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^{18}F-Fluorodeoxyglucose Uptake Level-Based Lymph Node Staging in Oropharyngeal Squamous Cell Cancer – Role of Molecular Marker Expression on Diagnostic Outcome

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Keywords

^{18}FDG-PET/CT · Oropharyngeal squamous cell cancer · Standard uptake value · Lymph node staging · Histology

Summary

Background: A prospective study was performed to assess standard uptake value (SUV)-level based ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET)/computed tomography (CT) lymph node staging in 33 patients with oropharyngeal squamous cell cancer (OSCC) out of a total of 99 patients with head-and-neck squamous cell cancer (HNSCC) and the role of nodal molecular marker expression in diagnostic outcome prediction. **Methods:** Preoperative nodal PET/CT staging in 123 lymph nodes was correlated with postoperative lymph node histology, which served as gold standard. Tissue samples were prepared for immunohistochemistry of the excised lymph nodes. **Results:** The negative and positive predictive values (NPV and PPV) of PET for correct lymph node assessment were 100% and 93%, respectively. There was a significant association between SUVmax and lymph node histology ($p < 0.0001$) and a significant linear correlation between SUVmax and nodal size (Pearson's correlation coefficient $r = 0.61336$, $p < 0.0001$). The molecular marker E-Cadherin was significantly overexpressed in lymph node metastases ($p < 0.0001$). Benign lymph nodes showed significant 2-fold Bcl2 overexpression ($p < 0.0001$). However, the molecular marker expression profiles were inhomogeneous and did not allow valuable diagnostic outcome prediction. **Conclusions:** SUV level-based ^{18}F-FDG-PET/CT lymph node assessment in OSCC still has to be considered as the most established and reliable staging tool. Lymph node molecular marker expression profiles need to be investigated further as they currently do not sufficiently contribute to therapy decision-making.

Introduction

Head-and-neck squamous cell cancer (HNSCC), irrespective of the primary tumor site, remains a diagnostic and therapeutic challenge even today. Despite multimodal treatment facilities comprising surgery and laser resection, chemotherapy and radiation, the 5-year overall survival in these patients is far lower than 50%, especially in advanced tumor stages with lymph node metastases [1]. The patient prognosis is influenced by the quality of pretherapeutic staging, which includes assessment of tumor spread, the extent of vascular infiltration, and the cervical nodal status [2, 3]. In the era of targeted cancer therapy, tumor detection at an early stage and follow-up care in due time are integral parts of the treatment protocol [4–6].

Although ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (PET)/computed tomography (CT) has not been implemented in the routine diagnostic workup of patients presenting with HNSCC, its benefit for initial node (N) staging and assess-

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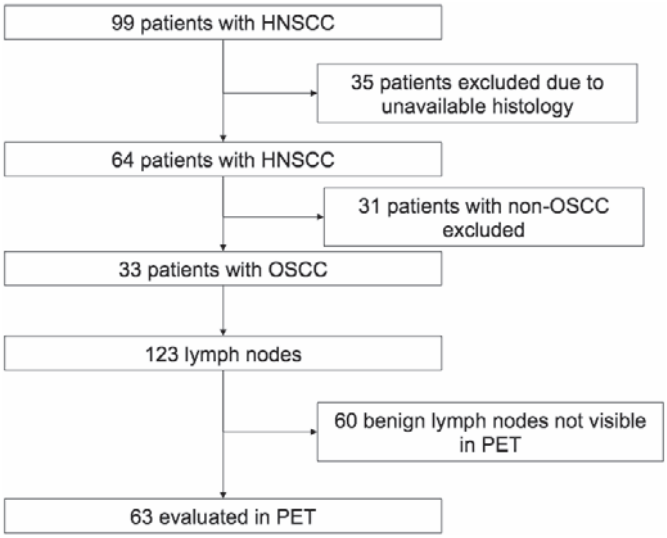

Fig. 1. Criteria for patient selection in this study.

ment of lymph node metastases recurrence has been reported in several studies [1, 7]. With sensitivities ranging from 87 to 95% and specificities of 80–98%, FDG-PET/CT has advanced to an established pre- and posttherapeutic imaging tool for cervical lymph node involvement in many centers [3, 8]. Limited information is available on the prognostic influence of maximum standard uptake values (SUVmax) of lymph node metastases on posttherapeutic response evaluation and the estimation of recurrence-free survival [1, 2, 9]. Variations in SUVmax levels and molecular marker expression patterns in lymph nodes may provide more accurate differentiation between benign and malignant lymph nodes. SUV levels and marker composition may also serve as parameter for outcome prediction [10, 11].

The aim of this prospective study was to combine initial 18F-FDG-PET/CT lymph node staging in oropharyngeal squamous cell cancer (OSCC) with the assessment of PET-SUV levels and molecular marker expression of the analyzed lymph nodes, with regard to their contribution to diagnostic outcome prediction in these patients.

Patients and Methods

Patients

From September 2009 until October 2011, a total of 99 patients with initial diagnosis of HNSCC underwent nodal staging with 18F-FDG-PET/CT at our institution. Prior to PET/CT, in all patients, a clinical examination and ultrasound of the neck was performed by an experienced head-and-neck surgeon. In 64 out of 99 patients with HNSCC, a postoperative correlation between the PET/CT findings and lymph node histology was available. 35 patients had to be excluded due to unavailable histology (no lymph node resection performed). 31 patients with non-OSCC tumor location site were excluded from the study. A total of 33 patients with exclusively OSCC were included for complete analysis: 21 male (64%) and 12 female patients (36%), mean age group 58.5 ± 7.8 years (range 40–73 years). For these 33 patients, 123 lymph node histologies were available. Out of these 123 findings, 63/123 lymph nodes were detected and evaluated by PET, whereas the remaining 60/123 lymph nodes were not visible in the PET scans due to lack of FDG uptake. The criteria for patient selection in this study are illustrated in figure 1. This prospective study was approved by our hospital institutional ethics review committee. All enrolled patients gave their written informed consent for staging PET/CT before undergoing neck dissection. The patient informed consent was waived by our institutional ethics board.

The treatment regimen for each patient was based on the decision of our institutional multidisciplinary tumor board conference. For all patients, a resection of the primary tumor with bilateral selective neck dissection and adjuvant radiochemotherapy (RCT) was planned.

According to the tumor board decision, all 33 patients underwent resection of their primary tumors. This was followed by a selective bilateral neck dissection of the levels 2, 3, and 4. Each neck level was resected individually. For every patient, the surgeon obtained a protocol sheet with a neck diagram before surgery. Within this neck diagram, the lymph node findings of PET/CT were plotted. This level-specific lymph node mapping enabled the surgeon to precisely mark the lymph nodes of every individual neck level for the pathologist.

After surgery, 28 patients underwent adjuvant RCT. 5 patients refused an additional adjuvant treatment. 1 patient who had not undergone adjuvant RCT developed a recurrence after 3 months and underwent repeated surgery. The other patients who developed a recurrence between 6 and 18 months of their follow-up period underwent chemotherapy with cisplatin and 5-fluorouracil (5-FU). Table 1 gives an overview of the clinical data of all 33 patients with OSCC.

^{18}F-FDG-PET/CT Imaging Protocol

Imaging was performed on a high-definition (HD) PET/CT scanner (Biograph mCT; Siemens Healthcare, Erlangen, Germany) according to a dedicated head-and-neck protocol, established in our diagnostic center. Patients received a weight-adapted intravenous (i.v.) injection of 250–300 MBq ^{18}F-FDG (ZAG Zyklotron AG, Karlsruhe, Germany) after a minimum fasting period of 6 h and at a blood glucose level of less than 140 mg/dl. After 60 min of resting, an attenuation-corrected low-dose extended-field-of-view (FOV) 780-mm CT was performed from the skull base to the pelvis, with 5 mm slice thickness and 3 mm increment (attenuation-corrected computed tomography (AC-CT) 50–70 mAs, 120 kV; B19f low dose for an ECT 5.0 sensor/3.0 mm increment, scan 5.0 mm, slice collimation 16 × 1.2 mm, 0.5 s/rotation, extended FOV > 80 mm). Scanning was performed with the patient in supine position, with arms down. The scan time was 3 min per bed position, and all emission images were acquired in 3-dimensional (3D) mode. Postprocessing of the PET images was obtained with a resolution-recovery 3D ordered subset expectation maximization (OSEM) algorithm (3 iterative steps, 24 subsets) on a 200 × 200 matrix. Subsequent to PET, every patient received a diagnostic contrast-enhanced biphasic CT from the skull base to the base of the lung (tube current 50–120 mAs, tube voltage 120 kV, slice collimation 16 × 1.2 mm, pitch 0.8, rotation time 0.5 s). The PET/CT lymph node findings and their SUVmax levels were allocated to cervical regions in the right and left neck and documented in an evaluation sheet, topographically defined according to the neck dissection nomenclature [12].

PET Analysis and Immunohistochemistry of Cervical Lymph Nodes

Measurement of the SUVmax levels of the cervical lymph nodes was performed with a scanner-integrated software on a dedicated workstation (Siemens Syngo TrueD). Individual regions of interest were drawn around the lymph nodes and the calculations were documented in an evaluation sheet. The dependence of the semi-quantitative parameter SUVmax on the scanner, the injected FDG dose, the patient weight and the reconstruction methods, and the difficulty in defining a cut-off level for benign and malignant lesions are documented in the literature [7, 11]. On the basis of our previous experience with PET/CT head-and-neck imaging and the manufacturer's hardware and software specifications, SUVmax levels < 3 were classified as benign lymph nodes whereas SUVmax levels ≥ 3 were characterized as lymph node metastases. Lymph node assessment was primarily based on PET-SUV levels in consideration of the corresponding CT slices. Suspicious lymph nodes in PET, histologically confirmed as malignant, were classified as true-positive findings. Unsuspicious lymph nodes in PET and corresponding SUV levels, histologically confirmed as benign, were classified as true-negative findings. Suspicious lymph nodes in PET, histologically non-malignant, were classified as false-positive

Table 1. Overview of the clinical data of all 33 patients with OSCC

No.	Patient code	Sex	Age[a], years	TNM[b]	Tumor site	ND level	RCTx	Recurr, months
1	O1	m	73	T3N1M0	Ton	2, 3, 4	no	none
2	O2	f	40	T1N1M0	Ton	2, 3, 4	no	3
3	O3	m	71	T3N2aM0	Ton, BT, SP2, 3, 4	yes	none	
4	O4	f	53	T2N1M0	Ton, SP	2, 3, 4	yes	none
5	O5	m	49	T3N0M0	BT	2, 3, 4	yes	18
6	O7	f	57	T2N1M0	Ton	2, 3, 4	yes	none
7	O8	m	67	T3N0M0	BT	2, 3, 4	yes	none
8	O9	m	46	T3N2bM0	BT	2, 3, 4	yes	18
9	O11	f	61	T2N2cM0	BT	2, 3, 4	yes	none
10	O13	m	58	T3N2bM0	BT	2, 3, 4	yes	18
11	O14	m	58	T3N0M0	Ton, BT, LPW	2, 3, 4	yes	none
12	O15	m	65	T3N2bM0	Ton, BT	2, 3, 4	yes	9
13	O18	f	53	T2N2bM0	Ton	2, 3, 4	yes	none
14	O20	m	65	T3N0M0	BT	2, 3, 4	yes	none
15	O21	m	54	T2N2bM0	Ton	2, 3, 4	yes	none
16	O23	f	52	T3N2bM0	Ton	2, 3, 4	yes	none
17	O25	f	54	T4N0M0	SP, LPW	2, 3, 4	yes	none
18	O26	m	61	T4N2cM0	BT	2, 3, 4	yes	9
19	O28	m	56	T3N2bM0	Ton	2, 3, 4	yes	none
20	O29	m	64	T2N2bM0	Ton	2, 3, 4	yes	none
21	O30	f	51	T2N2bM0	Ton	2, 3, 4	yes	none
22	O32	m	60	T4N2bM0	BT	2, 3, 4	yes	none
23	O34	m	52	T3N0M0	Ton, SP	2, 3, 4	no	none
24	O35	m	66	T3N1M0	Ton	2, 3, 4	no	none
25	O36	f	59	T2N0M0	Ton	2, 3, 4	yes	none
26	O38	f	60	T3N2cM0	SP	2, 3, 4	yes	6
27	O39	m	53	T2N3M0	To	2, 3, 4	yes	6
28	O40	m	53	T3N2bM0	SP	2, 3, 4	yes	none
29	O41	f	66	T4N2bM0	BT	2, 3, 4	yes	none
30	O43	m	70	T3N2cM0	SP, U	2, 3, 4	yes	none
31	O44	m	59	T2N1M0	Ton	2, 3, 4	no	none
32	O45	m	72	T3N2cM0	BT	2, 3, 4	yes	none
33	O48	m	59	T2N2cM0	Ton	2, 3, 4	yes	none

[a]Age at the time of study enrolment.
[b]TNM classification (Union for International Cancer Control (UICC) 2002).
ND = Neck dissection, RCTx = adjuvant radiochemotherapy, Recurr = recurrence given in months after surgery, m = male, f = female, Ton = tonsil, BT = base of tongue, SP = soft palate, U = uvula, LPW = lateral pharyngeal wall.

findings. Unsuspicious lymph nodes in PET, histologically confirmed as malignant, were classified as false-negative findings. All characterized lesions underwent intraoperative exploration and resection with histological correlation.

Tissue samples of 5–7 μm slice thickness (Leica CM 1900) were prepared for immunohistochemical analysis with antibodies against vascular endothelial growth factor (VEGF), E-Cadherin, epidermal growth factor receptor (EGFR), Ki-67, Cyclin D1, Bcl2, human papilloma virus type 16 (HPV-16) and HPV-18 in the lymph nodes. Individual antibodies and corresponding antigen retrieval mechanisms were applied for analysis (table 2). Immunostaining rates were classified into 4 scores (1 = no staining, 2 = 1–25% weak staining, 3 = 26–50% moderate staining, 4 = 51–100% intense staining).

Statistical Analysis

Assessment was done with SAS software release 9.3 (SAS Institute Inc., Cary, NC, USA). For each diagnostic parameter (i.e. PET and histological findings), sensitivity, specificity, and predictive values were analyzed together with the corresponding 95% confidence intervals. In order to compare 2 different diagnostic methods for lymph node characterization, sensitivities and specificities were compared with McNemar tests. Furthermore, the kappa index was calculated as a measure of agreement. For comparison of the 2 histology groups regarding SUVmax and lymph node size, Mann-Whitney tests were applied. The trend test according to Cochran and Armitage was used to compare the 2 histological groups regarding their tumor marker expression levels, which were classified into 4 scores. A potential linear relationship between lymph node SUVmax and size was analyzed with Pearson's correlation coefficient. Furthermore, logistic regression analysis and receiver operating characteristic (ROC) analysis were performed in order to determine an optimal SUVmax cut-off level for accurate prediction of benign and malignant lymph nodes.

Tumor-specific survival was calculated by Kaplan-Meier analysis. Differences and correlations were considered as statistically significant for test results with p values of < 0.05.

Results

Patient Characteristics

A total of 33 patients with exclusively OSCC were included for complete analysis (see 'Patients and Methods' and fig. 1). In most cases, advanced-stage OSCC with stage II n = 1 (3%), stage III n = 11 (33%), and stage IV n = 21 (64%) was diagnosed. Altogether, 123 lymph node histologies were available. Out of these 123 findings, 63 lymph nodes were detected and evaluated in PET, based on dedicated reading by 2 experienced radiologists and a preoperative mapping by 1 head-and-neck surgeon. 60 additionally excised lymph nodes in the surgical specimens were histologically benign. They had not been detected in preoperative PET due to lack of FDG uptake, which emphasizes the high negative predictive value (NPV) of PET/CT [13].

Results of Lymph Node Analysis

Of the 63 PET-visible lymph nodes, 23 (36.5%) were histologically benign, with 20 true-negative findings and 3 false-positive findings (specificity 87%, NPV 100%). 40/63 PET-diagnosed lymph nodes (63.5%) proved to be metastases and were correctly diagnosed as such (sensitivity 100%, positive predictive value (PPV) 93%). Comparing PET diagnosis with histological findings, a kappa value of 0.8944 and p = 0.0833 (McNemar test) were obtained. A SUVmax cut-off level of 3.0 was determined for the prediction of benign and malignant lymph nodes. SUVmax of the histologically benign lymph nodes was 3.4 ± 4.1 (1.4–22.0) and their average size was 8.9 ± 2.4 mm (5.0–14.0 mm). SUVmax of the malignant lymph nodes was 9.4 ± 7.1 (3.0–39.1), with an average size of 14.7 ± 9.2 mm (7.0–54.0 mm). The Mann-Whitney U test proved significantly lower SUVmax levels in benign compared to malignant lymph nodes (p < 0.0001) (fig. 2a). Benign lymph nodes were significantly smaller in size than nodal metastases (p = 0.0006) (fig. 2b). A linear relationship could be observed between SUVmax and lymph node size (Pearson's correlation coefficient r = 0.61336, p < 0.0001).

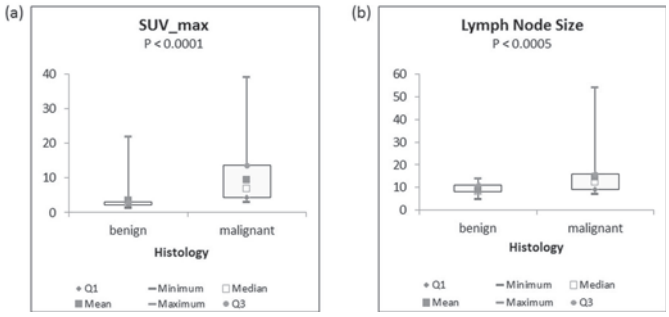

Fig. 2. Relation between SUVmax and lymph node histology (a) and nodal size and histology (b). Benign lymph nodes present with less average SUVmax levels and size than lymph node metastases.

Data on Immunohistochemistry

The tumor marker expression levels for VEGF, E-Cadherin, EGFR, Ki-67, Cyclin D1, Bcl2, HPV-16, and HPV-18 were very heterogeneous (fig. 3a–c). The p values of the Cochran-Armitage trend test for staining intensity and lymph node dignity were VEGF p = 0.9168, E-Cadherin p < 0.0001, EGFR p = 0.6029, Ki-67 p = 0.8205, Cyclin D1 p = 0.4612, Bcl2 p = 0.0001, HPV-16 p = 0.8227, and HPV-18 p = 0.1279. Hence, as a potential diagnostic tool for distinguishing between benign and malignant lymph nodes, only the expression levels of E-Cadherin and Bcl2 showed statistical significance justifying further consideration. Because of the statistically non-significant results for HPV-16 and -18, these markers were not considered for further diagnostic assessment.

In the lymph node metastases, E-Cadherin expression was significantly increased (with median values of 3 and 1, respectively) as

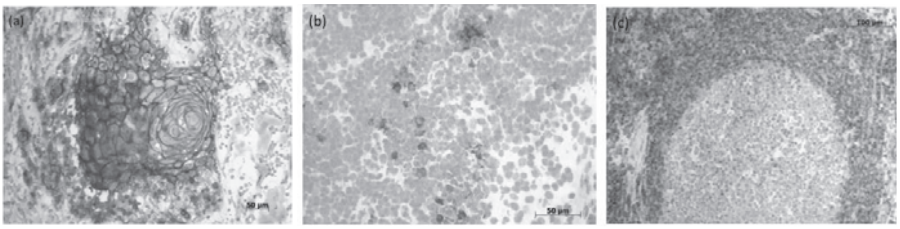

Fig. 3. Intensity of staining and immunomarker expression for E-Cadherin (a), Cyclin D (b), and Bcl2 (c) in lymphatic tissue. Scores: 1 = no staining, 2 = 1–25% weak staining, 3 = 26–50% moderate staining, 4 = 51–100% intense staining.

Table 2. Antibodies for immunohistochemical analysis

Antibody against	Type	Dilution	Antigen retrieval	Company
VEGF	polyclonal rabbit	1:200	citrate buffer	Santa Cruz, Heidelberg, Germany
E-Cadherin	monoclonal mouse	1:50	citrate buffer	Abcam, Cambridge, UK
EGFR	monoclonal mouse	1:100	citrate buffer	Santa Cruz, Heidelberg, Germany
Ki-67	monoclonal mouse	1:100	citrate buffer	DAKO, Hamburg, Germany
Cyclin D1	monoclonal mouse	1:100	citrate buffer	Abcam, Cambridge, UK
Bcl2	monoclonal mouse	1:50	citrate buffer	Acris, Herford, Germany
HPV-16	monoclonal mouse	1:100	citrate buffer	Abcam, Cambridge, UK
HPV-18	monoclonal mouse	1:20	citrate buffer	Abcam, Cambridge, UK

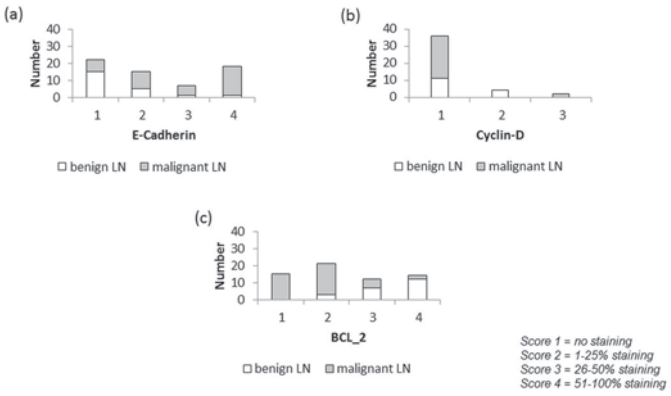

Fig. 4. Immunomarker expression patterns of E-Cadherin (a), Cyclin D (b), and Bcl2 (c) in benign lymph nodes and lymph node metastases of OSCC. Benign lymph nodes demonstrated insignificant expression of E-Cadherin and Cyclin D (1–25%) but significantly high levels of Bcl2 (51–100%). Lymph node metastases were characterized by a significantly high expression of E-Cadherin (51–100%), very moderate levels of Bcl2 (1–25%), and almost no evidence of Cyclin D.

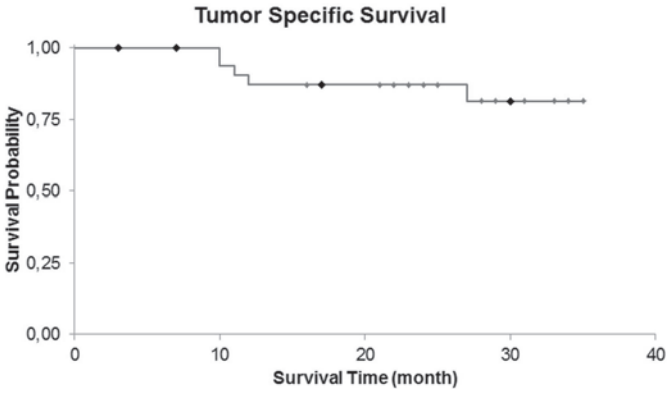

Fig. 5. Kaplan-Meier tumor-specific survival curve. 5 out of 9 patients died of tumor recurrence after 10 (2 ×), 11, 12, and 27 months. In 4 cases, non-cancer-related death occurred after 3, 7, 17, and 30 months. Data for tumor-specific survival after 35 months was available for 28 patients, including 4 patients with non-cancer-related death.

well as the SUVmax levels (median values 6.8 and 2.6, $p < 0.0001$ with Mann-Whitney U test). Benign lymph nodes with corresponding lower SUVmax levels showed significant overexpression of Bcl2 (median values 4 and 2, respectively) (fig. 4a–c).

Patient Survival Outcome

Out of the 33 patients with OSCC, 24 patients (73%) survived and 9 patients (27%) died during a mean follow-up period of 23.6 months (3–35 months, median 25 months). 5 out of 9 patients died of tumor recurrence after 10 (2 ×), 11, 12, and 27 months. In these 5 patients, advanced disease was diagnosed according to tumor/node/metastasis (TNM) criteria (T3: 4 ×, T4: 1 ×; N2b: 3 ×, N2c: 2 ×). In 4 cases, non-cancer-related death occurred after 3, 7, 17, and 30 months. These 4 patients presented with T3 stage OSCC with no nodal involvement in 3 cases and N2a stage in 1 patient. Data for tumor-specific survival time up to 35 months was available for 28 patients, including 4 patients with non-cancer-related death (fig. 5).

Discussion

Despite dedicated examination protocols for the diagnosis of HNSCC, these tumors represent a multifactorial disease with molecular and immunohistochemical heterogeneity. Precision medicine delivering targeted antiangiogenic tumor treatment seems to be a new approach to this tumor entity [10, 13, 14].

In this study, the key role of ^{18}F-FDG-PET/CT for lymph node staging in OSCC could be confirmed and also the value of the PET-SUV level as a prognostic marker, although in the clinical setting ^{18}F-FDG-PET/CT is still not considered a standard in HNSCC staging. An experienced head-and-neck surgeon will not base the therapy decision entirely on imaging findings, especially in patients with clinically negative neck. Kyzas et al. [15] performed a meta-analysis of 32 studies in HNSCC patients, demonstrating considerably decreased sensitivity and specificity of ^{18}F-FDG-PET alone in non-palpable occult cervical metastasis.

Nevertheless, there is increasing evidence suggesting the integration of combined ^{18}F-FDG-PET/CT for nodal staging, cancer of unknown primary (CUP) detection, and recurrence surveillance [16]. PET/CT scanner technology, dedicated imaging protocols, and high-quality postprocessing software are improving the diagnostic output in lymph node staging [6, 17]. Still, the 'N0 neck' continues to be a challenge despite increased diagnostic accuracy of up to 95%, by combining high-resolution PET with contrast-enhanced CT [18].

Assessment of the SUVmax has been feasible in many tumor entities, and its value is in agreement with several studies [11, 17, 19]. Some authors assume that the SUVmax is not a valuable parameter for outcome prediction [17, 20]. We are not of this opinion and agree with Kubicek et al. [1] that the SUVmax correlates with lymph node histology and size, which indeed has an impact on therapeutic patient management regarding surgical decision-making versus neoadjuvant or palliative chemotherapy. The high NPV of PET/CT could be demonstrated by the fact that 60 surgically excised lymph nodes did not at all show FDG uptake in PET and proved to be histologically benign. In this context, the prospective study by Liao et al. [21] has to be mentioned, which underlines the role of preoperative SUVmax analysis in patients with oral-cavity squamous cell cancer as a predictor of 5-year disease-free survival.

SUVmax is the most frequently applied semi-quantitative PET parameter for the assessment of lesion dignity, therapy response, and tumor recurrence in clinical routine [1, 6]. PET hardware features, patient body weight, blood glucose levels, and potential movement during scanning are known factors that influence SUVmax [17, 21]. For this reason there is currently no standardized SUVmax cut-off value available that allows clear delineation be-

tween benign and malignant lesions. Our cut-off value of SUVmax < 3/≥ 3 for benign/malignant lymph nodes was entirely based on experience with previous PET/CT studies in patients with HNSCC [17]. We also performed logistic regression analysis to confirm SUVmax as a useful predictor for lymph node dignity analysis; the cut-off value SUVmax < 3/≥ 3 resulted in best trade-off between sensitivity and specificity (Mc Nemar p value = 0.0082, kappa 0.74, sensitivity 100.0%, specificity 70%, PPV 85%, NPV 100%).

The assessment of molecular marker profiles in lymph node metastases in HNSCC, responsible for the aggressiveness of tumor growth, angiogenesis and metastatic spread, might represent a novel approach to diagnostic accuracy [22, 23]. Detection of individual biomarkers could help clinicians and head-and-neck surgeons to establish personalized medicine, distinguish therapy responders from non-responders and avoid unnecessary treatment-related morbidity.

In this study, we analyzed molecular markers in 63 excised lymph nodes. The number of 63 lymph nodes is moderate, but it has to be mentioned that dedicated reading and reporting between the radiologist, the head-and-neck surgeon, and the pathologist allowed for a precise level-to-level histological analysis of these surgically resected lymph nodes, which is rarely found in the literature [11, 23]. Besides the evaluation of SUVmax, nodal size and histology, the expression levels for VEGF, E-Cadherin, EGFR, Ki-67, Cyclin D1, Bcl2, HPV-16, and HPV-18 were assessed.

Unlike Baumann et al. [13], we could not prove significant nodal HPV-16 and -18 expression. It has to be mentioned that we only performed immunohistochemical analysis of lymphatic tissue and not of the primary tumor because that was not an integral part of our study. The fact that we had HPV-negative lymph nodes might be due to the fact that these OSCCs belong to the HPV DNA-negative group, the so-called tobacco-related HNSCCs, with poorer prognosis than the HPV-positive tumors [24, 25]. We did not find any correlation between the cellular proliferation marker Ki-67, SUVmax and lymph node histology. Ki-67 is a routinely used molecular marker with still unclear significance [25, 26].

Although VEGF is expressed in more than 80% of HNSCCs, this marker, responsible for tumor spread, metastatic potential, and treatment failure [6], was not evident in our nodal immunostainings and could therefore not be correlated with SUVmax and lymph node histology. The absence of significant VEGF expression may be linked to the fact that, in our 33 cases of OSCC, only 5 patients were potential therapy non-responders who died from tumor recurrence. EGFR expression was one of the first molecular markers of angiogenesis being targeted as an anticancer agent in HNSCC. We had poor results for EGFR expression, which did not allow correlation with SUV_{max} and lymph node histology. As overexpression of this marker is usually associated with reduced overall survival and tumor recurrence, it can be assumed that the lymph nodes associated with OSCC in our patients were not representative of aggressive tumor growth.

Out of the 3 molecular markers E-Cadherin, Cyclin D1, and Bcl2, we could unfortunately only prove statistically relevant results for E-Cadherin and Bcl2 (fig. 3a–c). We found a correlation between E-Cadherin expression and lymph node metastases in OSCC, which was confirmed by Mostaan et al. [27]. Downregulation of this adhesion molecule is responsible for loss of tumor cell differentiation, increased invasiveness and metastatic potential [28]. Malignant lymph nodes showed significant expression of E-Cadherin in our study, which might be responsible for well-differentiated OSCCs with lower tendency towards lymph node metastasis.

We can confirm the uncertain role of Cyclin D1 as a molecular marker in OSCC, as also reported by Maahs et al. [29]. Patients showed no relevant expression in benign and malignant lymph nodes so that it is most unlikely that Cyclin D1 could serve as prognostically valuable biomarker for therapy decisions in nodal metastases of HNSCC.

Significantly high expression levels of Bcl2 were found in the benign lymph nodes. Bcl2 represents an immunomarker that is also present in physiological oral mucosa [29]. Lacking expression in lymph node metastases adds to the assumption that well-differentiated OSCCs with less invasive potential and improved overall survival were diagnosed in our patients. At the same time, we discovered high expression levels of E-Cadherin in lymph node metastases, which have been made responsible for well-differentiated OSCC with less tendency of nodal spread.

In conclusion, in this study, we could confirm the tremendous contribution of ^{18}F-FDG-PET/CT to lymph node staging in OSCC. There is a correlation between SUVmax, lymph node histology and nodal size, which allows lymph node status prediction. Although the awareness of lymph node molecular marker expression and its effect on therapeutic management is growing, understanding the molecular marker patterns associated with HNSCC and OSCC still remains a challenge, and studies have to continue in search of the appropriate marker that can be integrated into clinical practice.

At present, SUV level-based ^{18}F-FDG-PET/CT and the associated high NPV of SUVmax in OSCC remains the most reliable diagnostic approach to lymph node staging and cannot be outperformed by nodal molecular marker expression profiles, which require further investigation.

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Disclosure Statement

The authors declare that they have no competing interests and no conflicts of interest.

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