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Individual patient data meta-analysis

Individual patient data meta-analysis of FMISO and FAZA hypoxia PET scans from head and neck cancer patients undergoing definitive radiochemotherapy

Sebastian Zschaeck [a,b,c,d,e,*], Steffen Löck [a,b,f], Frank Hofheinz [g], Daniel Zips [a,h,r], Lise Saksø Mortensen [i], Klaus Zöphel [b,c,j], Esther G.C. Troost [a,b,c,k,l,m,n], Simon Boeke [h], Mette Saksø [i], David Mönnich [h], Annekatrin Seidlitz [a,b,c], Jørgen Johansen [o], Tomas Skripcak [a,b], Vincent Gregoire [p], Jens Overgaard [i], Michael Baumann [a,b,c,k,q,1], Mechthild Krause [a,b,c,k,l,m,n,1]

[a] Department of Radiotherapy and Radiation Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden; [b] German Cancer Consortium (DKTK), partner site Tübingen, Germany, and German Cancer Research Center (DKFZ), Heidelberg, Germany; [c] OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Helmholtz-Zentrum Dresden – Rossendorf; [d] Charité - Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Radiation Oncology; [e] Berlin Institute of Health (BIH); [f] OncoRay – National Center for Radiation Research in Oncology, Biostatistics and Modeling in Radiation Oncology Group, Medical Faculty and University Hospital Carl Gustav Carus, Technische Universität Dresden, Helmholtz-Zentrum, Dresden – Rossendorf; [g] Helmholtz-Zentrum Dresden-Rossendorf, PET Center, Institute of Radiopharmaceutical Cancer Research; [h] Department of Radiation Oncology, University Hospital and Medical Faculty, Eberhard Karls University Tübingen, Germany; [i] Department of Experimental Clinical Oncology, Aarhus University Hospital, Denmark; [j] Department of Nuclear Medicine, Faculty of Medicine and University Hospital Carl Gustav Carus, Dresden; [k] Helmholtz-Zentrum Dresden - Rossendorf, Institute of Radiooncology – OncoRay; [l] National Center for Tumor Diseases (NCT), Partner Site Dresden, Germany: German Cancer Research Center (DKFZ), Heidelberg; [m] Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden; [n] Helmholtz Association/Helmholtz-Zentrum Dresden - Rossendorf (HZDR), Germany; [o] Department of Oncology, Odense University Hospital (OUH), Denmark; [p] St. Luc University Hospital, Brussels, Belgium; [q] German Cancer Research Center (DKFZ), Heidelberg, Germany; [r] German Cancer Consortium (DKTK), partner site Dresden, Germany, and German Cancer Research Center (DKFZ), Heidelberg, Germany

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ABSTRACT

Background and purpose: Tumor hypoxia plays an important role in head and neck squamous cell carcinomas (HNSCC). Various positron emission tomography (PET) tracers promise non-invasive assessment of tumor hypoxia. So far, the applicability of hypoxia PET is hampered by monocentric imaging trials with few patients.

Materials and methods: Multicenter individual patient data based meta-analysis of the original PET data from four prospective imaging trials was performed. All patients had localized disease and were treated with curatively intended radio(-chemo)therapy. Hypoxia PET imaging was performed with ^{18}F-Fluoromisonidazole (FMISO, 102 patients) or ^{18}F-Fluoroazomycin-arabinoside (FAZA, 51 patients). Impact of hypoxia PET parameters on loco-regional control (LRC) and overall survival (OS) was analyzed by uni- and multivariable Cox regression.

Results: Baseline characteristics between participating centers differed significantly, especially regarding T stage ($p < 0.001$), tumor volume ($p < 0.001$) and p16 status ($p = 0.009$). The commonly used hypoxia parameters, maximal tumor-to-muscle ratio (TMR_{max}) and hypoxic volume with 1.6 threshold ($HV_{1.6}$), showed a strong association with LRC ($p = 0.001$) and OS ($p < 0.001$). These findings were irrespective of the radiotracer and the same cut-off values could be applied for FMISO and FAZA ($TMR_{max} > 2.0$ or $HV_{1.6} > 1.5$ ml). The effect size of TMR_{max} was similar for subgroups of patients defined by radiotracer, p16 status and FDG-PET parameters for LRC and OS, respectively.

Conclusion: PET measured hypoxia is robust and has a strong impact on LRC and OS in HNSCC. The most commonly investigated tracers FMISO and FAZA can probably be used equivalently in multicenter trials. Optimal strategies to improve the dismal outcome of hypoxic tumors remain elusive.

Tumor hypoxia is a well-known adverse prognostic factor in various solid cancers and also showed predictive value in two *a posteriori* analyses of trials testing the addition of the hypoxia activated prodrug Tirapazamine to standard radiochemotherapy [1–3].

* Corresponding author at: Charité Centrum Tumormedizin CC14, Klinik für Radioonkologie und Strahlentherapie, Augustenburger Platz 1, 13353 Berlin, Germany.
E-mail address: Sebastian.Zschaeck@charite.de (S. Zschaeck).
[1] MB and MK share last authorship.

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Since hypoxia-activated prodrugs failed to demonstrate their superiority compared to standard treatment in three large phase III studies on various tumors including HNSCC without pre-selection on the basis of tumor hypoxia, the inclusion of a hypoxia-specific biomarker seems imperative [4–6]. Common positron emission tomography (PET) hypoxia tracers are imidazole derivatives, which accumulate in hypoxic cells. ^{18}F-Fluoromisonidazole (FMISO) was identified as a potential hypoxia tracer by Chapman and colleagues in 1979 [7,8]. Since then, FMISO has become the hypoxia tracer most extensively studied in various tumor sites.

One important limitation of FMISO is its low signal-to-noise ratio that requires a long image acquisition time and an interval between tracer injection and scanning of at least two, optimally four, hours [9]. This limitation led to the development of novel imidazole derivatives, often called second/third generation hypoxia tracers. For example, ^{18}F-Fluoroazomycin-arabinoside (FAZA) was developed to avoid the drawbacks of the high lipophilicity of FMISO [10], although not all comparative preclinical studies showed a clear superiority of the novel tracers [11]. Several publications assessed the prognostic role of FAZA in HNSCC 1.4 [12–15].

Clinical publications on hypoxia imaging share the common limitation that only small numbers of patients were evaluated. This has sometimes led to contradictory results, for example regarding the role of pre-therapeutic hypoxia, which did not show prognostic value in all studies [16]. Additionally, due to the low image contrast, there is usually the need for a reference volume. In HNSCC, the use of different reference structures such as deep neck muscles, larger vessels, cerebellum or blood for the normalization of tumor uptake hampers head to head comparison of different studies [17].

The aim of this multi-center analysis was to standardize reference volumes and evaluate the prognostic role of hypoxia PET imaging for HNSCC patients by central imaging re-analysis and centrally established cut-off values of all PET data.

Materials and methods

Patients, treatment and imaging

This is an analysis of patients treated with curatively intended definitive (chemo/cetuximab-) radiotherapy for HNSCC, who underwent pre-therapeutic hypoxia PET imaging with FMISO-PET (102 patients) or FAZA-PET (51 patients) within prospective imaging trials. Parts of the institutional data have been published by the respective institutions and detailed information about imaging parameters can be found there [13,16,18–20]. Inclusion criteria for this individual patient data meta-analysis was pre-therapeutic imaging of primary gross tumor volume (GTV, preferentially by FDG PET-CT) and pre-therapeutic imaging of hypoxia by either FMISO-/FAZA-PET and availability of corresponding CT or MRI to enable CT/MRI based co-registration of tumor delineation and hypoxia PET. Details on treatment and imaging of patients can be found in Supplemental Table 1.

Image analysis

All relevant images were centrally collected and analyzed by the same observer (SZ) using identical software and algorithms. The delineating observer was blinded to patient outcome.

Image analyses were performed using ROVER software (version 2.1.26; ABX, Radeberg, Germany). In a first step, co-registration of (FDG-PET)/CT and FMISO/FAZA-PET/CT was performed using the mutual information algorithm of ROVER. In a second step the co-registered CTs were checked for correct alignment and, if necessary, manually corrected with focus on a correct registration of the primary tumor by using adjacent anatomical landmarks. The following regions of interest (ROI) were delineated using the available CT and FDG-PET information: The primary tumor was semi-automatically delineated by the FDG-PET information whenever available. The software algorithm was used after generating a large spheroid around the primary tumor [21,22]. In a second step, central necrotic tumor areas or CT morphological suspicious areas were manually included to the primary tumor. If possible, only the primary tumor was delineated and neighboring affected lymph nodes were excluded from analysis. If the primary tumor and lymph nodes could morphologically not be discriminated, both were defined as the primary tumor. Additionally, cerebellum, carotid artery and muscle were delineated as reference volumes. Muscle was contoured using a spheroid of 16 mm diameter within the deep neck muscles, carotid as a spheroid of 12 mm within the carotid artery and, if within the field of view of the hypoxia PET, the cerebellum was delineated as a 28 mm diameter circle reaching for at least 3 planes of the cerebellum. Reference ROIs were delineated contralateral to the primary tumor whenever possible. The ROIs of the primary tumor and of the reference volumes were transferred to the hypoxia PETs for calculation of the following tumor to background (TBR) ratios: tumor-to-muscle (TMR), tumor-to-carotid (TCaR) and tumor-to-cerebellum (TCeR). Furthermore, thresholded hypoxic volumes (HV) were calculated. HV was volume-thresholded at a range of tumor-to-muscle threshold levels (e.g. $HV_{1.6}$ for a threshold $\geq$1.6 of the TMR_{mean}). Primary tumor volume, SUV_{max} and SUV_{mean} were re-calculated by ROVER for all hypoxia scans in 102 patients. For 11 patients from the Tübingen cohort and for 39 patients from the Aarhus/Odense cohort, the original SUV data calculated by the treating center had to be used due to missing information on injection time (Tübingen) or due to anonymization affecting DICOM information that would be necessary to calculate SUV values and thus missing possibility to re-calculate these values (Aarhus). Data on localisation, tumor volume and ratios (TMR, TCaR, TCeR) could be centrally calculated for all patients.

For displaying the topographical localization and quantification of the hypoxia distribution, maximal TMR values were allocated in a sagittal view with a 27-field matrix of the head and neck region of a representative patient. For inter-study comparison, the maximal TMR values were color decoded (green for lowest, yellow for intermediate, red for highest values) and located within the matrix together with the total number of patients.

Statistics

Categorical patient and tumor characteristics were compared between treatment groups by chi-squared tests. Differences in continuous parameters (e.g. the hypoxia-PET parameters) were evaluated by Mann-Whitney-U tests between two groups and by Kruskal-Wallis tests between more than two groups. Correlations between continuous variables were evaluated using the Spearman correlation coefficient R. Differences in correlation coefficients were tested for statistical significance by a bootstrapping procedure including 1000 bootstrap samples. The bootstrapping procedure was implemented in Python 2.7 and the other analyses were performed by SPSS 24 software (IBM Corporation, Armonk, NY). Survival analyses were performed for the endpoint locoregional tumor control (LRC), which was calculated from the first day of radiotherapy to the date of event or censoring. Survival curves were estimated by the Kaplan-Meier method. The impact of potential prognostic variables on the endpoints was evaluated using univariable Cox-regression. Multivariable Cox regression was applied to assess the independent prognostic information of hypoxia-PET parameters concerning other clinical parameters. For LRC, each hypoxia-PET parameter that was significant in univariable analysis was separately combined with T stage and p16 status, while for OS, it was combined with the logarithm of tumor

volume and p16 status. Patient stratification into groups of low and high risk of recurrence was performed using hypoxia-PET parameters. The optimal cut-off was chosen based on 1000 bootstrap samples of the cohort. For every possible cut-off, the bootstrap samples were stratified accordingly, and outcome of the patient groups was compared by log-rank tests. Cut-offs with a high fraction of significant results (power) for LRC were chosen as optimal cut-offs. Values close to the median were preferred in case of power >0.9. For all analyses, two-sided tests were applied and p-values <0.05 were considered statistically significant.

Results

Supplemental Fig. 1 shows a CONSORT diagram for the study: 153 patients with pre-treatment hypoxia imaging were analyzed (51 patients with FAZA and 102 patients with FMISO), 99 of these patients had additional pre-treatment FDG-PET scans available.

Clinical baseline characteristics as well as hypoxia-specific parameters differed significantly between centers. Patients from Tübingen and Aarhus/Odense presented more oropharyngeal carcinomas, the percentage of p16-positive tumors was highest in Aarhus/Odense (41%). cT stages were lowest in Aarhus/Odense (5% cT4 tumors) and highest in Dresden (61% cT4 tumors). Tumor volumes and hypoxia PET parameters also differed significantly between centers ($p < 0.001$ for tumor volume, hypoxia PET: SUV_{max}, SUV_{mean}, TBR_{max}, TBR_{mean} and various thresholded hypoxic volumes). Table 1 summarizes the baseline patient characteristics and comparison between centers.

Hypoxia SUV_{max} and SUV_{mean} of the primary tumor showed a high correlation to tumor to background (TBR) max and mean values, using deep neck muscles (TMR) or carotid (TCaR) for both hypoxia tracers. Fig. 1 shows a comparison of SUVs and TBRs. For cerebellum (TCeR), both tracers revealed notable differences due to the much lower FAZA retention within the cerebellum. To evaluate if background normalization was superior to SUV measurement alone, the correlation coefficient between TMR and TCaR was compared to the coefficients between SUV and TMR or TCaR, respectively. No significant increase in correlation could be shown.

The hypoxia PET parameters $SUV_{max/mean}$ and $TMR_{max/mean}$ showed a moderate correlation to tumor volume ($R \leq 0.6$), while

Table 1
Patient characteristics of participating centers. Absolute patient numbers and percentages (in brackets). All significant values are in bold.

	Parameter	Aarhus/Odense (39 patients)	Brussels (12 patients)	Dresden (69 patients)	Tübingen (33 patients)	p-value
		Number of patients (%)				
	Gender	8/31	3/9	8/61	4/29	0.44
	female/male	(80/20)	(75/25)	(88/12)	(88/12)	
	p16 status	23/16/0	3/2/7	38/5/26	14/2/17	**0.010**
	negative/positive/ missing	(59/41/0)	(25/17/58)	(55/7/38)	(42/6/52)	
	Tumor location	0/24/7/5/3/0	0/5/6/1/0/0	11/27/25/6/0/0	1/18/2/2/0/10	**0.001**
	oral cavity/ oropharynx/ hypopharynx/ larynx/nasopharynx/ missing	(0/61/18/13/8/0)	(0/42/50/8/0/0)	(16/39/36/9/0/0)	(3/55/6/6/0/30)	
	Grading	0/0/0/39	0/0/0/12	2/36/31/0	1/16/6/10	0.28
	1/2/3/missing	(0/0/0/100)	(0/0/0/100)	(3/52/45/0)	(3/49/18/30)	
	T stage	3/24/10/2/0	0/4/3/5/0	1/4/22/42/0	0/3/11/11/8	**<0.001**
	1/2/3/4/missing	(8/61/26/5/0)	(0/33/25/42/0)	(1/6/32/61/0)	(0/9/33/33/25)	
	N stage	9/5/24/1	2/0/9/1	7/7/52/3	8/3/21/1	0.62
	0/1/2/3	(23/13/61/3)	(17/0/75/8)	(10/10/76/4)	(24/9/64/3)	
	UICC stage	1/6/8/24/0	0/1/1/10/0	0/0/8/61/0	0/0/0/25/8	**0.002**
	I/II/III/IV/missing	(3/15/20/62/0)	(0/8/8/84/0)	(0/0/12/88/0)	(0/0/0/76/24)	
	Nimorazole	39/0	0/12	0/69	0/33)	**<0.001**
	yes/no	(100/0)	(0/100)	(0/100)	(0/100)	
	Concurrent systemic therapy	22/17	11/1	66/3	33/0	**<0.001**
	yes/no	(56/44)	(92/8)	(96/4)	(100/0)	
	Hypoxia tracer	FAZA	FAZA	FMISO	FMISO	
		Median (range)				
	Age (years)	61 (44–80)	56 (42–71)	55 (42–82)	57 (46–75)	0.12
	Dose (Gy)	66 (60–68)	70 (70–70)	72 (70–72)	71 (68–77)	**<0.001**
	Tumor volume (ml)	13.9 (3.83–91.3)	13.8 (1.78–67.7)	37.1 (4.5–403.4)	32.5 (1.88–83.3)	**<0.001**
Hypoxia PET parameters	$TCaR_{max}$	1.61 (1.00–3.21)	1.99 (1.04–3.00)	2.05 (1.39–3.77)	1.80 (1.12–4.45)	**<0.001**
	$TCaR_{mean}$	1.11 (0.81–1.83)	1.24 (0.57–1.52)	1.30 (0.89–1.98)	1.19 (0.85–2.43)	**<0.001**
	TMR_{max}	1.54 (1.10–3.47)	1.73 (0.88–3.15)	2.16 (1.33–5.03)	2.19 (1.29–4.35)	**<0.001**
	TMR_{mean}	1.10 (0.82–1.79)	1.09 (0.48–1.59)	1.38 (1.02–2.64)	1.32 (0.94–1.98)	**<0.001**
	$TCeR_{max}$	6.04 (4.27–9.94)	5.45 (2.88–9.42)	1.76 (1.25–3.47)	1.58 (1.07–3.14)	**<0.001**
	$TCeR_{mean}$	4.33 (2.97–5.65)	3.61 (1.57–5.33)	1.14 (0.92–1.88)	1.02 (0.81–1.29)	**<0.001**
	$HV_{1.4}$ (ml)	0.35 (0–27.2)	1.20 (0–14.7)	14.1 (0–315.0)	6.83 (0–53.2)	**<0.001**
	$HV_{1.6}$ (ml)	0 (0–16.0)	0.05 (0–6.26)	7.69 (0–274.0)	2.94 (0–30.1)	**<0.001**
	$HV_{1.8}$ (ml)	0 (0–13.1)	0.004 (0–4.46)	2.57 (0–236.6)	1.00 (0–22.6)	**<0.001**
	$HV_{2.0}$ (ml)	0 (0–8.80)	0 (0–2.94)	0.63 (0–203.0)	0.47 (0–16.6)	**<0.001**
	SUV_{max}	1.68 (1.07–3.99)	2.06 (1.20–3.02)	2.52 (1.62–5.23)	1.97 (0.64–4.08)	**<0.001**
	SUV_{mean}	1.15 (0.79–1.79)	1.26 (0.65–1.63)	1.59 (1.14–2.74)	1.36 (0.43–2.43)	**<0.001**
	FDG SUV_{max}	–	9.94 (2.95–14.0)	13.6 (6.85–47.8)	11.7 (6.23–21.8)	**0.001**
	FDG SUV_{mean}	–	4.71 (1.53–6.63)	6.78 (3.92–14.2)	4.60 (2.75–7.16)	**<0.001**

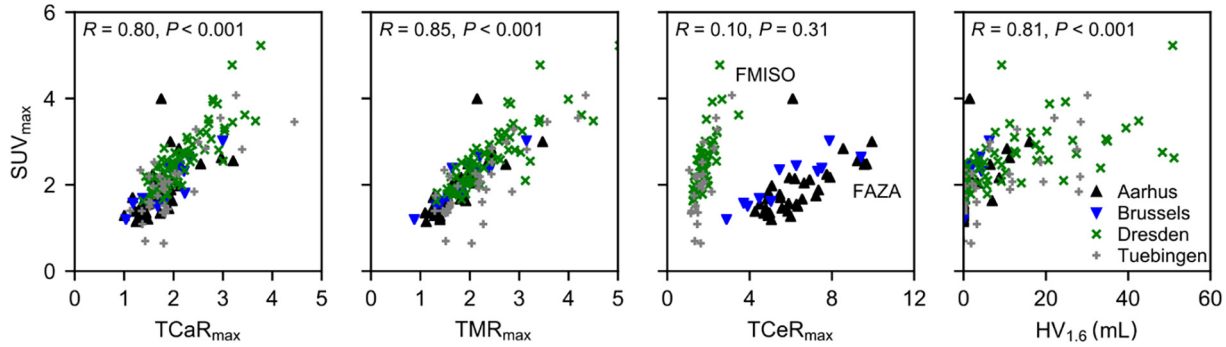

Fig. 1. Correlation between different maximal (max) tumor to background ratios: TCaR = tumor-to-carotid, TMR = tumor-to-muscle and TCeR = tumor-to-cerebellum and $HV_{1.6}$ (hypoxic volume with a threshold of 1.6 times of the mean deep neck muscle uptake) and maximal standardized uptake values (SUV_{max}). Applied tracers: FMISO (Tuebingen, Dresden) and FAZA (Aarhus, Brussels).

hypoxic volumes showed a stronger correlation ($R > 0.6$), see Supplemental Table 2. No significant differences of hypoxia parameters between different tumor grading (G1 and G2 versus G3) was observed but p16-positive tumors showed significantly lower TMR_{mean}, $HV_{1.8}$ und $HV_{2.0}$ values ($p = 0.041$, $p = 0.044$, $p = 0.028$, respectively). Tumor location was not associated with hypoxia parameters. Supplemental Fig. 2 shows the topographical distribution of maximum hypoxia tracer uptake in all patients.

Correlations between standard FDG-PET parameters and hypoxia PET TMR values were moderate, with $R = 0.33$ between FDG SUV_{max} and hypoxia TMR_{max} and $R = 0.52$ between FDG SUV_{mean} and hypoxia TMR_{mean}.

Several hypoxia parameters showed a strong association with worse LRC and OS in univariable Cox regression, as depicted in Tables 2 and 3. No parameter was significantly correlated to the occurrence of distant metastases (not shown). In addition, patients with higher T stage showed lower LRC, while OS was associated with p16 status and with the correlated parameters T stage, tumor volume, application of nimorazole and applied dose. In multivariable models including T-stage or tumor volume, p16 status and one hypoxia-PET parameter, most hypoxia-PET parameters remained significant for LRC and OS (Tables 2 and 3). In a subgroup analysis of 99 patients with additional pre-treatment FDG-PET imaging, TMR_{max} (HR = 1.73, $p = 0.007$), $HV_{1.6}$ (HR = 1.03, $p = 0.007$) and several other hypoxia parameters remained independent prognostic factors for LRC and also for OS even after inclusion of FDG SUV_{max} in multivariable analyses (Supplemental Table 3).

When analyzing HPV positive and negative tumors separately, similar results as for all tumors were obtained for HPV negative tumors (Supplemental Table 4). Of the 25 HPV-positive tumors only five developed a loco-regional recurrence. All of these recurrences occurred in patients with high hypoxia-PET values ($TMR_{max} > 2$, $HV_{1.6} > 8$ ml).

Table 2
Univariable and multivariable Cox regression for loco-regional tumor control. Multivariable analyses contained T stage (1–3 vs 4), p16 status (positive, negative or not available) and one univariably significant PET parameter; results are shown for the PET parameters only. All significant values are in bold.

	Parameter	Univariable analyses		Multivariable analyses	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value
	Gender (male vs female)	1.12 (0.52–2.43)	0.76		
	p16 status (negative vs positive)	0.50 (0.19–1.32)	0.16		
	Tumor location (overall chi-squared test)		0.56		
	Grading 1,2 vs 3	1.03 (0.47–2.25)	0.94		
	T stage 1–3 vs 4	1.95 (1.06–3.58)	**0.032**		
	N stage 0,1 vs 2,3	1.39 (0.69–2.83)	0.36		
	UICC stage I–III vs IV	1.45 (0.64–3.26)	0.37		
	Nimorazole (no vs yes)	0.69 (0.35–1.37)	0.29		
	Concurrent systemic therapy (no vs yes)	0.95 (0.42–2.13)	0.89		
	Tracer (FMISO vs FAZA)	0.72 (0.38–1.35)	0.40		
	Age (years)	1.00 (0.97–1.04)	0.89		
	Dose (Gy)	1.04 (0.95–1.15)	0.40		
	LN Tumor volume (ml)	1.28 (0.88–1.86)	0.20		
	LN FDG MTV (ml)	1.07 (0.68–1.68)	0.76		
Hypoxia PET parameters	$TCaR_{max}$	2.38 (1.47–3.85)	**<0.001**	1.97 (1.17–3.33)	**0.011**
	$TCaR_{mean}$	6.97 (1.95–24.9)	**0.003**	4.43 (1.14–17.2)	**0.032**
	TMR_{max}	1.97 (1.41–2.75)	**<0.001**	1.75 (1.21–2.53)	**0.003**
	TMR_{mean}	4.36 (2.02–9.38)	**<0.001**	3.27 (1.37–7.80)	**0.007**
	$TCeR_{max}$	1.02 (0.89–1.17)	0.79		
	$TCeR_{mean}$	0.99 (0.79–1.23)	0.90		
	$HV_{1.4}$ (ml)	1.02 (1.01–1.03)	**0.001**	1.01 (1.00–1.03)	**0.025**
	$HV_{1.6}$ (ml)	1.02 (1.01–1.04)	**0.001**	1.02 (1.00–1.03)	**0.043**
	$HV_{1.8}$ (ml)	1.02 (1.01–1.04)	**0.003**	1.02 (1.00–1.03)	0.080
	$HV_{2.0}$ (ml)	1.03 (1.01–1.05)	**0.003**	1.02 (1.00–1.05)	0.074
	SUV_{max}	1.60 (1.16–2.22)	**0.005**	1.46 (1.01–2.11)	**0.046**
	SUV_{mean}	2.45 (1.11–5.39)	**0.026**	1.74 (0.74–4.11)	0.20
	FDG SUV_{max}	0.98 (0.91–1.05)	0.60		
	FDG SUV_{mean}	1.02 (0.86–1.21)	0.86		

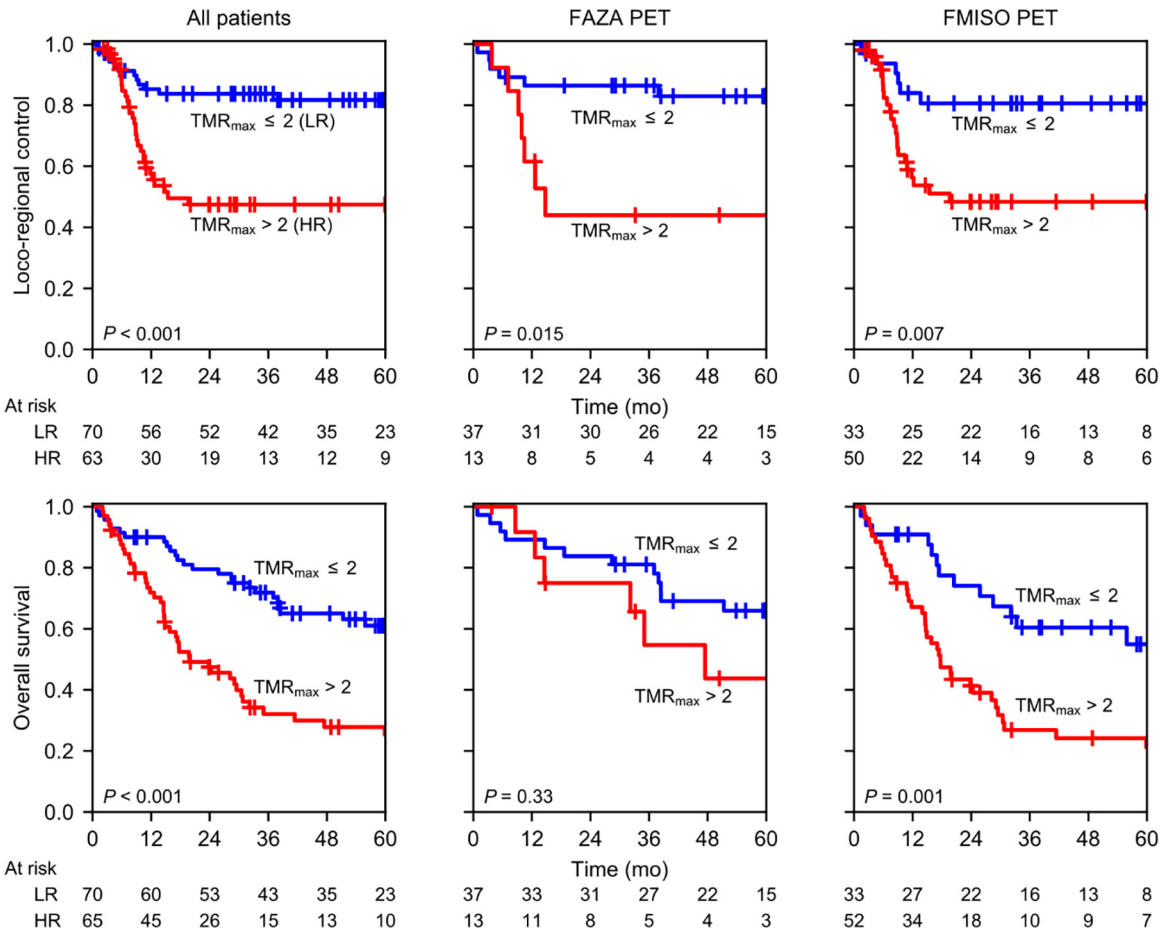

Fig. 2. Loco-regional tumor control probability (top) and overall survival (bottom) of all patients, stratified by TMR_{max} (cut-off > 2) shown for both hypoxia tracers (left), FAZA (middle) and FMISO (right).

Patients were stratified by the most widely used hypoxia parameter TMR_{max} (cut-off 2, LRC: power = 0.94, OS: power = 0.99). For all patients, but also separately for patients imaged by FAZA-PET and FMISO-PET, significant differences in LRC were observed (Fig. 2) with higher LRC in less hypoxic tumors. This translated into an improved OS for less hypoxic tumors, although this was not significant in patients with FAZA-PET imaging only (Fig. 2). Similar results were obtained when $HV_{1.6}$ was used for patient stratification (cut-off 1.5 ml, LRC: power = 0.99, OS: power = 0.99). The Forest plots (A) and (B) of Fig. 3 show that the effect size of the tracer accumulation defined by TMR_{max} is similar for different patient subgroups stratified by tracer, p16-status, FDG SUV_{max} and FDG defined metabolic tumor volume (MTV) for LRC and OS, respectively.

Since three participating centers employed treatment intensification (dose escalation up to 77 Gy or additional administration of the hypoxic cell sensitizer nimorazole), its impact on OS and LRC for patients presenting tumors defined as hypoxic upon central evaluation (TMR_{max} > 2) was analyzed. In this exploratory *post hoc* analysis neither nimorazole, nor moderate FMISO guided dose escalation (dose painting to hypoxic areas) led to an improved outcome compared to standard treatment, which may however be caused by the small sample size, in particular of the dose escalated patient group. Additionally concomitant application of the hypoxia enhanced cytostatic Mitomycin C did not improve outcome of patients classified as hypoxic (Fig. 3C and D).

Discussion

In this study the high prognostic value of PET measured hypoxia for HNSCC could be validated. To our knowledge this is the largest cohort of patients investigated by PET hypoxia imaging so far. Various baseline hypoxia parameters showed a strong association with loco-regional tumor control and overall survival. A common cut-off value to distinguish high-risk from low-risk patients could be applied irrespective of the chosen hypoxia tracer (FMISO or FAZA), which is an important pre-requisite for future multicenter interventional trials.

To our knowledge this is the first study to directly compare both hypoxia tracers and their prognostic value in patients. Using our pooled approach we were able to confirm the additional value of hypoxia specific PET imaging, as it is neither highly correlated to tumor volume nor to common parameters of FDG-PET. Additionally, upon multivariable analyses the prognostic value of hypoxia PET parameters was independent of established clinical risk-factors and FDG-PET parameters.

Methodologically, due to the low signal to noise ratio of hypoxia specific PET tracers, a reference ROI is important to ensure valid detection of hypoxia. Besides using muscles as reference, several other background ROIs have been proposed so far, including cerebellum, left ventricle or aorta [17,23,24]. In this analysis it was possible to validate the use of a deep neck muscle ROI as most appropriate and reliable background ROI, as it is always included

Table 3
Univariable and multivariable Cox regression for overall survival (OS). In univariable analysis, several highly correlated parameters were significantly related to OS. Multivariable analyses thus contained the logarithm of tumor volume, p16 status (positive, negative or not available) and one univariably significant PET parameter; results are shown for the PET parameters only. All significant values are in bold.

	Parameter	Univariable analyses		Multivariable analyses	
		HR (95% CI)	*p* value	HR (95% CI)	*p* value
	Gender (male vs female)	1.02 (0.56–1.85)	0.96		
	p16 status (negative vs positive)	0.25 (0.10–0.63)	**0.003**		
	Tumor location (overall chi-squared test)		0.50		
	Grading 1,2 vs 3	0.95 (0.54–1.67)	0.86		
	T stage 1–3 vs 4	2.15 (1.35–3.42)	**0.001**		
	N stage 0,1 vs 2,3	1.32 (0.78–2.22)	0.30		
	UICC stage I–III vs IV	1.81 (0.93–3.53)	0.082		
	Nimorazole (no vs yes)	0.41 (0.23–0.73)	**0.003**		
	Concurrent systemic therapy (no vs yes)	1.23 (0.65–2.34)	0.53		
	Tracer (FMISO vs FAZA)	0.44 (0.26–0.73)	**0.002**		
	Age (years)	1.02 (1.00–1.05)	0.066		
	Dose (Gy)	1.08 (1.01–1.16)	**0.031**		
	LN Tumor volume (ml)	2.00 (1.51–2.66)	**<0.001**		
	LN FDG MTV (ml)	1.83 (1.30–2.58)	**0.001**		
Hypoxia PET parameters	$TCaR_{max}$	2.46 (1.69–3.58)	**<0.001**	1.72 (1.11–2.68)	**0.015**
	$TCaR_{mean}$	7.70 (2.94–20.2)	**<0.001**	3.55 (1.22–10.3)	**0.020**
	TMR_{max}	1.96 (1.50–2.54)	**<0.001**	1.44 (1.04–1.98)	**0.027**
	TMR_{mean}	4.46 (2.45–8.11)	**<0.001**	2.16 (1.06–4.41)	**0.035**
	$TCeR_{max}$	0.89 (0.80–1.00)	**0.048**	1.03 (0.92–1.15)	0.63
	$TCeR_{mean}$	0.81 (0.68–0.96)	**0.017**	1.06 (0.87–1.30)	0.56
	$HV_{1.4}$ (ml)	1.02 (1.01–1.02)	**<0.001**	1.01 (1.00–1.02)	**0.006**
	$HV_{1.6}$ (ml)	1.02 (1.01–1.02)	**<0.001**	1.01 (1.00–1.02)	**0.015**
	$HV_{1.8}$ (ml)	1.02 (1.01–1.03)	**<0.001**	1.01 (1.00–1.02)	**0.034**
	$HV_{2.0}$ (ml)	1.02 (1.01–1.03)	**<0.001**	1.01 (1.00–1.02)	**0.062**
	SUV_{max}	1.65 (1.28–2.12)	**<0.001**	1.39 (1.03–1.88)	**0.031**
	SUV_{mean}	2.93 (1.62–5.33)	**<0.001**	1.88 (0.97–3.61)	0.060
	FDG SUV_{max}	1.07 (1.03–1.11)	**0.001**	1.05 (1.01–1.09)	**0.021**
	FDG SUV_{mean}	1.23 (1.11–1.37)	**<0.001**	1.18 (1.06–1.32)	**0.003**

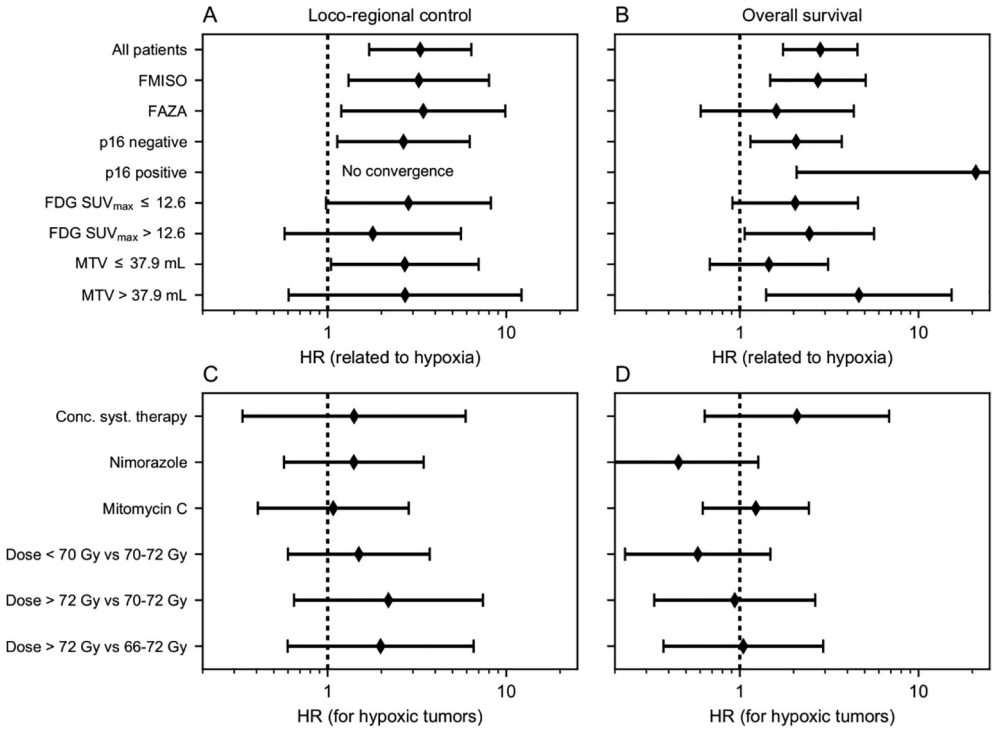

Fig. 3. Impact of pre-treatment hypoxia, defined as TMR_{max} > 2 according to hypoxia tracer, HPV status and FDG-PET parameters on loco-regional control (A) and overall survival (B). The plots show the hazard ratios of hypoxia (defined by TMR_{max} > 2) for different sub-groups of patients. Impact of different treatment regimes on loco-regional control (C) and overall survival (D) in the group of patients with tumors classified as being hypoxic (TMR_{max} > 2).

in the field of view (FOV) in HNSCC imaging. Additionally, a carotid artery ROI as a potential surrogate ROI of the arterial blood pool within the head and neck region showed similar results as the deep neck muscles. However anatomical restrictions make this ROI prone to partial volume effects and spill over from neighboring structures.

The strong association of pre-treatment hypoxia parameters with LRC and OS observed in this study shows that hypoxia PET

imaging delivers important additional prognostic information in HNSCC. This information appears complementary to known prognostic parameters including common FDG-PET imaging parameters such as SUV_{max}. In our analysis the prognostic value of hypoxia PET seems surprisingly robust. PET cut-off values were applicable in a multicenter environment, with patients differing regarding baseline and treatment characteristics. Furthermore, imaging protocols were not standardized between centers and two different hypoxia tracers have been used [25]. It is remarkable that despite these limitations, a centrally generated cut-off value could be established, that can be applied irrespective of the radiotracer to distinguish low and high risk patients (regarding LRC and OS). Besides the differences between treatment centers, another important limitation of this study is missing clinical/tumor parameters (especially grading and HPV status) for several patients. We tried to account for this by separate analysis of HPV positive and negative tumors and also restricted data analysis to patients with known tumor grading (Supplemental Table 5) and found no differences regarding the prognostic value of hypoxia PET parameters.

Although the low number of events in p16 positive patients did not allow firm conclusions, it is important to mention that all five recurrences of p16 positive tumors occurred in the high-risk group with hypoxia-PET parameters above the generated cut-off values in this study. Despite the lack of reliable statistical evidence, this finding should potentially be considered in the planning of treatment de-escalation trials in patients with macroscopic HPV positive HNSCC.

In a *post-hoc* analysis it was not possible to demonstrate an impact of nimorazole, mitomycin C or dose-escalation to hypoxic tumor regions on LRC or OS. This should, however, be interpreted cautiously and does not proof a lack of effect of these interventions. Several reasons may bias this observation: First, the respective interventions have usually only been performed in one of the participating centers. Thus, differences in baseline characteristics and other center effects can strongly impact these findings. Second, analysis of patients receiving dose-escalated radiotherapy is also limited due to the low number of only ten patients in the interventional arm. Third, the same holds true for the concomitant use of mitomycin C, a drug with increased efficacy under hypoxic conditions [26]. Mitomycin C has been applied routinely in one of the participating centers (Tübingen), but only as an alternative regime for patients with contraindications against cisplatinum in another center (Dresden). Thus, some of the patients may have been negatively selected.

Conclusion

Our results highlight an independent and robust prognostic value of hypoxia PET imaging in HNSCC patients undergoing definitive CRT. The applicability of cut-off values in this multicenter-study to distinguish low and high-risk patients makes hypoxia imaging via FMISO or FAZA a particularly suitable biomarker for future studies in HPV-negative HNSCC patients.

Clinical trial information

The respective studies were approved by the corresponding Institutional Ethics Committees and all patients signed a written informed consent. Additionally the analysis of the pooled pseudonymized data was approved by the Ethics Committee responsible for this analysis (university hospital Dresden, EK387102014) and the requirement to obtain informed consent was waived. The prospective imaging trials were registered at ClinicalTrials.gov, see ClinicalTrials identifier: NCT01017224, NCT02352792 and NCT00180180.

Authors contributions

SZ, SL and FH performed the analysis and drafted the manuscript, designed the figures and calculated the underlying statistics. DZ, LSM, KZ, ECGT, SB, MS, DM, AS, JJ, VG, JO, MK, MB, SZ were responsible for treatment, imaging data and follow up collection of patients as well as for evaluation of local datasets and adaptation for the meta-analysis. TS developed the database for data analysis. MK and MB provided the study idea, supervised the analysis and interpretation of the data and reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests

In the past 5 years, Dr. Baumann attended an advisory board meeting of MERCK KGaA (Darmstadt), for which the University of Dresden received a travel grant. He further received funding for his research projects and for educational grants to the University of Dresden by Teutopharma GmbH (2011–2015), IBA (2016), Bayer AG (2016–2018), Merck KGaA (2014–2030), Medipan GmbH (2014–2018). For the German Cancer Research Center (DKFZ, Heidelberg) Dr. Baumann is on the supervisory boards of HI-STEM gGmbH (Heidelberg). Dr. Baumann, as former chair of OncoRay (Dresden) and present CEO and Scientific Chair of the German Cancer Research Center (DKFZ, Heidelberg), was or is responsible for collaborations with a multitude of companies and institutions, worldwide. In this capacity, he has signed/signs contracts for his institute(s) and for the staff for research funding and/or collaborations with industry and academia, worldwide. In this role, he was/is further responsible for commercial technology transfer activities of his institute(s), including patent and other similar IP portfolios.

Dr. Baumann confirms that none of the above funding sources were involved in the preparation of this paper.

In the past 5 years, Dr. Krause received funding for her research projects by IBA (2016), Merck KGaA (2014–2018 for preclinical study; 2018–2020 for clinical study), Medipan GmbH (2014–2018). She is involved in an ongoing publicly funded (German Federal Ministry of Education and Research) project with the companies Medipan, Attomol GmbH, GA Generic Assays GmbH, Gesellschaft für medizinische und wissenschaftliche genetische Analysen, Lipotype GmbH and PolyAn GmbH (2019–2021). For the present manuscript, Dr. Krause confirms that none of the above mentioned funding sources were involved.

No other potential conflicts of interest relevant to this article exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2020.05.022.

References

[1] Rischin D, Hicks RJ, Fisher R, et al. Prognostic significance of [18F]-misonidazole positron emission tomography-detected tumor hypoxia in patients with advanced head and neck cancer randomly assigned to chemoradiation with or without tirapazamine: a substudy of Trans-Tasman Radiation Oncology Group Study 98.02. J Clin Oncol 2006;24:2098–104.

[2] Graves EE, Hicks RJ, Binns D, et al. Quantitative and qualitative analysis of [(18)F]FDG and [(18)F]FAZA positron emission tomography of head and neck

cancers and associations with HPV status and treatment outcome. Eur J Nucl Med Mol Imaging 2015;43:617–25.
[3] Horsman MR, Mortensen LS, Petersen JB, Busk M, Overgaard J. Imaging hypoxia to improve radiotherapy outcome. Nat Rev Clin Oncol 2012;9:674–87.
[4] Rischin D, Peters LJ, O'Sullivan B, et al. Tirapazamine, cisplatin, and radiation versus cisplatin and radiation for advanced squamous cell carcinoma of the head and neck (TROG 02.02, HeadSTART): a phase III trial of the Trans-Tasman Radiation Oncology Group. J Clin Oncol 2010;28:2989–95.
[5] DiSilvestro PA, Ali S, Craighead PS, et al. Phase III randomized trial of weekly cisplatin and irradiation versus cisplatin and tirapazamine and irradiation in stages IB2, IIA, IIB, IIIB, and IVA cervical carcinoma limited to the pelvis: a Gynecologic Oncology Group study. J Clin Oncol 2014;32:458–64.
[6] Tap WD, Papai Z, Van Tine BA, et al. Doxorubicin plus evofosfamide versus doxorubicin alone in locally advanced, unresectable or metastatic soft-tissue sarcoma (TH CR-406/SARC021): an international, multicentre, open-label, randomised phase 3 trial. Lancet Oncol 2017;18:1089–103.
[7] Chapman JD. Hypoxic sensitizers–implications for radiation therapy. N Engl J Med 1979;301:1429–32.
[8] Chapman JD, Franko AJ, Sharplin J. A marker for hypoxic cells in tumours with potential clinical applicability. Br J Cancer 1981;43:546–50.
[9] Abolmaali N, Haase R, Koch A, et al. Two or four hour [^{18}F]FMISO-PET in HNSCC. When is the contrast best?. Nukl Nucl Med. 2011;50:22–7.
[10] Kumar P, Stypinski D, Xia H, McEwan AJB, Machulla H-J, Wiebe LI. Fluoroazomycin arabinoside (FAZA): synthesis, 2H and 3H-labelling and preliminary biological evaluation of a novel 2-nitroimidazole marker of tissue hypoxia. J Label Compd Radiopharm. 1999;42:3–16.
[11] Sorger D, Patt M, Kumar P, et al. [18F]Fluoroazomycinarabinofuranoside (18FAZA) and [18F]Fluoromisonidazole (18FMISO): a comparative study of their selective uptake in hypoxic cells and PET imaging in experimental rat tumors. Nucl Med Biol 2003;30:317–26.
[12] Bollineni VR, Koole MJB, Pruim J, et al. Dynamics of tumor hypoxia assessed by 18F-FAZA PET/CT in head and neck and lung cancer patients during chemoradiation: possible implications for radiotherapy treatment planning strategies. Radiother Oncol 2014;113:198–203.
[13] Mortensen LS, Buus S, Nordsmark M, et al. Identifying hypoxia in human tumors: A correlation study between 18F-FMISO PET and the eppendorf oxygen-sensitive electrode. Acta Oncol 2010;49:934–40.
[14] Servagi-Vernat S, Differding S, Hanin F-X, et al. A prospective clinical study of ^{18}F-FAZA PET-CT hypoxia imaging in head and neck squamous cell carcinoma before and during radiation therapy. Eur J Nucl Med Mol Imaging 2014;41:1544–52.
[15] Saga T, Inubushi M, Koizumi M, et al. Prognostic value of PET/CT with (18)F-fluoroazomycin arabinoside for patients with head and neck squamous cell carcinomas receiving chemoradiotherapy. Ann Nucl Med 2015;30:217–24.
[16] Zips D, Zöphel K, Abolmaali N, et al. Exploratory prospective trial of hypoxia-specific PET imaging during radiochemotherapy in patients with locally advanced head-and-neck cancer. Radiother Oncol 2012;105:21–8.
[17] Muzi M, Peterson LM, O'Sullivan JN, et al. 18F-Fluoromisonidazole quantification of hypoxia in human cancer patients using image-derived blood surrogate tissue reference regions. J Nucl Med 2015;56:1223–8.
[18] Thorwarth D, Eschmann S-M, Holzner F, Paulsen F, Alber M. Combined uptake of [18F]FDG and [18F]FMISO correlates with radiation therapy outcome in head-and-neck cancer patients. Radiother Oncol 2006;80:151–6.
[19] Mortensen LS, Johansen J, Kallehauge J, et al. FAZA PET/CT hypoxia imaging in patients with squamous cell carcinoma of the head and neck treated with radiotherapy: results from the DAHANCA 24 trial. Radiother Oncol 2012;105:14–20.
[20] Hofheinz F, Pötzsch C, Oehme L, et al. Automatic volume delineation in oncological PET. Evaluation of a dedicated software tool and comparison with manual delineation in clinical data sets. Nukl Nucl Med. 2012;51:9–16.
[21] Hofheinz F, Langner J, Petr J, et al. An automatic method for accurate volume delineation of heterogeneous tumors in PET. Med Phys 2013;40:082503.
[22] Han K, Shek T, Vines D, et al. Measurement of tumor hypoxia in patients with locally advanced cervical cancer using positron emission tomography with 18F-fluoroazomyin arabinoside. Int J Radiat Oncol Biol Phys 2018;102:1202–9.
[23] Qian Y, Von Eyben R, Liu Y, et al. 18F-EF5 PET-based imageable hypoxia predicts local recurrence in tumors treated with highly conformal radiation therapy. Int J Radiat Oncol Biol Phys 2018;102:1183–92.
[24] Boellaard R, Rausch I, Beyer T, et al. Quality control for quantitative multicenter whole-body PET/MR studies: A NEMA image quality phantom study with three current PET/MR systems. Med Phys 2015;42:5961–9.
[25] Ludwig CU, Peng YM, Beaudry JN, Salmon SE. Cytotoxicity of mitomycin C on clonogenic human carcinoma cells is not enhanced by hypoxia. Cancer Chemother Pharmacol 1984;12:146–50.