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Correlation of PET Images of Metabolism, Proliferation and Hypoxia to Characterize Tumor Phenotype in Patients with Cancer of the Oropharynx

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Abstract

Spatial organization of tumor phenotype is of great interest to radiotherapy target definition and outcome prediction. We characterized tumor phenotype in patients with cancers of the oropharynx through voxel-based correlation of PET images of metabolism, proliferation, and hypoxia.

Methods—Patients with oropharyngeal cancer received ¹⁸F-fluorodeoxyglucose (FDG) PET/CT, ¹⁸F-fluorothymidine (FLT) PET/CT, and ⁶¹Cu-diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) PET/CT. Images were co-registered and standardized uptake values (SUV) were calculated for all modalities. Voxel-based correlation was evaluated with Pearson's correlation coefficient in tumor regions. Additionally, sensitivity studies were performed to quantify the effects of image segmentation, registration, noise, and segmentation on R.

Results—On average, FDG PET and FLT PET images were most highly correlated ($R_{\text{FDG:FLT}}=0.76$, range 0.53–0.85), while Cu-ATSM PET showed greater heterogeneity in correlation to other tracers ($R_{\text{FDG:Cu-ATSM}}=0.64$, range 0.51–0.79; $R_{\text{FLT:Cu-ATSM}}=0.61$, range 0.21–0.80). Of the tested parameters, correlation was most sensitive to image registration. Misregistration of one voxel lead to $\Delta R_{\text{FDG}}=0.25$, $\Delta R_{\text{FLT}}=0.39$, and $\Delta R_{\text{Cu-ATSM}}=0.27$. Image noise and reconstruction also had quantitative effects on correlation. No significant quantitative differences were found between GTV, expanded GTV, or CTV regions.

Conclusions—Voxel-based correlation represents a first step into understanding spatial organization of tumor phenotype. These results have implications for radiotherapy target definition and provide a framework to test outcome prediction based on pretherapy distribution of phenotype.

Keywords

Correlation; FDG; FLT; Cu-ATSM; PET

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Introduction

Traditionally, radiotherapy plans are designed to deliver uniform doses to target volumes defined on anatomical images obtained by computed tomography (CT). Recently, non-uniform radiation boosting of biological target volumes, often referred to as dose-painting, has been proposed to improve tumor control [1]. Biological imaging is uniquely suited to define biological target volumes due to its non-invasive, volumetric, and quantitative properties. Potential dose-painting strategies include boosting of metabolic [2], hypoxic [3], or proliferative regions [4], and the first clinical dose-painting study based on ^{18}F -fluorodeoxyglucose (FDG PET) has been reported [5].

The application of biological imaging to radiation oncology has greatly impacted diagnosis, staging, target definition, and treatment response assessment. In HNSCC, pre-therapy FDG uptake has been shown as a prognostic indicator of tumor response, patient survival, and local control [6]. Additionally, FDG PET has been shown to define gross tumor volumes that are more similar to resected surgical specimens than volumes defined by CT or magnetic resonance imaging (MRI) [7], leading to speculation that FDG PET would gain increasing prominence for target definition [8].

Much enthusiasm exists for targeting hypoxic tumor volumes. Hypoxia has historically been relevant to radiation oncology as relates to radioresistance, but more recently as a driver of malignant progression and a predictive indicator of poor treatment outcome [9]. A variety of hypoxia imaging modalities have been applied in the clinic, including nitroimidazoles, such as ^{18}F -fluoromisonidazole (FMISO), ^{18}F -fluoroazomycin arabinoside (FAZA), 2-(2-nitro-(1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)-acetamide (EF5), or (18F)-fluoroerythronitroimidazole (FETNIM), and ^{64}Cu -diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) PET. In particular, Cu-ATSM [11] has shown high discriminatory power in selecting patients that responded to treatment versus non-responders in prospective studies of non-small cell lung, cervical, rectal, and head and neck cancers [12–15].

Another phenotype of interest is cellular proliferation, due to repopulation of cells during radiotherapy. Proliferation is commonly imaged with ^{18}F -fluorothymidine (FLT) PET [16] and direct correlation between FLT uptake and Ki-67 labeling index from biopsy samples has been observed [17], indicating the potential of FLT as a surrogate of tumor proliferation. In head and neck cancers, FLT PET has been shown to measure proliferative response following radiotherapy [18].

Understanding the structure and interaction of these phenotypes may help form a consensus on biological targeting strategies as well as answer the fundamental question, why do two patients respond to therapy in two different ways? Quantitative spatial correlation of PET images represents a mathematical technique to evaluate the interrelation of physiological characteristics.

Although correlation coefficients have been employed to test interrelation between PET surrogates, many studies have correlated summary statistics [19–23]; for example, FDG SUV_{max} to FMISO SUV_{max} . Due to tumor heterogeneity, correlation at the subregion or voxel level provides a more accurate description of tumor phenotype. To that end, Rajendran *et al* correlated FMISO and FDG uptake at the voxel level in several cancer presentations and found site-specific differences in level of correlation, from $R=0.62$ for head and neck cancer to $R=0.32$ for soft tissue sarcoma [24]. Thorwarth *et al* performed similar work with FMISO and FDG in HNSCC and, while R values are not reported, noted heterogeneity in correlation [25]. Lohith *et al* correlated Cu-ATSM and FDG in lung cancers by drawing

multiple regions-of-interest (ROI) in the tumor region and found significant differences in PET uptake of adenocarcinoma versus squamous cell carcinoma [26].

In this work, we have characterized tumor phenotype in patients with HNSCC through voxel-based correlation of PET images of metabolism (FDG), proliferation (FLT), and hypoxia (Cu-ATSM). Additionally, to facilitate comparisons of imaging protocols between institutions, sensitivity studies have been performed to test the effect of image segmentation, noise, registration, and reconstruction on correlation values.

Materials and Methods

Patients

Thirteen patients with HNSCC enrolled in a trial to receive [^{18}F]FDG, [^{18}F]FLT, and [^{61}Cu]Cu-ATSM PET prior to the start of chemoradiation (Table 1). Two patients received only two scans and were excluded from correlation analysis. FDG PET and contrast-enhanced CT screening was performed per standard of care prior to FLT and Cu-ATSM imaging. p16 tumor immunostaining was available for 8 of the 11 patients in this analysis cohort and all 8 were p16 positive, suggesting HPV association. All patient imaging protocols were approved by Institutional Review Board and use of experimental tracers was approved by the Radiation Drug Research Committee at the University of Wisconsin.

Acquisition

Images were acquired on separate days over a span of less than two weeks per patient. Patients were imaged on a Discovery LS PET/CT scanner (GE, Waukesha, WI) and immobilized with thermoplastic masks during FLT and Cu-ATSM imaging; FDG PET was performed per routine clinical workflow and no immobilization was used. CT images were acquired for tumor localization and 2D PET acquisition was performed as follows: FDG PET/CT (whole body), 1 frame/bed position $\times$ 5 min/frame acquisition following 40–60 min uptake, 5.2 MBq/kg injection; FLT PET/CT (single bed position), 3 frames $\times$ 10 min/frame acquisition following 60 min uptake, 150 MBq injection; and Cu-ATSM PET/CT (single bed position), 3 frames $\times$ 15 min/frame acquisition following 120–180 min uptake, 110 MBq injection.

PET images were reconstructed with CT attenuation correction on scanner hardware. Default reconstruction parameters were 128 $\times$ 128 $\times$ 35 voxels, 3.91 $\times$ 3.91 $\times$ 4.25 mm³ voxel size, ordered-subset expectation-maximization (OSEM) reconstruction with 2 iterations, 28 subsets, 5 mm full-width-at-half-maximum (FWHM) loop-filtration, and 3 mm FWHM post-filtration.

Image Analysis

For all scans, CT images were rigidly co-registered and transformations were applied to corresponding PET images. PET acquisitions with multiple frames were time-averaged and normalized by injected dose and body weight to obtain SUV. GTV and CTV were drawn by the radiation oncologist on contrast-enhanced CT and FDG PET/CT images. The GTV was expanded by 2mm to define the GTV+2mm volume and all volumes were downsampled to PET resolution for analysis. The expanded GTV volume was used as the reference volume as it showed greatest visual conformity to uptake from the three PET tracers.

To characterize PET uptake, SUV_{mean} , SUV_{max} , and SUV_{peak} were calculated for all tracers. Pearson correlation coefficients (R) were calculated between summary statistics and statistical testing was performed using paired t-tests with $\alpha=0.05$. Voxel-based correlation

was performed on voxels in the analysis volume with $SUV > 0.0001$ to calculate R between PET images (Figure 1). Average correlations were obtained by taking R in quadrature.

Sensitivity Studies

Sensitivity studies were performed to quantify the effect of tumor segmentation, noise, and registration on R . For the segmentation study, correlation coefficients were compared in the GTV, GTV+2mm, and CTV volumes.

For the noise study, correlation values obtained with time-averaged images (default) were compared to correlation between individual frames (1×10 min frame for FDG, 3×10 min frames for FLT, and 3×15 min frames for Cu-ATSM).

For the registration study, correlation between co-registered images was compared to correlation between images translated by a single voxel (3.91×3.91×4.25 mm). This displacement was based on Zhang *et al*, who compared daily Tomotherapy MVCT for patients with head masks and found 90% confidence in setup errors of less than 5.5 mm, 3.5 mm, 5.0 mm in sagittal, coronal, and transverse directions [27].

For the reconstruction study, OSEM reconstruction parameters (Supplement 2) were varied on the GE scanner for 6 patients, including voxel grid (128×128 or 256×256), loop and post filtration (5 mm loop and 3 mm post or 2 mm loop and 2 mm post), and iteration number (2 or 5). Reconstruction A represents the default reconstruction at this institution.

Results

PET uptake was significantly different between all tracers (Table 1). FDG images ($SUV_{mean}=3.5\pm1.2$, $SUV_{max}=17.4\pm5.3$, $SUV_{peak}=11.3\pm3.1$) exhibited highest levels of uptake compared to FLT ($SUV_{mean}=2.3\pm0.8$, $SUV_{max}=10.6\pm2.9$, $SUV_{peak}=7.2\pm2.0$) and Cu-ATSM images ($SUV_{mean}=1.5\pm0.6$, $SUV_{max}=4.6\pm2.3$, $SUV_{peak}=3.2\pm1.6$). Correlation of ROI summary statistics between FDG:FLT yielded low correlation coefficients ($R=0.34$, 0.16, and 0.20 for SUV_{mean} , SUV_{max} , and SUV_{peak}); similar values were obtained for FDG:Cu-ATSM ($R=0.50$, 0.61, 0.29) and FLT:Cu-ATSM ($R=0.22$, 0.10, 0.16).

Voxel-based correlation values are displayed in Figure 2. Highest correlation values were obtained between FDG and FLT images ($R=0.76$, range 0.53–0.85); distributions of FDG:Cu-ATSM ($R=0.64$, range 0.51–0.79) and FLT:Cu-ATSM ($R=0.61$, 0.21–0.80) exhibited lower correlation (Supplement 1). Heterogeneity in R was greater for FDG:Cu-ATSM and FLT:Cu-ATSM than FDG:FLT. Voxel-based correlation coefficients were dissimilar to correlations based on ROI summary statistics and better corresponded to qualitative interpretation of images.

When comparing between different regions of interest, no significant differences were observed in correlation coefficients for GTV, expanded GTV, or CTV volumes (Figure 2). For FDG:FLT comparisons, average correlation in GTV, GTV+2mm, and CTV regions was 0.75, 0.76, and 0.76; for FDG:CuATSM, average correlation was 0.63, 0.63, and 0.64; and for FLT:CuATSM comparisons, average correlation was 0.60, 0.62, and 0.58, respectively. Visually, the expanded GTV volumes best encompassed the PET uptake.

When comparing frame-averaged images to individual image frames, correlation of individual frames led to $\Delta R/R=-0.20$ and an increase in range for all tracer comparisons (Figure 3, Supplement 1). R values decreased and variability in R increased with later frames, which correspond to lower radiotracer activity due to biological clearance and radioactive decay.

When comparing registered images to images shifted by one voxel, image translation resulted in $\Delta R/R = -0.30$ (range -0.72 to 0.09) across all tracers (Figure 3, Supplement 1). Decrease in R was significantly greater for translations of FLT images than for translations of FDG or Cu-ATSM images ($\Delta R_{FLT+} = -0.39$ compared to $\Delta R_{FDG+} = -0.25$ and $\Delta R_{Cu-ATSM+} = -0.27$, $p < 0.01$).

When comparing reference images to images reconstructed with different imaging parameters, correlation was highest with dense voxel grids, greater filtration widths, and low iteration number (Figure 3, Supplementary Table 1). Reconstruction with low filtration (2 mm loop, 2 mm post) led to $\Delta R/R = -0.20$ relative to the reference filtration (5 mm loop, 3 mm post). Reconstruction with five iterations led to $\Delta R/R = -0.10$ relative to two iterations. Reconstruction on 256×256 grids led to $\Delta R/R = 0.05$ relative to 128×128 grids. The highest average correlations were obtained at 256×256 voxels, 5 mm loop and 3 mm post filtration, and 2 iterations.

Discussion

In this study, high correlation between FDG and FLT uptake was observed ($R \sim 0.5-0.9$), suggesting that metabolic and proliferative phenotypes are highly correlated in cancers of the oropharynx. Correlations of FDG and FLT to Cu-ATSM exhibited wider variability ($R \sim 0.2-0.9$), indicating that hypoxia status is less clearly associated with metabolic or proliferative status. Correlation coefficients for FDG:Cu-ATSM correlation were similar to FDG:FMISO correlation reported in the literature [24] and the heterogeneity in correlation was similarly observed [25]. It should be noted that while Cu-ATSM and FMISO are both considered hypoxia-selective tracers, differences in retention mechanisms and uptake characteristics are such that some dissimilarity should be expected in spatial uptake [28]. Additionally, all patients with available p16 immunostains were strongly positive, suggesting HPV association. Therefore these imaging correlations may be particularly relevant for the rapidly expanding cohort of HPV-associated oropharynx cancer patients that comprise the majority of cases in most current oropharynx studies.

This technique represents a first step in quantifying phenotype interrelation in a way that may have clinical benefit. One application is prediction of clinical outcome or early response assessment. Shape-based characteristics of FDG uptake predicted response in patients with sarcoma [29]; in a similar manner, spatial correlation could be tested in clinical trials for outcome prediction. One hypothesis is that heterogeneity in tumor phenotype (i.e., lack of correlation between hypoxic and proliferative areas) will lead to poor patient prognosis.

Another potential application is radiotherapy target definition. Heterogeneity in correlation between FDG and Cu-ATSM uptake could indicate that conventional target volumes, usually defined by CT and FDG PET, may be missing hypoxic subregions. Also, the high correlation in FLT and FDG uptake reported here may allow rough comparison between dose-painting trials utilizing FDG or FLT target subvolumes.

Correlation analysis was performed in three distinct regions of interest to evaluate the sensitivity of this technique to the segmented volume. No significant differences were observed between GTV, GTV with 2mm expansion, and CTV volumes. Visually, the expanded GTV showed superior conformality to PET uptake versus GTV, likely due to the lower resolution of the PET images relative to CT. Use of expanded GTV also reduces partial volume effects due to resolution mismatch and could aid in capturing Cu-ATSM uptake, which was not explicitly considered in the GTV definition. Use of large volumes that did not conform visually to PET uptake, such as CTV, had a minor impact on

quantitative results because image voxels with $SUV < 0.0001$ were excluded from the analysis and the PET distributions tended to fall off quickly. However, such large volumes increase the possibility of measuring correlation from normal structures that are PET avid, such as the bone marrow for FLT. Going forward, it is recommended to use either expanded GTV or an auto-segmentation method, as well as an exclusion criteria on voxels with minimal uptake (as an absolute threshold or percent of SUV_{max}) for correlation analysis.

Unsurprisingly, correlation values were strongly affected by registration, as a displacement of a single voxel led to $\Delta R/R = -0.3$. Most error was recovered after image registration, but patient deformation remained a consideration even with thermoplastic mask immobilization; masks extended to the neck but not the shoulders, and modest registration of cervical nodes was required. Deformable registration could potentially improve image registration but such methods necessarily transform uptake values, leading to possibly severe impact on quantitative analysis and a need for further study. Translation of FLT images decreased correlation significantly more than translation of FDG or Cu-ATSM images, which may reflect distribution of cellular processes or uptake characteristics of the tracers.

Image noise and injected dose had an appreciable effect on correlation in this study. Injected FLT and Cu-ATSM doses were reduced relative to FDG doses due to protocol restrictions, so frames were averaged to improve signal to noise characteristics. Correlation of frame-averaged images increased average R values on the order of 0.15 and decreased variation in R relative to individual frames. While frame-averaging masks the true spatial distribution of the image, it reduces sensitivity to noise and motion.

Image reconstruction had quantitative impacts on image correlation. In general, correlation increased with voxel density and image filtration and decreased with number of OSEM iterations. Reconstruction effects were small on average (less than 0.10 fractional difference between reconstructions), although fractional differences up to 0.35 could be obtained in a worst-case scenario for individual patients. With sufficient control of imaging uncertainties, dense voxels and low filtration may better resolve underlying tracer distributions; however, effects on correlation must be considered for inter-institutional comparisons. The effect of OSEM iteration number on correlation is a less-direct relationship dependent on the underlying distribution; for example, see impact on image quantification in Barbee *et al* [30].

Correlation of summary statistics produced inconsistent results compared to other summary statistics and voxel-based correlation. Implementation of subregion-defining or clustering algorithms may improve the utility of correlation of summary metrics.

Conclusion

Correlation values were calculated between FDG, FLT, and Cu-ATSM images. FDG:FLT comparisons exhibited greatest correlations while Cu-ATSM had wide heterogeneity compared to FDG and FLT. No significant differences were measured when using GTV, expanded GTV, or CTV as the analysis volume. Additionally, the effects of image registration, noise, and reconstruction on R were quantified. While correlation coefficients are a simplistic interpretation of the vast biological heterogeneity of tumors, they represent a first step towards quantitative characterization of tumor phenotype. In general, correlation has potential value to target definition, outcome prediction, or response assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

PET/CT images of FDG, FLT, and Cu-ATSM uptake. In the top row, a patient with tonsillar disease exhibited high levels of correlation ($R=0.81$, 0.79 , and 0.80). In the bottom row, a patient with left lymph node involvement exhibited low levels of correlation ($R=0.56$, 0.60 , 0.42).

Figure 2. Correlation coefficients for GTV, expanded GTV, and CTV volumes. Although expanded GTV showed greatest visual concordance with PET uptake, no significant differences were measured between the three regions.

Figure 3.

Change in R due to imaging parameters. *Left:* Co-registered images versus images shifted by one voxel. Translation reduced correlation between all tracers, but translation of FLT images reduced correlation significantly more than translation of other images. *Center:* R values for frame-averaged images versus individual frames. Correlation values were uniformly lower for individual frames. The greatest reduction in correlation was seen in the last frame (lowest activity). *Right:* R values for images reconstructed with reference parameters versus images reconstructed with differing voxel grid, filtration, and OSEM iteration. Changes in reconstruction parameters affected all tracer comparisons similarly.

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Table 1

Patient characteristics, tumor staging, and PET uptake (BOT: base of tongue).

Pt	Sex	Age	Primary	Stage	FDG		FLT		Cu-ATSM	
					SUV _{mean}	SUV _{max}	SUV _{mean}	SUV _{max}	SUV _{mean}	SUV _{max}
1	M	75	R BOT	T4N2b	3.3	15.0	1.7	25.7	0.7	5.1
2	M	57	R tonsil	T2N2c	3.7	7.1	1.8	12.9	2.0	4.9
3	M	43	R tonsil	T2N2c	2.7	9.0	1.7	17.6	1.5	4.2
4	M	69	BOT	T4aN2c	2.5	12.0	2.1	17.0	1.9	7.9
5	M	56	BOT	T4aN2b	2.4	16.0	4.0	8.5	0.9	2.2
6	M	63	L tonsil	T2N2b	2.6	8.3	1.5	12.4	0.9	2.6
7	M	66	BOT	T2N2c	3.8	10.1	2.5	19.8	1.8	5.3
8	M	74	R BOT	T4aN2b	4.5	11.0	2.3	20.0	1.5	2.9
9	F	61	R BOT	T4N2b	5.6	16.4	2.9	7.1	1.3	3.1
10	M	44	BOT	T2N2b	2.2	15.6	1.4	10.1	0.9	2.9
11	M	55	L BOT	T2N2b	5.1	25.8	3.0	11.2	2.8	9.3
				mean	3.5	13.3	2.3	14.7	1.5	4.6
				SD	1.2	5.3	0.8	5.7	0.6	2.3