RESEARCH ARTICLE

Evaluation of Optimal Post-Injection Timing of Hypoxic Imaging with 18F-Fluoromisonidazole-PET/CT

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Abstract

Purpose: Positron emission tomography (PET)/computed tomography (CT) using 18F-fluoromisonidazole (FMISO) has been used as an imaging tool for tumour hypoxia. However, it remains unclear whether they are useful when scanning is performed earlier, e.g. at 2-h post-injection with a high sensitivity PET scanner. This study aimed to investigate the relationship between quantitative values in 18F-fluoromisonidazole (18F-FMISO)-PET obtained at 2- and 4-h post-injection in patients with head and neck cancer.

Procedures: We enrolled 20 patients with untreated locally advanced head and neck cancer who underwent 18F-FMISO-PET/CT scan between August 2015 and March 2018 at our institute. Image acquisition was performed 2 h and 4 h after 18F-FMISO administration using a combined PET/CT scanner. The SUVmax, SUVmean, SUVpeak, tumour-to-blood ratio (TBR), tumour-to-muscle ratio (TMR), metabolic tumour volume (MTV), and total lesion hypoxia (TLH) were measured in the region of interest of the primary tumour. We evaluated the between-image Spearman's rank correlation coefficients and percentage differences in the quantitative values. The locations of the maximum uptake pixel were identified in both scans, and the distance between them was measured.

Results: The mean (SD) SUVmax at 2 h and 4 h was 2.2(0.7) and 2.4(0.8), respectively. The Spearman's rank correlation coefficients (ρ) and mean (SD) of the percentage differences of the measures were as follows: SUVmax (0.97; 7.0 [5.1]%), SUVmean (0.97; 5.2 [5.8]%), SUVpeak (0.94; 5.3 [4.7]%), TBR (0.96; 14.2 [9.8]%), TMR (0.96; 14.7 [8.4]%), MTV (0.98; 39.9 [41.3]%), and TLH (0.98; 40.1 [43.4]%). There were significant between-scan correlations in all quantitative values. The mean (SD) distance between the two maximum uptake pixels was 7.3 (5.3) mm.

Conclusions: We observed a high correlation between the quantitative values at 2 h and 4 h. When using a combined high-quality PET/CT, the total examination time for FMISO-PET can be shortened by skipping the 4-h scan.

Key words: Head and neck cancer, Tumour hypoxia, FMISO-PET, Post-injection timing

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Introduction

Radiation therapy is crucially involved in managing head and neck squamous cell carcinoma (HNSCC). Although there have been therapeutic and technological advances, some patients present with persistent tumours after irradiation or develop locoregional failure, which results in significant morbidity and mortality [1]. Numerous factors affect tumour sensitivity to radiation therapy. Hypoxia, which is among the tumour microenvironments, is associated with poor sensitivity to radiation therapy. In 1953, Gray et al. reported that oxygen concentration strongly influenced the biological effects of ionizing radiation [2]. Several studies have reported that tumour hypoxia in head and neck cancer is associated with radioresistance; moreover, it is an adverse prognostic factor [3, 4]. ^{18}F-fluoromisonidazole (FMISO) is used for non-invasive tumour hypoxia evaluation; further, its uptake is associated with patient prognosis [5–8].

Several quantitative values in ^{18}F-FMISO positron emission tomography (PET) at 4-h post-injection have been reported as reproducible [9]. However, their usefulness after prior scanning, for example, at 2-h post-injection, remains unclear. Only one study has reported that images acquired at 4-h post-injection provided higher contrast than those acquired at 2-h post-injection [10]. The total examination time in ^{18}F-FMISO-PET can be shortened if the quantitative values at 2- and 4-h post-injection are equivalent.

We hypothesized that using a combined PET/computed tomography (CT) scanner, which has detectors that are more sensitive and yields high-quality images, could provide novel findings. This study aimed to assess the correlation of quantitative values obtained using ^{18}F-FMISO-PET with a high-quality PET/CT system at 2- and 4-h post-injection in patients with head and neck cancer.

Materials and Methods

Patients

We enrolled 20 patients with untreated locally advanced head and neck cancer (LAHNC) who underwent ^{18}F-FMISO-PET/CT scanning between August 2015 and October 2018 at our institute. This study was approved by the institutional review board of our hospital (Approval number C1053-1). All the patients provided written informed consent. Table 1 presents the characteristics of the patients. The median age of the patients was 68 years (range: 40–84 years); there were 14 men and 6 women. The 20 patients with LAHNC presented with the following: maxillary sinus cancer ($n = 1$), nasopharyngeal cancer ($n = 7$), oropharyngeal cancer ($n = 4$), hypopharyngeal cancer ($n = 7$), and laryngeal cancer ($n = 1$), without other metastatic diseases. The histology of 19 and 1 patient showed characteristics of squamous cell carcinoma and adenocarcinoma, respectively. Based on the Classification of Malignant Tumours, 7th

Table 1. Characteristics of the patients

No.	Age/sex	Cancer sites	Histology	Stage
1	66/M	Maxillary sinus cancer	Ad	IVB
2	72/F	Nasopharyngeal cancer	SCC	III
3	70/F	Oropharyngeal cancer	SCC	III
4	73/M	Oropharyngeal cancer	SCC	IVB
5	69/F	Hypopharyngeal cancer	SCC	III
6	72/M	Hypopharyngeal cancer	SCC	III
7	68/M	Nasopharyngeal cancer	SCC	IVA
8	59/M	Nasopharyngeal cancer	SCC	III
9	85/F	Nasopharyngeal cancer	SCC	IVB
10	64/M	Hypopharyngeal cancer	SCC	III
11	40/F	Nasopharyngeal cancer	SCC	II
12	76/F	Hypopharyngeal cancer	SCC	IVB
13	69/M	Hypopharyngeal cancer	SCC	II
14	68/M	Oropharyngeal cancer	SCC	IVB
15	62/M	Laryngeal cancer	SCC	III
16	65/M	Nasopharyngeal cancer	SCC	III
17	60/M	Hypopharyngeal cancer	SCC	IVB
18	46/M	Nasopharyngeal cancer	SCC	III
19	78/M	Hypopharyngeal cancer	SCC	IVB
20	69/M	Oropharyngeal cancer	SCC	IVA

Ad adenocarcinoma, *SCC* squamous cell carcinoma

edition of Union for International Cancer Control (UICC), we classified 2, 9, 2, and 7 patients as stage II, III, IVA, and IVB, respectively.

Data Acquisition

Image acquisition was performed at 2 h and 4 h after ^{18}F-FMISO administration. All images were acquired for 20 min in 3-dimensional mode using a dedicated PET/CT scanner (Discovery IQ, GE Healthcare) with 5 circular BGO ($Bi_4Ge_3O_{12}$) detectors. The sensitivity was 22 kcps/MBq, which was 2.4 times higher than our conventional PET/CT scanner. Image reconstruction was performed using VuePoint HD. The axial resolution was full width at half maximum of 5.6 mm, at 1 cm from the centre of the field of view.

Image Analysis

^{18}F-FMISO-PET images were quantitatively analysed. Although a threshold for the hypoxia definition remains unestablished, we defined a volume of interest where the standardized uptake value (SUV) was ≥ "blood mean + 0.5 × standard deviation (SD)", which was modified from a previous study [9], as the metabolic tumour volume (MTV) for calculating PET parameters. We calculated the SUVmax, SUVmean, SUVpeak, tumour-to-blood ratio (TBR), tumour-to-muscle ratio (TMR), metabolic tumour volume (MTV), and total lesion hypoxia (TLH) from the volume of interest containing the primary tumour. The SUVmax was calculated as the maximal single voxel of activity concentration/(injected dose/body weight). The SUVmean was calculated as the average SUV within the MTV, while the SUVpeak was calculated as the maximum average SUV within a 1-

cm^3 spherical volume. The TBR was derived as the maximum tumour concentration divided by the blood concentration in the cervical artery measured in the axial image at the hyoid level. We did not perform invasive blood sampling for TBR analysis. The TMR was defined as the tumour uptake divided by the uptake of the posterior cervical muscle in the axial image at the hyoid level. We analysed the average value of each 10 muscle lesions and the blood uptake in the region of interest (ROI) with a diameter of 0.4 cm. Subsequently, the physiological accumulation region identified in other modalities was manually excluded from the MTV. The TLH was calculated as the MTV × SUVmean.

CT and ^{18}F-FMISO-PET images were co-registered on a workstation (MIM, version 6.5.3). The locations of the maximum uptake pixels on the FMISO2h and FMISO4h images were identified, and the distance between them was measured to evaluate the location reproducibility of ^{18}F-FMISO uptake.

Statistical Analysis

Regarding the statistical analysis of the reproducibility of ^{18}F-FMISO uptake and MTV, we evaluated the Spearman's rank correlation coefficients of these quantitative values. We also calculated the percentage difference as the difference between both values divided by their average values. A correlation coefficient of more than 0.7 indicates a high correlation. Statistical significance was set at an αvalue of 0.05. All statistical analyses were performed using Microsoft Excel software, version 16.11.

Results

The mean (SD) ^{18}F-FMISO injection dose was 327.5 (132.8) MBq (range: 166.3–601.0 MBq); moreover, the time interval between injection and scan start was 126.0 (11.9) min (range: 114–167 min) for FMISO2h and 245.9 (13.4) min (range: 226–263 min) for FMISO4h.

Table 2 shows the SUVmax, SUVmean, SUVpeak, TBR, TMR, MTV, and TLH. The Spearman's rank correlation coefficients (ρ) of the parameters and the mean (SD) of the percentage difference were as follows: SUVmax (0.97; 7.0 [5.1]%), SUVmean (0.97; 5.2 [5.8]%), SUVmean (0.94; 5.3 [4.7]%), TBR (0.96; 14.2 [9.8]%), TMR (0.96; 14.7 [8.4]%), MTV (0.98; 39.9 [41.3]%), and TLH (0.98; 40.1 [43.4]%) (Fig. 1). There was a high between-scan correlation of the quantitative values ($P < 0.01$).

The mean (SD) distance between the maximum uptake locations of both scans was 7.3 (5.3) mm (range: 0.9–22.1 mm). This distance was smaller than the PET/CT scanner resolution in 11 out of 20 patients. Figure 2 presents a selected axial image of the representative cases.

Discussion

We observed a high correlation between the quantitative values of ^{18}F-FMISO-PET/CT scans at 2- and 4-h post-

Table 2. Parameters of FMISO2h and FMISO4h

	SUVmax		SUVmean		SUVpeak		TBR		TMR		MTV (mL)		TLH (mL)	
	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h	2 h	4 h
1	2.7	2.8	2.1	2.1	2.4	2.5	2.1	2.2	1.9	2.1	49.9	46.3	106.3	98.6
2	3.2	3.8	2.3	2.6	2.7	3.1	2.1	2.9	2.4	3.0	3.1	2.5	7.2	6.5
3	2.0	2.2	1.9	1.9	1.9	1.9	1.2	1.5	1.2	1.5	0.6	0.7	1.1	1.4
4	2.2	2.5	1.8	1.9	2.0	2.2	1.8	2.4	1.7	2.2	15.4	8.1	27.0	15.0
5	1.9	1.8	1.7	1.7	1.7	1.5	1.2	1.3	1.3	1.2	1.1	0.1	1.9	0.1
6	1.8	1.9	–	–	1.6	1.6	1.8	1.8	1.5	1.6	–	–	–	–
7	3.4	3.2	2.5	2.4	2.9	2.8	2.5	2.5	2.3	2.2	3.1	4.2	7.7	10.0
8	3.9	4.6	2.6	2.9	3.2	3.8	3.4	4.4	3.0	4.1	10.2	8.4	26.8	24.6
9	1.5	1.6	–	–	1.4	1.4	1.2	1.6	1.3	1.6	–	–	–	–
10	1.5	1.5	1.3	1.3	1.4	1.3	1.5	1.8	1.3	1.6	1.7	1.4	2.2	1.8
11	2.0	1.8	1.7	1.5	1.8	1.6	1.6	1.7	1.7	1.8	2.0	1.9	3.4	2.8
12	3.4	3.4	2.5	2.5	2.9	2.9	2.7	2.8	2.8	3.5	7.6	5.5	18.7	13.5
13	1.6	1.7	1.5	1.4	1.5	1.5	1.4	1.6	1.3	1.4	1.2	1.5	1.8	2.2
14	1.9	2.1	1.7	1.7	1.8	1.8	1.4	1.6	1.4	1.7	9.5	4.1	16.1	7.0
15	1.7	1.7	1.6	1.4	1.5	1.4	1.3	1.5	1.4	1.5	0.2	0.3	0.3	0.5
16	2.3	2.5	1.9	2.0	2.0	2.1	1.7	2.0	2.3	2.8	2.6	2.5	5.0	5.1
17	1.7	1.8	1.6	1.5	1.5	1.5	1.2	1.5	1.1	1.3	1.5	1.5	2.3	2.2
18	1.9	1.8	1.6	1.5	1.7	1.5	1.6	1.7	1.4	1.4	15.1	6.8	24.2	10.1
19	1.7	1.9	1.5	1.6	1.6	1.6	1.4	1.5	1.3	1.5	2.5	1.0	3.7	1.7
20	2.6	2.6	2.1	2.0	2.4	2.4	1.9	2.2	2.0	2.2	2.1	2.9	4.5	5.8
mean	2.2	2.4	1.9	1.9	2.0	2.0	1.8	2.0	1.7	2.0	7.2	5.5	14.5	11.6
SD	0.7	0.8	0.4	0.5	0.5	0.7	0.6	0.7	0.5	0.8	11.4	10.2	24.0	22.0
% dif	7.0±5.1		5.3±4.7		5.2±5.8		14.2±9.8		14.7±8.4		39.9±41.3		40.1±43.4	
P^a	0.03		NS		NS		<0.01		<0.01		<0.01		0.03	

[a]As determined using the Mann-Whitney U test. *NS* not significant
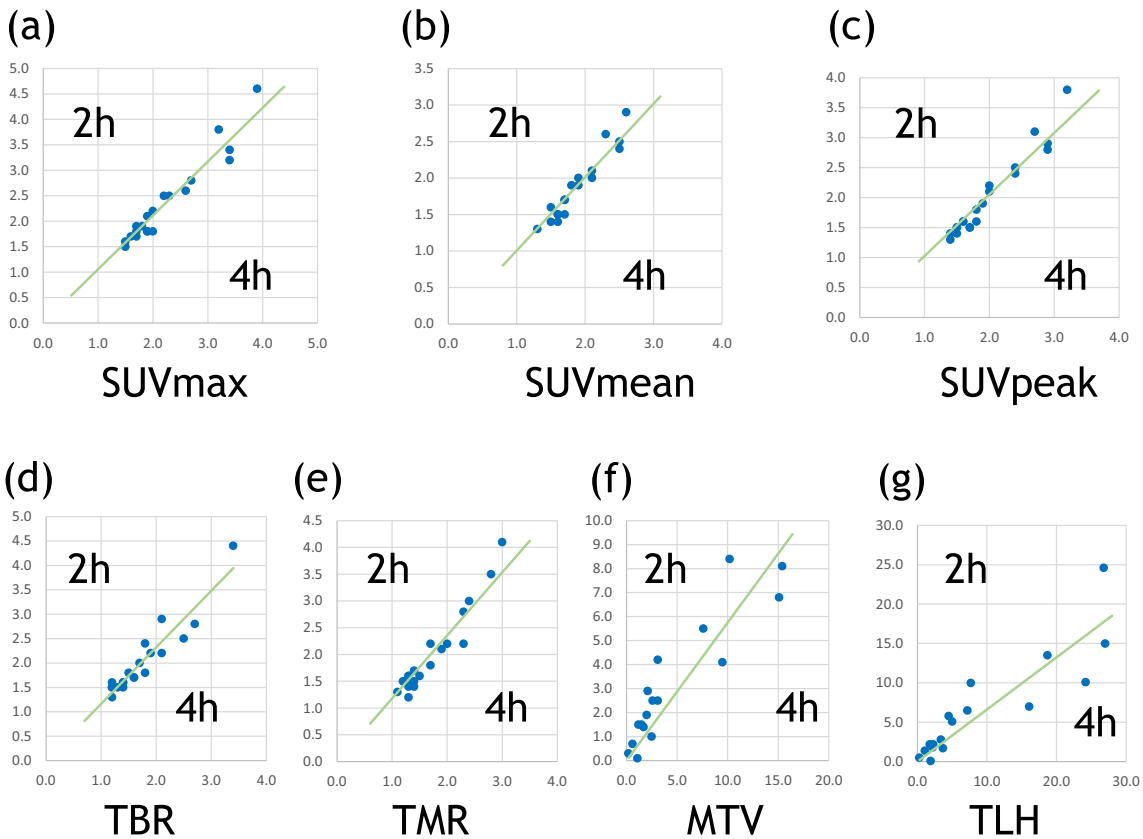
Fig. 1. Scatterplots of FMISO2h vs FMISO4h parameters. (**a**) SUVmax, (**b**) SUVmean, (**c**) SUVpeak, (**d**) TBR, (**e**) TMR, (**f**) MTV, (**g**) TLH. The lines show the modelled linear fit of these parameters.

injection, with few patients presenting a moderate difference in volumetric parameters.

^{18}F-FMISO-PET images obtained at 4-h post-injection were considered as more suitable for reproducibility evaluation compared with images acquired less than 3 h due to slow blood clearance of ^{18}F-FMISO and low contrast [5–7, 11–14].

Additionally, Gagel et al. [15] and Zimmy et al. [16] compared pO2 measurements with 2-h ^{18}F-FMISO PET data to pO2 probe measurement in head and neck cancer. From these studies, it is equivocal as to which timepoint is better for ^{18}F-FMISO imaging. A previous study by Okamoto et al. [9] compared the quantitative values of two ^{18}F-FMISO-PET/CT
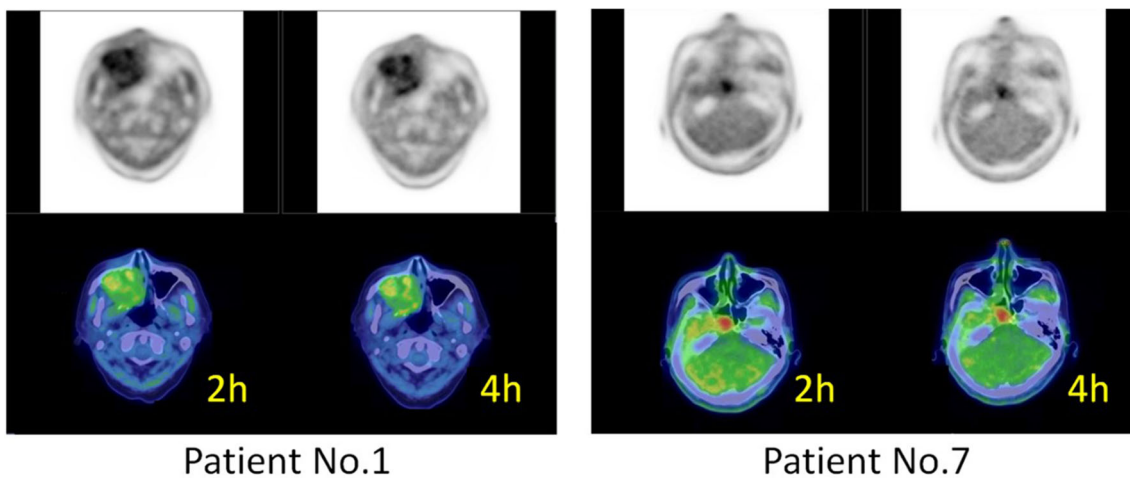

Fig. 2. Axial images of FMISO2h and FMISO4h for representative patients. Patient 1 had maxillary sinus cancer, and patient 7 had nasopharyngeal cancer.

images obtained at 48-h intervals, including SUVmax, TBR, TMR, and hypoxic volume (HV), and reported high reproducibility of these quantitative values at 4 h post-injection. Another previous study [10] directly compared the ^{18}F-FMISO images acquired at 2- and 4-h post-injection. It was concluded that images acquired after 4 h had a higher contrast than those acquired after 2 h. The discrepancy between this study and ours could arise from two aspects: the difference in analysis method which uses absolute value or correlations and the sensitivity of PET scanners. The present study found a high correlation between quantitative values obtained at 2 h and 4 h using a current combined PET/CT scanner, which has highly sensitive detectors and can yield high-quality images. Further examination is needed for the evaluation of the two scanners. Specifically, there was a high between-scan correlation in quantitative values, including SUVmax, TBR, and TMR. Moreover, there was a high between-scan correlation in volumetric values, including MTV and TLH. Further studies with a larger population should confirm the reproducibility of two ^{18}F-FMISO PET/CT images acquired at intervals of more than 2-h post-injection. This could shorten the total examination time in ^{18}F-FMISO-PET, which results in a decrease in the patient burden caused by this examination.

^{18}F-FMISO has an imidazole ring and is reductively activated in hypoxic cells [17]. It is the most common PET radiotracer for tumour hypoxia imaging; however, its high lipophilicity and slow clearance kinetics require up to 2- or 3-h post-injection to obtain contrast [18]. The biological half-life of this tracer is 12–13 h [19, 20]. A previous study concluded that images acquired after 4 h of uptake and washout provided higher contrast compared with images acquired after 2 h [10]. In the present study, the correlation of SUVmax and the subtraction evaluation were indicative of the reproducibility of FMISO2h and FMISO4h.

A previous study reported that the oxygen enhancement ratio was approximately 3 under 20 mmHg [21], which decreased to 2.0–2.8 at an oxygen tension of 3–20 mmHg. Therefore, compared with normoxic areas, areas of FMISO accumulation are radioresistant. Regarding oxygen tension in areas of FMISO accumulation, an animal study using pig liver reported reduced FMISO when pO2 was less than 15 mmHg [22]. In that study, the SUV of FMISO was 1.57, which corresponded to a pO2 of 20 mmHg. Various thresholds of TBR, TMR, and SUVmax have been provisionally determined [23]. The present study used an SUV ≥ "blood mean + 0.5 ×SD" as the threshold since it is adaptive and suitable for hypoxic volume delineation. We did not draw off the volume using a SUVmax threshold of 50 %, 60 %, or 1.6. A universal threshold separating the background from the hypoxic region is required to define the target for dose escalation. Future large-scale studies should examine the optimal cut-off value of ^{18}F-FMISO-PET uptake for estimating the hypoxic state and predicting therapy outcome for HNC.

The hypoxic area in a tumour is characterized by chronic and acute hypoxia [24, 25]. Acute hypoxia can change every

few hours or days and therefore could alter the ^{18}F-FMISO uptake distribution. Mönnich et al. [26] reported that acute hypoxia did not affect PET imaging reproducibility in simulations. In the present study, there were no significant differences in SUVmax, TBR, TMR, MTV, and TLH. This observed reproducibility is suggestive of the insignificant effect of transient acute hypoxia on ^{18}F-FMISO studies at two intervals. Future studies should assess whether acute hypoxia can be captured at this PET resolution.

Our final goal is determining the dose escalation for a hypoxic region to improve the radiotherapy outcome. There have been numerous reports on parameters regarding the hypoxic region as a prognostic factor [27, 28]. Moreover, there have been several studies on dose painting based on the spatial distribution of hypoxia [29, 30]. These studies have shown that hypoxia-targeted radiotherapy using dose painting is a feasible technique for increasing tumour control without a concomitant increase in normal tissue complications. A small pilot study described the outcome of patients with HPV-related oropharyngeal cancer whose radiation dose was de-escalated from 70 to 60 Gy in grossly involved lymph nodes [31]. In de-escalation treatment strategies, functional imaging for selectively de-escalating nodes to 60 Gy has been confirmed as safe. These dose painting planning requires the feasibility and reproducibility of functional imaging.

In the FDG-PET protocol, imaging at 2-h post-injection was considered to yield good contrast and high diagnostic ability compared with imaging at 1-h post-injection [32]. However, scans performed at later times show degraded image quality due to physical F-18 decay. In routine clinical FDG-PET examinations, a 1-h uptake phase protocol is adopted since its diagnostic ability is not inferior. Therefore, despite the good contrast of FMISO, which peaks after 4 h, the high correlation could allow a shorter 2-h imaging protocol upon clinical application. Regarding the absolute value, SUV was non-significant, while TBR or TMR had significant differences mainly due to decreased background activity resulting from decay and washout. Whether a 4-h timepoint has improved tumour-to-background contrast is not necessarily relevant from a biological perspective. These differences in TBR or TMR could allow qualitative FMISO2h evaluation and may also be useful in quantitative analysis. The shortened protocol could allow differentiation of the hypoxic tumour region from the background; however, care must be taken when extracting the volume and using volumetric parameters for dose painting.

Conclusion

Our preliminary data demonstrated a higher correlation between quantitative values obtained at 2- and 4-h post-injection. Using the novel high-quality PET/CT, the total examination time in FMISO-PET can be shortened by skipping the scan at 4 h.

Presentation

This work was presented at the 2017 Annual Meeting of the European Society of Radiation Oncology, Vienna, Austria.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Approval number C1053-1) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent

Written informed consent was obtained from all individual participants included in the study.

Consent for Publication

The authors affirm that human research participants provided informed consent for publication.

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