

Video Article

Semi-quantitative Assessment Using [^{18}F]FDG Tracer in Patients with Severe Brain Injury

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Abstract

Patients with severe traumatic brain injury (sTBI) have difficulty knowing whether they are accurately expressing their thoughts and emotions because of disorders of consciousness, disrupted higher brain function, and verbal disturbances. As a consequence of an insufficient ability to communicate, objective evaluations are needed from family members, medical staff, and caregivers. One such evaluation is the assessment of functioning brain areas. Recently, multimodal brain imaging has been used to explore the function of damaged brain areas. [^{18}F]-fluorodeoxyglucose positron emission tomography-computed tomography ([^{18}F]FDG-PET/CT) is a successful tool for examining brain function. However, the assessment of brain glucose metabolism based on [^{18}F]FDG-PET/CT is not standardized and depends on several varying parameters, as well as the patient's condition. Here, we describe a series of semiquantitative assessment protocols for a region-of-interest (ROI) image analysis using self-produced [^{18}F]FDG tracers in patients with sTBI. The protocol focuses on screening the participants, preparing the [^{18}F]FDG tracer in the hot lab, scheduling the acquisition of [^{18}F]FDG-PET/CT brain images, and measuring glucose metabolism using the ROI analysis from a targeted brain area.

Video Link

The video component of this article can be found at <https://www.jove.com/video/58641/>

Introduction

Patients with sTBI are presented with unforeseeable neurological difficulties over the course of rehabilitation that include motor deficits, sensory deficits, and psychiatric instability¹. Although clinical assessment is generally performed verbally, patients with sTBI such as unresponsive wakefulness syndrome or minimally conscious state have particular difficulty in knowing whether they are accurately expressing their thoughts and emotions because of disorders of consciousness, disrupted higher brain function, and verbal disturbances^{2,3}. Family members, medical staff, and caregivers are sometimes confounded by unforeseeable neurological changes or the lack of response that can result from insufficient communicatory ability^{4,5}.

Recently, multimodal brain imaging has been used to explore regional brain function^{6,7,8,9}. The brain is the main consumer of glucose-derived energy, with glucose metabolism providing approximately 95% of the adenosine triphosphate (ATP) required for the brain to function¹⁰. The uptake of [^{18}F]-fluorodeoxyglucose (FDG) is a marker for the uptake of glucose by brain tissue. [^{18}F]FDG-PET/CT can detect [^{18}F]FDG uptake and is, therefore, a useful tool for examining brain function¹¹. In general, [^{18}F]FDG image analysis is divided into two categories: ROI analysis and voxel-based analysis (VBA)¹². Previous reports show that ROI analysis is preferred for studying specific regions of traumatic injury. This is because VBA (such as statistical parametric mapping [SPM]) requires coregistration and normalization to a standard brain, which does not work well in cases of TBI due to brain tissue deformation such as brain atrophy, swelling, enlargement, and shrinking of ventricular space^{7,12}. Although various algorithms and software have been developed for analyzing magnetic resonance imaging (MRI) data, metals used in neurosurgical and orthopedic surgery generate noise artefacts^{7,12,13}. Recently, the use of photomultipliers with PET/CT devices has improved the spatial resolution of PET/CT-derived brain images¹⁴. The current protocol focuses on semi-quantitatively measuring glucose uptake via ROI analysis in [^{18}F]FDG-PET/CT using self-produced [^{18}F]FDG tracers in patients with sTBI.

Protocol

This study was performed in compliance with the institutional review board (approval No. 07-01) and adhered to the tenets of the Declaration of Helsinki. Informed consent for medical record and brain image use was obtained from the patients' legal representatives. The study was conducted after approval by the institutional ethics committee (2017-14). This protocol was made following the guidelines of the Japanese Society of Nuclear Medicine and European Association of Nuclear Medicine as a reference^{15,16}.

1. Screening of the Participants

1. Obtain informed consent to use the medical records and brain images of the patients from the patients' legal representatives. A Glasgow Coma Scale score ≤ 8 at the time of accident must have been recorded in each patient's medical record^{17,18,19}.
2. Hold neurology, psychology, and multi-disciplinary staff conferences every six months to assess clinical manifestations.
Note: Conference members should include medical staff such as medical doctors, nurses, physical therapists, occupational therapists, speech therapists, nutritionists, and medical social workers. Be sure to constantly check whether patients can communicate (verbally or nonverbally) and make decisions for themselves because arousal state and neurological status are typically unstable.
3. Conduct clinical assessments of the auditory function, visual function, motor function, oromotor/verbal function, communication function, arousal state, facial expression, and other relevant functions, using standard assessment batteries such as the Coma Recovery Scale-Revised (CRS-R), the Nociception Coma Scale, and the Wessex Head Injury Matrix^{20,21,22}.
4. Schedule [¹⁸F]FDG-PET/CT scans for the patients who are medically stable and can safely participate in examinations. Only schedule those who have provided informed consent or whose legal representatives have provided informed consent, as stated in the informed consent form. Schedule [¹⁸F]FDG-PET/CT image acquisition near the day of clinical assessment.

2. Preparation of the [¹⁸F]FDG Tracer in the Hot Lab

1. In the hot lab, begin to manufacture reagent kits for the automated production of FDG tailored to the FDG synthesizer (see **Table of Materials**). Be sure to use the automatic program to check the mobility of the pumping system in the FDG synthesizer and to ensure that air does not leak from the reagent kit. Sterilize the contact area of the machine (this is the start time).
Note: Be sure to check the radiation monitor in the hot lab and use the portable radiation dosimeters to check the radiation levels of each person before they enter the hot lab.
2. Check the volume of [¹⁶O]-water and [¹⁸O]-water and the volume of helium, hydrogen, and nitrogen in the gas tank. Check whether the tap water temperature for primary cooling is under 25 °C and that for secondary cooling is under 22 °C. Use all water in the closed system (30 min after the start) for production.
3. Begin the preliminary irradiation of [¹⁶O]-water in the cyclotron (1 h after the start). Check the monitor to be sure that 2 - 3 mL of [¹⁶O]-water is irradiated in optimal conditions (e.g., 20 μ A, 5 min) in the target area of the cyclotron. After irradiation, install the vial of [¹⁶O]-water into a radioisotope dose calibrator and measure the level of radioactivity (see **Table of Materials**).
Note: The radioactive decay should be calculated using the following formula.

$$N(t) = N(0) \times \left(\frac{1}{2}\right)^{\frac{t}{T}}$$

Here,

N(t) is the number radioactive nuclei at $t = t$ seconds;

N(0) is the number radioactive nuclei at $t = 0$ seconds;

T = the half-life.

4. Begin the irradiation of [¹⁸O]-water in the cyclotron (1 h 30 min after the start). Set the bombardment time for up to 20 min and the energy of the impinging protons to 16.5 MeV.
5. Start the FDG synthesizer according to the operator manual²² (2 h after the start). A modified procedure is given below.
 1. After the irradiation, use helium gas to transfer 2 - 3 mL of the [¹⁸O]-water from the cyclotron to the polypropylene receiver of the FDG synthesizer.
 2. Hook syringes onto the corresponding syringe drivers, pressurize reagent vials, dissolve the 1,3,4,6-Tetra-O-acetyl-2-O-trifluoromethanesulfonyl- β -D-mannopyranose in one vial (7 ± 0.2 mL) of acetonitrile (purity $\geq 99.5\%$), and rinse the cassette with acetonitrile.
 3. After the bombardment, transfer the irradiated [¹⁶O]-water and [¹⁸O]-water to the FDG synthesizer.
Note: Once the synthesis has started, the irradiated [¹⁸O]-water moves through an anion exchange cartridge (see **Table of Materials**). Be sure to condition and convert the cartridge to the carbonate before the synthesis.
 4. After transferring the eluent containing the [¹⁸F]activity without liquid into the reaction vessels, allow the solvents to evaporate until dry. During the drying process, add small amounts of acetonitrile to the reaction vessel 3x (each time, add 80 μ L). Perform the evaporation at 95 °C under nitrogen flow and vacuum.
 5. Add the mannose triflate precursor (25 mg) to the dry residue after dissolving it in about 3.5 mL of acetonitrile (with a purity of $\geq 99.5\%$). A nucleophilic substitution reaction occurs at 85 °C in the FDG synthesizer.
 6. As a preliminary purification, mix the labeled solution with 26 mL of distilled water. Send about 4 mL of the diluted labeling solution back to the reaction vessel to recover the remaining activity. Pass the solution through the reverse-phase cartridge (see **Table of Materials**). Rinse the cartridge containing the trapped labeled precursor 4x using 10 mL, 10 mL, 13 mL, and 13 mL of distilled water on the successive washes.

7. Convert the acetylated compound (labeled precursor) into FDG within the cartridge *via* alkaline hydrolysis, using 750 μ L of 2 N NaOH for 2 min at room temperature.
8. After hydrolysis, collect the alkaline FDG solution in 7 mL of water and mix it with the neutralization solution (5 mL of citrate buffer and 1 mL of 2 N HCl).
9. Purify the resulting neutralized FDG solution.
 1. Pass the neutralized FDG solution through a second reverse-phase cartridge (see **Table of Materials**), retaining the partially hydrolyzed compounds and nonpolar by-products.
 2. Pass it through an Alumina N cartridge (see **Table of Materials**), retaining the last traces of unreacted [18 F]fluoride ions. Then, pass it through a 0.22- μ m filter.
 3. Rinse the cassette and cartridges, filter with 3 mL of water to recover the residual FDG that is left in the lines and, then, drain the FDG into the final vial, which contains 15 - 17 mL of liquid.
10. Perform a qualitative analysis of the [18 F]FDG tracer (2 h 30 min after the start).
 1. Visually observe the vial. Confirm that it is transparent and that it does not include any particles.
 2. Measure the amount of liquid using a Roberval's balance (should be 15 - 17 mL).
 3. Measure the radioactivity and half-life using a radioisotope dose calibrator (the same as in step 2.3, see **Table of Materials**) (criterion: 105 - 115 min).
 4. Dispense 0.5 mL from the vial. Perform a radiochemical purity test *via* carbohydrate analysis. Use columns of 3.9 x 300 mm for high-performance liquid chromatography (see **Table of Materials**) to detect the peak radioactivity (over 95).
Note: A single peak means high purity.
 5. Measure the pH (pH 5.0 - 8.0) by using pH test paper (see **Table of Materials**). Measure the residual 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (see **Table of Materials**) (< 40 ppm) using test paper (see **Table of Materials**). Measure the endotoxins with the appropriate endotoxin-measuring device through absorbance measuring (see **Table of Materials**) (0.25 EU/mL). Do a test for sterility (finding no bacteria after 8 d at 37 °C).
11. Fill the vial covered by lead and tungsten with the [18 F]FDG tracer at a dosage of 5 MBq/kg body weight.
12. Transfer the [18 F]FDG tracer from the hot lab to the working room (3 h 25 min after the start).

3. Time Course for the Acquisition of the [18 F]FDG-PET/CT Brain Images

1. Schedule the patients. Be sure to inform the staff to stop nutrition and feeding *via* gastrostomy. Do not stop providing water. The patients should fast starting 7 h before the image acquisition.
2. Prepare the intravenous route for [18 F]FDG tracer administration. Secure a 22- to 24-G needle with 5 mL of heparin sodium (10 units/mL) on one of the lower limbs before entering the radiation-controlled area.
3. Have the patients lie down on a light stretcher before entering the radiation-controlled area. Bring the patients to the radiation-controlled area and wait for 30 min, in silence, while medical staff are on stand by.
4. Recheck the patency of the intravenous route by drawing blood with a 10-mL syringe. Measure the blood-glucose levels with a glucose meter.
5. After transferring the [18 F]FDG tracer from the hot lab to the working room, set it up in the auto-dispensing and injection system (see **Table of Materials**).
6. Recheck the following information (*via* the medical staff): patient ID number, name, birthday, height, and body weight; the name of the tracer, the amount of tracer (water with 3.5 mL of [18 F]FDG tracer + 12 mL of saline), the programmed radioactivity (5 MBq/kg), the time of injection, the [18 F]FDG tracer-lot number, the injection speed (normally, 0.3 mL/s), and the level of radioactivity that was measured in the hot lab.
7. Record the automatic measurement of preinjected radioactivity that appears on the display of the auto-dispensing and injection system.
8. Inject the [18 F]FDG tracer *via* the intravenous route prepared in step 3.2 (3 h 30 min after the start).
9. Record the residual volume of the [18 F]FDG tracer, which is shown automatically on the display of the auto-dispensing and injection system.
10. Have the patients wait in the waiting room of the radiation-controlled area for 50 min.
11. Transfer the patients from the waiting room to the PET/CT machine (see **Table of Materials**). Record the brain images for 10 min (4 h 30 min after the start).
Note: The imaging parameters for [18 F]FDG-PET/CT images are 10 min list mode. Reconstruct the data from 10-min bins. The data under 3 min are not used because the low-intensity signals are not adequate. Set the image reconstruction parameters: a block sequential regularized expectation maximization reconstruction algorithm (see **Table of Materials**); the matrix size = 192; the field of view = 25 cm; β -value: 100 - 200; z-axis filter: none.
12. After taking the images, check the injection area for extravasation. Discard all urine if the patient has a urinal catheter with urine bag.
13. Remove the patient from the radiation-controlled area (4 h 50 min after the start).
Note: See **Figure 1** for a schematic of the time schedule of events (patient procedure and synthesis of the [18 F]FDG tracer).

4. Analysis of the [18 F]FDG-PET/CT Images

1. Evaluate all image data for a standardized uptake value (SUV) measurement using the imaging software (see **Table of Materials**).
2. Select the patients.
3. Assign the data to the **MM oncology** workflow.
4. Click the button for **Functional** browsers.
5. Click the **VOI (volume of interest) threshold** button.
6. Set the VOI sphere to the three-dimensional browser.
Note: The maximum SUV (SUVmax) and mean SUV (SUVmean) are automatically measured for the VOI according to the chosen SUVmax threshold. Be sure to draw a border around the targeted VOI on the browser using the three-dimensional sphere, excluding other targets, extraocular muscles, and the scalp because they tend to disturb the set SUV threshold. Check the target area on axial, coronal, and sagittal slices.

7. After selecting all the right settings, click the **Edit the measure** button.
8. Change the threshold value (e.g., 50%) of the VOI and click **OK**.
9. Record the SUVmax, SUVmean, target volume, and threshold of the target area, which are automatically measured.
10. To sterically visualize the glucose metabolism of the whole-brain surface, use the software (see **Table of Materials**) to set a color map for the [^{18}F]FDG-PET/CT images based on blood glucose.
11. Finally, compare the clinical assessment with the [^{18}F]FDG-PET/CT images.

Representative Results

A 63-year-old man who had been run over by a car while cycling was brought to the emergency room *via* ambulance. The examination revealed a Glasgow Coma Scale score of 7 (eye opening = 1, best verbal response = 2, best motor response = 4), anisocoria (right: 2 mm, and left: 3 mm), and a negative corneal response¹⁷. A CT of the head showed subarachnoid and intracranial hemorrhage and a skull fracture of the left zygoma, temporal bones, and parietal bones. The patient had no medical history and was managed conservatively. After nine months, he was admitted to the Rehabilitation Center for Traumatic Apallics Chiba. Examination at admission revealed a Coma Recovery Scale (Revised) score of 6 (auditory function = 0 [none]; visual function scale = 1 [visual startle]; motor function scale = 3 [localization to noxious stimulation]; oromotor/verbal function scale = 1 [oral reflexive movement]; communication scale = 0 [none]; arousal scale = 1 [eye opening with stimulation]) and spontaneous eye opening, but no evidence of language comprehension or expression²⁰. Additionally, we saw no spontaneous limb movement, except for that associated with a change of systemic muscle tonus. We observed positive blink responses to loud sounds near his ear. He was regarded as having unresponsive wakefulness syndrome (previously referred to as vegetative state) by multi-disciplinary conferences.

To investigate thalamic activity for the possibility of neurological recovery, [^{18}F]FDG-PET/CT was performed 13 months after the accident. [^{18}F]FDG tracer was injected at a 242.4-MBq level of radioactivity.

Figure 2A shows that the glucose metabolism in the left thalamus was lower than in the right thalamus (right thalamus: SUVmax = 9.44, SUVmean = 5.93; left thalamus: SUVmax = 6.79, SUVmean = 4.53). The laterality ratio for SUVmax ($\text{SUVmax}_{\text{left}} / \text{SUVmax}_{\text{right}}$) was $6.79/9.44 = 0.72$. Based on a previous report²⁴, this suggested that the patient might become psychiatrically unstable over the clinical course.

Additionally, an overall view of the whole-brain [^{18}F]FDG-PET/CT images showed that the peak glucose metabolism was in the left basal ganglia. Further, an examination of the three-dimensional brain-surface image showed that the glucose metabolism in the right frontal and parietal areas was higher than in the corresponding regions of the left hemisphere (see **Figure 2C**). Based on these data, clinical manifestations such as a level of wakefulness, motor activity, language comprehension and expression, visual and auditory cognition, facial expression, and psychiatric state can be compared with SUV values for the targeted brain area.

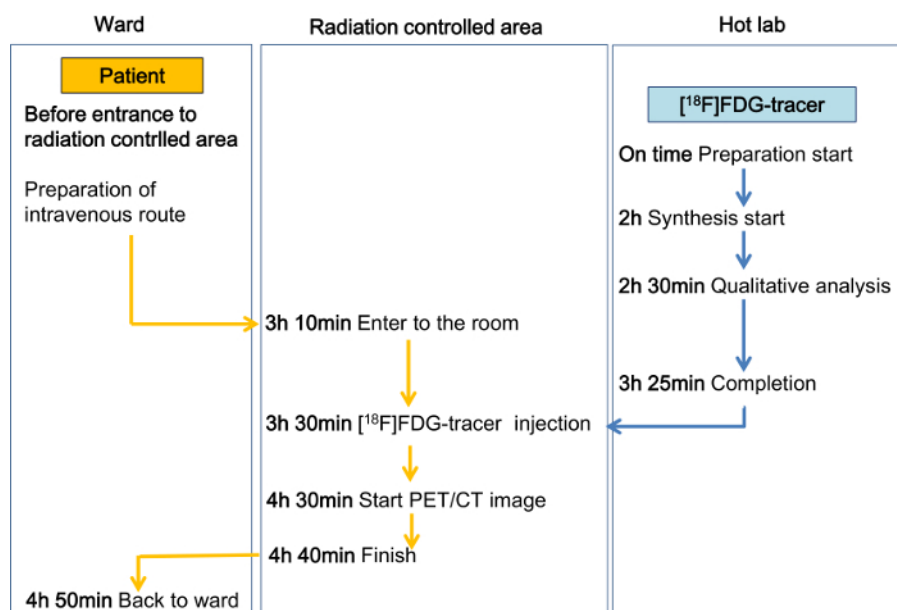


Figure 1: Schematic diagram of the time schedule for patient procedures and synthesis of [^{18}F]FDG tracer. [^{18}F]FDG: Fluorine-18 fluoro-2-deoxyglucose. [Please click here to view a larger version of this figure.](#)

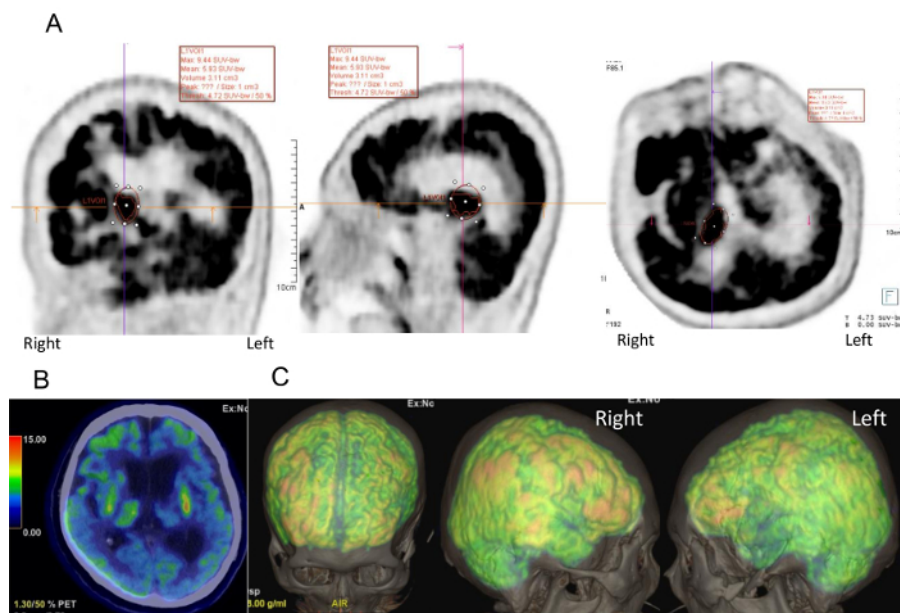


Figure 2: Representative ^{18}F FDG-PET/CT brain image. (A) This panel shows a measurement of the right thalamic glucose metabolism viewed using the three-dimensional image browser. (B) This panel shows representative color-mapped images after ^{18}F FDG-PET and CT fusion. The blood glucose level at the time of the scan (maximum 15 g/mL) is depicted as red with a 50% SUVmax threshold. (C) This panel shows representative three-dimensional brain-surface ^{18}F FDG-PET images. The reddish regions have a higher glucose metabolism than the greenish regions. The blood glucose level at the time of scan (maximum 8 g/mL) is shown in red. (C) Images were constructed using advanced visualization software. ^{18}F FDG: ^{18}F -fluoro-deoxyglucose; PET/CT: positron emission tomography/computed tomography. [Please click here to view a larger version of this figure.](#)

Discussion

This protocol provides the means to conduct a series of brain-glucose metabolic assessments with ^{18}F FDG-PET/CT using self-produced ^{18}F FDG tracer at a single institution.

The production of ^{18}F FDG tracer follows the procedure described in the FDG synthesizer operator manual; however, caution is necessary regarding three points. First, the bombardment time and energy (step 2.5) should be adjusted according to the number of patients. Second, attention should be paid to the tube for 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane because it can easily become stopped up by the crystallization of 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane. Third, the hook of syringes (step 2.5.2) should be handled carefully because it tends to break.

Clinical assessment must be handled with caution. The condition of patients with sTBI is typically unstable due to fluctuations in awareness and mood, especially during the chronic stage. Therefore, multidisciplinary regular conferences (e.g., every six months) are needed to verify the patient status. Otherwise, clinical signs can be overlooked by the examiners^{19,20,21,22}. To prevent misdiagnosis, several scoring systems, such as the Coma Recovery Scale-Revised and the Wessex Head Injury Matrix, should be used^{20,22}. However, it is likely that these clinical assessments cannot be performed on the same day as the ^{18}F FDG-PET/CT.

Another point of caution is that patients can sometimes make unforeseen movements during image acquisition, such as muscle tonus or sudden epileptic seizures. Because anesthetic sedation can influence brain glucose metabolism, this protocol does not include a method for sedation¹³. Therefore, the possibility that image acquisition might be interrupted or needs to be suspended is unavoidable and should be prepared for.

The automated SUVs for single voxels corresponding to extraocular muscles and the scalp may include outliers. Further, the automated VOI using the imaging software can become less anatomically accurate depending on the SUV threshold and spatial resolution of the CT. Additionally, if only a small amount of ^{18}F FDG tracer accumulates, we should distinguish the focal active area from the surrounding tissues on the browser. However, assessment via PET/CT alone is essential because most sTBI patients have neurosurgical and orthopedic surgical metal in their bodies, making MRI impossible.

Although preparing the equipment for ^{18}F FDG tracer production in advance is necessary, the delivery of the tracer makes it easy to use in clinical studies that lack facilities with a cyclotron²⁵. This ^{18}F FDG PET/CT approach for patients with sTBI has the potential to identify injured brain areas and residual brain function, which can be used for determining therapeutic targets. In the future, this protocol should be modified for use with advanced PET/CT imaging.

Disclosures

The authors have nothing to disclose.

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