatory lesions.

FDG-PET for Imaging Infectious/Inflammatory Disease

While infectious/inflammatory lesions may interfere with definitive diagnoses of malignancy, reports are emerging with evidence for the usefulness of FDG-PET for

detecting sites of infection and monitoring infection in patients with known or suspected infectious/inflammatory disease. These reports consist primarily of small numbers of patients and the authors universally conclude that more data is needed. However, the early evidence supports the conclusion that FDG-PET detects infections with high sensitivity and can be used to monitor infection/inflammation. The success of FDG-PET in accurate detection and monitoring of infectious/inflammatory disease has been reported for sarcoidosis,^{118;126} bacterial infections,^{26;27;127} and fungal infections.^{26;128;129} FDG-PET has some advantages over traditional nuclear imaging techniques used to image infection and inflammation such as ⁶⁷Ga or Tc-labeled anti-granulocyte antibody.²⁷ These advantages include more rapid time to diagnosis, higher sensitivity, and absence of the need for technical, time-consuming radiopharmaceutical labeling procedures.²⁷

Summary

Positron emission tomography provides information regarding physiologic and metabolic processes in the body. FDG-PET is useful where CT has known limitations, such as distinguishing benign from malignant lesions³⁸. Fluorine-18-fluorodeoxyglucose is used in PET to detect malignancies based on their increased glucose metabolism. The amount of FDG accumulating in a lesion can be quantified using the standardized uptake value. The standardized uptake value is a semi-quantitative measure of glucose metabolism. Malignant lesions are identified by SUVs that are typically higher than the SUVs of benign lesions. FDG-PET is highly sensitive for identifying malignancies, but specificity is lower due to uptake of FDG by inflammatory lesions. Inflammatory lesions,

particularly granulomatous lesions, have high intensity uptake of FDG and may be misdiagnosed for malignancy. False positive diagnoses for pulmonary malignancy frequently result due to high intensity uptake of FDG by fungal infections. However, more information is needed to draw firm conclusions regarding uptake of FDG by fungal-associated inflammatory lesions.

CHAPTER 3

A Technique for Creating Localized Subcutaneous *Blastomyces* Granulomas in Rats

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Abstract

Subcutaneous *Blastomyces* abscesses were induced in rats following injection of 10^8 and 10^{10} organisms. Nodules first appeared after 4-7 days and reached 2-15 mm in diameter by 7-28 days after inoculation. No systemic spread of *Blastomyces* organisms was observed. Live yeasts were recovered from all lesions. Experimentally induced lesions were characterized by a necrotic center surrounded by a layer of viable yeast and granulomatous inflammation.

Introduction

Blastomycosis is the systemic fungal disease caused by *Blastomyces dermatitidis*.¹³⁰ The most common route of infection is via inhalation, with subsequent dissemination from the lungs to other organs.¹³⁰ Alternatively, blastomycosis may result from local inoculation of *Blastomyces* organisms into the skin via trauma or a wound.¹³⁰

The pathology of blastomycosis, host and pathogen factors in disease, host immune response to infection, and evaluation of therapeutic agents have been widely studied using animal models of pulmonary and disseminated blastomycosis.¹³¹⁻¹³⁹ The most extensively used species for studying experimental blastomycosis has been the mouse.¹³⁹ Experimental blastomycosis has been created in mice using both the yeast and spore forms of *Blastomyces dermatitidis*.^{131;133;137-143} The organisms have been administered intravenously, intratracheally, intraperitoneally, and intranasally.^{133;137;140-143} Other species used for the study of experimental blastomycosis include hamsters, rabbits, guinea pigs, dogs, and rats.^{134;141;144-147}

Skin lesions are common with blastomycosis, but there are few reported models of cutaneous experimental blastomycosis. Salfelder described subcutaneous inoculation of *Blastomyces dermatitidis* in hamsters.¹⁴⁶ Hamsters were inoculated subcutaneously in the foreleg or over the abdomen with 10 to 10⁶ organisms. Sixteen of 34 hamsters formed grossly visible subcutaneous nodules. Twenty-four of 34 hamsters had histologic inflammation with yeast cells. Rats inoculated with 10⁴ and 10⁶ yeast organisms most

consistently showed gross or histologic lesions. Twenty-one days after inoculation the lesions were characterized histologically as histiocytic granulomas with central necrosis and abscessation. Yeasts were present in all lesions. No hematogenous spread was observed.

Landay et al inoculated hamsters subcutaneously with 10^5 yeast organisms and 10^5 fungal mycelia.¹⁴⁵ All hamsters formed abscesses at the site of injection, ranging from 0.1-1.5 cm in diameter. In contrast to Salfelder, these investigators did observe disseminated disease after subcutaneous inoculation with this dose that was fatal in some animals. The authors suggested this might be due to differences in virulence between different isolates of *B. dermatitidis*.

We were unable to find a report of a method for producing experimental cutaneous blastomycosis in rats. This paper describes a technique for producing localized subcutaneous *Blastomyces* nodules in rats.

Materials and Methods

Animals

Thirty-nine young, adult male Sprague-Dawley rats were used for the study. Rats were housed at the University of Tennessee College of Veterinary Medicine Laboratory

Animal Facility. Commercial rat chow and water were available ad libitum. The University of Tennessee Animal Care and Use Committee approved all protocols.

Preparation and Administration of the Blastomyces Inoculum

All handling procedures for inoculum preparation were conducted in accordance with the Centers for Disease Control/National Institute of Health recommended biosafety guidelines for Biosafety Level II/III agents. An isolate of *Blastomyces dermatitidis* (MI 99-427) from a clinical case of blastomycosis in a dog was used. Growth conditions and methods for inoculum preparation were those described by Chang et al. Briefly, *B. dermatitidis* was maintained in the yeast form at 35° C on Middlebrook 7H10 medium containing oleic acid/albumin/dextrose/catalase. Fresh, 4 day-old cultures were harvested in sterile phosphate buffered saline (PBS) containing 0.1 % bovine serum albumin. Yeast were washed by centrifugation (30 minutes at 1500 g) and suspended in PBS with 0.1 % bovine serum albumin. To obtain yeast in primarily single and doublet form the suspension was repeatedly aspirated through a 25 gauge needle. Serial dilutions were prepared in PBS with 0.1 % bovine serum albumin, and yeasts in diluted samples were counted microscopically with a hemocytometer. A clump of yeast was counted as one cell in the microscopic assay. The volume of the concentrated suspension was adjusted to obtain the desired yeast concentration. As a control, 4 replicate samples (10 uL) from each yeast dilution was plated on Columbia Agar plates containing 5 % sheep blood and incubated at 35° C to determine the number of colony forming units (CFU). In each case the CFU closely paralleled the microscopic count ($\pm 0.5 \log_{10}$).

Administration of the Blastomyces Inoculum

All rats were inoculated subcutaneously in the region over the distal right or left tibia. Hair was clipped from the site prior to inoculation. Rats were anesthetized with isoflurane by mask for injection. Rats were examined daily for pain or lameness, and signs of systemic disease including lethargy, decreased appetite etc. The injection site was examined twice weekly and presence of visual or palpable nodules recorded.

Dose of Inoculum

Four rats were used to test various dose concentrations. One rat was inoculated with each of the following doses: 10^4 yeast organisms, 10^5 yeast organisms, 10^8 yeast organisms, and 10^{10} yeast organisms. Ten additional rats were inoculated with 10^8 organisms, and six additional rats were inoculated with 10^{10} organisms to confirm the efficacy of these doses.

Histologic Character of Nodules and Evaluation for Systemic Blastomycosis

The remaining 19 rats were inoculated with 10^8 or 10^{10} organisms. The rats were euthanized with intravenous or intraperitoneal sodium pentobarbital 7, 10, 14, 21, or 28 days after inoculation to evaluate the histologic character of the *Blastomyces* nodules at varying time points.

A total of 30 rats were inoculated with 10^8 organisms. Twenty-eight of the 30 rats formed nodules. Of these 28 rats, 10 rats were euthanized 7 days after injection, 7 rats were euthanized 10 days after injection, 2 rats were euthanized 14 days after injection, and 9 rats were euthanized 21 days after injection. A total of 7 rats received 10^{10} organisms. Six of the 7 rats formed nodules. Of these 6 rats, 1 was euthanized 21 days after injection, and 5 were euthanized 28 days after inoculation.

The *Blastomyces* nodules were dissected from surrounding subcutaneous tissue and placed in formalin for histopathologic examination. A complete post-mortem examination was done. Samples of heart, lungs, liver, spleen, kidney and testis were placed in formalin.

Formalin fixed tissues were processed routinely for histologic examination. Serial sections from each tissue were stained with hematoxylin and eosin (HE) and Gomori's methenamine silver (GMS) stain and examined by a pathologist (PNB).

Recovery of Yeast Organisms

To confirm the presence of live yeast organisms, a sample from each nodule was submitted for culture. Samples were minced into fine pieces and inoculated onto Columbia Agar containing 0.5% sheep blood and incubated at 35° C, and onto Sabouraud's Agar with and without 100 ug/ml gentamicin, 50 ug/ml chloramphenicol,

and 500 ug/ml cycloheximide, and incubated at 30° C. Mold to yeast conversion by subculture on Brain Heart Infusion Agar at 35° C was used to confirm identification.

Results

Clinical Findings

No adverse clinical signs were observed in any of the rats. Rats were bright, alert and active throughout the duration of the study. Rats did not exhibit any signs of pain or lameness at the inoculation site.

Dose of Inoculum

No nodules were palpated in rats receiving 10^4 or 10^5 yeast organisms. Nodules were present 21 days after inoculation in rats receiving 10^8 and 10^{10} organisms.

Inoculating additional rats with each of the 2 higher doses was done to confirm the efficacy of these doses. Nodule formation was observed in 5/6 additional rats inoculated with 10^{10} organisms. Seven of 10 additional rats inoculated with 10^8 organisms formed nodules. Two of the 3 rats that did not form nodules were inoculated a second time. These rats were inoculated approximately 2 months after the first injection, in the opposite leg. Both rats formed nodules following the second injection. These 2 nodules are included in subsequent results.

Based on these results, a dose of 10^8 organisms was used for the remaining 19 rats.

Eighteen of these 19 rats formed nodules.

In summary, 28/30 rats receiving 10^8 organisms formed nodules. Six of 7 rats receiving 10^{10} organisms formed nodules. A total of 34/39 rats in the study formed nodules.

Gross Appearance of Nodules

Visual or palpable evidence of nodule formation was noted as early as 3 days after inoculation. Nodules appeared in most rats by 4 days after inoculation, and all 34 rats had visible or palpable nodules by 7 days after inoculation. Nodules harvested after 7, 10, or 14 days ranged in diameter from 2-5 mm. Nodules harvested after 21 or 28 days ranged in diameter from 2 -15 mm (Figure 3).

Nodules were confined to the subcutaneous space in all rats except for 1 rat with a 1 cm diameter nodule that drained through the skin.

Recovery of Yeast Organisms

The presence of viable yeast organisms was confirmed by culture from all recovered nodules.

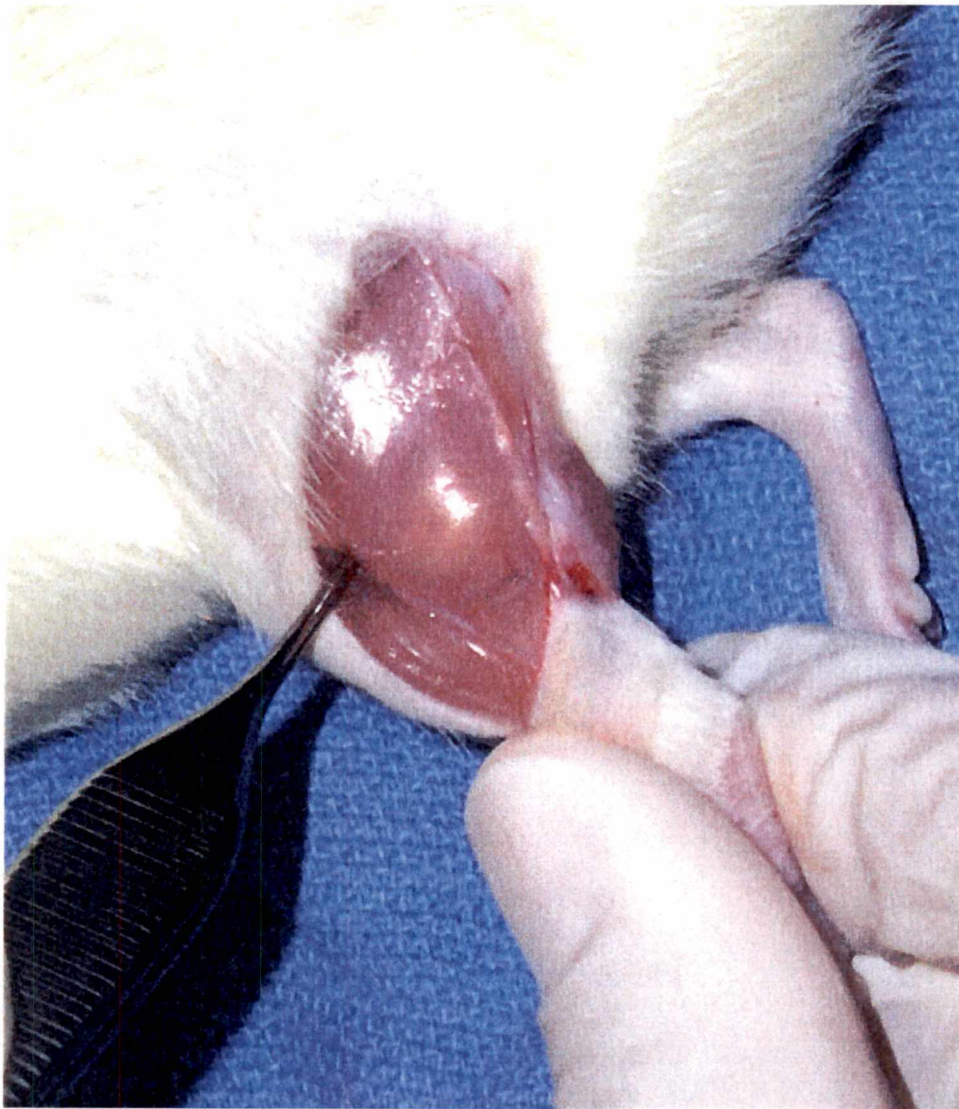


Figure 3. Subcutaneous *Blastomyces* nodule in a rat 7 days after inoculation with 10^8 yeast organisms.

Histologic Character of Nodules and Evaluation for Systemic Blastomycosis

No gross lesions of thoracic or abdominal organs were seen on post mortem examination.

No *Blastomyces* organisms were observed in any rat on histologic evaluation of heart, lung, liver, spleen, kidney and testes.

Nodules harvested 7, 10, 14, and 21 days after inoculation with 10^8 organisms were histologically similar. The subcutaneous nodules extended into the adjacent musculature. The nodules were characterized by a center portion comprised of abundant cellular debris with eosinophilic (degenerate) yeast organisms consistent in morphology with *Blastomyces dermatitidis* (Figure 4). The necrotic center region was surrounded by a layer of yeast organisms admixed with inflammatory cells. The inflammatory cells included numerous macrophages, lymphocytes, plasma cells, and few neutrophils and eosinophils. In some rats, this layer also contained multinucleated giant cells, fibroplasia, and angiogenesis. On hematoxylin and eosin stained sections the yeast organisms in this layer had a basophilic central area with a clear capsule and were presumed to be viable. Most of the yeast organisms were extracellular, with occasional yeasts within macrophages and giant cells. The size of the necrotic center and the width of the yeast and inflammatory layer varied between rats. Subjectively, nodules harvested after 21 days had a larger necrotic center and fewer viable yeast organisms. The lesions were characterized histologically as severe chronic locally extensive granulomatous and lympho-plasmacytic myositis with intralesional fungal yeast, consistent with a focal fungal granuloma and associated tissue reaction.

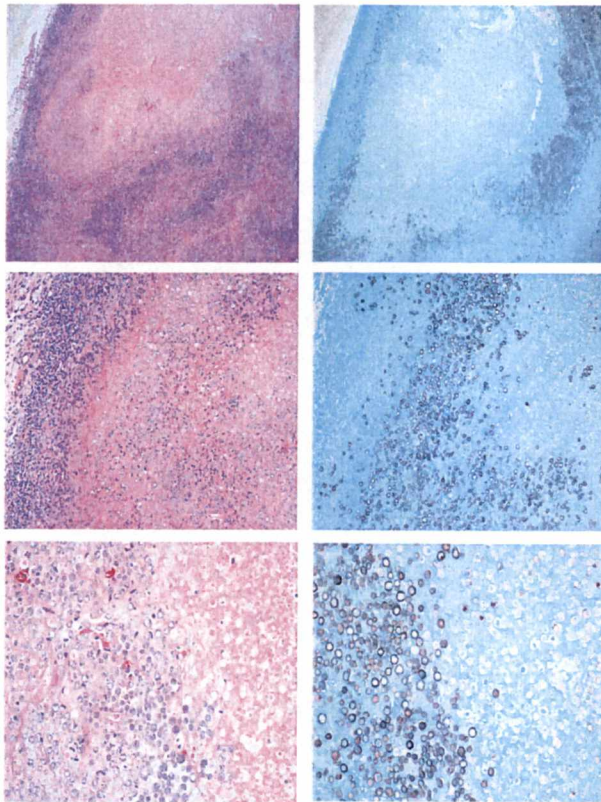


Figure 4. Hematoxylin and eosin (Left) and Gomori's methenamine silver (Right) stained sections of a subcutaneous *Blastomyces* nodule in a rat 7 days after inoculation with 10^8 yeast organisms. (Top) 2.5x magnification. (Center) 10x magnification. (Bottom) 20x magnification. The lesion is characterized by a central region containing necrotic cellular debris with degenerate yeast organisms. Adjacent to the necrotic center is a layer of viable yeast organisms and inflammatory cells. Closest to the necrotic center this layer is predominantly composed of yeast organisms. The proportion of admixed inflammatory cells increases toward the periphery of the layer such that the outermost edge is composed almost exclusively of inflammatory cells. The inflammatory layer is composed of macrophages, lymphocytes, plasma cells, neutrophils and eosinophils. Gomori's methenamine stain highlights yeast organisms.

Nodules harvested 28 days after inoculation with 10^{10} organisms were histologically similar to the previously described nodules, except that lesions subjectively appeared to be composed of a relatively larger portion of central necrosis and very few viable yeast organisms.

Discussion

This study describes a simple, reliable technique for inducing subcutaneous localized *Blastomyces* abscesses in rats. Doses of 10^8 and 10^{10} organisms consistently resulted in nodule formation at the site of inoculation by 7 days after inoculation, with no evidence of systemic spread of blastomycosis. Doses of 10^8 and 10^{10} organisms resulted in formation of nodules in a high proportion of the rats in this study. These doses are higher than doses used to successfully create subcutaneous *Blastomyces* granulomas in hamsters.^{145,146} Doses of 10^4 and 10^5 organisms failed to induce nodule formation in a small number of rats in this study. A larger number of organisms may have been required because rats are less sensitive to infection with *Blastomyces* than are hamsters.¹⁴¹ It is possible that doses less than those used in this study but greater than those used in the hamsters may have induced nodule formation in the rats of this study. The doses of 10^8 and 10^{10} organisms used in this study were arbitrary choices. Because the purpose of the study was to identify a dose that would consistently induce nodule formation, and the chosen doses provided the desired results, other doses were not evaluated.

The histologic character of the subcutaneous *Blastomyces* nodules in the rats of this study was similar to that of the subcutaneous nodules described in hamsters.¹⁴⁶ The granulomatous/pyogranulomatous nature of the inflammation in the experimentally induced nodules in this study is similar to that described for naturally occurring *Blastomyces*-associated inflammatory lesions. The makeup of the experimentally induced granulomas, with a central zone of abscessation surrounded by granulomatous inflammation, is similar to the microabscesses occasionally seen in naturally occurring blastomycosis.^{130;148-150} However, most lesions of naturally occurring blastomycosis are characterized by pyogranulomatous lesions composed of neutrophils, macrophages and multinucleated giant cells with occasional yeast dispersed throughout the inflammatory cells.^{130;148-150} The large central area of necrosis surrounded by clusters of yeast organisms seen in the experimental lesions of the rats in this study is not typical of naturally occurring lesions. Differences between the organization of these experimental lesions and naturally occurring lesions may be due to the mode of infection, or to the difference in ages of lesions. Blastomycosis in people and animals is typically not diagnosed until the disease has been present for weeks or months, compared with the lesions in this study that were only days old.^{130;149;150}

There was no evidence of systemic disease in any of the rats of this study. This may have been due to the doses used, or to the strain of *Blastomyces dermatitidis* used. The same strain was used for all rats in this study. We cannot conclude that systemic spread would not occur using a different strain of *Blastomyces*.

Conclusions

Subcutaneous *Blastomyces* abscesses can be induced in rats following injection of 10^8 and 10^{10} organisms. Nodules first appear after 4-7 days and reach 2-15 mm in diameter by 7-28 days after inoculation. No systemic spread of *Blastomyces* organisms was observed in this study. Live yeasts were recovered from all lesions. Experimentally induced lesions are characterized by a necrotic center surrounded by a layer of viable yeast and granulomatous inflammation.

CHAPTER 4

Comparison of Uptake of Fluorine-18-Fluorodeoxyglucose by Fungal-Associated Inflammation with Non-Fungal Inflammation and Neoplasia in Experimentally Induced Lesions in Rats

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Abstract

The granulomatous inflammatory lesions of fungal infections, tuberculosis, and sarcoidosis may be misdiagnosed as tumors on positron emission tomography (PET) using fluorine-18-fluorodeoxyglucose (FDG). The standardized uptake value (SUV) of fungal lesions can be as high as for neoplastic lesions. The objective of this study was to characterize FDG uptake by fungal-associated inflammation: to describe the distribution

of FDG uptake in *Blastomyces*-induced granulomas, and to compare uptake of FDG in fungal-associated inflammation with uptake by non-fungal inflammation and malignancy. In 18 rats (Group 1), subcutaneous *Blastomyces* granulomas and subcutaneous turpentine abscesses were induced. In another 7 rats (Group 2), subcutaneous *Blastomyces* granulomas and subcutaneous lymphomas were induced. The rats received 2 mCi FDG intravenously 1 hour before euthanasia and harvesting of the lesions. The lesions were weighed, and activity was counted in a well counter. The differential uptake ratio (DUR) for lesions and a sample of semitendinosus muscle were calculated. Frozen tissue sections of *Blastomyces* lesions and turpentine abscesses were placed in contact with radiographic film for macroautoradiography. The median (range) DURs for the *Blastomyces* granulomas, turpentine abscesses, and muscle from the rats in Group 1 were 1.9 (1.1-2.6), 0.9 (0.6-1.4), and 0.2 (0.1-0.5), respectively. There was a significant difference in the DURs among the three tissue types ($p < 0.001$). Pairwise multiple comparisons indicated significant differences between the DURs of *Blastomyces* granulomas and muscle ($p < 0.05$), *Blastomyces* granulomas and turpentine abscesses ($p < 0.05$), and turpentine abscesses and muscle ($p < 0.05$). The median (range) DURs for the *Blastomyces* granulomas, lymphomas, and muscle from the rats in Group 2 were 1.8 (1.2-3.4), 1.9 (1.0-4.0), and 0.2 (0.1-0.3), respectively. There was a significant difference between the median DURs from the three tissue types ($p = 0.001$). Pairwise multiple comparisons indicated significant difference between the DURs of *Blastomyces* granulomas and muscle ($p < 0.05$), and lymphomas and muscle ($p < 0.05$). There was no significant difference between the DURs of *Blastomyces* granulomas and lymphomas. Macroautoradiographs of the *Blastomyces* granulomas revealed intense uptake of FDG in

the region occupied by the yeast organisms and the granulomatous inflammation. Uptake of FDG by the yeast organisms was suspected but not proven in this study. The intensities of FDG uptake by the granulomatous inflammatory region of the *Blastomyces* granulomas and by the inner inflammatory layer of the turpentine abscess were significantly higher than the intensity of the outer inflammatory layer of the turpentine abscess. There was no significant difference between the intensities of uptake of FDG by the *Blastomyces* granuloma and the inner layer of the turpentine abscess. Results of this study show that intense uptake of FDG is associated with the region occupied by yeast organisms and the granulomatous inflammatory response of *Blastomyces* granulomas. The DUR of experimentally induced fungal-associated inflammation (*Blastomyces* granulomas) was higher than for experimentally induced non-fungal inflammation (turpentine abscesses). The DUR of *Blastomyces* granulomas was similar to experimentally induced lymphomas. The marked inflammatory response associated with the experimental lymphomas may have increased the uptake of FDG and the DUR.

Introduction

Positron emission tomography (PET) using fluorine-18-fluorodeoxyglucose (FDG) is widely used for imaging tumors.^{8;10;23;36;38;39} FDG-PET is particularly valuable for distinguishing malignant from benign disease.^{1-7;15-18} The amount of FDG accumulating within a lesion can be measured from the PET images using the semi-quantitative standardized uptake value (SUV).^{8;10} Malignant lesions typically have a higher SUV than do benign lesions.^{1-7;15-18} Lesions with SUVs above the cutoff threshold, typically 2.5-

3.5, are considered malignant. Lesions with SUVs below the threshold are considered benign.^{8;10}

FDG-PET has a reported sensitivity of 90 to 100 % for detecting malignant pulmonary nodules.^{1-7;15-18} However, FDG-PET is not 100% specific; inflammatory lesions with SUVs in the malignant range may be misdiagnosed as malignancy.¹⁹⁻²⁴ Most false positive benign inflammatory lesions have SUVs only slightly above the cutoff value, in the low range for malignancy.

Granulomatous inflammatory lesions resulting from fungal infection, tuberculosis, and sarcoidosis can have SUVs in the range for malignancy thereby resulting in a false positive diagnosis for malignancy.^{1;2;4-7;16;17;28-30} This finding is not surprising since macrophages accumulate 3-4 times more FDG than tumor cells.²⁰ In studies of cancer patients, the SUV for fungal lesions misdiagnosed for malignancy can be as high as for malignant lesions.^{2;4-6;16;28;30} It is not known whether uptake of FDG by the fungal organisms contributes to uptake of FDG by fungal lesions. Not all fungal lesions have high SUVs; many fungal lesions have SUVs below the cutoff for malignancy and are readily identified as benign.¹⁶

There are too few reports of fungal lesions imaged with FDG-PET to draw firm conclusions regarding the uptake of FDG by fungal inflammation. The objective of this study was to characterize the uptake of FDG by fungal-associated inflammation: to describe the distribution of FDG uptake in *Blastomyces*-induced granulomas, and to

compare uptake of FDG in fungal granulomas with uptake by non-fungal inflammation and tumors. When administered subcutaneously to rats, *Blastomyces dermatitidis* yeast organisms, turpentine, and malignant rat lymphoma cells produce *Blastomyces* nodules, turpentine abscesses, and lymphoma, respectively. *Blastomyces dermatitidis* is a fungal organism that is the causative agent of blastomycosis in people and animals. The inflammatory response associated with experimental and naturally occurring *Blastomyces* infection is typically characterized as pyogranulomatous or granulomatous. This study compared uptake of FDG by *Blastomyces* granulomas with uptake by turpentine abscesses and lymphomas in rats.

Materials and Methods

Animals

Young adult male Sprague Dawley and Wistar Furth rats were used for this study. Rats were housed at the University of Tennessee College of Veterinary Medicine Laboratory Animal Facility. Commercial rat chow and water were available ad libitum. The University of Tennessee Animal Care and Use Committee approved all protocols.

Blastomyces Granulomas

Blastomyces granulomas were created in 19 adult male Sprague Dawley rats (Group 1) and 10 adult male Wistar Furth rats (Group 2) by subcutaneous inoculation of 10^8 yeast

organisms over the distal tibia. The yeast suspension was prepared from a clinical isolate of *Blastomyces dermatitidis* obtained from a dog with systemic blastomycosis at the University of Tennessee College of Veterinary Medicine. Rats were injected randomly in the right or left limb. Rats were anesthetized with halothane by mask for injection. Rats were examined daily for signs of systemic disease including lethargy, decreased appetite, etc., and for pain or lameness at the injection site. Granulomas were grown for 7-14 days (Group 1) or 10 days (Group 2), at which time they were harvested.

Turpentine Abscesses

All rats in Group 1 were inoculated with 0.1 ml turpentine subcutaneously under the loose skin overlying the triceps muscle. Rats were injected randomly in the right or left limb. Rats were anesthetized with halothane by mask for turpentine injection. Buprenorphine (0.15 mg, intraperitoneally) was administered at the time of turpentine inoculation. Rats were examined twice daily for evidence of pain at the turpentine inoculation site as evidenced by lameness. Buprenorphine (0.15 mg, IP, q12-24h) was administered as needed. Abscesses were harvested 4 days after injection of turpentine.

Lymphomas

All rats in Group 2 were inoculated with 1×10^8 (C58NT) D rat lymphoma cells subcutaneously over the flank on the side opposite the *Blastomyces* inoculation site. (C58NT) D lymphoma cells are rat lymphoma cells derived from a Gross virus-induced

Wistar-Furth (WF) rat T-cell lymphoma.¹⁵¹⁻¹⁵³ When these cells are inoculated subcutaneously in WF rats a tumor forms 6-8 days after inoculation, reaching a peak size of approximately 1 cm diameter 10-12 days after inoculation.^{152;153} Rats were anesthetized with halothane by mask for inoculation. The rats were examined twice weekly for signs of illness or discomfort at the inoculation site. Lymphomas were harvested 10 days after inoculation.

Measurement of FDG Accumulation Using the Differential Uptake Ratio

On the day of harvest, rats received 2 mCi FDG intravenously (IV). After one hour to allow for localization of FDG within tissues, rats were humanely euthanized with IV sodium pentobarbital. The rats were weighed. The fungal granuloma, turpentine abscess or lymphoma, and a sample of semitendinosus muscle (from the limb opposite the *Blastomyces* granuloma) were dissected away from surrounding tissue. The granulomas, abscesses, lymphomas, and muscle samples were weighed, then counted for 60 seconds in a sodium iodide well counter interfaced to a multi-channel analyzer with the peak set to the 511 keV photopeak of F-18 gamma photons.

The efficiency of the well counter was determined using an FDG standard in 1 ml of saline. The average of the cpm/mCi was used as the conversion factor to determine dose of FDG administered to the rats in cpm.

The DUR is a semi-quantitative measure of FDG accumulation analogous to the SUV.

The equation for the DUR is:

$$\text{DUR} = \frac{\text{Tissue activity (cpm)}/\text{tissue weight (g)}}{\text{Dose of FDG (cpm)}/\text{animal body weight (g)}}$$

The DUR was calculated for the *Blastomyces* granulomas, turpentine abscesses, lymphomas, and muscles. The DUR was calculated for all lesions in all rats within 10 minutes of euthanasia.

Histopathologic Evaluation

To characterize the histologic nature of the lesions, a pathologist examined formalin-fixed tissue sections of the *Blastomyces* granulomas, turpentine abscesses, and lymphomas. The tissues were prepared routinely and stained with hematoxylin and eosin. The nature of the inflammatory responses of the *Blastomyces* granulomas and turpentine abscesses was described. A subjective evaluation was made to compare the appearance of the *Blastomyces* granulomas in the Sprague-Dawley rats (Group 1) with the *Blastomyces* granulomas from the Wistar Furth rats (Group 2). The histologic character of the lymphomas was described.

Recovery of Yeast Organisms from Infected Rats

A sample of the granuloma was minced into fine pieces and inoculated onto Columbia Agar containing 0.5% sheep blood and incubated at 35° C, and onto Sabouraud's Agar with and without 100 ug/ml gentamicin, 50 ug/ml chloramphenicol, and 500 ug/ml cycloheximide, and incubated at 30° C. Mold to yeast conversion by subculture on Brain Heart Infusion Agar at 35° C was used to confirm identification.

Macroautoradiography

Macroautoradiography was used to determine the distribution of FDG uptake in *Blastomyces* granulomas and to compare the intensity of FDG uptake between the inflammatory responses of the *Blastomyces* granulomas and the turpentine abscesses. Immediately after radioactivities of the *Blastomyces* granulomas and turpentine abscesses were measured in the well counter the tissues were sectioned in half. One half of each lesion was placed in formalin for histopathologic evaluation. The remaining half was frozen in isopentane that was cooled in liquid nitrogen. The frozen tissues were sectioned into multiple 8 um-thick sections on a cryostat at -20° C. Tissue sections were placed on glass slides, air dried, then placed in direct contact with Kodak Biomax MS Imaging Film (Eastman Kodak Company, Rochester, New York). Tissue sections from the granuloma and turpentine abscess from the same rat were placed in contact with the same piece of film. The film was placed in x-ray cassettes, exposed overnight and

developed in an automated processor. The tissue sections were then stained with hematoxylin and eosin or Gomori's methenamine silver (GMS) stain.

Image Analysis

Macroautoradiographs were digitized. The corresponding tissue sections were photographed with a digital camera. Using image-analysis software (SigmaScan Pro 5.0, SPSS Science, Chicago, IL), a region of interest (ROI) was placed over the areas of highest intensity activity on the macroautoradiograph. To establish a consistent ROI size, the upper and lower intensity limits of the visually drawn ROIs were recorded and used to determine the average isocontour limits of the ROIs for each ROI. The mean $\pm$ sd isocontour limits for the ROIs from the *Blastomyces* granulomas was $55 \pm 0.06\%$, and for the ROIs from the turpentine abscesses was $52 \pm 0.08\%$. The mean of these two values was 54%. ROIs were redrawn by the computer for each region of inflammation using this 54% isocontour limit. The ROIs were superimposed over the corresponding tissue sections to establish the location of the uptake of FDG. The image-analysis software calculated the mean intensity for each region of inflammation as defined by the 54% isocontour limit ROI.

Statistical Analysis

All data was tested for normality and appropriate parametric or non-parametric statistical methods were employed based on the results of the normality test. Kruskal-Wallis one-

way analysis of variance was used to compare the DURs of the *Blastomyces* granulomas, turpentine abscesses, and muscle samples (Group 1), the DURs of the *Blastomyces* granulomas, lymphomas, and muscle samples (Group 2), and the intensities for each type of inflammation calculated from the macroautoradiographs. Pairwise multiple comparison procedures (Dunn's method) were used to identify the group(s) that differed from the others. A t-test was used to compare the DURs of the *Blastomyces* granulomas between Groups 1 and 2, and to compare the DURs of the muscle samples between Groups 1 and 2. For all tests, significance was set at $p < 0.05$.

Results

Changes in Protocol/Animal Numbers

On the day of harvest there was no visible *Blastomyces* nodule in 1 rat from Group 1. Consequently, the DUR was measured for 18 rats in Group 1.

Because of inability to obtain FDG on the planned day of harvest, 2 rats in Group 2 had their lesions harvested on Day 13, rather than Day 10. By day 13, tumors had regressed in the 3 remaining rats and were not available for harvest. Thus, the DUR was measured for 7 rats rather than 10 rats in Group 2.

Differential Uptake Ratios

The (mean $\pm$ sd) DURs for the *Blastomyces* granulomas, turpentine abscesses, and muscles for rats from Group 1 are shown in Figure 5. The (mean $\pm$ sd) DURs for the *Blastomyces* granulomas, lymphomas, and muscle for rats from Group 2 are shown in Figure 6.

The median (range) DURs for the *Blastomyces* granulomas, turpentine abscesses, and muscle from the rats in Group 1 were 1.9 (1.1-2.6), 0.9 (0.6-1.4), and 0.2 (0.1-0.5), respectively. There was a significant difference between the DURs from the three tissue types ($p < 0.001$). Pairwise multiple comparisons indicated significant differences between the DURs of *Blastomyces* granulomas and muscle ($p < 0.05$), *Blastomyces* granulomas and turpentine abscesses ($p < 0.05$), and turpentine abscesses and muscle ($p < 0.05$). The relative uptake ratios for *Blastomyces* granuloma:muscle, turpentine abscess:muscle, and *Blastomyces* granuloma:turpentine abscess were 8.9, 4.2, and 2.1, respectively.

The median (range) DURs for the *Blastomyces* granulomas, lymphomas, and muscles from the rats in Group 2 were 1.8 (1.2-3.4), 1.9 (1.0-4.0), and 0.2 (0.1-0.3), respectively. There was a significant difference between the median DURs from the three tissue types ($p = 0.001$). Pairwise multiple comparisons indicated significant differences between the DURs of *Blastomyces* granulomas and muscle ($p < 0.05$), and lymphomas and muscle ($p < 0.05$). There was no significant difference between the DURs of *Blastomyces*

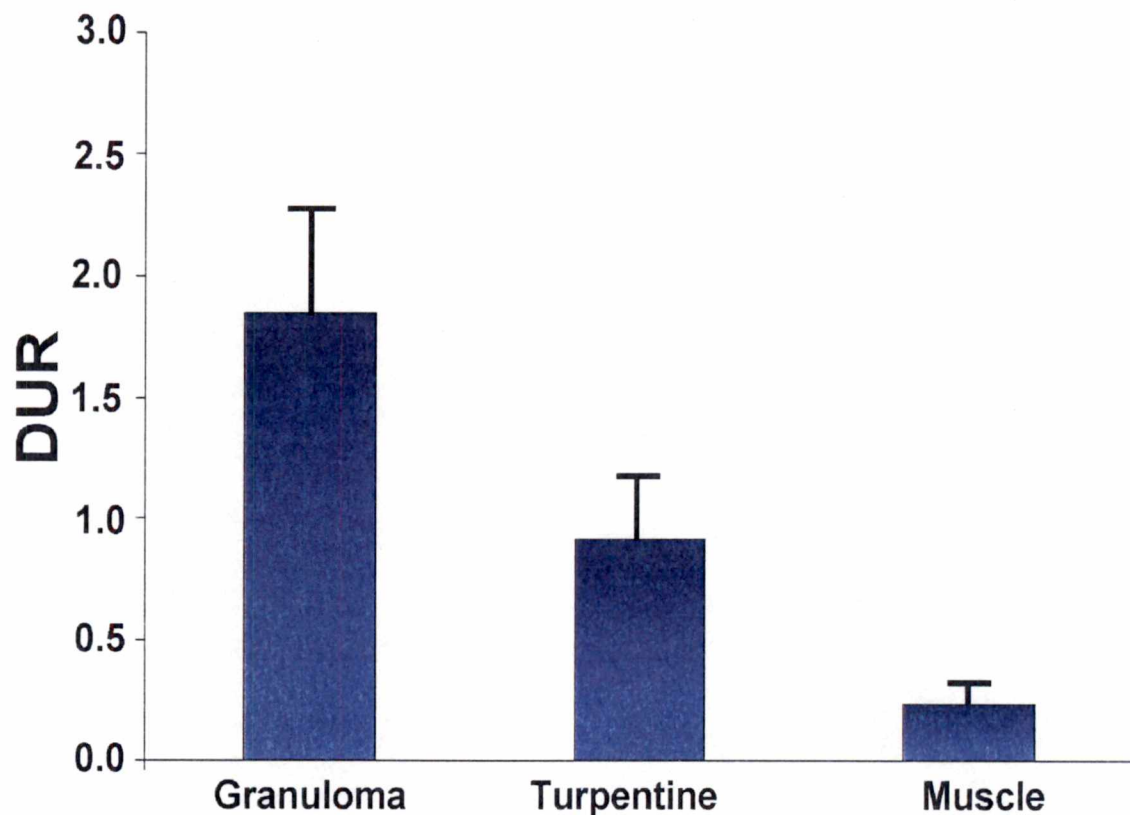


Figure 5. Mean differential uptake ratios of fluorine-18-fluorodeoxyglucose for *Blastomyces* granulomas, turpentine abscesses, and semitendinosus muscle from 18 rats. There was a significant difference between the DURs from the 3 tissue types ($p < 0.001$). Pairwise multiple comparisons indicated significant differences between the DURs of *Blastomyces* granulomas and muscle ($p < 0.05$), *Blastomyces* granulomas and turpentine abscesses ($p < 0.05$), and turpentine abscesses and muscle ($p < 0.05$). The relative uptake ratios for *Blastomyces* granuloma:muscle, turpentine abscess:muscle, and *Blastomyces* granuloma:turpentine abscess were 8.9, 4.2, and 2.1, respectively.

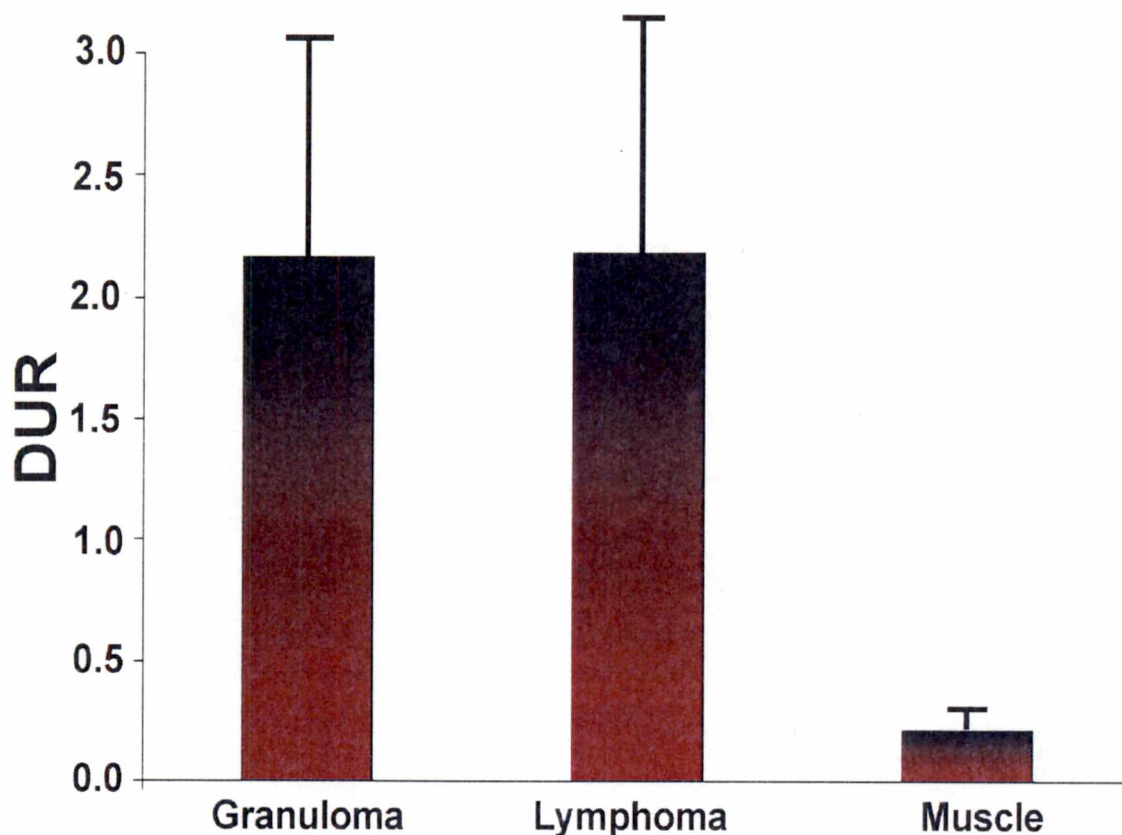


Figure 6. Mean differential uptake ratios of fluorine-18-fluorodeoxyglucose for *Blastomyces* granulomas, lymphomas, and semitendinosus muscle from 7 rats. There was a significant difference between the median DURs from the three tissue types ($p = 0.001$). Pairwise multiple comparisons indicated significant differences between the DURs of *Blastomyces* granulomas and muscle ($p < 0.05$), and lymphomas and muscle ($p < 0.05$). There was no significant difference between the DURs of *Blastomyces* granulomas and lymphomas. The relative uptake ratios for *Blastomyces* granuloma:muscle, lymphoma:muscle, and *Blastomyces* granuloma:lymphoma were 9.3, 11.2, and 1.2, respectively.

granulomas and lymphomas. The relative uptake ratios for *Blastomyces* granuloma:muscle, lymphoma:muscle, and *Blastomyces* granuloma:lymphoma were 9.3, 11.2, and 1.2, respectively.

There was no significant difference between the DURs of *Blastomyces* granulomas in Groups 1 and 2 ($p = 0.236$). There was no significant difference between the DURs of muscles in Groups 1 and 2 ($p = 0.701$). Because there were no significant differences between the DURs of the *Blastomyces* granulomas and muscles between the 2 groups, it was considered valid to compare the turpentine abscesses and lymphomas. Using the Mann-Whitney Rank Sum Test, the median DUR of the lymphomas was significantly greater than the median DUR of the turpentine abscesses ($p < 0.001$). The relative uptake ratio for lymphoma:turpentine abscess was 2.1.

Histopathologic Evaluation

Histopathologic evaluation of the *Blastomyces* granulomas revealed a central region consisting of necrotic cellular debris and degenerate yeast organisms (Figure 7). The relative size of this region varied between rats. Surrounding the central necrotic region was a layer of viable yeast organisms and inflammatory cells. Clusters of yeast organisms were admixed with numerous macrophages, and occasional multinucleated giant cells, lymphocytes, plasma cells, eosinophils, and neutrophils. The innermost portion of the yeast/inflammatory layer was comprised primarily of yeast organisms. Increased numbers of inflammatory cells were admixed with the yeasts towards the outer

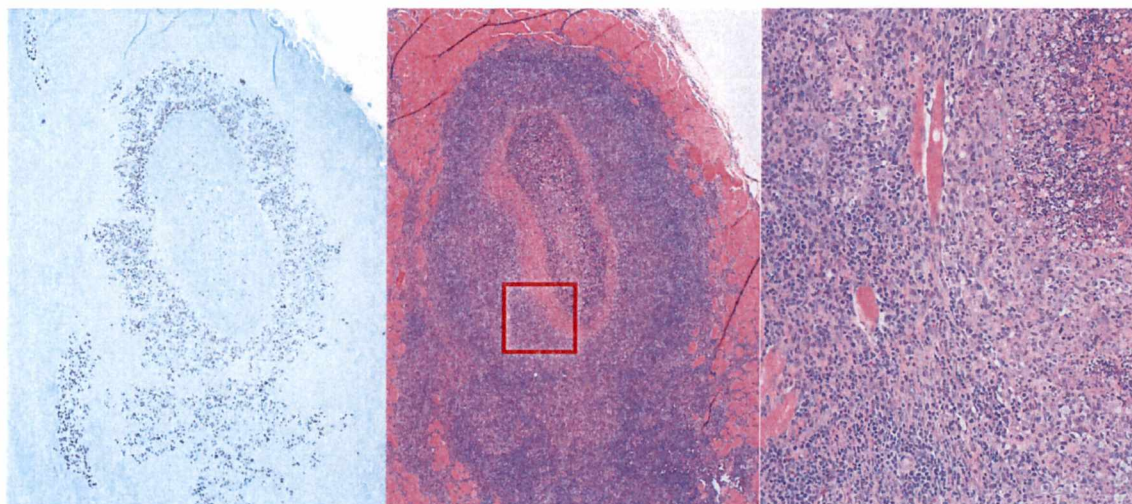


Figure 7. Formalin fixed tissue sections from a subcutaneous *Blastomyces* granuloma from a rat at 2.5x magnification (Center, Left) and 10x magnification (Right). (Center) Hematoxylin and eosin stained section shows central portion consisting predominantly of necrotic debris and degenerate yeast. A layer of inflammatory cells and yeast organisms surrounds the central necrotic region. (Right) Enlarged area of center image. (Left) Gomori's methenamine silver stain of center image highlights yeast organisms. Yeast organisms predominate at the innermost portion of the yeast/inflammatory layer. Progressively increased numbers of inflammatory cells are mixed with the yeasts such that the outermost portion of the layer is predominated by inflammatory cells

edge of the inflammatory layer such that the outermost portion of this layer was comprised of only rare yeast and predominantly inflammatory cells. The number of neutrophils varied between rats, ranging from few to moderate. In some lesions there was scant fibroplasia and angiogenesis. Overall, the lesions were typified by severe, chronic, granulomatous and lympho-plasmacytic inflammation, with some lesions that could be characterized as pyogranulomatous. Subjectively, there was no obvious histologic difference between granulomas in the Sprague Dawley rats compared with the Wistar Furth rats.

Histopathologic evaluation of the turpentine abscesses revealed a central region containing a moderate, lacey fibrin network, minimal cellular debris, and basophilic foreign material (Figure 8). Two distinctly different layers of inflammation surrounded this central region. Immediately surrounding the turpentine center was a narrow band of leukocytes consisting principally of viable and degenerate neutrophils on the innermost rim, with degenerate and viable neutrophils and mononuclear leukocytes at the periphery. Bordering this layer was a wide rim of granulation tissue. The turpentine lesions were characterized as subacute, severe, suppurative and necrotizing cellulitis.

The lymphomas were characterized by a central region composed of sheets of malignant lymphocytes, with significant areas undergoing necrosis and apoptosis (Figure 9). The appearance of the central area was typical of large cell/lymphoblastic lymphoma. A thick band of inflammatory tissue composed of numerous small blood vessels and neovascularization, fibroblasts, and leukocytes, surrounded the tumor. Macrophages,

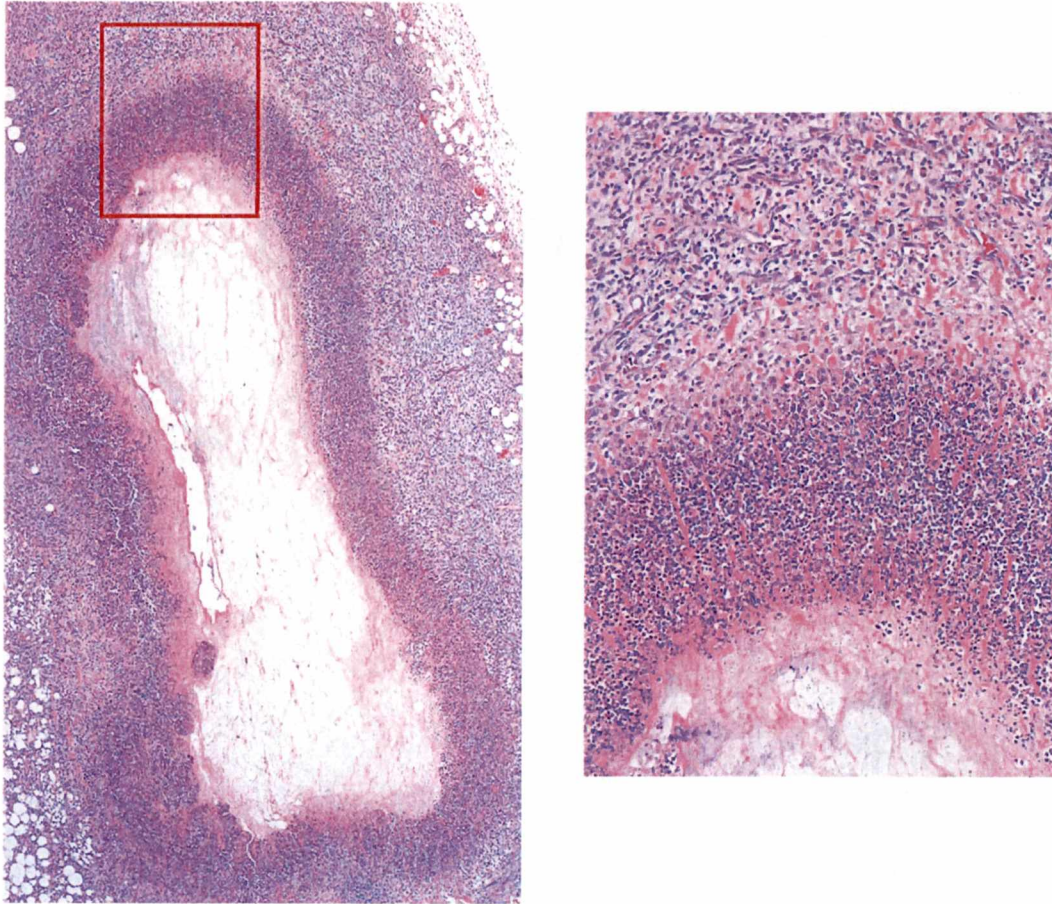


Figure 8. Formalin fixed tissue section from a subcutaneous turpentine abscess from a rat at 2.5x magnification (Left) and 10x magnification (Right). (Left) The central region contains a lacey fibrin network, scant cellular debris, and basophilic foreign material. Immediately adjacent to the central region is a narrow band of leukocytes, consisting principally of degenerate and viable neutrophils. Toward the periphery of this band mononuclear cells are also present. Bordering this layer is a wide rim of granulation tissue. (Right) Enlarged area of image at left. Hematoxylin and eosin.

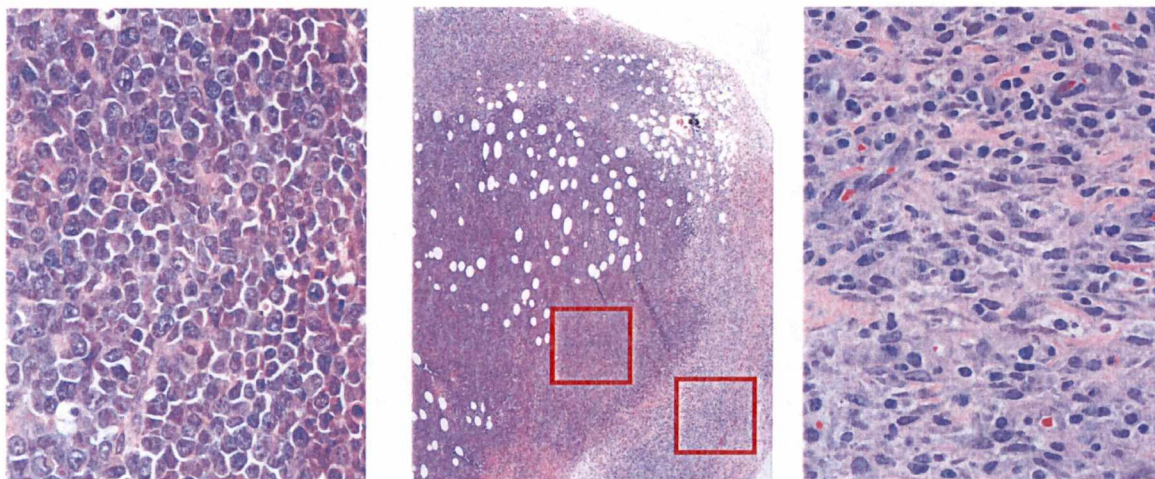


Figure 9. Formalin fixed tissue section from a subcutaneous lymphoma from a rat at 2.5x magnification (Left) and 10x magnification (Left, Right). (Center) The central region is composed of sheets of malignant lymphocytes, typical of large cell/lymphoblastic lymphoma. A thick band of inflammatory tissue surrounds the tumor. (Left) Enlarged area of central region composed of malignant lymphocytes. (Right) Enlarged area of surrounding inflammatory tissue. Hematoxylin and eosin.

lymphocytes, and neutrophils were present in varying proportions. This inflammatory reaction appeared similar to the inflammatory reaction incited by the yeast. It was estimated that the inflammatory reaction comprised 10 - 40% of the entire lesion.

Recovery of Yeast Organisms from Infected Rats

Live yeasts were recovered from all 18 granulomas.

Macroautoradiography

The highest intensity uptake of FDG on the macroautoradiographs of the *Blastomyces* granulomas corresponded to the region occupied by the granulomatous inflammatory reaction and the yeast organisms (Figure 10). There was minimal FDG accumulation in the central necrotic region or in adjacent muscle. The liquid center of the granulomas was lost from some tissue sections during frozen sectioning. In those sections, absence of activity in the central region might have been due to lack of tissue on that portion of the slide. However, there were only rare viable cells or yeasts in the necrotic center and the absence of activity in this region was attributed to lack of viable cells in those sections where large portions of the necrotic center remained on the slide. Macroautoradiographs of the turpentine abscesses showed no uptake in the central region. An intense band of activity was present in the area of the neutrophilic inflammatory layer. There was lesser intensity uptake of FDG associated with the layer of granulation tissue (Figure 11).

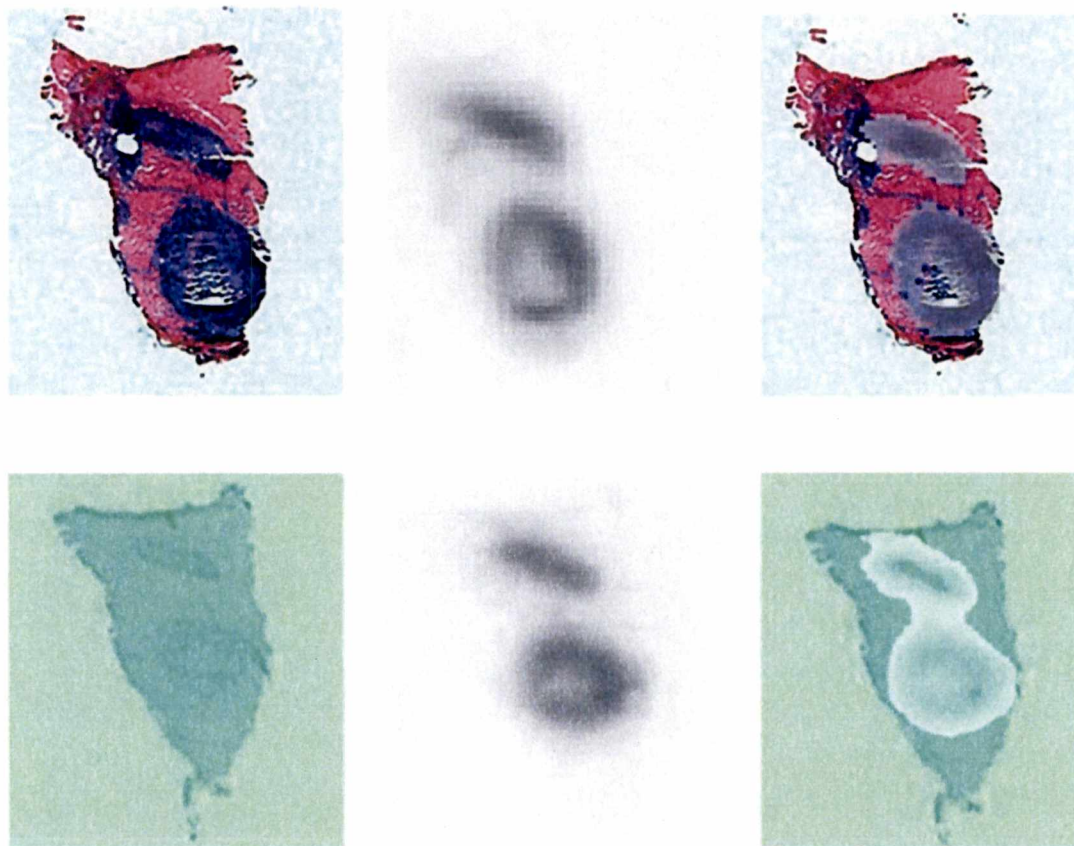


Figure 10. Frozen sections and corresponding macroautoradiographs from a subcutaneous *Blastomyces* granuloma and a subcutaneous turpentine abscess in a rat. (Top) Hematoxylin and eosin stained tissue section of a *Blastomyces* nodule (left) with corresponding macroautoradiograph (center), and macroautoradiograph superimposed over tissue section (right). The area of highest intensity activity on the macroautoradiograph corresponds to the region occupied by the granulomatous inflammatory reaction and yeast organisms. There is little activity in the central necrotic region or adjacent muscle tissue. (Bottom) Gomori's methenamine silver stain of serial section of *Blastomyces* nodule from Top (left), corresponding macroautoradiograph (center), and macroautoradiograph superimposed over tissue section (right). The area of highest intensity activity on the macroautoradiograph includes and extends beyond the region occupied by the yeast organisms.

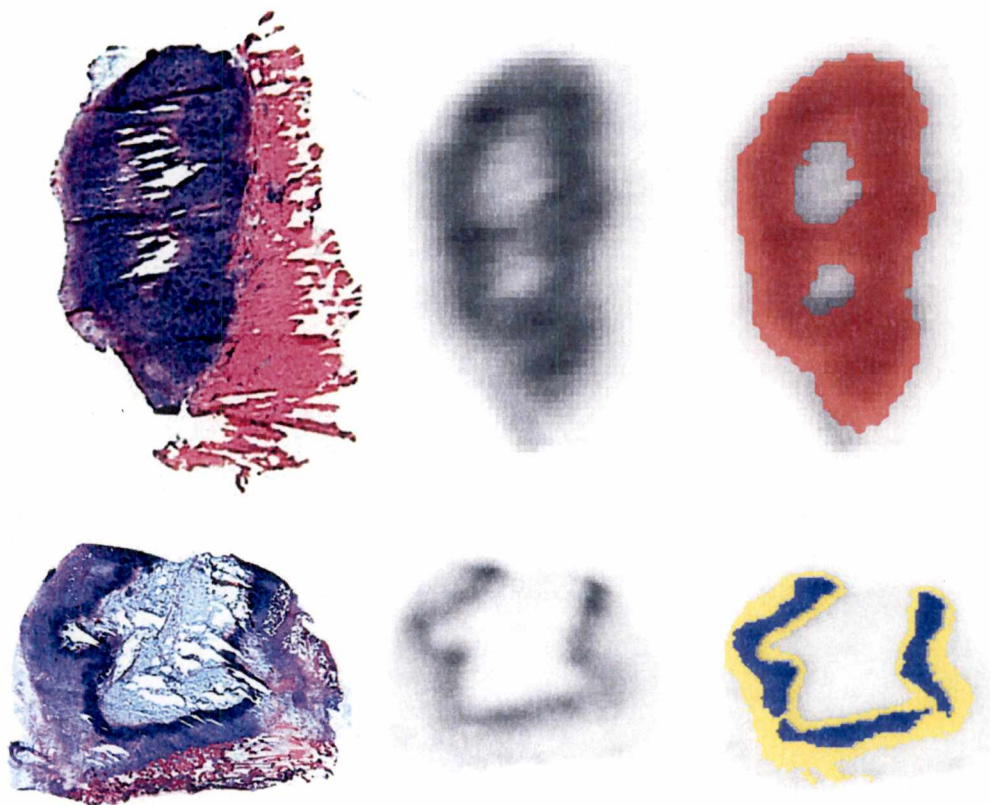


Figure 11. Hematoxylin and eosin stained tissue sections, corresponding macroautoradiographs, and 54 % isocontour regions of interest defining the inflammatory layers of a *Blastomyces* granuloma (Top) and a turpentine abscess (Bottom) from a rat. (Top) The red ROI corresponds to the area of granulomatous inflammation +/- yeast organisms. (Bottom) An intense band of activity is present in the area of the neutrophilic inflammatory layer. Lesser intensity uptake of FDG is present over the region of the granulation tissue. The blue ROI corresponds to the inner layer of inflammation. The yellow ROI corresponds to the granulation tissue.

On the macroautoradiographs of the *Blastomyces* granulomas, a single ROI was placed that encompassed the region of high intensity uptake representing the granulomatous inflammation and yeast organisms (Figure 11). Two ROIs were drawn on the macroautoradiographs of the turpentine abscesses: one encompassing the highest intensity inner layer of neutrophilic inflammation, the other including the outer, lesser intensity region of granulation tissue (Figure 11).

The median (range) intensity for the region of granulomatous inflammation of the *Blastomyces* granulomas was 100 (46-135). The median (range) intensities for the inflammatory layers of the turpentine abscess were 80 (67-99) for the inner layer of neutrophilic inflammation, and 52 (32-59) for the outer layer of granulation tissue. There was a significant difference between the median intensities of the three types of inflammation ($p < 0.001$). Pairwise multiple comparisons indicated significant differences between the intensities of the *Blastomyces* granuloma and the outer layer of the turpentine abscess, ($p < 0.05$), and the inner and outer layers of the turpentine abscess ($p < 0.05$). There was no significant difference between the intensities of the *Blastomyces* granuloma and the inner layer of the turpentine abscess.

Discussion

Results of this study show that *Blastomyces*-induced inflammatory lesions have high uptake of FDG. Superimposition of the macroautoradiographs over the corresponding tissue sections indicated that the uptake is associated with the region occupied by the

granulomatous inflammatory reaction and the yeast organisms. It was not possible in this study to show with certainty that the yeast organisms were accumulating FDG. It might be expected that this would occur, because yeasts have the same glycolytic pathways as eukaryotic cells.^{154;155} Yeast glycolytic pathways are very active such that yeast can acquire the energy they require through growth using fermentation alone.¹⁵⁴⁻¹⁵⁶ Carbohydrate metabolism in some yeasts is similar to carbohydrate metabolism in malignant cells, such that yeast metabolism has been used as model for tumor metabolism.^{65;156} Both yeast and malignant cells have high rates of glycolysis, even in the presence of unlimited oxygen.⁶⁵ Both glucose-sensitive yeasts and tumor cells do not respond to the typical negative feedback mechanisms of oxidative respiration.⁶⁵ Finally, when their oxidative capacity is overwhelmed, yeasts secrete ethanol and malignant cells secrete lactate (the byproduct from the corresponding pathway to ethanol formation in yeast).⁶⁵

Given that yeast organisms are highly glycolytic, it is reasonable to hypothesize that uptake of FDG by the yeast organisms may have contributed to the high intensity uptake of FDG in the region of the *Blastomyces* granulomas occupied by the granulomatous inflammation and the yeast organisms. Alternatively, activity in the region occupied by the yeast organisms may have been due to inability to resolve uptake by yeasts from inflammatory cells on the macroautoradiographs. Finally, the yeast organisms may contribute indirectly to high uptake of FDG by fungal lesions by producing a factor that causes macrophages to become highly activated, increasing their utilization of glucose.

Even if yeast organisms were proven to accumulate FDG, it is unlikely that they contribute significantly to uptake by naturally occurring lesions. The large clusters of yeast present in these experimental lesions are not typical of lesions of naturally occurring blastomycosis, and the significance of uptake of FDG by yeast organisms to overall uptake of FDG by naturally occurring fungal lesions is not known. Yeast organisms typically are scarce in naturally occurring lesions,¹⁴⁸ suggesting that uptake of FDG by yeast would play a minimal role in overall uptake of FDG by the lesion.

Possible reasons for increased uptake of FDG by the *Blastomyces* granulomas compared with the turpentine abscesses include differences in cell types, differences in cell density, or uptake by the *Blastomyces* organisms. The *Blastomyces* granulomas were predominantly composed of macrophages, lymphocytes, and plasma cells, with few neutrophils, whereas the turpentine abscesses were comprised of predominantly neutrophils and granulation tissue. Uptake of FDG is due in part to the number of viable cells present, and macrophages have high intensity accumulation of FDG.²⁰ Greater uptake of FDG by the *Blastomyces* granulomas might be due to the predominance of macrophages in the inflammatory reaction. However, results of the macroautoradiography showed that uptake by the granulomatous inflammation +/- yeast organisms of the *Blastomyces* granulomas was not significantly greater than uptake by the neutrophilic inner layer of the turpentine abscess. The histologic character of the turpentine abscesses in this study was similar to that seen by Kubota et al. Kubota et al further subdivided the histologic character of the inner layer into what they referred to as a neutrophil layer and a marginal zone. The neutrophil layer included live and dead

neutrophils, and the marginal zone comprised neutrophils, macrophages, young fibroblasts and endothelial cells. These microscopic subdivisions could not be distinguished using macroautoradiography in this study, but using microautoradiography, Kubota et al demonstrated that the highest uptake of FDG was seen in the marginal zone, followed by the neutrophil layer, then the granulation tissue. Uptake of FDG is in part dependent on the number of viable cells present. Given the high uptake of FDG in the marginal zone documented by Kubota, and the lack of difference in intensity of uptake between the inner neutrophil layer of the turpentine abscesses and the granulomatous inflammation of the *Blastomyces* granulomas observed in this study, the higher DUR of the *Blastomyces* granulomas could simply be due to presence of a greater proportion of cells with high FDG uptake in the *Blastomyces* granulomas compared with the turpentine abscesses. That is, whereas the entire inflammatory reaction associated with the *Blastomyces* lesion demonstrated high FDG uptake, only the narrow inner layer of the turpentine abscess did.

The absence of uptake of FDG in the central necrotic region was similar to that seen in experimental infectious foci caused by inoculation with *E. coli*, in which the highest uptake of ^3H -FDG on autoradiographs was seen in the inflammatory cell infiltrate (composed of neutrophils, macrophages, and remnants of muscle fibers) surrounding the central region of bacteria and necrosis.²⁷ In both the *Blastomyces*-induced lesions of this study and the *E. coli*-induced abscesses, little uptake was associated with the abscess centers comprised of large amounts of necrotic debris.²⁷

In this study, the DUR was used to compare uptake of FDG by fungal-associated inflammation with uptake by non-infectious inflammation and neoplasia. Initially, the SUV from FDG-PET scans of the rats was to be used to make this comparison. However, the lesions were too small and could not be adequately resolved by the PET scanner (data not shown).

The DUR of *Blastomyces* granulomas was approximately twice that of uptake by turpentine abscesses. For this study, *Blastomyces* granulomas were used to represent fungal-associated inflammation, and turpentine abscesses were used to represent non-fungal inflammation to compare uptake by fungal-associated inflammation with uptake by other benign inflammatory disease. Results observed using *Blastomyces* and turpentine might not be representative of behavior of all fungal-associated inflammation or benign inflammatory lesions. Results of this study do, however, support the literature suggestion that fungal-associated inflammatory lesions can have higher FDG uptake than other benign lesions comprised predominantly of neutrophils.^{6;16;27} This study did not investigate the difference in uptake of FDG by other non-fungal granulomatous lesions such as tuberculosis or sarcoidosis.

The highest DURs for individual lesions in this study were observed in the *Blastomyces* granulomas. However, the median DUR of *Blastomyces* granulomas was as high, but not higher, than the median DUR for malignant lymphomas in this study. A confounding factor, however, was the presence of the marked inflammatory response associated with the experimental lymphomas. This illustrates one of the limitations of using

experimental models. This inflammatory response is not characteristic of naturally occurring lymphoma.^{149;157} (C58NT) D lymphoma cells are described as being “highly immunogenic”, thus, in retrospect, the marked inflammatory response associated with the experimental lesions would be expected. Uptake of FDG by the inflammatory response may have elevated the overall DUR of the tumors. However, the role of uptake of FDG by the inflammatory response associated with the lymphoma was not assessed.

Macroautoradiography would have been valuable but was not done as we had not anticipated the magnitude of the inflammatory reaction. Because of the limitations of the experimental model, we cannot draw conclusions about what would result by comparing naturally occurring diseases. Naturally occurring lymphoma lacks the inflammatory response associated with experimental lesions, and uptake of FDG by *Blastomyces*-associated inflammation may be greater than for lymphoma. Some naturally occurring tumors are, however, composed of a large degree of tumor-associated inflammation.^{20;149;152} In this instance, results of this experimental study may be accurately representative of naturally occurring neoplastic disease. That is, uptake of FDG by fungal-associated inflammation is as high, but not higher than, for neoplastic disease. The impact of tumor-associated inflammatory cells on interpretation of FDG-PET images has been debated. Kubota et al showed higher uptake of FDG by tumor-associated macrophages than by viable tumor cells, stressing the importance of considering the role of inflammatory cells when interpreting FDG-PET images. Conversely, Brown et al demonstrated little uptake of FDG by tumor-associated inflammatory cells, and concluded that uptake of FDG on FDG-PET images predominantly reflects tumor uptake.

In this study, uptake of FDG by lymphomas was approximately twice the uptake by turpentine abscesses. The greater uptake of FDG by lymphomas compared with turpentine abscesses supports the accepted principle that malignant disease typically has greater uptake of FDG than benign disease.^{3-6;8;10;15;16;28}

By using these experimental lesions to represent naturally occurring disease, there are limitations to the conclusions that can be made. Experimentally induced lesions and naturally occurring lesions differ which may impact on uptake of FDG. Secondly, the presence of two different immunogenic lesions in one animal may alter the nature of the lesions. Finally, one cannot use results from this study to generalize regarding all types of fungal-associated inflammation compared to all types of non-fungal inflammation and all tumor types. However, results of this study do provide evidence that a similar study in naturally occurring disease would be worthwhile.

Conclusions

Results of this study show that *Blastomyces* granulomas typically have high uptake of FDG associated with the region of the granulomatous inflammatory reaction and *Blastomyces* yeast organisms. The DUR of experimentally induced fungal-associated inflammation (*Blastomyces* granulomas) was higher than for experimentally induced non-fungal inflammation (turpentine abscesses). The DUR of *Blastomyces* granulomas was as high, but not higher than for experimentally induced lymphomas. The marked inflammatory response associated with the experimental lymphomas contributed to the

high uptake of FDG, which did not allow us to see a difference between uptake of FDG by *Blastomyces* granulomas and lymphomas. *Blastomyces*-associated inflammation may have greater uptake of FDG compared with lymphomas in naturally occurring disease.

CHAPTER 5

Fluorine-18-Fluorodeoxyglucose Accumulation in *Blastomyces dermatitidis*-Associated Inflammation in a Dog

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Abstract

Whole-body PET imaging with FDG was performed in a dog with blastomycosis. Prior to FDG- PET scanning, blastomycotic lesions had been identified in both eyes, lungs, and tracheobronchial lymph nodes. Increased FDG uptake was present in these sites in addition to multiple organs without previous clinical evidence of disease. All sites of

FDG uptake were confirmed histologically to be blastomycotic granulomas. FDG-PET scanning may be useful for defining the extent of fungal disease and detecting persistent foci of infection.

Introduction

Positron emission tomography (PET) using fluorine-18 fluorodeoxyglucose (FDG) is widely used for tumor imaging.^{8;10;23;36;38;39} Tumor cells are distinguished from normal cells by their increased FDG uptake. Non-neoplastic lesions may also have increased FDG uptake.¹⁹⁻²⁴ Accumulation of FDG can occur in various inflammatory lesions, including fungal infections with *Histoplasma*, *Cryptococcus*, *Aspergillus* and *Blastomyces*.^{2;4-6;16;28;30}

The utility of FDG-PET specifically for assessing fungal disease has not been extensively explored. Uptake of FDG by fungal lesions has been encountered primarily when this uptake resulted in false positive diagnosis for tumor.^{2;4-6;16;28;30} Blastomycosis is common in dogs in Tennessee, providing a model for investigating FDG-PET imaging of fungal disease. We report on a dog with significant accumulation of FDG in sites of histologically confirmed lesions containing *Blastomyces dermatitidis*.

Case Report

A 4-year-old male Doberman Pinscher was referred to the University of Tennessee College of Veterinary Medicine because of acute onset of blindness of one week duration. The dog had severe bilateral chronic anterior uveitis, secondary glaucoma in the right eye, and corneal perforation of the left eye. Ocular blastomycosis was suspected based on these findings in conjunction with positive serology for *Blastomyces dermatitidis* antibodies. There were no other significant abnormalities noted on complete physical examination.

A complete blood count and serum biochemical profile were normal. On thoracic radiographs there was tracheobronchial and cranial mediastinal lymphadenomegaly, and diffuse moderate to severe pulmonary interstitial infiltrates, consistent with fungal infection (Figure 12).

Both eyes were enucleated. Severe pyogranulomatous panophthalmitis, filtration angle obstruction from exudate and fibrin, retinal detachment, and intralesional yeasts were present in both eyes. Corneal perforation and iris bombe were present in the left eye. Yeast morphology was consistent with *Blastomyces sp.*

Two days later an FDG-PET scan was performed with an ECAT 921 scanner (CTI PET Systems, Knoxville, TN). The dog was imaged 1 hour after a 5 mCi dose of FDG was administered intravenously. Whole-body images were obtained with the dog in left

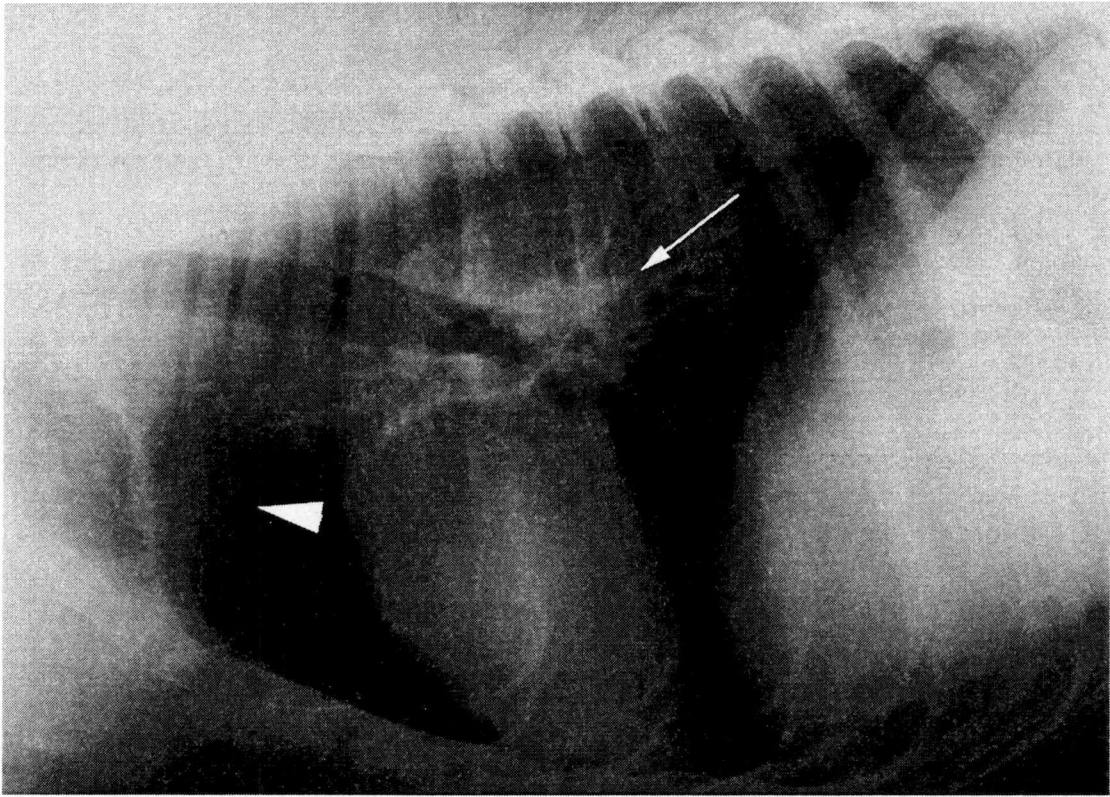


Figure 12. Lateral thoracic radiograph of a dog with blastomycosis. Tracheobronchial lymph nodes (arrows) and cranial mediastinal lymph nodes (arrowheads) are enlarged. There are diffuse, moderate to severe interstitial lung opacities throughout all lung fields.

lateral recumbency by acquiring six adjacent bed positions with 47 image planes each for an 84 cm total axial field of view.

Multiple areas of increased FDG accumulation were evident on the PET scan (Figure 13). Significant uptake of FDG was present in lymph nodes (tracheobronchial, mediastinal, and superficial cervical), lung, bone (carpus and elbow), prostate, right testis, and skin. One day after the PET scan, fine needle aspirates were collected from left and right superficial cervical lymph nodes. The aspirates contained pyogranulomatous inflammation and budding yeasts consistent with *Blastomyces dermatitidis*.

The dog was treated with Itraconazole (5 mg/kg PO twice daily). One day after starting treatment the left elbow and right carpus became acutely painful. Radiographically there was disruption of the trabecular pattern within the proximal olecranon and mild antebrachial carpal joint swelling of the left forelimb. The lameness resolved after the dog was treated with aspirin (325 mg PO twice daily). Twelve days following the PET scan he was euthanized due to sudden onset of coma. A complete post mortem examination was performed.

Multiple firm 5-10 mm diameter intradermal nodules along the dorsal midline, corresponding to areas of accumulation of FDG on the PET scan, were identified as follows: one medial to the right ear, one between the dorsal scapulae, one from the cranial thorax at the level of T3-4, one from the caudal thorax at the level of T10-11, and one from the cranial lumbar area. The mandibular, axillary, superficial cervical,



Figure 13. Whole-body FDG-PET scan of a dog with disseminated blastomycosis (planar view). Multiple sites of FDG accumulation are evident. *Blastomyces* organisms were found in all sites of FDG uptake. Normal accumulation of FDG in brain, kidneys and urinary bladder is seen.

popliteal, caudal deep cervical, and cranial mediastinal lymph nodes were firm and enlarged bilaterally. All of the tracheobronchial lymph nodes were firm, enlarged, and had coalesced to form a multinodular 8 x 8 x 6 cm hilar mass. There were firm 1-3 mm diameter nodules disseminated throughout all lung lobes. The prostate was firm, multinodular, and asymmetrically enlarged with the left lobe being slightly larger than the right. The right testis was within the scrotum and was unremarkable. The left testis was intraabdominal and approximately 1/2 the size of the right.

Histopathologic evaluation revealed pyogranulomatous inflammation secondary to blastomycosis in all grossly affected tissues. Mesenteric lymph nodes and both thyroid lobes were grossly unremarkable but had histologic evidence of disease. Lesions were characterized by large numbers of degenerate and viable neutrophils, foamy macrophages and cellular debris. Within the infiltrate were round-oval 7-15 um diameter thick-walled yeasts. In the brain there was severe hydrocephalus of the lateral and third ventricles with blastomycotic granulomatous ventriculitis. There was blastomycotic villous arthritis in the left elbow joint with disruption of lamellar trabecular bone in the radius.

Discussion

Increased accumulation of FDG can occur in areas of inflammation.¹⁹⁻²⁴ Increased FDG uptake occurs in fungal lesions, including *Histoplasma*, *Cryptococcus*, *Aspergillus* and *Blastomyces*.^{2;3;5;6;16;28;30} Reports of FDG accumulation in blastomycotic lesions have been limited to cases in which high FDG uptake in pulmonary lesions due to

blastomycosis resulted in false positive diagnosis for tumor.^{5,6} This case demonstrates that histologically confirmed extrapulmonary blastomycotic lesions can also have significant uptake of FDG. Accumulation of FDG in infectious inflammatory lesions is attributed to uptake by inflammatory cells;^{20,24,27} it is not known whether there is uptake of FDG by the infectious organisms themselves. Further analysis of the pattern and intensity of uptake of FDG in fungal-associated lesions may help to distinguish fungal disease from neoplasia.

Whole-body FDG-PET scanning in this dog with blastomycosis identified widespread accumulation of FDG in tissues without clinical evidence of disease. Prior to the FDG-PET scan, blastomycotic lesions had been identified in the eyes, lung, and tracheobronchial lymph nodes, which are common sites of infection in dogs. In addition to these sites, the PET scan identified disease in superficial cervical lymph nodes, bone, prostate, right testicle, and skin, all of which were unremarkable on physical examination. Gross pathologic lesions corresponding with areas of accumulation of FDG on the FDG-PET scan in the sites listed above were confirmed histologically as blastomycotic infiltrates. In people with blastomycosis, the lung is the most commonly infected organ. Extrapulmonary lesions are most often observed in skin, bone, and the genitourinary tract, although these organs are affected much less frequently than the lung.¹⁴⁹ The majority of human patients have involvement of only one organ, most commonly the lung. It is interesting to speculate that people may have more extensive extrapulmonary disease than previously recognized, which could be detected if a sensitive method was used to identify sites of infection. FDG-PET scanning may help define the extent of

fungal disease and monitor response to therapy; persistent foci of infection could be detected to identify patients requiring longer courses of therapy.

Canine blastomycosis shares many common clinical features with blastomycosis in people.¹⁵⁰ In dogs, the lung is the portal of entry for *Blastomyces* organisms and the majority of dogs (85%) will have pulmonary lesions.¹⁵⁰ Skin lesions occur in 20-40% of affected dogs, and bone involvement is observed in up to 30% of cases.¹⁵⁰ Ocular disease occurs in up to 40% of dogs with blastomycosis.¹⁵⁰ Other less commonly affected tissues in dogs include the urogenital tract, central nervous system, and nasal cavity.¹⁵⁰ Dogs appear to be more susceptible to infection than people and have a shorter prepatent period.¹⁵⁰ In endemic areas, the incidence of blastomycosis in dogs may be 10 times greater than in people.¹⁵⁰ Because of the similarities between canine and human blastomycosis and the availability of dogs with naturally occurring disease, dogs may serve as a useful model for evaluating FDG-PET scanning of fungal disease in people.

CHAPTER 6

Comparison of Uptake of Fluorine-18-Fluorodeoxyglucose in Dogs with Naturally Occurring Blastomycosis and Lymphoma

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Abstract

FDG-PET is a highly sensitive technique for detecting malignant disease based on increased uptake of FDG by malignant lesions. The technique lacks specificity because accumulation of FDG is not specific for malignancy. Uptake of FDG occurs in lesions caused by the fungal agents *Histoplasma*, *Cryptococcus*, *Aspergillus*, and *Blastomyces*. Based on limited reports, fungal lesions can have remarkably high intensity uptake of FDG. This study compared the SUV of naturally occurring lesions of blastomycosis with

the SUV of naturally occurring lymphoma in dogs and evaluated the usefulness of whole-body FDG-PET for imaging disseminated fungal disease. Five dogs with naturally occurring blastomycosis and three dogs with lymphoma underwent whole-body FDG-PET. In dogs with blastomycosis and dogs with lymphoma, FDG-PET detected all known sites of disease. In addition, numerous sites of disease were detected on the FDG-PET images that were not detected clinically. The SUV was calculated for 13 lesions from the 5 dogs with blastomycosis. The (mean $\pm$ sd) SUV for the blastomycosis lesions was 7.7 $\pm$ 2.0. The SUV was calculated for 17 lesions from the 3 dogs with lymphoma. The mean SUV for lymphomas was 4.8 $\pm$ 1.8. There was a significant difference between the mean SUV of lesions from dogs with blastomycosis and the mean SUV of lesions from dogs with lymphoma ($p < 0.001$). There was overlap between the SUVs of *Blastomyces*-associated lesions and lymphomas, but a cutoff SUV of 7.0 was 100% specific for *Blastomyces* lesions. On follow-up FDG-PET images after itraconazole treatment of dogs with blastomycosis, there was no evidence of FDG uptake in any previous sites of disease in 3/5 dogs. These 3 dogs remain clinically disease-free 7, 9, and 11 months after completion of therapy. FDG-PET indicated persistent sites of infection in the remaining 2 dogs. FDG-PET is useful for determining the extent of disease and response to therapy in dogs with blastomycosis. Lesions associated with blastomycosis exhibit high uptakes of FDG. The SUV for *Blastomyces*-associated lesions are as high or higher than for malignant lymphoma. Because of the similarities in canine and human blastomycosis and lymphomas, similar results would be predicted in human patients. In regions where blastomycosis is endemic, *Blastomyces* granulomas

should be considered a differential diagnosis for lesions with high intensity uptake of FDG.

Introduction

Whole-body positron emission tomography (PET) using fluorine-18-fluorodeoxyglucose (FDG) is widely used for tumor imaging.^{8;10;23;36;38;39} Tumor cells have increased numbers of glucose transporters and increased activity of glycolytic enzymes, resulting in increased cellular glucose metabolism.⁸⁻¹² Fluorine-18-fluorodeoxyglucose, a glucose analogue, is transported into cells and is phosphorylated by hexokinase in the same manner as glucose.^{8;10;13;14} Phosphorylated FDG is trapped within cells but, unlike glucose, it cannot undergo further metabolism.^{8;10;13;14} Thus the amount of FDG trapped within the cells of a tissue is a reflection of the rate of the hexokinase reaction, which at steady state represents the glycolytic rate of the tissue.^{8;10;13;14} This trait is exploited for imaging tumors: FDG-PET can identify tumors by their increased uptake of FDG relative to surrounding normal tissues.^{8;10;13;14}

FDG-PET is a highly sensitive technique for detecting malignancies based on increased uptake of FDG by malignant lesions.^{1-7;15-18} The technique lacks specificity, however, because accumulation of FDG is not specific for malignancy. Any tissue with increased glucose utilization will exhibit increased uptake of FDG.¹⁹⁻²⁴ For example, inflammatory cells use increased amounts of glucose during chemotaxis and phagocytosis, so inflammatory lesions demonstrate increased uptake of FDG.^{21;24-27} Usually the uptake of

FDG by benign lesions is significantly less than for malignant lesions.^{1;2;4-7;16;17;28-30}

Accumulation of FDG can be measured semi-quantitatively using the standardized uptake value (SUV).^{8;10} A threshold SUV of 2.5-3.5 is used to distinguish benign from malignant disease; lesions having an SUV below this value are considered benign, and lesions with an SUV above this value are characterized as malignant.^{8;10} Benign lesions such as abscesses, fibrosis, and benign tumors generally have an SUV of 1-3.^{1-7;15-18} Malignant lesions typically have SUVs ranging from 3-20.^{1-7;15-18}

Inflammatory lesions with SUVs over 2.5 will occasionally be mistaken for malignancies. Many of these false positive lesions are the granulomatous inflammatory reactions of tuberculosis, sarcoidosis, and fungal infections.^{1-7;15-18} Uptake of FDG by granulomatous lesions is typically higher than for suppurative inflammatory lesions, and is attributed to avid uptake of FDG by macrophages.²⁰ Lesions caused by the fungal agents *Histoplasma*, *Cryptococcus*, *Aspergillus*, and *Blastomyces* commonly accumulate FDG.^{2;4-6;16;28;30} While inflammatory lesions typically have SUVs only in the low range for malignancy, SUVs as high as 10 occur in fungal-associated inflammatory lesions.⁵

The limited reports of FDG uptake by fungal lesions typically involve patients misdiagnosed as having malignancy.^{2;4-6;16;28;30} Further information is needed to draw firm conclusions regarding uptake of FDG by fungal-associated inflammatory lesions. In experimental *Blastomyces*-induced lesions in rats, the differential uptake ratios (DUR) of *Blastomyces* granulomas were as high as for experimentally induced subcutaneous lymphomas. However, there was a marked inflammatory response associated with the

experimental lymphomas that is not typical of the naturally occurring disease. We suspect that uptake of FDG by these inflammatory cells contributed significantly to the DURs of the tumors.

The purpose of this study was to compare the SUV of naturally occurring *Blastomyces* with the SUV of naturally occurring sites of lymphoma in dogs. Blastomycosis is a systemic fungal disease caused by *Blastomyces dermatitidis*, commonly seen in dogs in Tennessee. The canine disease mimics that seen in people, but occurs about 10 times more frequently in dogs than in people in the same endemic region, thereby providing an accessible model to study the disease.¹⁵⁰ We previously showed that whole body FDG-PET could detect *Blastomyces*-associated inflammatory lesions in a dog with disseminated blastomycosis. Lymphoma is a common malignancy of dogs that resembles non-Hodgkin's lymphoma in people. We hypothesized that fungal lesions would have a higher SUV than malignant lesions, and that differentiation between malignant and fungal lesions could be achieved by determining an additional SUV cutoff value. Lesions with SUVs above this value would most likely be fungal lesions, whereas lesions with SUVs below this value would be most consistent with malignancy. Also, we wanted to determine the usefulness of whole body FDG-PET scans in imaging disseminated fungal disease.

Materials and Methods

Animals

Dogs participating in this study were client-owned pets presented to the University of Tennessee College of Veterinary Medicine Veterinary Teaching Hospital for evaluation of suspected blastomycosis or lymphoma. The following criteria were used to identify suitable dogs for inclusion into the study: a definitive diagnosis was required prior to undergoing FDG-PET imaging; dogs could not have been treated for their disease; dogs had to be considered safe candidates for general anesthesia; dogs had to have a normal blood glucose concentration and no history of diabetes mellitus. Informed consent was obtained from all owners. The diagnosis of blastomycosis was based on histopathologic or cytologic evaluation of granulomatous or pyogranulomatous inflammatory lesions containing *Blastomyces* organisms, or positive *Blastomyces* serology in the presence of compatible historical, physical, and radiographic signs. The diagnosis of lymphoma was based on histologic or cytologic examination of enlarged lymph nodes.

PET Imaging Protocol

Dogs with blastomycosis underwent whole body FDG-PET scanning prior to and after treatment (itraconazole, 5 mg/kg/day for 60 days). Dogs with lymphoma underwent whole body FDG-PET prior to therapy only.

Dogs were fasted overnight (minimum 12 hour fast) prior to FDG-PET. Approximately 185 MBq (5 mCi) of FDG was given intravenously. After a 60-minute uptake period, dogs were anesthetized and positioned in left lateral recumbency for the duration of the scan. Dogs were premedicated with butorphanol (0.2 mg/kg IV) and acepromazine (0.05 mg/kg IV). Anesthesia was induced with pentobarbital to effect (10-15 mg/kg IV) and maintained with 50 mg boluses of pentobarbital as needed to sustain a light plane of anesthesia.

Scans were obtained using an ECAT 921 camera (Siemens CTI Co., Knoxville, TN). This 3-ring system has a 17-cm field of view and produces 47 contiguous image planes with plane spacing of 3.5 mm and in-plane resolution of 3.5-mm full-width-half-maximum. Images were acquired in sequential bed positions and joined in a single display. Whole body images were obtained using 3-7 bed positions (10 minutes in each bed position) to include the entire dog's body. Transmission scanning was performed for 3 minutes in each bed position using a ^{68}Ge ring source. Images were corrected for attenuation and reconstructed using filtered back projection with a Hann filter and 0.4 px^{-1} cutoff value.

Image Analysis

Images were read by a nuclear medicine physician (GTS) unaware of the diagnosis or known sites of disease. Foci of increased FDG uptake were identified by visual

inspection. Clinical, cytologic, and radiographic examinations were used to corroborate the findings on the FDG-PET images.

Standardized uptake values (SUVs) were computed using a computer software program that converts attenuation and decay corrected (to the time of injection) images into SUV images on a pixel-by-pixel basis. The SUV was calculated using the following equation:

$$\text{SUV} = \frac{\text{Tissue radioactivity (MBq/g)}}{\text{Injected dose (MBq) / Body weight (g)}}$$

The tissue radioactivity was calculated by:

$$\text{Tissue radioactivity (MBq/g)} = \text{ECAT cps/px} * \text{ECF (MBq/g/ECAT cps/px)}$$

Where the ECAT calibration factor (ECF) is known from a standard quality control phantom.

The SUV was calculated for all lesions measuring > 2 cm diameter. Lesions < 2 cm diameter were excluded to minimize inaccuracy of SUVs due to partial volume effects. Transverse or coronal 3.5-mm slices were used for SUV determination. An elliptical region of interest (ROI) was placed around the entire lesion in all sequential slices in which the lesion was visualized. The maximum SUV for the lesion was used for analysis.

Statistical Analysis

The body weight, blood glucose concentration, dose of FDG, and time to imaging were compared between the dogs with blastomycosis and the dogs with lymphoma using the appropriate parametric (Student's t-test) or non-parametric (Mann-Whitney Rank Sum Test) method. The SUV for fungal inflammatory lesions was compared with the SUV for lymphoma lesions using the Student's t-test. Significance for all tests was set at $p < 0.05$. A receiver operating characteristic (ROC) curve was used to establish a threshold SUV to distinguish *Blastomyces* lesions from lymphoma.

Results

Animals

During the study interval, 5 dogs with blastomycosis and 3 dogs with lymphoma met the study inclusion criteria. A variety of breeds was represented. In 4/5 dogs with blastomycosis, the diagnosis was made based on cytologic evaluation of fine needle aspirates indicating pyogranulomatous inflammation with intralesional *Blastomyces* yeast organisms. In the remaining dog, the diagnosis was based on compatible historical, physical, and radiographic signs in conjunction with positive *Blastomyces* serology. Ages of the 5 dogs with blastomycosis were 1, 2, 2, 8, and 8 years. The mean $\pm$ sd body weight for the 5 dogs was 30.3 $\pm$ 7.2 kg. The 3 dogs with lymphoma were 4, 6, and 7 years old, with a mean $\pm$ sd body weight of 18 $\pm$ 9 kg. All dogs had serum blood

glucose concentrations in the normal range at the time of presentation. There were no significant differences between the body weights or blood glucose concentrations from the dogs with blastomycosis compared with the dogs with lymphoma.

Imaging

The mean $\pm$ sd doses of FDG administered to the dogs with blastomycosis and the dogs with lymphoma were 200 ± 35 MBq (5.4 ± 0.9 mCi) and 205 ± 26 MBq (5.5 ± 0.7 mCi), respectively. The median (range) time from injection of FDG to initiation of imaging in dogs with blastomycosis was 75 (55 – 135) minutes. The median (range) time between injection of FDG and onset of imaging was 80 (75 – 80) minutes in dogs with lymphoma. There were no significant differences in dose of FDG or time from injection of FDG to imaging between the 2 groups of dogs.

Mild, bilaterally symmetrical uptake of FDG was evident in the salivary glands, orbits, and thyroid glands in all scans and was interpreted as normal for dogs. In dogs with blastomycosis and dogs with lymphoma, FDG-PET detected all known sites of disease. In addition, numerous sites of disease were detected on the FDG-PET images that were not detected clinically. In dogs with blastomycosis, increased FDG uptake was identified in lymph nodes, lung, skin, testicles, bone, and sphenoid sinus (Figures 14 and 15). Asymmetric uptake of FDG was present in the orbits of 1 dog. In dogs with lymphoma, increased uptake of FDG was present in mandibular lymph nodes, prescapular lymph nodes, axillary lymph nodes, popliteal lymph nodes, and abdominal lymph nodes

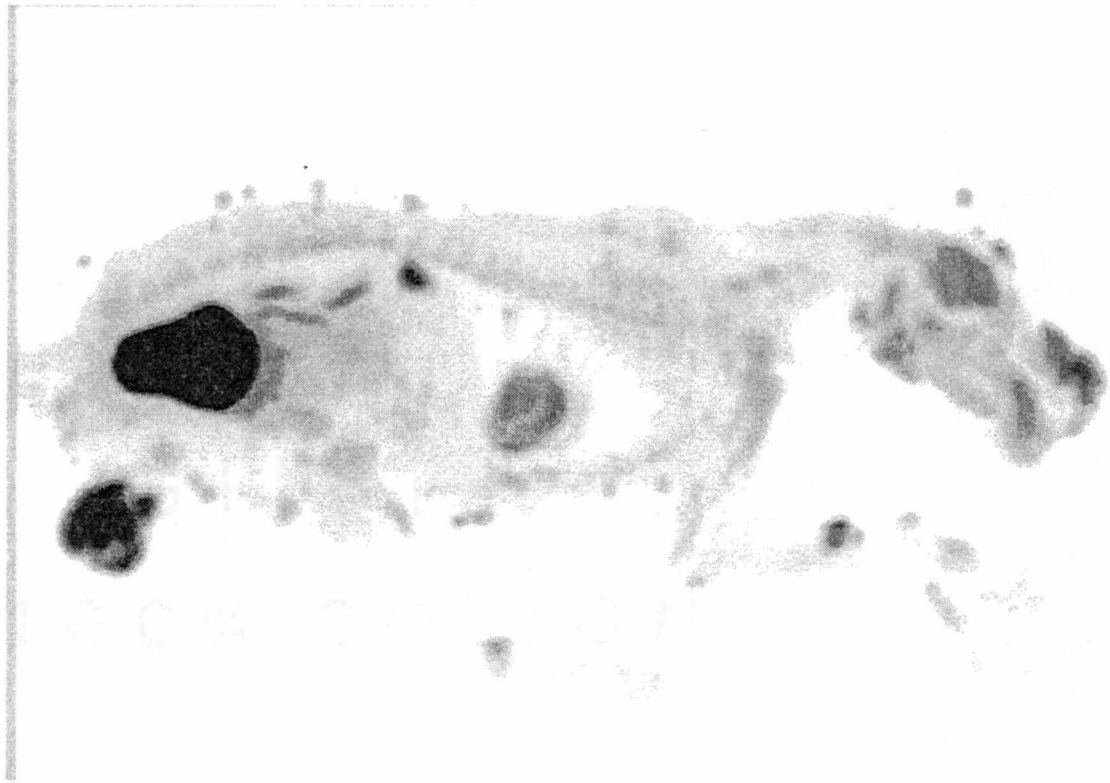


Figure 14. Whole-body FDG-PET scan of a dog with blastomycosis (planar view).

There is high intensity uptake of FDG associated with soft tissue infection of the testicles. The SUV of this lesion was 8.0. Note multiple sites of FDG uptake associated with lesions of the skin, nasal planum, and lymph nodes. Many of these sites of infection were unrecognized prior to doing the scan. Typical high intensity uptake is seen in the brain, renal pelves, and bladder. The mild intensity uptake in the regions of the orbits, mandibular salivary glands, and thyroid lobes was seen in all scans and characterized as normal for dogs.



Figure 15. FDG-PET scan of a dog with blastomycosis (planar view). There is high intensity uptake of FDG associated with *Blastomyces* lesions of the peribronchial lymph nodes, lung, mandibular lymph nodes, and the sphenoid sinus. There is mild intensity uptake associated with *Blastomyces* lesions of the skin and popliteal lymph nodes. Many of these sites of infection were unrecognized prior to doing the scan. The SUV of the enlarged peribronchial lymph node is 11.2. The SUV of the pulmonary granuloma is 9.1.

(Figure 16). Accessible sites of uptake were subsequently confirmed to have cytologic or radiographic characteristics of blastomycosis or lymphoma, with the exception of the sphenoid sinus, which was not further evaluated. Anterior uveitis, partial retinal detachment, and a retinal granuloma were consistent with the presence of ocular blastomycosis in the right eye of the dog with asymmetrical orbital uptake.

The SUV was calculated for 13 lesions from the 5 dogs with blastomycosis. The mean SUV for the blastomycosis lesions was 7.7 ± 2.0 . The SUV was calculated for 17 lesions from the 3 dogs with lymphoma. The mean SUV for lymphomas was 4.8 ± 1.8 . The mean SUV of *Blastomyces* lesions was significantly higher than the mean SUV of lymphomas ($p < 0.001$; Figure 17). There was overlap between the SUVs of *Blastomyces*-associated lesions and lymphomas, but a cutoff SUV of 7.0 gave 100% specificity for *Blastomyces* lesions (Figure 18). Eight of 13 *Blastomyces* lesions had SUVs above 7.0, and none of the 17 lymphomas had SUVs above 7.0.

The follow-up FDG-PET images after 60 days of treatment in dogs with blastomycosis revealed no evidence of FDG uptake in any previous sites of disease in 3/5 dogs (Figure 19). These 3 dogs remain clinically healthy to date, 7, 9, and 11 months after completing treatment. Of the remaining 2 dogs, 1 dog had uptake remaining in the right lung, although the lesion appeared smaller than previously. This dog also had new foci of FDG uptake in the mandibular nodes and the right prescapular lymph node. Previous lesions in the peribronchial lymph nodes, right popliteal lymph node, and skin were no longer visible (Figure 20). The last dog had uptake remaining in the left lung and peribronchial



Figure 16. Whole-body FDG-PET scan of a dog with lymphoma (planar view). There is high intensity uptake of FDG associated with the mandibular, prescapular, axillary, and popliteal lymph nodes. The SUVs for the lesions in this dog ranged from 4.5 – 7.0.

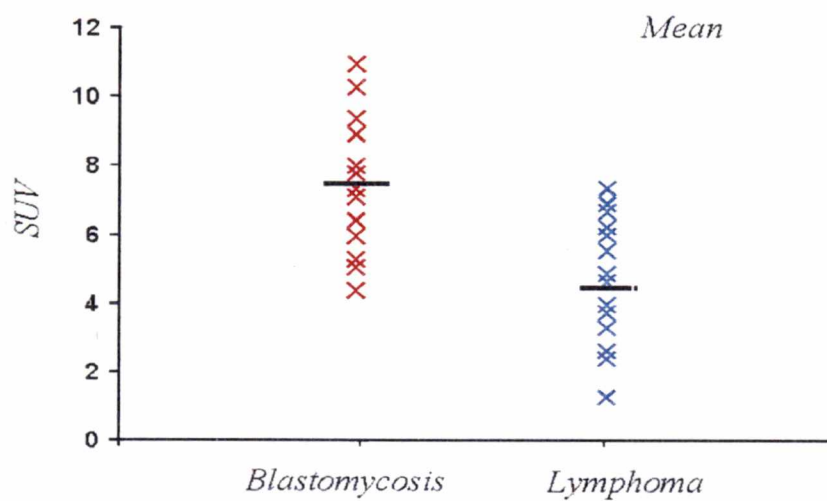


Figure 17. Graph of SUVs from 13 lesions from 5 dogs with blastomycosis and 17 lesions from 3 dogs with lymphoma. The mean SUV for *Blastomyces* lesions was significantly greater than the SUV for lymphomas ($p < 0.001$).

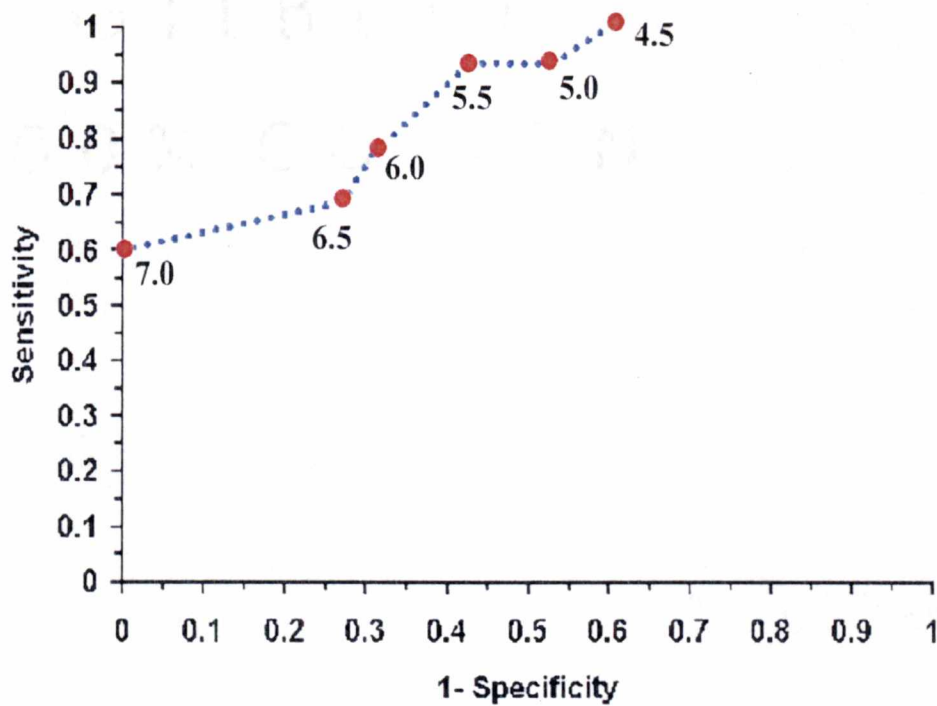


Figure 18. Receiver operator characteristic curve for determining threshold SUV to distinguish *Blastomyces* lesions from lymphomas. A cutoff SUV of 7.0 gives 100% specificity for *Blastomyces* lesions.

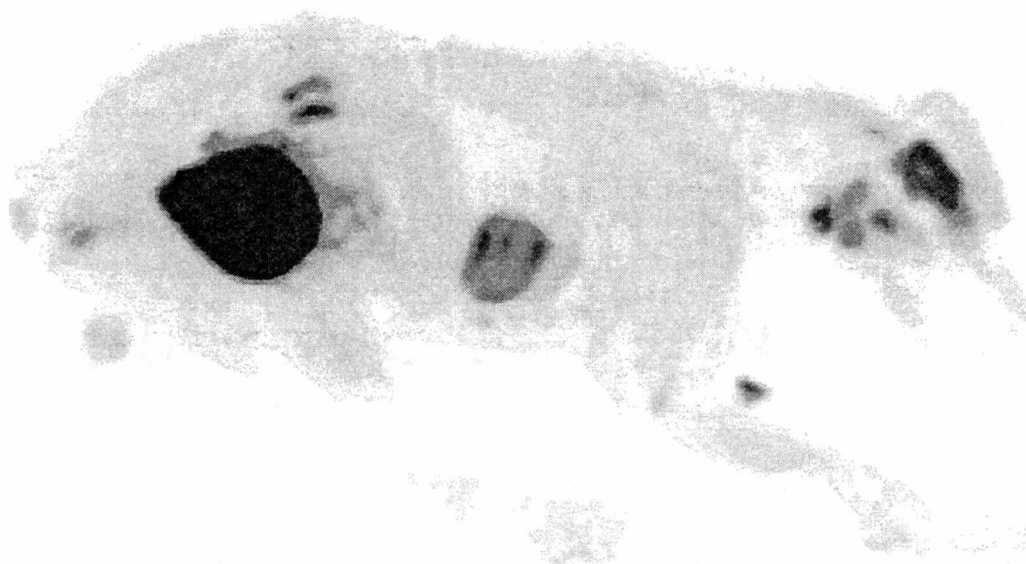


Figure 19. Whole-body FDG-PET scan of the dog in Figure 14 (planar view) after treatment for blastomycosis. Previous sites of FDG uptake are no longer visualized. Typical mild intensity uptake of FDG is present in the region of the orbits, mandibular salivary glands, and thyroid lobes. The focal high intensity uptake over the front limb is the injection site. This dog is clinically disease-free 7 months after completion of itraconazole treatment.

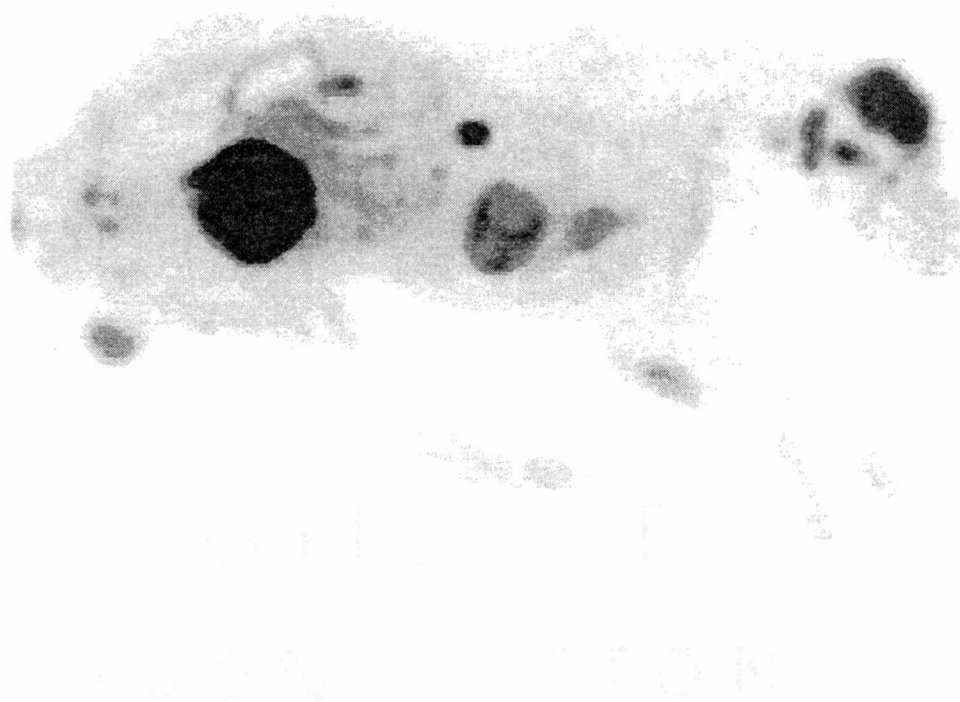


Figure 20. Whole-body FDG-PET scan of the dog in Figure 15 (planar view) after treatment for blastomycosis. There is resolution of many lesions noted previously. The pulmonary granuloma is still visible. Uptake can be seen in the right prescapular lymph node that was not present on the initial scan. This dog had ocular blastomycosis, which commonly acts as a nidus for continued infection in dogs. An additional course of therapy was recommended.

lymph nodes, although the intensity of uptake was less than prior to therapy.

Continuation of therapy with itraconazole was recommended for the 2 dogs with persistent foci of uptake. Follow-up FDG-PET scans were not available after the second course of therapy.

Discussion

This study showed that naturally occurring fungal lesions associated with *Blastomyces dermatitidis* in dogs consistently have high uptake of FDG. FDG-PET reliably detected known sites of *Blastomyces*-associated granulomas. More importantly, FDG-PET identified numerous lesions in tissues that were not detected on physical examination or thoracic radiographs. We previously reported similar findings in one dog with disseminated blastomycosis that died. In that dog, all sites of FDG uptake were confirmed histopathologically to be inflammatory lesions caused by *Blastomyces dermatitidis*. The value of FDG-PET for detecting lesions and defining the extent of disease in dogs with blastomycosis is now evident. In dogs and people, the lung is considered to be the most common site of infection with *Blastomyces*.^{130;148-150} Detection of multiple extrapulmonary lesions in sites that were unremarkable on clinical examination prompts speculation that dogs have more extensive extrapulmonary disease than previously recognized. This may be true for human patients with blastomycosis, and extrapulmonary lesions may be identified if a sensitive method for detection is used. All dogs of this study were successfully treated, and without post-mortem examinations we cannot know whether FDG-PET failed to detect lesions not seen on radiographs or

detected on physical examination. However, the results of this study support a role for FDG-PET in defining the extent of disease in patients with blastomycosis.

High intensity uptake of FDG by lesions of blastomycosis may be due to high uptake of FDG by macrophages. Many lesions characterized by a granulomatous inflammatory reaction exhibit high accumulation of FDG.^{1-3;5-7;16;28;30} Macrophages have been shown to accumulate 3-4 times as much FDG as malignant cells.²⁰ Uptake of FDG by the yeast organisms may contribute to overall uptake of FDG by fungal lesions. However, few organisms are usually identified in lesions of naturally occurring *Blastomyces* lesions,¹⁴⁸ suggesting that uptake of FDG by the yeast organisms plays only a small role in overall uptake of FDG by *Blastomyces* lesions.

In this study of naturally occurring diseases in dogs, the mean SUV for *Blastomyces* lesions was greater than the mean SUV for lymphomas. The SUV was calculated for 13 lesions from 5 dogs with blastomycosis and for 17 lesions from 3 dogs with lymphoma. Use of a t-test was considered valid for the comparison of SUVs between *Blastomyces* lesions and lymphomas because the 2 variables (dogs with blastomycosis and dogs with lymphoma) were independent. In the dogs of this study, 8 of the 13 *Blastomyces* lesions had SUVs above 7.0, and none of the 17 lymphomas had SUVs above 7.0. A cutoff SUV of 7.0 gave 100% specificity for *Blastomyces* lesions. This suggests that using a cutoff value, above which lesions are considered to be most likely fungal disease, and below which lesions are considered to be malignant, could improve specificity of FDG-PET for

distinguishing *Blastomyces* lesions from lymphomas. Further investigation is required to see if this holds true for other fungal lesions and other malignancies.

Determining when to discontinue antifungal treatment can be difficult in dogs and people with blastomycosis. Patients may appear clinically and radiographically disease-free, but have a recurrence of blastomycosis at a later date.¹⁵⁰ Relapse following appropriate therapy occurs in up to 20-25% of dogs with blastomycosis, usually within a year of completing treatment.¹⁵⁰ FDG-PET, in addition to defining extent of disease, may also be useful for monitoring response to therapy. The small number of cases in this study did not allow us to statistically assess the ability of post-treatment FDG-PET scans to predict likelihood of relapse in dogs with blastomycosis. However, follow-up studies correctly identified 3/5 dogs in this study as having complete resolution of disease. These 3 dogs remain clinically disease-free 7, 9, and 11 months following therapy. FDG-PET identified improved but persistent disease in the remaining 2 dogs. Neither of these dogs was considered cured based on persistence of lesions on thoracic radiographs, and additional therapy would have been recommended without the information from the FDG-PET images. However, in one dog, FDG-PET indicated development of new disease foci that would not have otherwise been detected, in the face of resolving disease at the original sites. This dog had ocular blastomycosis, which frequently acts as a nidus for continued infection in dogs. FDG-PET would be useful in such cases to monitor persistent foci of infection that required prolonged therapy.

This study was done using dogs with naturally occurring blastomycosis and lymphoma. However, because of the similarities of these diseases in dogs and people, the results are probably applicable to people. In dogs and people, the lung is the site of entry and the primary site of disease in cases of blastomycosis.^{130;149;150} Common extrapulmonary sites of infection, including skin, bone, and genitourinary tract are the same in both species.^{130;149;150} The geographic distribution of blastomycosis in dogs parallels that of people, however dogs are more susceptible to infection, and have a shorter prepatent period.¹⁵⁰ Asymptomatic and self-limiting presentations are common in people. It is unknown how often this occurs in dogs.^{130;149;150} However in dogs, as in people, treatment is recommended for patients with clinical signs associated with the disease.¹⁵⁰ Canine lymphoma is one of the most common neoplasms in dogs.^{158;159} Canine lymphomas are classified using either the Kiel or Working Formulation classification systems developed for human non-Hodgkin's lymphomas.¹⁵⁹ The majority of canine lymphomas are B-cell lymphomas similar to medium and high-grade non-Hodgkin's lymphomas in people.¹⁵⁹ In both dogs and people, the clinical presentation is similar, with > 80% of cases being presented for asymptomatic superficial lymphadenopathy.^{157;159;160} In dogs, multicentric lymphoma is the most common presentation.¹⁵⁹

Conclusions

FDG-PET is useful for determining the extent of disease and response to therapy in dogs with blastomycosis. Lesions associated with blastomycosis exhibit high uptake of FDG.

The SUV for *Blastomyces*-associated lesions may be as high or higher than for malignant lymphoma. Because of the similarities in canine and human blastomycosis and lymphomas, similar results would be predicted in human patients. In regions where blastomycosis is endemic, *Blastomyces* granulomas should be considered a differential diagnosis for lesions exhibiting high intensity uptake on FDG-PET images.

CONCLUSIONS

Results of this project showed that localized subcutaneous *Blastomyces dermatitidis* lesions could be created in rats. Accumulation of FDG by inflammatory lesions caused by the fungal agent *Blastomyces dermatitidis* was higher than for non-fungal inflammatory lesions (turpentine abscesses) and as high as for malignant tumors (lymphomas) in experimentally created lesions in rats. The uptake of FDG in the experimentally created *Blastomyces* lesions was associated with the region occupied by the granulomatous inflammatory reaction and the yeast organisms. The mean (+/- sd) SUV for naturally occurring lesions of blastomycosis in dogs was 7.7 +/- 2.0, and was higher than the mean SUV for naturally occurring lymphomas in dogs of 4.8 +/- 1.8. A cutoff SUV of 7.0 gave 100% specificity for blastomycosis. Lesions caused by the fungal agent *Blastomyces dermatitidis* typically show high intensity uptake of FDG in experimentally created lesions in rats and naturally occurring lesions in dogs. Whole-body FDG-PET can reliably detect sites of *Blastomyces*-associated inflammation in dogs. Similar results would be expected in people. Blastomycosis should be considered a differential diagnosis for lesions with high intensity uptake of FDG on PET scans in regions where blastomycosis is endemic.

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