Journal of Visualized Experiments

Semi-quantitative assessment using [18F]FDG tracer in patients with severe brain injury

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE58641R2
Full Title:	Semi-quantitative assessment using [18F]FDG tracer in patients with severe brain injury
Keywords:	Glucose metabolism; brain injury; FDG-PET; [18F]FDG; TBI; PET/CT; traumatic brain injury
Corresponding Author:	Tomohiro Yamaki Chiba Ryogo Center Chiba, JAPAN
Corresponding Author's Institution:	Chiba Ryogo Center
Corresponding Author E-Mail:	yamakitomohiro@hotmail.com
Order of Authors:	Tomohiro Yamaki
	Shinji Onodera
	Tomoki Uchida
	Yoshihiro Ozaki
	Kazuaki Yokoyama
	Haruko Henmi
	Mizuho Kamezawa
	Miyoko Hayakawa
	Daisuke Itou
	Nobuo Oka
	Masaru Odaki
	Yasuo Iwadate
	Shigeki Kobayashi
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	3-30-1 Isobe, Mihamaku, Chiba-shi, Chiba 261-0012, Japan

TITLE:

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

AUTHORS AND AFFILIATIONS:

Tomohiro Yamaki[1,2], Shinji Onodera[2], Tomoki Uchida[2], Yoshihiro Ozaki[2], Kazuaki Yokoyama[3], Haruko Henmi[2], Mizuho Kamezawa[2], Miyoko Hayakawa[2], Daisuke Itou[1], Nobuo Oka[1,2], Masaru Odaki[1], Yasuo Iwadate[4], Shigeki Kobayashi[1]

[1]Division of Neurosurgery, Rehabilitation Center for Traumatic Apallics Chiba, National Agency for Automotive Safety and Victims' Aid, Chiba, Japan
[2]Division of PET imaging, Rehabilitation Center for Traumatic Apallics Chiba, National Agency for Automotive Safety and Victims' Aid, Chiba, Japan
[3]Tokyo Nuclear Services Co. Ltd., Tokyo, Japan
[4]Department of Neurological Surgery, Graduate School of Medicine, Chiba University, Chiba, Japan

Corresponding Author:

Tomohiro Yamaki (t-yamaki@chiba-ryougo.jp)
Tel: +81-43-277-0061

Email Addresses of Co-authors:

Shinji Onodera (s-onodera@chiba-ryougo.jp)
Tomoki Uchida (t-uchida@chiba-ryougo.jp)
Yoshihiro Ozaki (y-ozaki@chiba-ryougo.jp)
Kazuaki Yokoyama (k-yokoyama-tns@chiba-ryougo.jp)
Haruko Henmi (pet-nurse@chiba-ryougo.jp)
Mizuho Kamezawa (m-kamezawa@chiba-ryougo.jp)
Miyoko Hayakawa (m-hayakawa@chiba-ryougo.jp)
Daisuke Itou (d-ito@chiba-ryougo.jp)
Nobuo Oka (noka-nsu@umin.net)
Masaru Odaki (odaki-nsu@umin.net)
Yasuo Iwadate (iwadatey@faculty.chiba-u.jp)
Shigeki Kobayashi (kobasige@green.ocn.ne.jp)

KEYWORDS:

Glucose metabolism, brain injury, FDG-PET, [^{18}F]FDG, TBI, PET/CT, traumatic brain injury

SUMMARY:

[^{18}F]-fluorodeoxyglucose (FDG) positron emission tomography-computed tomography is useful for studying glucose metabolism related to brain function. Here, we present a protocol for an [^{18}F]FDG tracer set-up and semiquantitative assessment of the region-of-interest analysis for targeted brain areas associated with clinical manifestations in patients with severe traumatic brain injury.

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.

INTRODUCTION:
Patients with sTBI are presented with unforeseeable neurological difficulties over the course of rehabilitation that include motor deficits, sensory deficits, and psychiatric instability[1]. 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 function[10]. 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 function[11]. 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 images[14]. 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

1.1. 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].

1.2. 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.

1.3. 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].

1.4. Schedule [^{18}F]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 [^{18}F]FDG-PET/CT image acquisition near the day of clinical assessment.

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

2.1. 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.

2.2. Check the volume of [^{16}O]-water and [^{18}O]-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.

2.3. Begin the preliminary irradiation of [^{16}O]-water in the cyclotron (1 h after the start). Check the monitor to be sure that 2 - 3 mL of [^{16}O]-water is irradiated in optimal conditions (*e.g.*, 20 µA, 5 min) in the target area of the cyclotron. After irradiation, install the vial of [^{16}O]-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.

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

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.

2.4. Begin the irradiation of [^{18}O]-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.

2.5. Start the FDG synthesizer according to the operator manual[22] (2 h after the start). A modified procedure is given below.

2.5.1. After the irradiation, use helium gas to transfer 2 - 3 mL of the [^{18}O]-water from the cyclotron to the polypropylene receiver of the FDG synthesizer.

2.5.2. 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 ≥ 99.5%), and rinse the cassette with acetonitrile.

2.5.3. After the bombardment, transfer the irradiated [^{16}O]-water and [^{18}O]-water to the FDG synthesizer.

Note: Once the synthesis has started, the irradiated [^{18}O]-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.

2.5.4. After transferring the eluent containing the [^{18}F]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 µL). Perform the evaporation at 95 °C under nitrogen flow and vacuum.

2.5.5. 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 ≥ 99.5%). A nucleophilic substitution reaction occurs at 85 °C in the FDG synthesizer.

2.5.6. 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.

2.5.7. Convert the acetylated compound (labeled precursor) into FDG within the cartridge *via* alkaline hydrolysis, using 750 µL of 2 N NaOH for 2 min at room temperature.

2.5.8. 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).

2.5.9. Purify the resulting neutralized FDG solution.

2.5.9.1. Pass the neutralized FDG solution through a second reverse-phase cartridge (see **Table of Materials**), retaining the partially hydrolyzed compounds and nonpolar by-products.

2.5.9.2. 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-µm filter.

2.5.9.3. 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.

2.5.10. Perform a qualitative analysis of the [^{18}F]FDG tracer (2 h 30 min after the start).

2.5.10.1. Visually observe the vial. Confirm that it is transparent and that it does not include any particles.

2.5.10.2. Measure the amount of liquid using a Roberval's balance (should be 15 - 17 mL).

2.5.10.3. 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).

2.5.10.4. 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.

2.5.10.5. 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).

2.5.11. Fill the vial covered by lead and tungsten with the [^{18}F]FDG tracer at a dosage of 5 MBq/kg body weight.

2.5.12. 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

3.1. 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.

3.2. 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.

3.3. 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.

3.4. Recheck the patency of the intravenous route by drawing blood with a 10-mL syringe. Measure the blood-glucose levels with a glucose meter.

3.5. 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**).

3.6. 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.

3.7. Record the automatic measurement of preinjected radioactivity that appears on the display of the auto-dispensing and injection system.

3.8. Inject the [^{18}F]FDG tracer *via* the intravenous route prepared in step 3.2 (3 h 30 min after the start).

3.9. Record the residual volume of the [^{18}F]FDG tracer, which is shown automatically on the display of the auto-dispensing and injection system.

3.10. Have the patients wait in the waiting room of the radiation-controlled area for 50 min.

3.11. 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.

3.12. After taking the images, check the injection area for extravasation. Discard all urine if the patient has a urinal catheter with urine bag.

3.13. 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

4.1. Evaluate all image data for a standardized uptake value (SUV) measurement using the imaging software (see **Table of Materials**).

4.2. Select the patients.

4.3. Assign the data to the **MM oncology** workflow.

4.4. Click the button for **Functional** browsers.

4.5. Click the **VOI (volume of interest) threshold** button.

4.6. 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.

4.7. After selecting all the right settings, click the **Edit the measure** button.

4.8. Change the threshold value (*e.g.*, 50%) of the VOI and click **OK**.

4.9. Record the SUVmax, SUVmean, target volume, and threshold of the target area, which are automatically measured.

4.10. 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.

4.11. 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 response[17]. 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 expression[20]. 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 ($SUVmax_{left}$/ $SUVmax_{right}$) was 6.79/9.44 = 0.72. Based on a previous report[24], 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 AND TABLE LEGENDS:

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.

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.

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 sedation[13]. 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 cyclotron[25]. 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.

ACKNOWLEDGMENTS:
The authors wish to thank Dr. Uchino in Sousen hospital for all procedures. The authors also thank Adam Phillips from the Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:
1. Godbolt, A.K. *et al*. Disorders of consciousness after severe traumatic brain injury: a Swedish-Icelandic study of incidence, outcomes and implications for optimizing care pathways. *Journal of Rehabilitation Medicine*. **45** (8), 741-748 (2013).

2. Klingshirn, H. *et al.* Quality of evidence of rehabilitation interventions in long-term care for people with severe disorders of consciousness after brain injury: A systematic review. *Journal of Rehabilitation Medicine*. **47** (7), 577-585 (2015).

3. Fischer, D.B., Truog, R.D. What is a reflex? A guide for understanding disorders of consciousness. *Neurology*. **85** (6), 543-548 (2015).

4. Klingshirn, H. *et al.* RECAPDOC - a questionnaire for the documentation of rehabilitation care utilization in individuals with disorders of consciousness in long-term care in Germany: development and pretesting. *BMC Health Services Research.* **18** (1), 329 (2018).

5. Stéfan, A., Mathé, J.F., SOFMER group. What are the disruptive symptoms of behavioral disorders after traumatic brain injury? A systematic review leading to recommendations for good practices. *Annals of Physical and Rehabilitation Medicine.* **59**, 5-17 (2016).

6. Liu, S. *et al.* Multimodal neuroimaging computing: a review of the applications in neuropsychiatric disorders. *Brain Informatics.* **2** (3), 167-180 (2015).

7. Wong, K.P. *et al*. A semi-automated workflow solution for multimodal neuroimaging: application to patients with traumatic brain injury. *Brain Informatics.* **3** (1), 1-15 (2016).

8. Chennu, S. *et al.* Brain networks predict metabolism, diagnosis and prognosis at the bedside in disorders of consciousness. *Brain*. **140** (8), 2120-2132 (2017).

9. Di Perri, C. *et al.* Neural correlates of consciousness in patients who have emerged from a minimally conscious state: a cross-sectional multimodal imaging study. *The Lancet Neurology*. **15** (8), 830-842 (2016).

10. Erecińska, M., Silver, I.A. ATP and brain function. *Journal of Cerebral Blood Flow & Metabolism*. **9** (1),2-19 (1989).

11. Lundgaard, I. *et al.* Direct neuronal glucose uptake heralds activity-dependent increases in cerebral metabolism. *Nature Communications.* **6**, 6807 (2015).

12. Byrnes, K.R. *et al.* FDG-PET imaging in mild traumatic brain injury: a critical review. *Frontiers in Neuroenergetics.* **5**, 13 (2014).

13. Mortensen, K.N. *et al.* Impact of Global Mean Normalization on Regional Glucose Metabolism in the Human Brain. *Neural Plasticity*. **2018**, 6120925 (2018).

14. Wagatsuma, K. *et al.* Comparison between new-generation SiPM-based and conventional PMT-based TOF-PET/CT. *Physica Medica*. **42**, 203-210 (2017).

15. Fukukita, H. *et al.* Japanese guideline for the oncology FDG-PET/CT data acquisition protocol: synopsis of Version 2.0. *Annals of Nuclear Medicine*. **28** (7), 693-705 (2014).

16. Varrone, A. *et al.* European Association of Nuclear Medicine Neuroimaging Committee. EANM procedure guidelines for PET brain imaging using [18F]FDG, version 2. *European Journal of Nuclear Medicine and Molecular Imaging.* **36** (12), 2103-2110 (2009).

17. Teasdale, G. Jennett, B. Assessment of coma and impaired consciousness. A practical scale. *The Lancet.* **2** (7872), 81-84 (1974).

18. Valadka, A.B. Injury to the cranium. In *Trauma*. Edited by Moore, E.J., Feliciano, D.V., Moore, E.E., 377-399, McGraw-Hill. New York, NY (2000).

19. Carney, N. *et al.* Guidelines for the Management of Severe Traumatic Brain Injury, Fourth Edition. *Neurosurgery*. **80** (1), 6-15 (2017).

20. Giacino, J.T., Kalmar, K., Whyte, J. The JFK Coma Recovery Scale-Revised: measurement characteristics and diagnostic utility. *Archives of Physical Medicine and Rehabilitation*. **85** (12), 2020-2029 (2004).

21. Schnakers, C. *et al.* The Nociception Coma Scale: a new tool to assess nociception in disorders of consciousness. *Pain*. **148** (2), 215-219 (2010).

22. Shiel, A. *et al*. The Wessex Head Injury Matrix (WHIM) main scale: a preliminary report on a scale to assess and monitor patient recovery after severe head injury. *Clinical Rehabilitation.* **14** (4), 408-416 (2000).

23. GE Healthcare. *TRACERlabMX$_{FDG}$ operator manual, Version 1* (March 2003).

24. Yamaki, T. *et al.* Association between uncooperativeness and the glucose metabolism of patients with chronic behavioral disorders after severe traumatic brain injury: a cross-sectional retrospective study. *BioPsychoSocial Medicine*. **12**, 6 (2018).

25. Schwaiger, M., Wester, H.J. How many PET tracers do we need? *Journal of Nuclear Medicine*. **52**, Suppl 2, 36S-41S (2011).

Figure 1

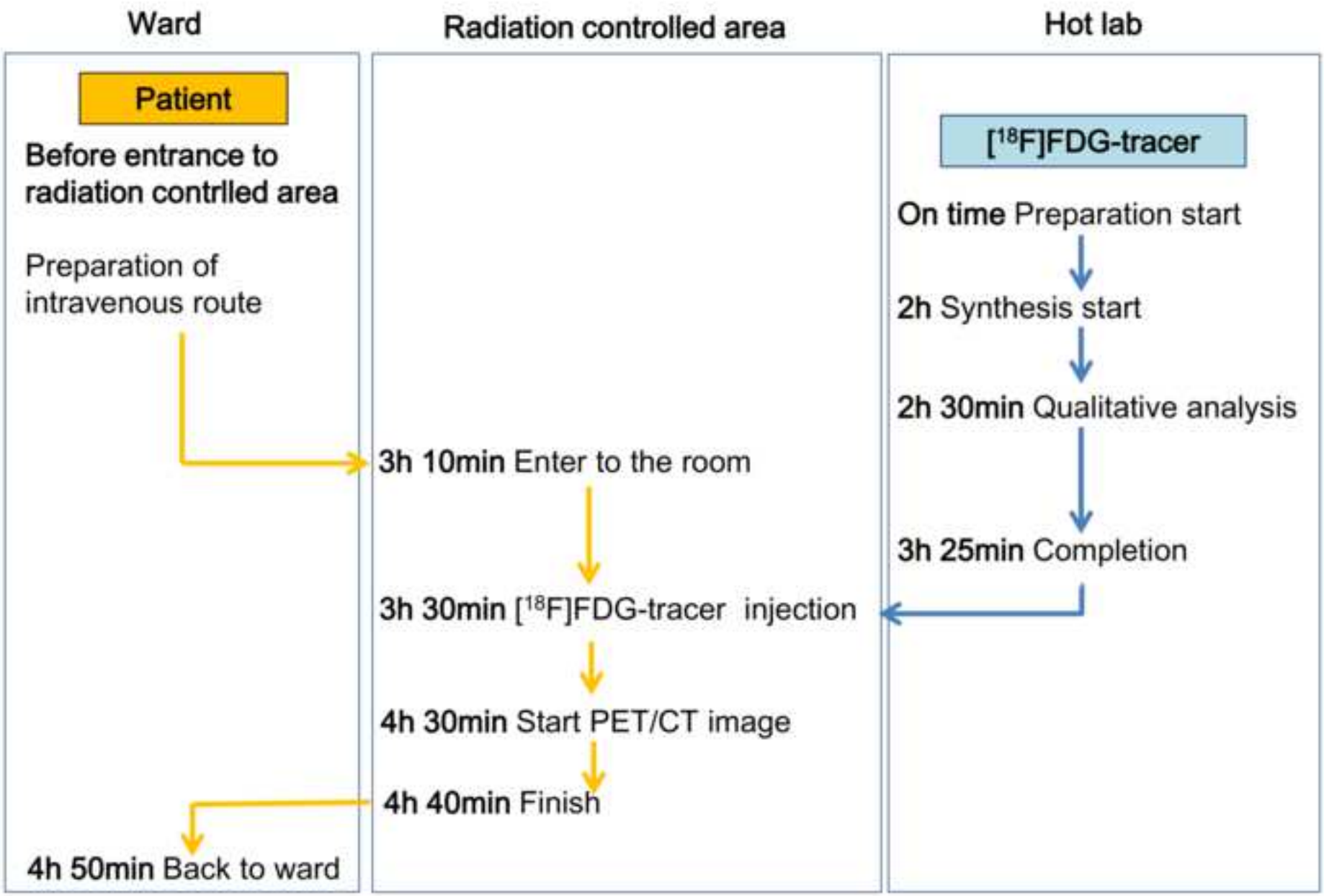

Figure 2

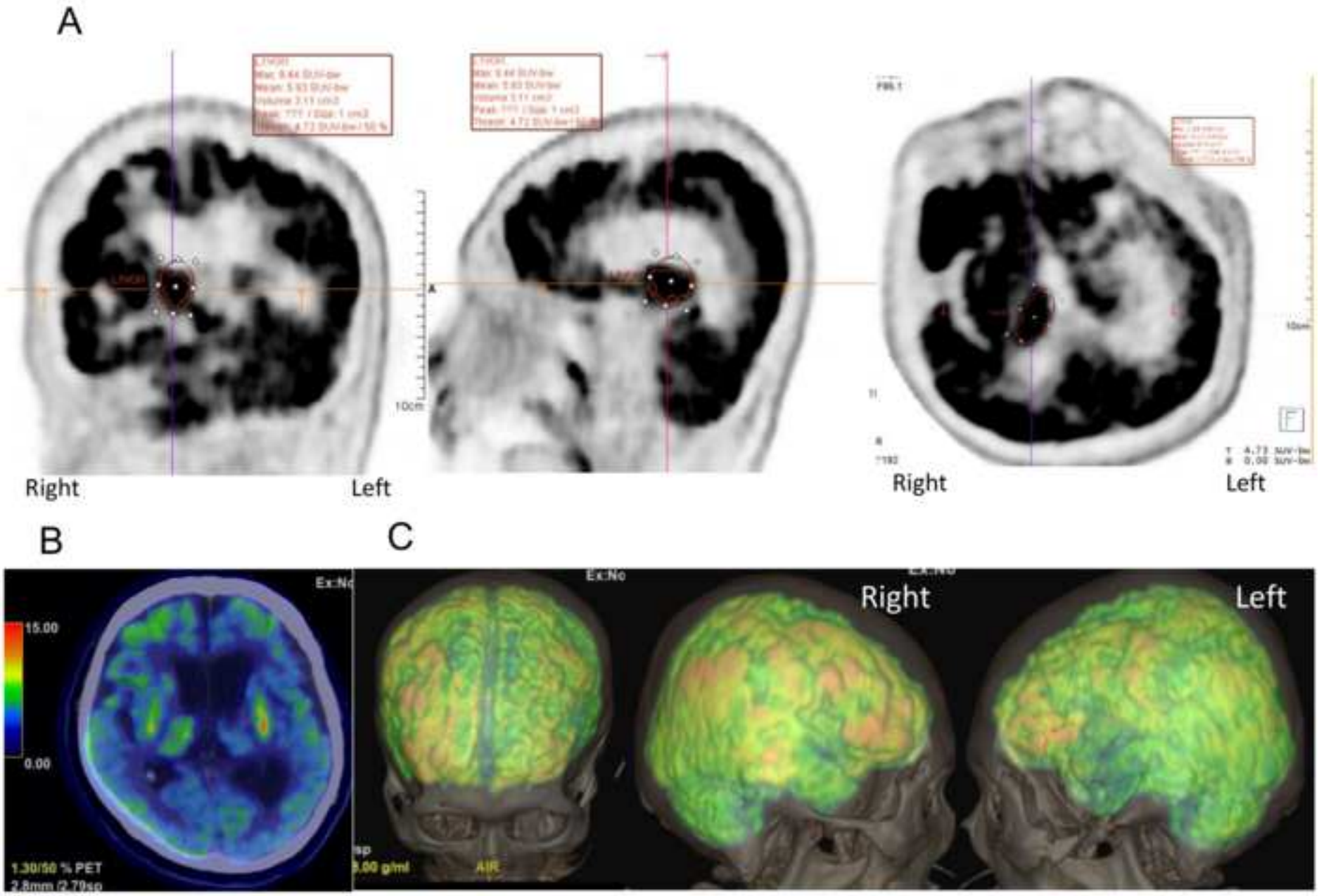

Table of Materials

Click here to access/download;Table of Materials;20180817JoVE_Materials.xls

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
20ml syringe	Terumo	SS-20ESZ	
10ml syringe	Terumo	SS-10ESZ	
1ml syringe	Terumo	SS-01T	
Protective plug	Top	ML-KS	
Three-way cock L type 180°	Terumo	TS-TL2K	
Extension tube	Top	X1-50	
Indwelling needle 22G or 24G	Terumo	SR-OT2225C	
Tegaderm transparent dressing	3M	1624W	
Hepaflash 10U/ml 10ml	Terumo	PF-10HF10UA	
Auto dispensing and injection system	Universal Giken Co., Ltd.	UG-01	
Fluid for auto dispensing and injection system	Universal Giken Co., Ltd.	UG-01-001	
Millex-GS Syringe Filter Unit	Millipore	SLGSV255F	
Air needle	Terumo	XX-MFA2038	
Check valve	Hakko	23310100	
Saline 500ml	HIKARI pharmaceutical Co., Ltd.	18610155-3	
Yukiban 25x7mm	Nitto	3252	
Elascot No.3	Alcare	44903221	
Presnet No.3 27x20mm	Alcare	11674	
Steri Cotto a 4x4cm	Kawamoto	023-720220-00	
StatstripXp3	Nova Biomedical	11-110	
Statstrip Glucose strips	Nova Biomedical	11-106	
JMSsheet	JMS	JN-SW3X	
Injection pad	Nichiban	No.30-N	
Stepty	Nichiban	No.80	
Advantage Workstation	GE Healthcare	Volume Share 7. version 4.7	

Discovery MI PET/CT	GE Healthcare	
EV Insite	PSP	
GE TRACERlab MX_{FDG} synthesizer		
reagent kit	ABX	K-105TM
TRACERlab MX_{FDG} cassette	GE Healthcare	P5150ME
Extension tube	Universal Giken Co., Ltd	AT511-ST-001
TSK sterilized injection needle 18x100	Tochigiseiko	AT511-ST-004
TSK sterilized injection needle 18x60	Tochigiseiko	AT511-ST-002
TSK sterilized injection needle 21x65	Tochigiseiko	AT511-ST-003
Seal sterile vial -N 5ml	Mita Rika Kogyo Co., Ltd.	SSVN5CBFA
k222 TLC plate	Universal Giken Co., Ltd.	AT511-01-005
Anion-cation test paper	Toyo Roshi Kaisha	7030010
Endospecy ES-24S set	Seikagaku corporation	20170
Sterile evacuated vial	Gi phama	10214
5ml syringe	Terumo	SS-05SZ
Extension tube	Top	X-120
Finefilter F	Forte grow medical Co.Ltd.	F162
Millex FG	Merck	SLFG I25 LS
Vented Millex GS	Merck	SLGS V25 5F
Injection needle 18x38	Terumo	NN-1838R
Injection needle 21x38	Terumo	NN-2138R

Water-18O	Taiyo Nippon Sanso	F03-0027
Distilled water	Otsuka phrmaceutical	
Hydrogen gas G1	Hosi Iryou Sanki	
Helium gas G1	Hosi Iryou Sanki	
Nitrogen G1	Hosi Iryou Sanki	
TRACERlabMX$_{FDG}$	GE Healthcare	
Sep-Pak Light Accell Plus QMA	WATERS	
Sep-Pak Plus tC18	WATERS	
Sep-Pak Plus Alumina N	WATERS	
HPLC with 3.9 X 300 mm columns	WATERS	
US-2000	Universal Giken CO. Ltd.	
Kryptofix222	Merck	
EG Reader SV-12	Seikagaku Corporation	
UG-01	Universal Giken Co., Ltd.	
syngo.via	Siemens Healthineers	
Advantage Workstation Volume Share 7, version 4.7	GE Healthcare	
Q clear	GE Healthcare	
CRC-15PET dose calibrator	CAPINTEC, INC.	

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Semi-quantitative assessment of brain glucose metabolism via ^{18}F-FDG-PET/CT in patients with brain injury

Author(s): Tomohiro Yamaki, Shinji Onodera, Tomoki Uchida, Yoshihiro Ozaki, Kazuaki Yokoyama, Haruko Henmi, Mizuho Kamezawa, Miyoko Hayakawa, Daisuke Itou, Nobuo Oka, Masaru Odaki, Yasuo Iwadate, Shigeki Kobayashi

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/author) via: ☑ Standard Access ☐ Open Access

Item 2 (check one box):

☑ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name: Tomohiro Yamaki

Department: Division of Neurosurgery , Division of PET imaging

Institution: Rehabilitation Center for Traumatic Apallics Chiba (Chiba Kyogo Center)

Article Title: Semi-quantitative assessement of brain glucose metabolism via 18F-Fluoro-deoxyglucose position emission tomography-computed tomography in patients with brain injury

Signature: Tomohiro Yamaki Date: 18 June 2018

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Alisha DSouza, Ph.D.
Senior Review Editor
Journal of Visualized Experiments

18 August, 2018

Dear Dr. DSouza

Thank you for your email [dated 9 August] enclosing the editors' comments. Our responses are given in a point-by-point manner below. Change to the manuscript is shown in track the changes within the manuscript to identify all of the edits.

Response to the comments from the Editorial Comments

Comment 1: This sounds a bit awkward. Consider deleting this?

Response: Thank you for your suggestion. We have changed the article title to **"Semi-quantitative assessment using [^{18}F]FDG tracer in patients with severe brain injury"**

Comment 2: The manuscript will benefit from thorough language revision as there are a number of grammatical errors throughout. Please have a proficient English-speaker thoroughly review the manuscript and edit any errors.

Response: Thank you for your suggestion. This manuscript was checked by Edanz Research Editing Group (www.edanzediting.com/ac) again

Comment 3: Some steps were edited for clarity. Highlighting was adjusted to meet JoVE's style requirements.

Response: I really appreciate your editing and highlighting. I agree all parts.

Comment 4: Reference?

Response: In accordance with editor 's comment, we have added the **REFERENCE 17 (p.3, lines 101)**.

Comment 5: Unhighlighted due to lack of filmable content.

Response: I agree it.

Comment 6: Please ensure that the hot lab is accessible for filming. Please mention notes of caution regarding appropriate shielding and personnel protection.

Response: Our hot lab is accessible for filming. We conjecture that the cameraman will be

exposed to radiation about 10μSv, which means 5-10 percent of simple X ray. In accordance with editor 's comment, we have added the **PROTOCOL 2.1. (p.3, lines 130-132)**

⇒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.

Comment 7: Unclear what is to be done and what we would show, please clarify.

Response: In accordance with editor 's comment, we have added the **PROTOCOL 2.1. (p.3, lines 130-132)**

⇒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.

Comment 8: What is checked for?

Response: In accordance with editor 's comment, we have added the **PROTOCOL 2.2. (p.4, lines 133-134)**

⇒2.2. Check the volume of [^{16}O]-water and [^{18}O]-water and the volume of helium, hydrogen, and nitrogen in the gas tank. Check the tap water temperature for primary cooling under 25 degrees and that that for secondary cooling is under 22 degrees.

Comment 9: What is done to prepare the cyclotron and gas tank?

Response: In accordance with editor 's comment, we have added the **PROTOCOL 2.2. (p.4, lines 134-135)**

⇒Check the tap water temperature for primary cooling under 25 degrees and that that for secondary cooling is under 22 degrees.

.

Comment 10: What is done here? In order to film this, exact actions must be described. Please elaborate. Mention any relevant settings.

Response: In accordance with editor 's comment, we have added the **PROTOCOL 2.3. (p.4, lines 138-145)**

⇒2.3. Begin preliminary irradiation of [16O]-water in the cyclotron (1 h after start). Check the monitor to be sure that 2–3ml of [16O]-water Is irradiated in optimal conditions (e.g., 20 □A, 5 min) in the target area of the cyclotron. After irradiation, enter 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 formula: $N(t) = N(0) \times (1/2)t/T$

N(t) is the number radioactive nuclei at t = t seconds; N(0) is the number radioactive nuclei at t = 0 seconds; T is the half-life.

Comment 11: What is done here? In order to film this, exact actions must be described. Please elaborate. Mention any relevant settings.

Response: In accordance with editor 's comment, we have added the **PROTOCOL 2.4. (p.4, lines 147-148)**

⇒2.4. Begin irradiation of [^{18}O]-water in the cyclotron (1 h 30 min after start). Set bombardment time for up to 20 min and the energy of the impinging protons to 16.5 MeV.

Comment 12: Prepare how exactly? Do you simply mean, load 2-3 ml 18O water? Where is the water loaded? In what kind of container?

Response: In accordance with editor 's comment, we have added the **PROTOCOL 2.5.1. (p.4, lines 153-154)**

⇒2.5.1. After irradiation, use helium gas to transfer 2–3 ml of the [18O]-water from the cyclotron to the polypropylene receiver of the FDG synthesizer.

Comment 13: How? What I done here? What would we film?

Response: In accordance with editor 's comment, we have added the **PROTOCOL 2.5.1. (p.4, lines 156-158)**

⇒2.5.2. 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 1 vial (7 ± 0.2 ml) of acetonitrile (purity ≥ 99.5%), and rinse the cassette with acetonitrile.

The cameraman can film the procedure on the monitor of the FDG synthesizer.

Comment 14: What is the precursor?

Response: In accordance with editor 's comment, we have changed the **PROTOCOL 2.5.1. (p.4, lines 157)**

⇒1,3,4,6–Tetra–O–acetyl–2–O–trifluoromethanesulfonyl–β–D–mannopyranose

Comment 15: What volume? 1 vial is vague.

Response: In accordance with editor 's comment, we have changed the **PROTOCOL 2.5.1. (p.4, lines 157-158)**

⇒1 vial (7 ± 0.2 ml)

Comment 16: %?.

Response: In accordance with editor 's comment, we have changed the **PROTOCOL 2.5.1. (p.4, lines 157-158)**

⇒purity ≥ 99.5%

Comment 17: There appear to be missing steps before this as there is some discontinuity..
Response: In accordance with editor ’s comment, we have added highlighted color area of **PROTOCOL 2.5.4.**
The cameraman can film the procedure on the monitor of the FDG synthesizer.

Comment 18: %?.
Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.5. (p.4, lines 173)**
⇒purity ≥ 99.5%

Comment 19: Unclear. Is this in the synthesizer?.
Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.5. (p.4, lines 173-174)**
⇒A nucleophilic substitution reaction occurs at 85 °C in the FDG synthesizer.

Comment 20: Ultrapure? Distilled? De-ionized?
Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.6. (p.4, lines 176)**
⇒26 ml of distilled water

Comment 21: Ultrapure? Distilled? De-ionized?
Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.6. (p.5, lines 180)**
⇒using 10 ml, 10 ml, 13 ml, and 13 ml of distilled water

Comment 22: There is a discontinuity in the highlighting. Please ensure continuity between highlighted steps. Please highlight relevant steps for filming from 2.5.3-2.5.9.
Response: In accordance with editor ’s comment, we have added highlighted color area from **PROTOCOL 2.5.4. - 2.5.9.3.**

Comment 23: If relevant, cite a reference for how this is done. Please add a bit more details to describe what is done (for filming purposes).
Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.10.3. (p.5, lines 207-208)**

⇒Measure the radioactivity and half-life using a radioisotope dose calibrator the same as step 2.3.(see table of materials) (criterion: 105–115 min).

Comment 24: Unclear what is meant. Please describe the actions in detail.

Comment 25: Unclear what we would film here. Please describe all the steps..

Response: In accordance with editor ’s comment, we have edited for clarity and changed the **PROTOCOL 2.5.10.3. -2.5.10.5 (p.5, lines 207-216)**

2.5.10.3. Measure the radioactivity and half-life using a radioisotope dose calibrator the same as step 2.3.(see table of materials) (criterion: 105–115 min).

2.5.10.4. Dispense 0.5 ml from the vial.

2.5.10.5. Perform a radiochemical purity test via carbohydrate analysis. Use 3.9 × 300 mm columns for high-performance liquid chromatography (see table of materials) to detect the peak radioactivity (over 95).

Note: A single peak means high purity.

Comment 26: How is this done? What would we film here?

Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.10.6. (p.5, lines 219-220)**

⇒ 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)

Comment 27: How is this done? What would we film here? All steps need to be described in the text.

Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.10.6. (p.5, lines 220 - p.6, lines 221)**

⇒Measure the endotoxins with the appropriate endotoxin-measuring device through absorbance measuring (see table of materials) (0.25 EU/ml).

Comment 28: Unclear what the incubation conditions would be.

Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 2.5.10.6. (p.6, lines 221)**

⇒finding no bacteria after 8 days at 37°

We think that we had better set the humidity in future

Comment 29: Food and water?

Response: In accordance with editor ’s comment, we have changed the **PROTOCOL3.1. (p.6, lines 233-234)**

⇒Do not stop taking water. Patients should fast starting 7 hours before image acquisition.

Comment 30: Which parameters? Please describe.

Response: In accordance with editor ’s comment, we have deleted the words.

Comment 31: Place where?

Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 3.2. (p.6, lines 236-238)**

⇒Secure a 22–24 G needle with 5 ml heparin sodium (10 units/ml) on the lower limbs before entering the radiation-controlled area.

Comment 32: Mention volume and needle gauge.

Response: In accordance with editor ’s comment, we have changed the **PROTOCOL 3.4. (p.6, lines 244-245)**

⇒Recheck the patency of the intravenous route by drawing blood with a 10 ml syringe without a needle.

Comment 33: Please add the step where the ROIs/VOIs are drawn.

Response: In accordance with editor’s comment, we have changed the following text in the **PROTOCOL 4.4 (p.7, lines 300-303)**

⇒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.

Comment 34: Unclear what is being said. Please revise for grammar.

Response: In accordance with editor’s comment, we have changed the following text in the **PRPTOCOL 4.8 (p.7, lines 312-313)**

⇒4.8. To sterically visualize glucose metabolism of the whole brain surface, use the software (see table of materials) to set a color map for the [18F]FDG-PET/CT images based on blood glucose.

Comment 35: Reference?

Response: In accordance with editor’s comment, we have added the **REFERENCE 17 (p.8, lines 319)**

Comment 36: Reference?

Response: In accordance with editor's comment, we have added the **REFERENCE 20 (p.8, lines 329)**

Comment 37: It looks like the opposite is the case in 2c. Please indicate the right and left hemispheres in the figures

Response: Thank you for your suggestion. In accordance with editor 's comment, we have changed the **Figure 2C** and indicate the right and left hemispheres

Comment 38: Please mark the right and left sides of the brain on the panels.

Response: In accordance with editor 's comment, we have added the marking of the right and left sides of the **Figure 2**

Comment 39: The text and markings are too small to see clearly. Please increase the line weights and font sizes. Please translate the marking to English as well to allow a reader to interpret this.

Response: In accordance with editor 's comment, we have changed the font size language and of the **Figure 2**. The font size of **Figure 2A** was fixed in this program, so I enlarged the picture

Comment 40: Unclear what is meant, needs revision.

Response: In accordance with editor's comment, we have changed the following text in the **DISCUSSION (p.9, lines 374-377)**

⇒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 crystallization of 4,7,13,16,21,24-Hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane.

Comment 41: Unclear what is meant, needs revision.

Response: In accordance with editor's comment, we have changed the following text in the **DISCUSSION (p.9, lines 378-379)**

⇒Third, the hook of syringes (PROTOCOL 2.5.2) should be handled carefully because it tends to be broken.

Comment 42: But you previously say that the main reason behind your protocol is that MRI has artefacts from metal implants. This is a bit confusing

Response: In accordance with editor's comment, we have changed the following text in the

DISCUSSION (p.10, lines 404-405)

⇒In the future, this protocol should be modified for use with advanced PET/CT imaging.

Comment 43: Please do not abbreviate journal titles. Please follow this format: Godbolt A. K., Deboussard C. N., Stenberg M., Lindgren M., Ulfarsson T., Borg J. Disorders of consciousness after severe traumatic brain injury: a Swedish-Icelandic study of incidence, outcomes and implications for optimizing care pathways. Journal of Rehabilitation Medicine. 45 (8), 741-748, (2013).

Response: In accordance with editor's comment, I have corrected the references throughout the manuscript.

We wish to thank the editor again for the valuable comments.

We look forward to a publication of our manuscript in Journal of Visualized Experiments.

Tomohiro Yamaki M.D., Ph.D.,

3-30-1 Isobe, Mihama-ku, Chiba 261-0012 Japan, Division of Neurosurgery and Division of PET imaging

Rehabilitation Center for Traumatic Apallics Chiba (National Agency for Automotive Safety and Victims' Aid)

Email: t-yamaki@chiba-ryougo.jp; yamakitomohiro@hotmail.com

Phone: +81-43-277-0061; Fax: +81-43-277-2259