Radiotherapy and Oncology 130 (2019) 97–103

Contents lists available at ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

FMISO-PET-based lymph node hypoxia adds to the prognostic value of tumor only hypoxia in HNSCC patients

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ARTICLE INFO

Article history:
Received 11 April 2018
Received in revised form 10 September 2018
Accepted 12 September 2018
Available online 4 October 2018

Keywords:
Hypoxia
FMISO-PET
Lymph node
Locally advanced HNSCC
Prognostic biomarker

ABSTRACT

Purpose: This secondary analysis of the prospective study on repeat [^{18}F]fluoromisonidazole (FMISO)-PET in patients with locally advanced head and neck squamous cell carcinomas (HNSCC) assessed the prognostic value of synchronous hypoxia in primary tumor (Tu) and lymph node metastases (LN), and evaluated whether the combined reading was of higher prognostic value than that of primary tumor hypoxia only.

Methods: This analysis included forty-five LN-positive HNSCC patients. FMISO-PET/CTs were performed at baseline, weeks 1, 2 and 5 of radiochemotherapy. Based on a binary scale, Tu and LN were categorized as hypoxic or normoxic, and two prognostic parameters were defined: Tu-hypoxia (independent of the LN oxygenation status) and synchronous Tu-and-LN-hypoxia. In fifteen patients with large LN ($N = 21$), additional quantitative analyses of FMISO-PET/CTs were performed. Imaging parameters at different time-points were correlated to the endpoints, i.e., locoregional control (LRC), local control (LC), regional control (RC) and time to progression (TTP). Survival curves were estimated using the cumulative incidence function. Univariable and multivariable Cox regression was used to evaluate the prognostic impact of hypoxia on the endpoints.

Results: Synchronous Tu-and-LN-hypoxia was a strong adverse prognostic factor for LC, LRC and TTP at any of the four time-points ($p \leq 0.004$), whereas Tu-hypoxia only was significantly associated with poor LC and LRC in weeks 2 and 5 ($p \leq 0.047$), and with TTP in week 1 ($p = 0.046$). The multivariable analysis confirmed the prognostic value of synchronous Tu-and-LN-hypoxia regarding LRC (HR = 14.8, $p = 0.017$). The quantitative FMISO-PET/CT parameters correlated with qualitative hypoxia scale and RC ($p < 0.001$, $p \leq 0.033$ at week 2, respectively).

Conclusions: This secondary analysis suggests that combined reading of primary tumor and LN hypoxia adds to the prognostic information of FMSIO-PET in comparison to primary tumor assessment alone in particular prior and early during radiochemotherapy. Confirmation in ongoing trials is needed before using this marker for personalized radiation oncology.

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Patients with locally advanced head and neck squamous cell carcinoma (HNSCC) have a prognosis with a 3-year overall survival rate of approx. 40% after primary radiochemotherapy (RCT) with loco-regional relapse being the prime cause of treatment failure [1,2]. In order to improve treatment outcome for high risk HNSCC patients, local radiation dose escalation and/or intensified (concurrent) systemic treatment strategies have been developed [3–8]. In

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https://doi.org/10.1016/j.radonc.2018.09.008
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the era of personalized medicine, patient selection based on prognostic biomarkers is potentially avoiding both under- and overtreatment. Tumor cell hypoxia is one of the most promising prognostic markers in HNSCC, as hypoxia correlates with poor outcome following R(C)T and surgery, and hypoxia-modification was shown to improve treatment results [5,9–16].

The adverse impact of hypoxia on treatment outcome was initially shown with invasive Eppendorf electrode measurements in accessible primary tumors (Tu) or lymph node metastases (LN). This invasive technique is limited regarding spatial information and repeatability [17–21]. Positron emission tomography (PET) using hypoxia-related markers, e.g., [^{18}F]fluoromisonidazole (FMISO) and [^{18}F]fluoroazomycinarabinoside (FAZA), enables non-invasive imaging of the entire tumor metabolism at repeat time-points [10,22–29]. (Pre)clinical data have shown that hypoxia-PET readings vary depending on the level of oxygenation, that radiation dose escalation is feasible, and that FMISO-PET findings are of prognostic value [3,27,30–37].

Recently, we have successfully validated the prognostic value of FMISO-PET/computed tomography (CT) imaging of the primary tumor for loco-regional tumor control (LRC) in advanced stage HNSCC patients [38]. Until now, the majority of the analyses has focused on the prognostic value of hypoxia-PET measured in the primary tumor or index lesions [10,21–27,38,39]. Therefore, in our study we qualitatively and quantitatively assessed FMISO-PET-based hypoxia in Tu and LN at baseline and during the course of RCT. The aim was to evaluate whether combined assessment of FMISO-PET in Tu and LN increased the prognostic value of FMISO-PET in comparison with the Tu assessment only, as well as to investigate the correlation between quantitative LN parameters and regional control (RC).

Patients and methods

Patients

Between July 2006 and August 2013, 50 advanced stage HNSCC patients were evaluated in the prospective Dresden FMISO-PET imaging trial (NCT00180180). Of these, 45 patients diagnosed with metastatic regional lymph nodes ($\geq$N1) were included in this secondary analysis. All patients had histologically-proven, (functionally) irresectable HNSCC and provided written informed consent. Further inclusion criteria and approval by authorities and the local Ethics Committee have previously been described [22,38].

Work-up, radiochemotherapy and follow-up

The protocol for staging, treatment, imaging and follow-up has previously been described in detail [22,38]. Briefly, the total radiation dose of 72 Gy to the Tu and affected LN was combined with concurrent chemotherapy consisting of intravenous 5–fluorouracil with cisplatin, or with mitomycin C [1,22].

Image acquisition and analysis

Patients received pre-therapeutic (baseline) [^{18}F]fluorodeoxyglucose (FDG-) and FMISO-PET/CT as well as FMISO-PET/CT after 8–10 Gy (week 1), 18–20 Gy (week 2) and 50–60 Gy (week 5). The pre-treatment FDG-PET/CT scans analyzed in this study were acquired 60 min post injection (p.i.), and the FMISO-PET scans 4 h p.i. (except for one data set with a 2 h p.i. scan only). Details on imaging protocol, registration and image analysis were described in [22,38,40–42]. The gross tumor volume of the Tu (GTV_{Tu}) and affected LN (GTV_{LN}), as well as their sum (GTV_{total}), were delineated on the pre-treatment CT taking into account clinical findings as well as the FDG-positive volume automatically segmented using an adaptive thresholding algorithm [43,44]. To improve the analyses of the FMISO-PET/CT scans prone to therapy-induced longitudinal changes, an ellipsoidal volume of interest (VOI) was placed around each GTV_{Tu} and GTV_{LN} in each scan. The background activity for subsequent qualitative and quantitative analyses was assessed within an ellipsoidal VOI ($Background_{VOI}$) in the deep neck muscles (Fig. 1 A-D) [22].

Qualitative hypoxia analysis

One investigator, blinded for treatment outcome, performed the qualitative analysis of hypoxia for each Tu and LN VOI using a visual binary scale: "hypoxic" defined as FMISO uptake higher than $Background_{VOI}$ and "normoxic" defined as equal to/lower than $Background_{VOI}$ (Fig. 1 A-D) [30,36,45,46]. To ensure reproducibility of the scoring system, all FMISO-scans were evaluated twice with an interval of approximately 10 weeks, reaching an agreement rate of 94%. In case of unequivocal findings, visual binary scoring was discussed with a second observer. The results of binary hypoxia were validated against quantitative FMISO-PET/CT readings performed in a subset of patients (see next paragraph).

Two parameters were defined based on the qualitative hypoxia scale: Tu-hypoxia (patients with hypoxic Tu) and synchronous Tu- and LN-hypoxia (patients with a hypoxic primary tumor and at least one hypoxic lymph node). Based on these parameters two analyses were performed: (a) patients with Tu-hypoxia versus patients with Tu normoxia, irrespective of the LN oxygenation status; (b) patients with Tu- and LN-hypoxia versus Tu and/or LN normoxia (both tumor and LN normoxia, Tu normoxia and LN hypoxia, or Tu hypoxia and LN normoxia).

Quantitative hypoxia analysis for validation of the qualitative parameters

In order to avoid underestimation of quantitative FMISO-PET parameters due to the partial volume effect in small lesions, the quantitative analysis was only performed in large lymph node metastases defined as FDG-PET/CT positive volume >5 ml (volume of sphere structure with diameter >2 cm using automatic FDG-PET segmentation). In patients fulfilling this criterion, quantitative FMISO-PET parameters, i.e., peak standardized uptake value [SUV_{peak}; the mean SUV within $5 \times 5 \times 5$ voxels (1.26 ml) of highest FMISO uptake], peak tumor-to-background-ratio (TBR_{peak}; ratio of the SUV_{peak} in Tu or LN and the mean SUV in $Background_{VOI}$), as well as hypoxic volumes (HV) encompassing those voxels with SUVs above different thresholds of multiples of the mean SUV in $Background_{VOI}$, 1.4 or 1.6 ($HV_{1.4}$, $HV_{1.6}$, respectively) were extracted from LN VOIs, and SUV_{peak} and TBR_{peak} were extracted from Tu VOI (for validation of binary hypoxia scale only, see below). Validation of the binary hypoxia scale was performed using Mann–Whitney-*U* tests comparing differences of SUV_{peak} and TBR_{peak} values extracted from Tu and LN VOIs between groups defined by the binary hypoxia score.

Endpoints and statistics

The primary endpoint of the prospective study was LRC and secondary endpoints were freedom from distant metastases (DM), overall survival (OS), local control (LC, defined as absence of progression or recurrence in Tu), regional control (RC, defined as absence of progression or recurrence in LN) and time-to-progression (TTP; defined as time to any progression). The endpoints were calculated from the first day of radiotherapy to the date of event or censoring [22,38]. Corresponding survival curves were estimated by the Kaplan–Meier method for OS and by the cumulative incidence function accounting for the competing risk

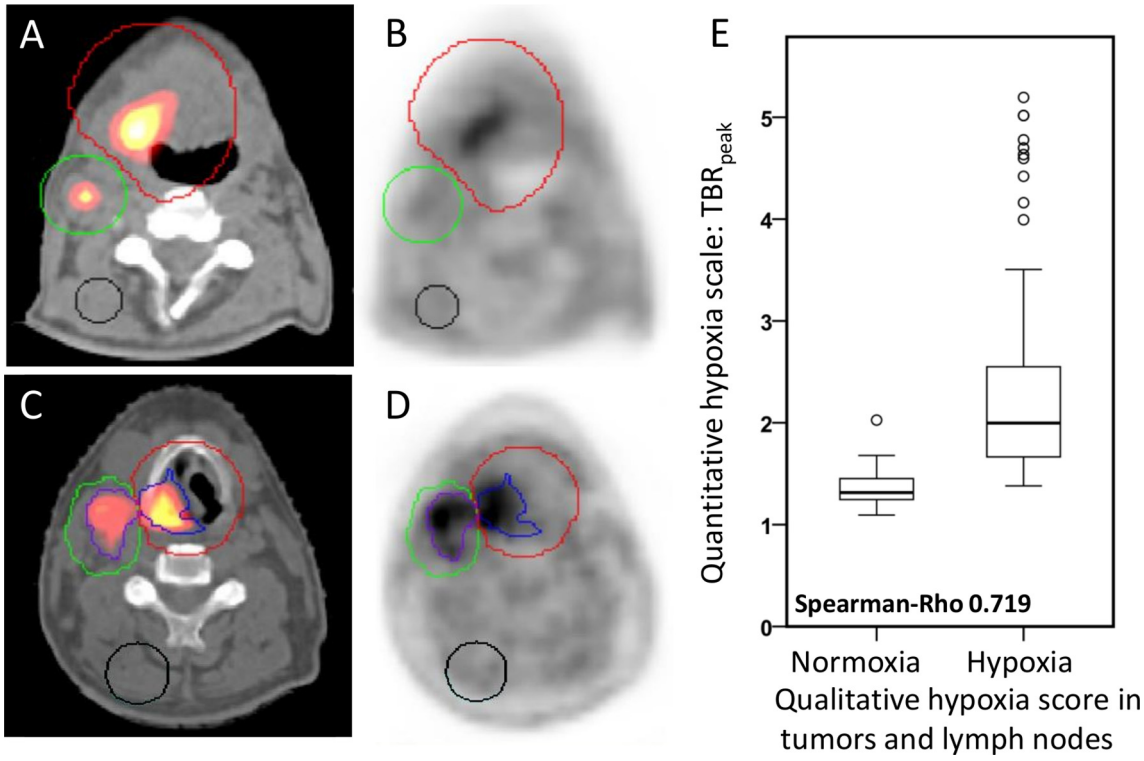

Fig. 1. FDG-PET/CT (A,C) and FMISO-PET (B,D) in two patients with metabolically active FDG-avid primary tumor and lymph node metastasis. (A) FDG-avid primary tumor and lymph node metastasis, (B) hypoxia in the primary tumor only. (C) + (D) Synchronous FDG- and FMISO-uptake in both primary tumor and lymph node [red – primary tumor volume of interest (VOI), green – lymph node VOI, blue – gross tumor volume of primary tumor (GTV_{Tu}), purple – GTV of lymph node (GTV_{LN})]. (E) Box-and-whiskers plots showing the discriminative power of the qualitative binary hypoxia scale (normoxia *vs.* hypoxia) when correlated to the quantitative FMISO-PET/CT parameter TBR_{peak} in primary tumors and LNs in all patients with lymph node metastases >5 ml on FDG-PET/CT. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of death for the other endpoints. Log rank tests (OS) and Gray's test (other endpoints) were performed for patient groups stratified by qualitative hypoxia parameters. Univariable Cox regression was used to assess the impact of prognostic factors on the endpoints. Clinical factors found to be of significance on univariable analysis and qualitative hypoxia parameters were included in multivariable analyses. Mann–Whitney-*U* tests were used to compare continuous parameters between two groups. The statistical analyses were performed with SPSS Statistics 22 (IBM Corporation, Armonk, NY). Two-sided tests were performed and *p*-values <0.05 were considered statistically significant.

Results

The patient and treatment characteristics of the 45 patients included in this secondary analysis are summarized in Table 1. The median follow-up of these patients was 21 months (range 1–65 months). At the time of analysis, 13 (29%) patients were alive with 38 months median follow-up of (range 24–66 months). The 2-year and 3-year OS rates were 49% and 33%, respectively, and the cumulative incidence of loco-regional recurrences was 33% at both time points. Disease progression was observed in 19 (42%) patients, of whom 18 patients presented with synchronous Tu-and-LN-hypoxia in the baseline FMISO-PET/CT scan (Table 2).

Validation of qualitative hypoxia scale

Assessment of the two parameters of the qualitative hypoxia scale is shown in Fig. 1. Highly significant differences in the quantitative FMISO-PET image parameters SUV_{peak} and TBR_{peak} were found between groups defined by the qualitative binary hypoxia scale ($p < 0.001$; Fig. 1E). For normoxic *vs.* hypoxic Tu and LN combined, the median SUV_{peak} and TBR_{peak} were 1.48 *vs.* 2.32, and 1.32 *vs.*1.97, respectively.

Table 1
Patient, tumor and treatment characteristics ($n = 45$).

Characteristic	Value
Age [mean, (range)]	55 (42–74)
Male/female	38/7
Primary site	
Oral cavity/oropharynx/hypopharynx/larynx	8/18/15/4
cT-stage	
cT2/cT3/cT4a/T4b	1/15/24/5
cN-stage	
cN1/cN2a/cN2b/cN2c/cN3	6/2/11/24/2
Histological grade	
G1/G2/G3	2/24/19
UICC-stage	
III/IVa/IVb	5/35/5
HPV: p16-status (positive/negative/not available)	2/37/6
History of tobacco use (yes/no)	42/3
Mean radiation dose (range)	72 Gy (69*-72 Gy)
Mean overall treatment time (range)	42 days (40 – 65 days)
Chemotherapy cisplatin plus 5-FU/mitomycin C plus 5-FU	39/6†

Abbreviations: cT, clinical tumor stage; cN, clinical nodal stage; G, grade; UICC, Union International Contre le Cancer; HPV, human papilloma virus.
* One patient prematurely terminated treatment due to health-related issues.
† One patient received both cisplatin (2 cycles) and mitomycin C (1 cycle).

Prognostic value of qualitative FMISO readings

In the cohort, 24 patients presented with Tu- and LN-hypoxia, whereas 21 patients were allocated to the Tu and/or LN normoxia group (17 with hypoxic primary tumor and normoxic LN, 3

Table 2
Tumor and LN hypoxia status as detected by qualitative FMISO-PET reading at baseline, in weeks 1, 2 and week 5 in all patients with progression during follow up; 1 – hypoxia, 0-normoxia.

Patient	Baseline		Week 1		Week 2		Week 5		Progression
	Tu	LN	Tu	LN	Tu	LN	Tu	LN	
1	1	1	1	1	NA	NA	0	1	LRR
2	1	1	1	1	1	1	0	0	LR, DM
3	1	1	1	1	1	0	1	0	LR
4	1	1	NA	NA	1	1	1	1	LR
5	1	1	1	1	1	1	0	1	LR
6	1	1	1	1	1	1	1	1	LR
7	1	1	1	1	1	1	1	1	LR
8	1	1	1	1	1	0	1	1	LRR
9	1	1	1	1	1	1	1	1	LR
10	1	1	NA	NA	1	1	0	1	LRR
11	1	0	1	0	1	0	0	0	LR
12	1	1	1	1	1	1	0	0	LR, RR*, DM
13	1	1	1	1	0	0	0	0	LR
14	1	1	1	1	1	1	0	0	LR
15	1	1	1	1	1	1	1	1	RR, DM
16	1	1	1	1	0	1	0	0	DM
17	1	1	NA	NA	0	1	0	0	DM
18	1	1	1	1	0	0	0	0	DM
19	1	1	1	1	0	0	0	0	DM

Abbreviations: LR, local recurrence; LRR, loco-regional recurrence; DM, distant metastases; RR, regional recurrence; *, regional recurrence outside of FMISO-PET/CT field of view; NA, not available for FMISO-PET/CT scan is missing.

patients with normoxic tumor and normoxic LN, and one patient with normoxic tumor and hypoxic LN). The various patterns of reoxygenation during radiochemotherapy can be found in Figs. S1–2.

Patients with synchronous Tu-and-LN-hypoxia at any of the four FMISO-PET time-points were found to have worse LC, LRC and TTP compared to patients with Tu and/or LN normoxia ($p \leq 0.004$; Fig. 2 and Table 3). Conversely Tu-hypoxia, the parameter based on hypoxia assessment of the primary tumor only, was of prognostic relevance for LRC and LC in weeks 2 and 5, and for TTP at all of the per-treatment time points ($p \leq 0.047$, Fig. 2 and Table 3). Synchronous Tu-and-LN-hypoxia based on pre-treatment FMISO-PET correlated with poor LC, LRC, TTP and DM while Tu-hypoxia only did not ($p < 0.001$, $p < 0.001$, $p < 0.001$ and $p = 0.009$, respectively, *vs.* $p = 0.18$, $p = 0.16$, $p = 0.091$ and $p = 0.37$, respectively; Table 3). Finally, Tu-hypoxia was found to be associated with poor overall survival at all time points, whereas Tu-and-LN-hypoxia was at baseline, weeks 2 and 5 (Table 3).

On univariable Cox analysis, the GTV_{total} and the number of hypoxic LN were significantly associated with LRC at baseline ($p = 0.02$, HR = 1.01; and $p = 0.01$, HR = 1.5, respectively), whereas the other parameters assessed showed no significant correlations (Table S1). On multivariable Cox analysis, including GTV_{total} and the number of hypoxic LN, synchronous Tu-and-LN-hypoxia was confirmed as prognostic factor ($p = 0.017$, HR = 14.8, Table S1).

Prognostic value of quantitative FMISO-analysis

The quantitative analysis of FMISO-PET parameters was possible in 15 patients with 21 lymph nodes exceeding the minimum required volume. The quantitative FMISO-PET parameters measured in these large LN during RCT statistically significantly correlated with RC at numerous FMISO-PET time-points (Table 4). The parameters $HV_{1.4}$ and $HV_{1.6}$ at baseline, at weeks 1 and 5 were significantly larger in LN developing a regional recurrence (LN non-responder) than in those with a complete regional remission during follow-up (responder; Table 4 and Fig. S3). In accordance with the findings for the primary tumors by Zips et al. [22] and Löck et al. [38], LN responders and non-responders revealed different reoxygenation patterns and the risk of a regional recurrence was higher in large LN with higher level of or with persisting hypoxia (Table 4 and Fig. S3).

Discussion

This is the largest analysis focusing on the prognostic value of synchronous hypoxia in the primary tumor and lymph node metastases based on repeat FMISO-PET imaging in advanced stage HNSSC patients. The results of this secondary analysis of a prospective imaging trial showed that the combined assessment of tumor and lymph nodes hypoxia improves the prognostic value of hypoxia PET/CT imaging at several time-points prior to and during RCT [22,38]. Since the nature of this investigation was explorative, these findings need to be validated in an independent cohort.

Past research has mainly focused on assessing the prognostic value of hypoxia-PET imaging or invasive Eppendorf measurements of one index lesion, most often the primary tumor [10,17,20–26,38]. In this secondary analysis we showed that synchronous primary tumor and lymph node hypoxia at baseline and during RCT is highly prognostic for worse LRC and LC and that it is so at an earlier time-point compared to tumor hypoxia only, which is found in the second week of RCT. The per-treatment prognostic factor is in accordance with preclinical data suggesting that the hypoxic volume correlates with local tumor control after 20 Gy but not at baseline [47]. Previously, few authors reported on differences in the oxygenation status of primary tumors and lymph node metastases in individual patients [10,28,29,48,49] but correlations with the clinical outcome are rare [46]. The incidence of synchronous Tu-and-LN-hypoxia prior to initiation of RCT of 44–58% corresponds well to the values in our cohort (53%). Servagi-Vernat et al. [28] acquired FAZA-PET imaging in twelve patients prior to and in the second and fourth weeks of RCT, but did not correlate the PET-readings with outcome. In the cohort ($N = 20$) reported by Lee et al. [46], almost exclusively consisting of oropharyngeal cancer patients, the 3-year LC rate was 100% and consequently FMISO-imaging performed 4 weeks after start of RCT did not correlate with patient outcome. The forty-five patients included in the prospective clinical study on radiochemotherapy in combination with the hypoxic cytotoxin tirapazamine by

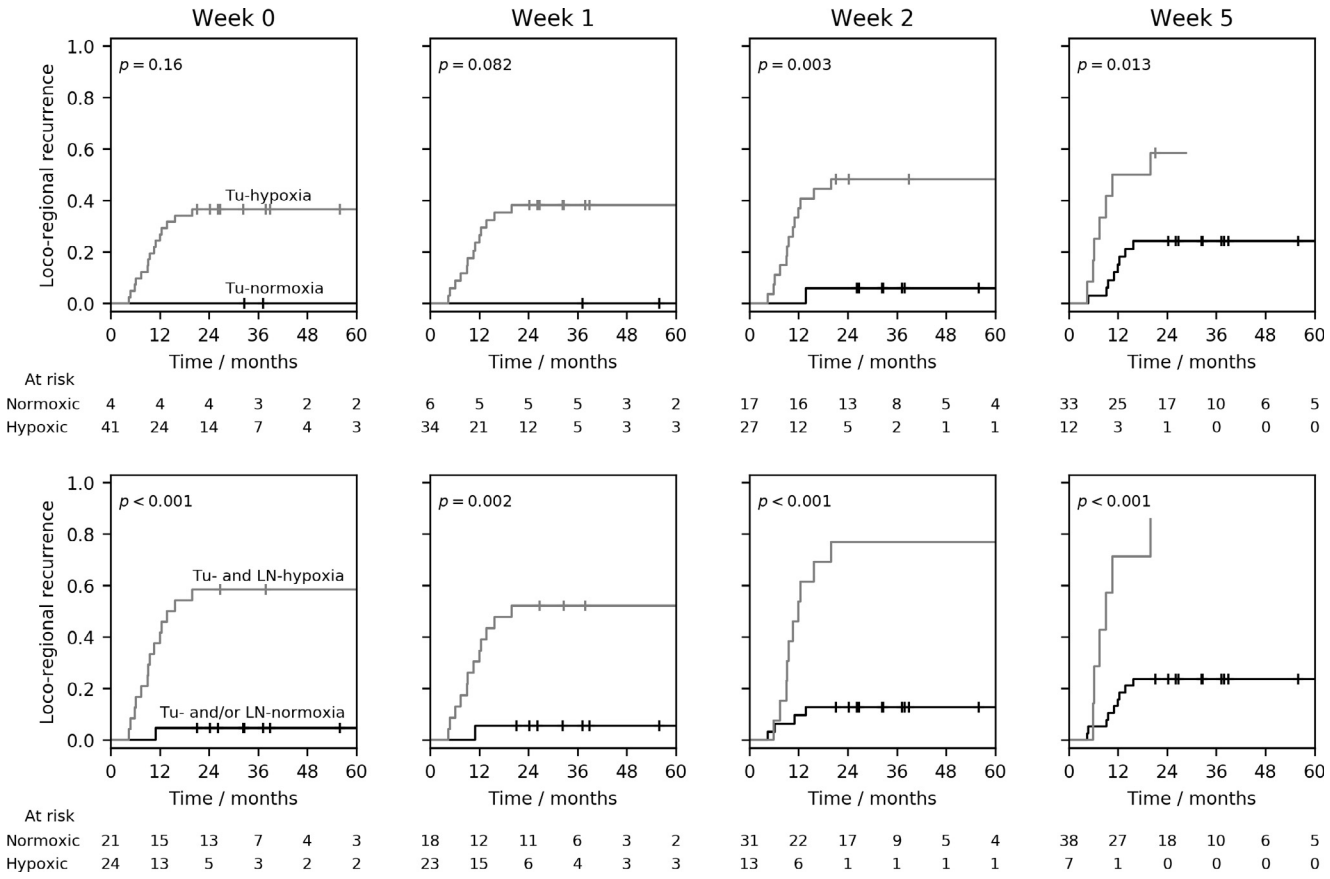

Fig. 2. Cumulative incidence function for loco-regional recurrences after radiochemotherapy for cohorts of HNSCC discriminated by hypoxia qualitatively determined in the primary tumor (Tu; top row) *versus* primary tumor and lymph nodes (LN; bottom row) at four time-points before and during treatment. Tu-hypoxia was of significant prognostic value for LRC in weeks 2 and 5, whereas synchronous Tu-and-LN-hypoxia revealed prognostic significance at all time points.

Table 3
Time-points of FMISO-PET/CT acquisition, number of qualitatively assessed primary tumors and LNs, and prognostic value of the hypoxia parameters at the respective time points (cumulative incidence corrected for competing risks; *p*-values < 0.05 presented in bold).

	Baseline	Week 1	Week 2	Week 5
Number of assessed patients = primary tumors	*n* = 45	*n* = 40	*n* = 44	*n* = 45
Number of assessed LN on FMISO PET/CT	103	95	101	103
Local control				
Tu-hypoxia	0.18	0.098	**0.006**	**0.047**
Tu-and-LN-hypoxia	**<0.001**	**0.004**	**<0.001**	**0.003**
Loco-regional control				
Tu-hypoxia	0.16	0.082	**0.003**	**0.013**
Tu-and-LN-hypoxia	**<0.001**	**0.004**	**<0.001**	**<0.001**
Time-to-progression				
Tu-hypoxia	0.091	**0.046**	0.14	0.100
Tu-and-LN-hypoxia	**<0.001**	**0.001**	**<0.001**	**0.001**
Freedom from distant metastases				
Tu-hypoxia	0.37	0.28	0.33	0.47
Tu-and-LN-hypoxia	**0.009**	**0.022**	0.40	0.93
Overall survival				
Tu-hypoxia	**0.014**	**0.050**	**<0.001**	**0.003**
Tu-and-LN-hypoxia	**0.036**	0.12	**0.011**	**0.008**

Abbreviations: Tu-hypoxia, primary tumor hypoxia (independent of LN oxygenation); Tu-and-LN-hypoxia, synchronous primary tumor and lymph node hypoxia assessed by the qualitative hypoxia scale.

Rischin et al. [30] had detectable hypoxia in either the tumor or lymph nodes or both in 71% of the patients. The authors revealed that FMISO-based primary tumor hypoxia in the non-tirapazamine receiving patients was associated with a high risk of locoregional failure but they did not investigate prognostic value of synchronous tumor and LN hypoxia.

We observed a reoxygenation kinetics in lymph nodes similar to that published by other groups [28,49]. Noteworthy, the quantitative FMISO-PET, measurements of sufficiently large LN performed on the PET/CT-scan obtained in the second week of radiochemotherapy correlated with regional control. In agreement with previously published results based on Eppendorf electrode

Table 4
Results of the quantitative analysis: FMISO-PET/CT parameters measured in large LNs ($N = 21$) for regional control: univariable Cox test (top) and Mann–Whitney U test (bottom), both at baseline and during radiochemotherapy (p-values <0.05 are given in bold).

FMISO-PET/CT parameter in LN-metastases ($N = 21$)		Baseline		Week 1		Week 2		Week 5	
		p	HR (95% CI)	p	HR (95 % CI)	p	HR (95 % CI)	p	HR (95 % CI)
SUV_{peak}		0.142	1.40 (0.89–2.19)	0.64	2.61 (0.94–7.19)	**0.016**	3.43 (1.26–9.31)	**0.006**	12.8 (2.09–79.8)
TBR_{peak}		0.13	1.61 (0.88–2.95)	**0.037**	2.24 (1.05–4.77)	**0.020**	2.83 (1.18–6.8)	**0.012**	8.75 (1.61–47.6)
$HV_{1.4}$		**0.021**	1.02 (1.00–1.01)	**0.017**	1.04 (1.01–1.06)	**0.033**	1.03 (1.00–1.06)	0.058	1.03 (1.00–1.07)
$HV_{1.6}$		**0.034**	1.03 (1.00–1.06)	**0.035**	1.04 (1.00–1.07)	**0.032**	1.03 (1.00–1.07)	0.073	1.04 (1.00–1.09)
		p	Average (SE)	p	Average (SE)	p	Average (SE)	p	Average (SE)
SUV_{peak}	Responder	**0.015**	2.4 (0.2)	0.079	2.1 (0.2)	**0.049**	1.8 (0.1)	**0.001**	1.5 (<0.1)
	Non-Responder		3.5 (0.4)		3.2 (0.7)		3.5 (0.7)		2.4 (0.2)
TBR_{peak}	Responder	**0.040**	2.1 (0.5)	0.13	1.9 (0.2)	0.080	1.7 (0.1)	**0.003**	1.4 (0.1)
	Non-Responder		3.1 (0.5)		3.3 (0.9)		3.1 (0.8)		2.0 (0.2)
$HV_{1.4}$	Responder	**0.040**	15.1 (6.2)	**0.035**	10.3 (4.7)	0.22	6.8 (2.7)	**0.008**	1.4 (0.6)
	Non-Responder		53.5 (21.6)		52.3 (21.0)		37.4 (20.7)		17.6 (12.0)
$HV_{1.6}$	Responder	**0.032**	10.1 (4.5)	**0.046**	4.8 (2.0)	0.12	2.9 (1.3)	**0.003**	0.2 (0.1)
	Non-Responder		35.4 (14.9)		29.8 (16.8)		28.3 (17.7)		11.4 (9.1)

Abbreviations: LN, lymph node; HR, Hazard Ratio; CI, confidence interval; SE, standard error; SUV, standardized uptake value; TBR, tumor-to-background-ratio; HV, hypoxic volume; Responder, LN with regional control; Non-Responder, LN with regional failure.

measurements, we observed that patients with at least one hypoxic LN at pretreatment FMISO-PET, have a significantly worse LRC and a higher rate of DM, and that LRC worsened with increasing number of hypoxic LNs [18,19,50]. Importantly, in our cohort no regional recurrence occurred in any of the lymph nodes ($N = 82$) not taken into account for the quantitative analysis based on the volumetric cut-off criteria (data not shown).

In agreement with the recently published data of the primary results of the present study by Löck et al. and with Mortensen et al. [10], hypoxia determined in the primary tumor before start of treatment was not of significant prognostic value for LC. This is in contrast to results by other groups that found a correlation between baseline FMISO-PET parameters measured in the FDG-positive tumor volume and LC [23,26,27]. Welz et al. [31] have recently published the planned interim analysis of their dose-escalation study (NCT02352792) in 25 advanced stage HNSCC patients based on pre-treatment FMISO-PET imaging. Toxicity was favorable and LRC increased from 44% in ten patients with hypoxic primary tumors receiving standard RCT to 70% in ten patients with hypoxic primary tumors receiving dose-escalated RCT. Based on the findings of our own prospective explorative and validation phase II study [38], we have designed a randomized phase II multicenter trial. Patients with residual tumor hypoxia, as depicted by repeat FMISO-PET imaging prior to and in the second week of RCHT, will receive either a standard dose or an escalated dose to the entire tumor volume using photons or particles. This cohort will be the ideal population for validation of the findings on synchronous primary and lymph node hypoxia before and during RCT presented here.

In summary, the role of measuring PET-hypoxia and its optimal timing as a prognostic and predictive marker for radiotherapy is still subject of controversy and ought to be further examined. Taken together the results presented here on the prognostic value of synchronous primary tumor and lymph node hypoxia are promising, and they will, together with other biomarkers, serve as tools for personalized radiation oncology [51–53].

In conclusion, FMISO-based synchronous primary tumor and lymph node hypoxia in advanced stage HNSCC patients undergoing radiochemotherapy was found to be of high prognostic relevance prior to and during radiochemotherapy. This finding ought to be validated in ongoing prospective clinical trials prior to using it for personalized radiation oncology.

Conflict of interest statement

There are no conflicts of interest to declare.

Acknowledgements

The authors thank Dr. Rosalind Perrin for her thoughtful comments and suggestions during this study. Prof. Howard Thames is gratefully acknowledged for the statistical support. The authors thank Dr. Kristin Gurtner for her comments on the patients' lymph node status. This study was supported by the German Federal Ministry of Education and Research (BMBF contract 03ZIK42/OncoRay).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.radonc.2018.09.008.

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