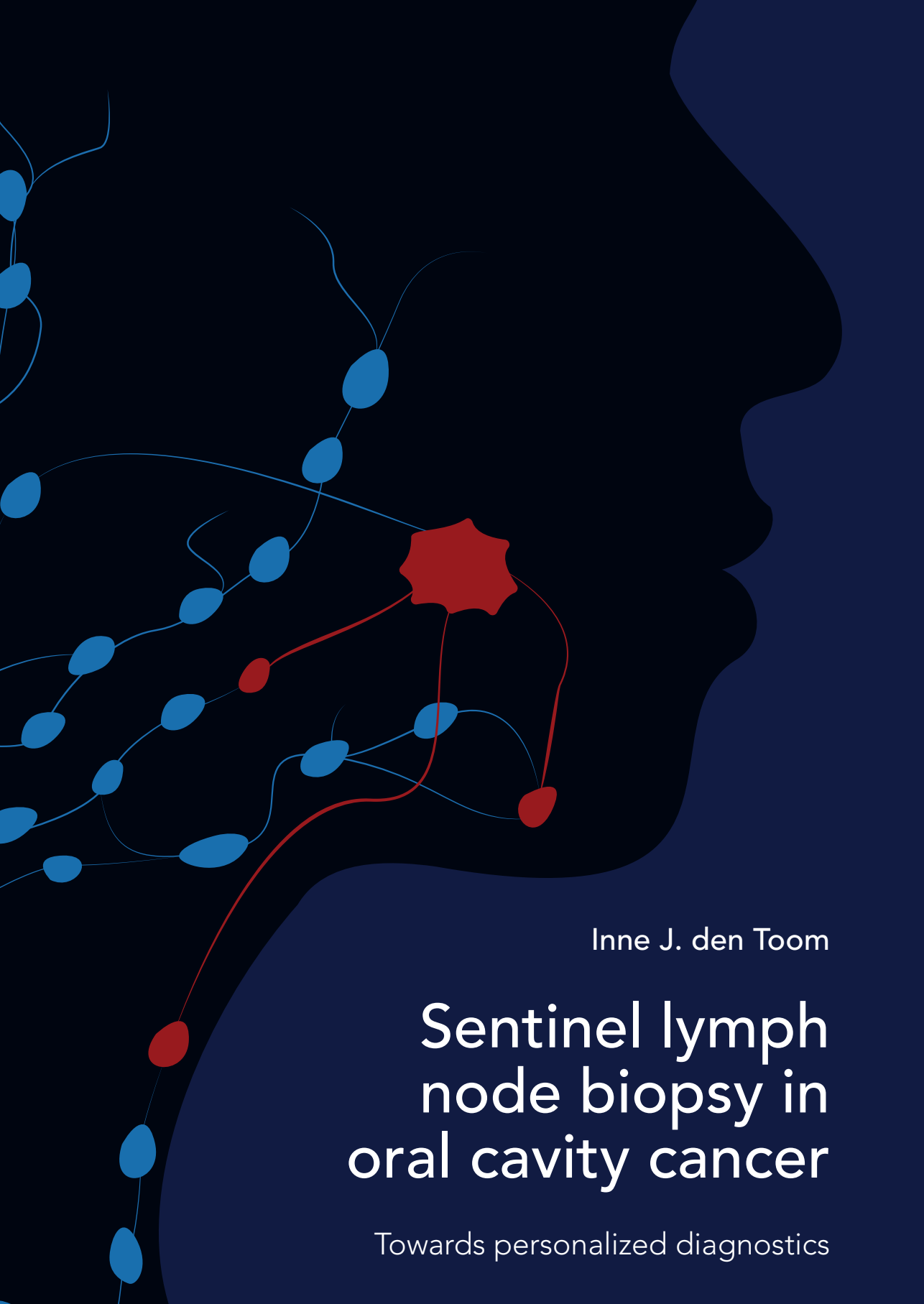

Inne J. den Toom

Sentinel lymph node biopsy in oral cavity cancer

Towards personalized diagnostics

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Towards personalized diagnostics

De schildwachtklierprocedure voor mondholtecarcinomen

Op weg naar diagnostiek op maat

(met een samenvatting in het Nederlands)

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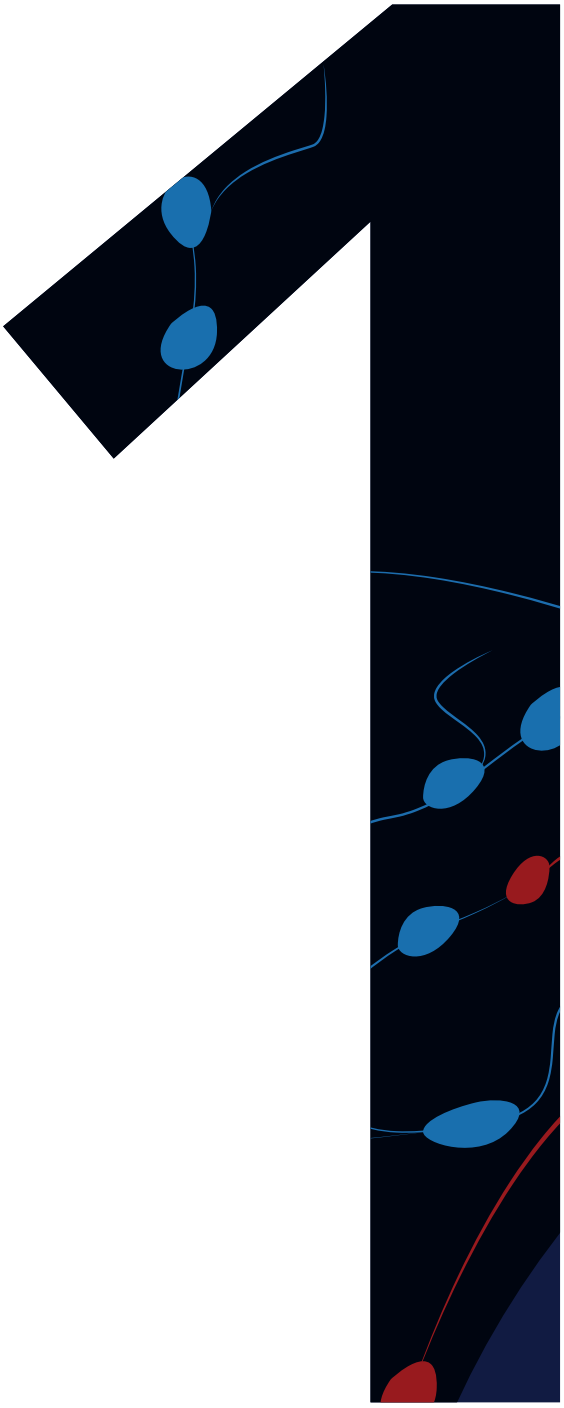
CONTENTS



General introduction

GENERAL INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) arises from the epithelium of the upper aerodigestive tract and is mostly located in the oral cavity, pharynx and larynx. The development of HNSCC is strongly associated with risk factors as tobacco smoking and alcohol consumption, however genetic predisposition and human papilloma virus (especially in the oropharynx subsite) are also known as important key factors. HNSCC is the 9th most common cancer type worldwide, representing 3.9% of the total cancer incidence.[1] In the Netherlands approximately 3000 patients are newly diagnosed each year.[2]

To determine the stage of HNSCC the TNM-classification system of the Union for International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC) is used.[3,4] With this classification the extent of tumour (T-stage), the presence of nodal metastases (N-stage) and distant metastases (M-stage) are established. The TNM-staging is based on physical examination, imaging and histopathological analysis after an eventual surgical procedure (Table 1).

Staging of patients is of essential value for therapeutic and prognostic purposes (Table 2). Advanced stage disease (stage III/IV) is unfortunately diagnosed in two-thirds of all HNSCC patients. Prognosis of these patients is much worse compared to the one-third of the patients presenting with early stage disease (stage I/II). Treatment modalities for HNSCC patients are surgery, radiotherapy and chemotherapy and a combination of these is frequently used in advanced stage disease. Recently, immunotherapy is introduced as breakthrough therapy in a variety of tumour types and its potential value for HNSCC seems promising.[5,6]

Despite the increasing knowledge of biological characteristics of tumours and the improvements of treatment modalities, survival rates of HNSCC patients have not improved evidently over the last decades.[7]

This chapter describes the management of patients with early stage oral cavity cancer and a clinically node negative neck (cT1-2,N0). Besides, the concept of the sentinel lymph node biopsy procedure will be explained. Finally, the aim and outline of this thesis will be explicated and the other chapters of this thesis will be introduced.

Table 1. Eighth edition of TNM classification system for lip and oral cavity cancer of the Union for International Cancer Control and American Joint Committee on Cancer (UICC-AJCC)

TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour ≤ 2 cm in diameter and ≤ 5 mm depth of invasion
T2	Tumour ≤ 2 cm in diameter and between > 5 mm and ≤ 10 mm depth of invasion Tumour > 2 cm but ≤ 4 cm in diameter, with ≤ 10 mm depth of invasion
T3	Tumour > 4 cm in diameter or > 10 mm depth of invasion
T4a	(lip) Tumour invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin
T4a	(oral cavity) Tumour invades through cortical bone of the mandible or maxillary sinus, or invades the skin of the face
T4b	(lip and oral cavity) Tumour invades masticator space, pterygoid plates, or skull base, or encases internal carotid artery
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastasis in a single ipsilateral lymph node, ≤ 3 cm in greatest dimension without extranodal extension
N2a	Metastasis in a single ipsilateral lymph node, > 3 cm but ≤ 6 cm in greatest dimension without extranodal extension
N2b	Metastasis in multiple ipsilateral lymph nodes, none > 6 cm in greatest dimension, without extranodal extension
N2c	Metastasis in bilateral or contralateral lymph nodes, none > 6 cm in greatest dimension, without extranodal extension
N3a	Metastasis in a lymph node > 6 cm in greatest dimension without extranodal extension
N3b	Metastasis in a single or multiple lymph nodes with clinical extranodal extension
M0	No distant metastases
M1	Distant metastases

Table 2. Staging of patients according to the TNM classification

Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1	M0
Stage IVA	T4a	N0, N1	M0
	T1, T2, T3, T4a	N2	M0
Stage IVB	Any T	N3	M0
	T4v	Any N	M0
Stage IVC	Any T	Any N	M1

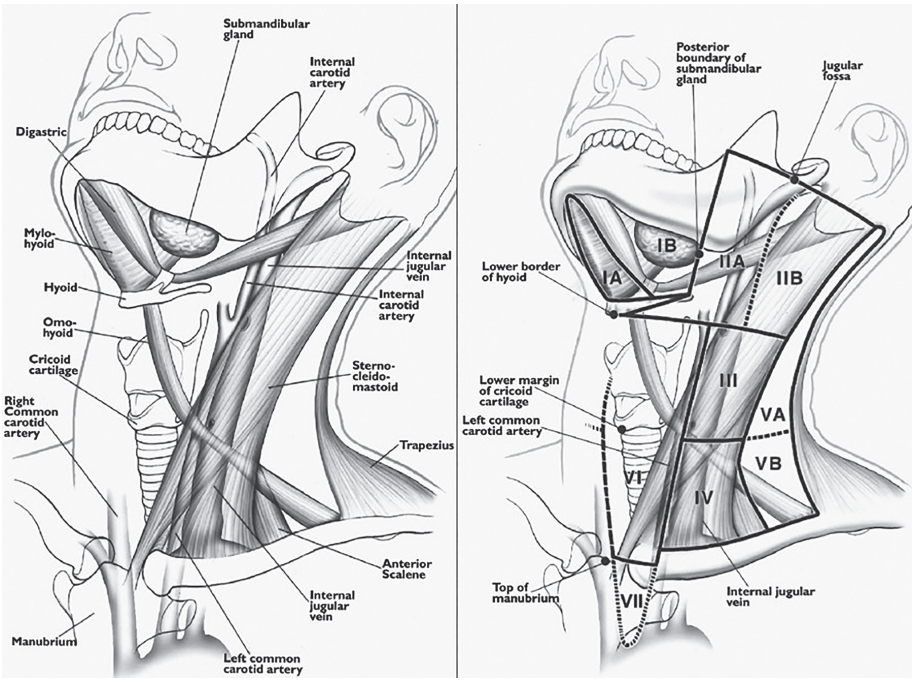


Figure 1. Neck levels. [10]

Level I Submental region (Ia), submandibular region (Ib)
Level II Subdigastric region; anterior (IIa), and posterior from accessory nerve (IIb)
Level III Midjugular region
Level IV Low jugular region
Level V Posterior triangle
Level VI Prelaryngeal and pre- and paratracheal region
Level VII Superior mediastinal nodes

Cervical lymph node metastases

The anatomy of the cervical lymphatic system was firstly described by Rouvière in 1932.[8] Currently, lymph nodes are mapped according to the American Academy of Otolaryngology – Head and Neck Surgery (AAO-HNS) (Figure 1).[9]

It is well known that cervical lymph node metastases are a major prognostic factor in patients with HNSCC.[11,12] Presence of regional metastases increases the risk of having distant metastases (from 7% to 47%, in case of > 3 positive nodes) and reduces in general survival nearly by half. Therefore, it is for prognosis and treatment planning of essential value to determine nodal involvement accurately. Palpation by clinicians detect only clinical manifest lymph node metastases and proved to be inferior compared to conventional imaging techniques, such as computerized tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and ultrasonography (US).[13] These imaging modalities focus on size and homogeneity criteria, which both not always reflecting presence of malignancy correctly. A large variation still exists in criteria to define lymph nodes containing metastases on imaging, suggesting that none of the above mentioned imaging modalities suffices adequately. US-guided fine-needle aspiration cytology (USgFNAC) shows a higher sensitivity and specificity, but also has its limitations.[14,18] USgFNAC is strongly dependent on the experience and skills of the ultrasonographer and cytopathologist. Thereby, sensitivity will always be limited due to sampling error. Taking into account all limitations of palpation and imaging, patients with a clinically and radiologically negative neck (cN0) still has a substantial risk of having occult metastases in the neck.

How to treat patients with a risk of occult metastases is a largely discussed topic for decades, especially in patients whose neck not have to be treated as part of management of the primary tumour. In early stage oral cavity cancer (cT1-T2N0) the neck remains generally untreated, considering that the primary tumour could be resected transorally and no reconstruction is necessary. The incidence of occult metastases is reported to be around 30%.[19,22] A long lasting dilemma is ongoing to manage the clinically negative neck in this population. It could be an option to treat all patients prophylactically with an elective neck dissection, or to let the neck untreated and pursue patients in a stringent "watchful waiting" policy.

Watchful waiting strategy

If absence of metastasis is confirmed by USgFNAC, the watchful waiting (WW) strategy consists of transoral excision of the primary tumour alone with thorough observation

of the neck. Patients are strictly monitored by clinical examination and USgFNAC on regular basis during the first 2 years. During follow-up 28% of the patients developed regional metastases.[23] In this last mentioned study of Flach et al., WW patients with metastases during follow-up did not show lower overall- and disease-specific survival curves compared to patients receiving an elective neck dissection which contains metastases. In contrary, D'Cruz et al. showed in a randomized controlled trial a significant lower overall and disease free survival in patients treated therapeutically (neck dissection performed when clinically manifest metastases were observed) compared to the elective neck dissection group.[24] Unfortunately, no disease-specific survival rates were presented in this study. In general, this Indian study could probably not well be compared to European (and American) literature due to the substantial different pre-treatment work-up and follow-up.[25]

Elective neck dissection

The principle of removing lymph nodes electively is based on the high number of occult metastases in early stage oral cancer. In 1994 Weiss et al. performed a decision analysis to identify a threshold value (20%) from where treatment is mandatory.[21] However, that threshold value was based on old-fashioned techniques and theories, while nowadays other factors (e.g. patients preferences) will also play an important role in decision making.[26]

One of the most important risk factors for having occult metastases is depth of invasion of the primary tumour. A depth of invasion of ≥ 4 mm is associated with an increased risk of having occult disease and generally elective neck dissections (END) are performed from this depth on.[27,28]

An advantage of elective treatment is that theoretically all potential nodal metastases are removed and most often surgical removal of small occult disease cures enough, without the need for adjuvant radiotherapy. However, with the previously described risk of 30% of having occult disease, the majority of patients are overtreated with an END, causing unnecessary morbidity.[29,30] Even when the spinal accessory nerve is spared, shoulder complaints are reported from 22% to 39%. Deterioration in shoulder function has a considerable negative impact on quality of life. Another disadvantage of END could be the removal of a physical barrier preventing distant metastases in case of local recurrences or second primary tumours, which are common in head and neck cancer patients.[31,32]

To prevent unnecessary overtreatment with elective neck dissection (or potentially delayed treatment in the watchful-waiting strategy), diagnostics has to be improved to decrease the high number of patients with occult nodal disease.

Sentinel lymph node biopsy

Although Herophilus thought it were blood vessels, in essence he was the first who identified the existence of lymph vessels 300 B.C.[33] Thomas Bartholin (1616–1680) from Denmark gave the lymph vessels their name.[34] Rudolf Virchow, in the nineteenth century, formulated the theory that lymph nodes filter particulate matter from lymph.[35] In retrospect, this filtering effect can be seen as one of the first principles upon which the theory of the sentinel lymph node biopsy (SLNB) procedure is conceived.

In 1977, Ramon Cabañas was the first who identified sentinel lymph nodes (SLNs) on lymphoscintigraphy treating patients with penile cancer. Lymphatic channels draining into the iliac lymph nodes without draining into the SLN were never demonstrated, nor were the inguinal-femoral lymph nodes involved in the absence of SLN involvement.[36] Nowadays, the sentinel lymph node biopsy (SLNB) procedure had been applied in a variety of tumour types, including head and neck cancer during the last decades.

The concept of the SLNB procedure is based on an orderly and predictable pattern of lymphatic drainage within a nodal basin (Figure 2).

Theoretically, the SLNB procedure aims to identify the first draining lymph node (the sentinel lymph node), which is most likely to harbour metastases. Therefore, SLNs allows to reflect histopathologically the rest of the nodal basin. If a SLN contains metastases, other lymph nodes within the nodal basin could be also affected. A histopathologically tumour-negative SLN precludes the presence of lymphatic metastases. The SLNB procedure consists of preoperatively identification on imaging, surgical removal and extensive histopathological examination of SLN(s) (Figure 3).[37]

In the standard procedure, firstly, a radioactive agent is peritumourally injected directly before imaging. In the Netherlands (and generally throughout Europe), normally 2-4 injections are given with ^{99m}Technetium labelled nanocolloid ([^{99m}Tc]Tc-nanocolloid), though other agents are also described for SLN identification.[38,39] Migration to and uptake in (sentinel) lymph nodes of this agent could be visualized using a gamma camera or single photon emission computed tomography-computed tomography (SPECT/CT), both referred to as lymphoscintigraphy. Planar dynamic and

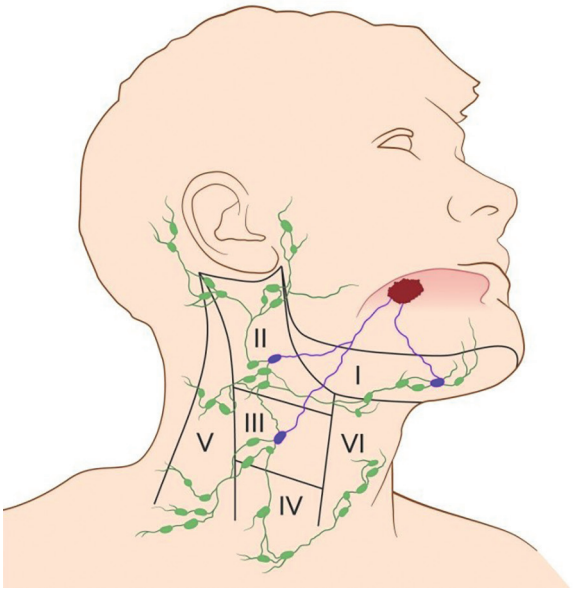


Figure 2. Lymphatic drainage of a tongue tumour showing 3 SLNs (purple)

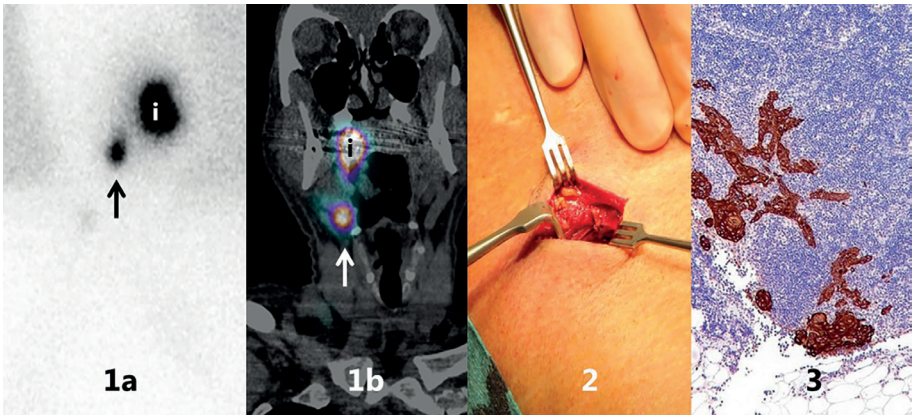


Figure 3. SLNB procedure. (1a) planar static lymphatic imaging after peritumoural injection of [^{99m}Tc]Tc-nanocolloid in a patient with tongue carcinoma, followed by SPECT-CT imaging (1b). Both showing a hotspot of the injection site (i), as well as a SLN (arrow) on the ipsilateral side of the neck. (2) Surgical removal of the SLN by a small incision. (3) Histopathological examination by step-serial sectioning, immunohistochemistry comprising additional staining with pancytokeratin antibody (AE1/AE3), showing metastatic disease.

static lymphoscintigraphic images are performed directly post injection, while the SPECT/CT is generally performed approximately 2 hours post injection. Hot spots on lymphoscintigraphy are classified as SLNs or second echelon lymph nodes. Only hotspots considered to be a SLN are image-guided marked on the skin using a cobalt marker. In most centers, the imaging procedure is performed one day prior to surgery.[37] SLNs could be lymphoscintigraphically visualized in 90-100% of the patients with early stage oral cavity cancer.[40,42]

Optionally, blue dye could be injected directly before surgery to aid SLN localization and harvesting.[43] However, in the largest singlecenter and multicenter reports so far, the use of patent blue is increasingly excluded from the procedure because of the limited additional value of the only blue nodes and surgical guidance towards SLNs could also be done reliably with [^{99m}Tc]Tc-nanocolloid only.[44,45] Even so, blue dye can cause difficulties by blurring the surgical resection margins.

During surgery a handheld gamma probe guides the surgeon to radioactive SLNs. Small incision(s) are made, taking into account that a potential additional neck dissection has to be performed in case of a positive SLNB. High (> 90%) surgical detection rates were reported to harvest visualized (and thus marked) SLNs.[40.42] Harvested SLNs will be extensively histopathological examined by step-serial sectioning with additional immunohistochemical keratin stainings.[37] According to the classification of Hermanek et al. metastases are categorized based on size into isolated tumour cells (≤ 0.2 mm), micrometastasis (> 0.2 mm and ≤ 2 mm) or macrometastasis (> 2 mm).[46] In case of a metastasis containing SLN (a positive SLN) an additional (selective) neck dissection will be performed in a second surgical procedure. In selected cases the neck can also be primarily treated by radiotherapy.

Although European guidelines exist many small adaptions of the aforementioned procedure have been used.[37,47] In the most recent meta-analysis with 66 studies comprising 3566 patients a pooled sensitivity of 87% (95% CI: 85-89%) and a pooled negative predictive value of 94% (95% CI: 93-95%) was found with an area under the curve of 0.98 (95% CI: 0.97-0.99).[41] In the Netherlands, the SLNB procedure has shown promising results and was implemented in the national guidelines since 2016.[48,49]

It is essential to realize when starting with the SLNB procedure validation of this technique should be done with an elective neck dissection, because of a steep learning curve of 10-20 procedures.[45,50] Consequently in literature, first studies describing the

SLNB technique used that neck dissection as reference standard for the accuracy of the SLNB procedure.[50,51] However, it is well known that with routine histopathological examination (as performed in neck dissection specimens), micrometastatic tumour deposits can be missed in up to 15% compared to the step-serial sectioning and immunohistochemistry techniques (as used in SLNs).[52,53] Therefore, in validation studies with neck dissections as reference standard, one must be aware that some negative cases could be erroneously classified as true negative since some micrometastases may not be detected in the neck dissection specimen.

Building on this theory of potentially missed metastases, it should be noted that literature with END as histopathological staging method may be less reliable than presented. As aforementioned in this chapter, depth of invasion of the primary tumour is the most promising predictive factor for nodal metastases.[12,27,28,54] This is also reflected in the new 8th TNM classification in which depth of invasion is now incorporated as determinant for clinical and pathological T staging (Table 1).[3,4,55,56] Of note, depth of invasion has to be measured according to Moore et al.; "from a theoretical reconstructed normal mucosal line to the deepest extent of growth".[57] Based on the histopathological findings in the END specimens, often a cut-off value of ≥ 4 mm depth of invasion is determined to perform END.[27,28,58] However, SLNB allows us to histopathologically examine lymph nodes with the highest risk of metastases more precise than routine examination of all lymph nodes in END. In SLNB-negative patients, a watchful waiting strategy of the neck renders the opportunity for isolated tumour cells and micrometastases (probably missed by routine histopathological examination of an END specimen) to become clinical manifest. Therefore, SLNB can serve as a more accurate reference standard than END for the evaluation of tests predicting the presence of lymph node metastases.

Challenges in sentinel lymph node biopsy

In oral cavity cancer lymphoscintigraphy reveals often more than one sentinel lymph node. Due to the complex anatomy of the lymphatic system in the neck multiple foci are usually visible and, subsequently, multiple SLNs are harvested. In other words, not every hotspot (radioactivity containing lymph node) is a SLN. Although the theory behind its procedure is clear, in practice interobserver variabilities shown moderate agreement in defining SLNs.[59] During the last decades, SPECT-CT has increasingly been used for localisation of SLNs, also in oral cancer since 2003.[60] SPECT-CT shows its value especially for tumours with close proximity to the SLN and complex lymphatic regions which is the case in the head-and-neck region.[61] Although SPECT-

CT is useful in this region, the problem of close proximity between the injection spot and SLNs has not been solved yet. Particularly in floor of mouth tumours, the short distance between SLN(s) in level I and the primary tumour could result in the "shine-through" phenomenon (Figure 4). The large injection spot on lymphoscintigraphy will "overshine" the uptake in the SLN, which may not be visualized and thus not be identified. Intraoperative detection of potential SLNs in this area is difficult because the gamma probe will not differentiate between radioactivity of the injection spot or SLN. As a result, frequently a lower accuracy has been reported for SLNB in floor of mouth tumours compared to other locations in the oral cavity.[44,49,50,62]

Consequently, a lot of research has been conducted to improve the accuracy for floor of mouth tumours.[63,67] One of the possibilities to reduce the "shine-through" phenomenon is to decrease the amount of radioactivity near the injection spot, and secondly, to bind selectively to SLNs. In contrast to [^{99m}Tc]Tc-nanocolloid, the new radioactive agent [^{99m}Tc]Tc-tilmanocept (Lymphoseek ®) has been specifically designed for SLN identification. [^{99m}Tc]Tc-tilmanocept is a receptor targeted (CD206 on

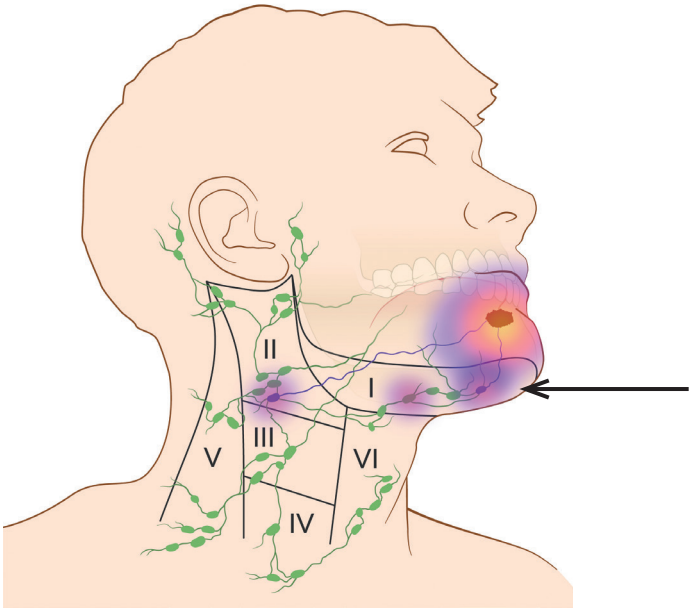


Figure 4. "Shine-through" phenomenon. Radiation flare of the primary tumour overshines the hotspot of sentinel lymph node in close proximity to the primary tumour (arrow).

macrophages) SLN detection agent (Figure 5).[68,70] Due to its proposed rapid clearance from the injection site, rapid uptake and high retention within SLNs, as well as low uptake by the remaining (higher echelon) lymph nodes, [^{99m}Tc]Tc-tilmanocept may be of benefit in floor of mouth tumours and other head and neck tumours with complex drainage patterns and close spatial relation to the SLN.[71,72] A multicenter validation study using [^{99m}Tc]Tc-tilmanocept for SLNB in head and neck squamous cell carcinoma showed an SLN identification rate of 97.6%, a false negative rate of 2.56% and a negative predictive value of 97.8%. Of note, these high figures were also obtained in floor of mouth cancers.[73]

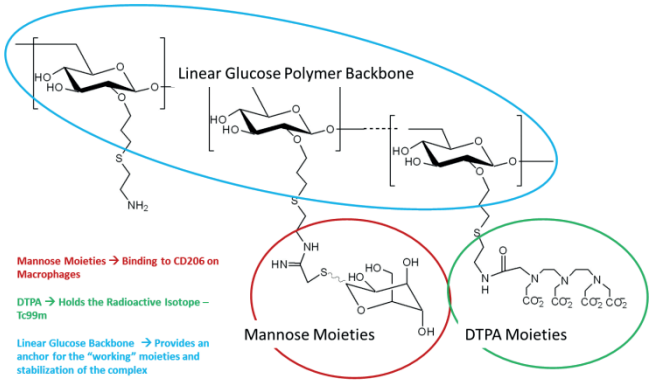


Figure 5. [^{99m}Tc]Tc-tilmanocept structure and functional elements

OUTLINE OF THIS THESIS

The first part (chapters 2 and 3) will present the current status of the SLNB procedure in the Netherlands. The second part (chapter 4, 5, 6) is about defining potential applications of the SLNB procedure. Finally, in chapter 7 and 8, improvements of the current technique will be explicated.

In chapter 2 we described the accuracy of the SLNB procedure in a singlecenter study in the Netherlands, presenting the identification rate, sensitivity and negative predictive value. Also, we perform survival analysis in general and according to the size of metastasis in the SLN.

In chapter 3 the largest cohort of SLNB procedures in oral cavity cancer worldwide is presented due to a multicenter study design of 5 Dutch head and neck centers. Interestingly, the accuracy of the SLNB procedure will be compared to a cohort of patients who had undergo END before the SLNB was introduced. By absence of a randomized controlled trial in literature, this study provides the highest evidence of the effectiveness of both procedures in early stage oral cancer.

Chapter 4 describes one of the advantages of the SLNB procedure, by classifying the metastases based on their size. Due to the extensive histopathological examination very small tumour deposits (isolated tumour cells, micrometastasis) are detectable. The influence of such metastases on additional neck dissections and survival is presented in a cohort of 199 patients, and available literature is evaluated.

Depth of invasion has been determined as important prognostic factor and has recently been incorporated in T-stadium of the 8th TNM classification. Most often, depth of invasion has been correlated to lymph node metastasis of END specimens. In chapter 5, we evaluated the risk of having nodal metastasis correlated to depth of invasion, using the SLNB procedure as gold standard of nodal disease.

The SLNB procedure has increasingly been implemented during the last years in oral cancer, however, regularly only patients with an untreated neck are eligible. Chapter 6 describes the applicability of the SLNB procedure in patients who are previously treated in the neck.

Chapter 7 illustrates the additional value of the SPECT-CT as imaging modality for identification of SLNs. We compare planar lymphoscintigraphy to SPECT-CT by scoring the number of SLNs, the pathology of SLNs and investigate if the SPECT-CT provides additional important anatomical information about the localisation of SLNs pre-operatively.

To reliably compare a new radioactive agent for SLN identification ([^{99m}Tc]Tc-tilmanocept), we conducted a head to head comparative prospective study in which 20 patients received the routinely used tracer ([^{99m}Tc]Tc-nanocolloid) as well as the new tracer ([^{99m}Tc]Tc-tilmanocept). The results of this non-inferiority study are described in chapter 8.

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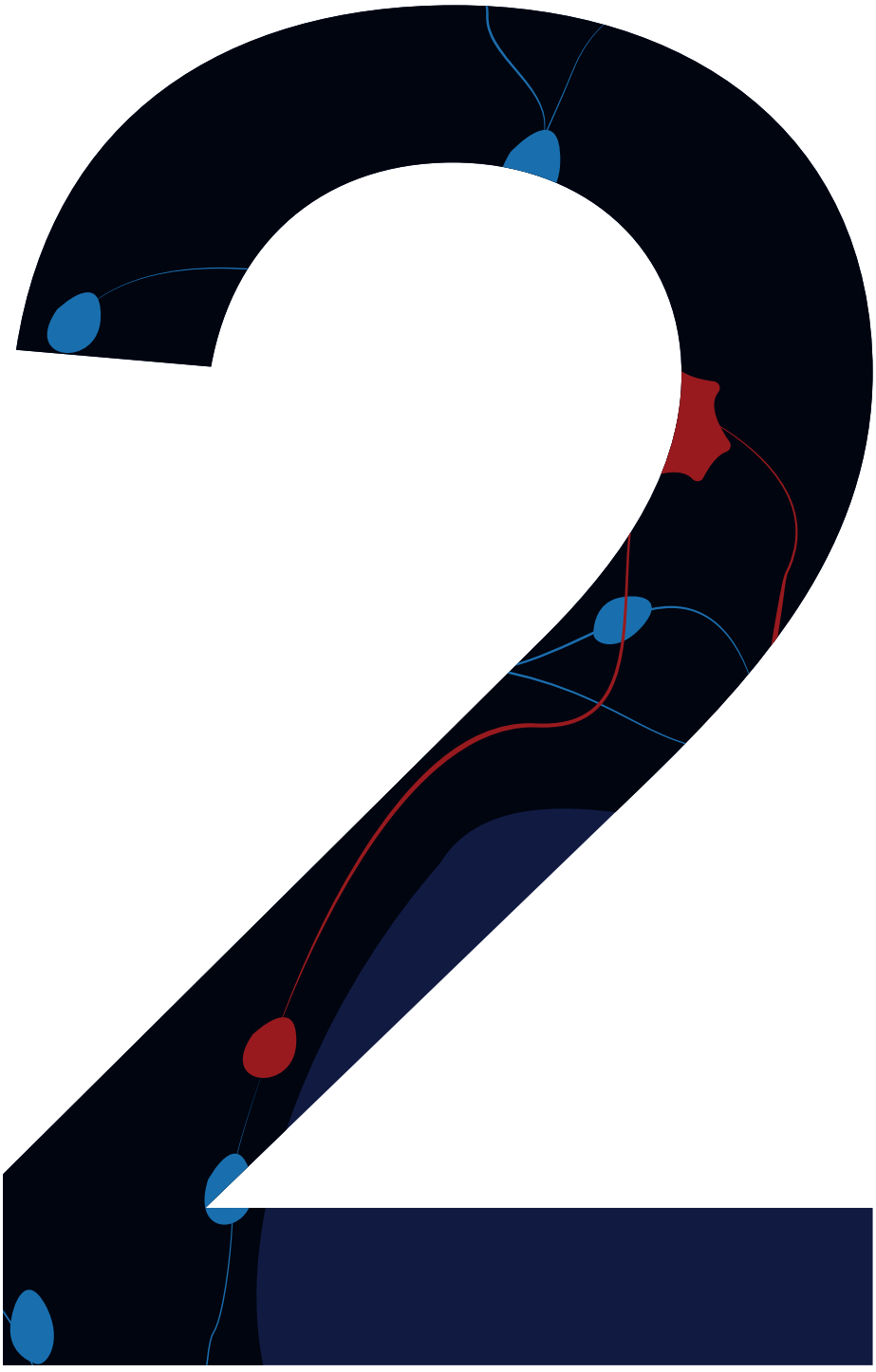

Sentinel lymph node biopsy for early stage oral cavity cancer: the VU University Medical Center experience

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ABSTRACT

Background Sentinel lymph node biopsy (SLNB) in head and neck cancer is recently introduced as staging technique for patients diagnosed with a T1-T2 oral squamous cell carcinoma and clinically negative (cN0) neck. We report one of the largest singlecenter series.

Methods Retrospective analysis of 90 previously untreated patients, who underwent a SLNB procedure between 2007 and 2012. A neck dissection was only performed after a positive SLNB.

Results The lymphoscintigraphic identification rate was 98% (88/90) and surgical detection rate 99% (87/88). The upstaging rate was 30%. Sensitivity of SLNB was 93% and the negative predictive value was 97%. Overall survival (OS) and disease-free survival (DFS) for SLNB negative were 100% and 84% and for SLNB positive patients 73% and 88%, respectively.

Conclusions SLNB is a reliable diagnostic staging technique for the clinically negative neck in patients with early stage (cT1-T2N0) oral squamous cell carcinoma.

INTRODUCTION

One of the most important prognostic factors in T1-T2 oral squamous cell carcinoma is the presence of nodal metastases in the neck.[1,6] Clinical staging by palpation followed by imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound-guided fine-needle aspiration cytology (USgFNAC) does not seem reliable enough to detect early nodal metastases, because occult (micro) metastases are still present in about 30% of the node negative (cN0) patients.[2,5-15]

According to Weiss et al. a risk more than 20% of occult metastases warrants elective neck dissection (END), although leading to overtreatment in up to 80% of patients.[16] A wait and scan policy may also be proposed in selected cases, since delayed lymph node metastases may still be cured by salvage surgery. However, this strategy means more extended neck dissection (ND) and more often adjuvant radiotherapy for patients with delayed metastases as compared patients with occult metastases undergoing END.[17] With both strategies some patients are unnecessarily overtreated.[18,19] Therefore, more reliable staging procedures are desirable.

Sentinel lymph node biopsy (SLNB) is a reliable diagnostic procedure for staging of the cN0 neck and identifying patients with nodal metastatic disease.[14,20,24] First, studies analysed the value of SLNB assisted neck dissection, considering a ND as the reference (gold) standard.[25,26] However, follow-up (no ND if SLNB is negative) is a more representative reference standard than (routine) histopathological examination of (elective) neck dissections.[27]

The standard SLNB procedure starts with peritumoural injection of a [^{99m}Tc]Tc-labelled colloidal tracer, drainage mapping by lymphoscintigraphy (LSG) and the injection of blue dye intraoperatively. Radioactive, as detected by a gamma probe, and/or blue lymph nodes are harvested during a surgical procedure. Using step-serial sectioning and immunohistochemistry as histopathological evaluation it is possible to detect micrometastases and isolated tumour cells (ITC) with higher sensitivity compared to traditional histopathological evaluation after END.[12]

When used routinely in clinical practice SLNB positive diagnosed patients undergo an subsequent ND, while patients with a negative SLNB are carefully observed, without the disadvantage of undergoing unnecessary surgery.

In this study the results of SLNB alone in 90 patients with cT1-2N0 oral squamous cell carcinoma treated in the VU University Medical Center were evaluated.

Patients and methods

From February 2007 until October 2012, 90 consecutive patients with cT1-2N0 tumours of the oral cavity were recruited for SLNB. Patients and tumour characteristics are presented in Table 1. After approval of the Institutional Review Board and Ethics Committee, informed consent was obtained until SLNB was performed as standard procedure in our institution. SLNB was performed according to the EANM/SENT joint practice guidelines.[12] The patients were staged as cN0 by palpation and negative USgFNAC. The day before surgery, in four quadrants peritumoural injections of ^{99m}Tc (mean 100.3 MBq, range 40-175 MBq, in total) labelled nanocolloidal albumin (Nanocoll; GE Healthcare; The Netherlands) were given. Immediately following injections drainage was visualized using dynamic LSG, followed by (late) static LSG imaging. In 57/88 patients SPECT-CT was performed as (late) static LSG imaging method. After identification of the sentinel lymph nodes (SLNs), locations were marked on the skin using a ^{57}Cobalt marker and confirmed using a handheld gamma probe (Europrobe II; Eurorad, Strasbourg, France). At the start of the surgery 1 ml of patent blue V dye was injected at four quadrants around the primary tumour. During surgery, radioactive SLNs as detected by the handheld gamma probe, and/or blue SLNs were harvested. Histopathological examination of SLNs consisted of step-serial sectioning at intervals of 150 μm for the entire lymph node. At each level, staining with haematoxylin-eosin (HE) and pan-cytokeratin antibody (AE 1/3) was performed. If a metastasis was diagnosed, the size of the metastasis was classified as isolated tumour cells (ITC, size ≤ 0.2 mm), micrometastasis (size > 0.2 mm and ≤ 2 mm) or macrometastasis (size > 2 mm).[23] SLNB positive patients underwent ND in a second surgical procedure, whereas SLNB negative patients were three-monthly followed up by physical examination and USgFNAC of the neck in the first year of follow-up. Median follow-up was 18 months (range 2-62).

Statistical analysis

The Chi-square test and Fisher-Exact test were used to compare means between SLNB positive and SLNB negative patients. Survival analysis (overall survival (OS), disease-free survival (DFS) and disease-specific survival (DSS)) and comparison between SLNB negative and SLNB positive patients was derived by the Kaplan-Meier method and log-rank test, followed by Bonferroni correction in multiple comparisons. A value of $p < 0.05$ was considered statistically significant. Statistical analysis was carried out in SPSS 20 for Windows (IBM, Chicago, IL) in cooperation with a statistician.

Table 1. Data of demographic and tumour-related patient characteristics

		Histopathological status of SLNB	
Characteristic	Overall (%)	Negative (%)	Positive (%)
Patients, n (%)	87 (100%)	61 (70%)	26 (30%)
Gender, n (%)			
Male	45 (52%)	33 (54%)	12 (46%)
Female	42 (48%)	28 (46%)	14 (54%)
Median age (y) (range)	60 (29-86)	59 (32-81)	60 (29-86)
Tumour location, n (%)			
Tongue	54 (62%)	37 (61%)	17 (65%)
Floor of mouth	23 (26%)	17 (28%)	6 (23%)
Buccal mucosa	3 (3%)	3 (5%)	0
Inferior alveolar process	4 (5%)	2 (3%)	2 (8%)
Soft palate	3 (3%)	2 (3%)	1 (4%)
Clinical T stage, n (%)			
T1	51 (59%)	42 (69%)	9 (35%)
T2	36 (41%)	19 (31%)	17 (65%)
Follow-up, (*m*) (range)			
Observation time	18 (2-62)	15 (3-61)	21 (2-62)

Abbreviations: SLNB, sentinel lymph node biopsy; FOM, floor of mouth

RESULTS

SLNB was successful in 87 of 90 (97%) patients. In 2 patients, lymphoscintigraphy failed to visualize SLNs (identification rate 98% (88/90)), while in one patient the SLN could not be found intraoperatively (surgical detection rate 99% (87/88)). Blue dye was used in 83/88 (94%) patients.

In total 229 SLNs (median 2, range 1-9) were surgically removed. A histopathologically positive SLNB was found in 26/87 (30%) of patients. Five patients were diagnosed with ITC, 12 with micrometastasis and 9 with macrometastasis. A positive SLNB was followed by (selective) neck dissection in 25/26 (96%) patients. One patient with isolated tumour cells was treated by radiotherapy needed for adverse pathological findings of the primary tumour. In 5/25 (20%) patients who underwent a subsequent ND additional metastatic lymph nodes were found. In all these patients the SLNB

contained macrometastasis (Table 2). One patient with a lateralized T1 tumour of the tongue demonstrated ITC in a SLNB in the contralateral neck.

Table 2. Additional metastases in neck dissection specimen related to metastasis type in SLNB

Metastasis in SLNB	Neck dissection				
	Selective I-III	Selective I-IV	Modified Radical	Total, n (%)	Metastases in ND specimen
Isolated tumour cells	2	2	0	4 (15%)	0
Micrometastasis	4	4	4	12 (46%)	0
Macrometastasis	0	3	6	9 (36%)	5 (56%)
Total	6	9	10	25	5 (20%)

SLNB, sentinel lymph node biopsy; ND, neck dissection

Table 3. Data of patients with regional recurrence during follow-up

SLNB	Recurrence time (months)	Management
Positive	6.8	Treated with salvage neck dissection and CRT. Alive and disease free.
Positive	4.7	Treated with CRT. Deceased by distant metastasis.
Positive	2.2	During PORT, contralateral metastasis were found. Treated with salvage neck dissection and PORT. Deceased by distant metastasis.
Negative	51.3	Patient withdrew from follow-up for 4 years. Salvage neck dissection for N3 neck disease. Patient refused PORT. He is still alive.
Negative	3.1	Treated with salvage neck dissection and PORT. Alive and disease free.

SLNB, sentinel lymph node biopsy; CRT, chemoradiotherapy; NED, no evidence of disease; PORT, postoperative radiotherapy

Table 3 summarizes the recurrences during follow-up. In 3/26 (12%) SLNB positive patients a nodal metastasis recurred, for which additional treatment was indicated. In 2 of these patients the metastasis recurred in the dissected neck, the other patient with a well lateralized tumour developed nodal metastasis in the contralateral neck. Two of them died of disease after treatment, one patient (with metastasis in the dissected neck) remained disease free for 18 months.

Of the 61 SLNB negative patients, 2 patients (3%) developed regional metastasis in the neck after 3 and 51 months of follow-up, respectively. In the first patient, the metastasis was detected by USgFNAC during follow-up and after ND and postoperative radiotherapy this patient is alive and disease free. The other patient withdrew from follow-up visits (physical examination and repeated USgFNAC), but showed up after 51 months with a large ulcerative nodal metastasis (N3) in the neck. After salvage ND he refused adjuvant chemoradiation. He is still alive. The regional metastases of both patients were found in other neck levels compared to the SLNs.

SPECT-CT was added to imaging procedure in 57/88 (65%) patients. In these 57 patients none of them developed a regional recurrence during follow-up (median 10 months). The sensitivity and negative predictive value (NPV) of SLNB were 93% (26/28) and 97% (59/61), respectively. Regarding primary tumour site, the accuracy of SLNB in floor of mouth (FOM) tumours compared to other tumour sites in the oral cavity appeared to be lower (sensitivity 86% vs. 95%, NPV 94% vs. 98%), although not statistically significant ($p = 0.44$ and $p = 0.48$ respectively) (Table 4).

Table 4. Results of SLNB in T1-T2 oral carcinoma with separate analysis for floor of mouth carcinoma

Tumour location	SLNB +	N +	Sens.	TN	SLNB -	NPV
FoM	6	7	86%*	16	17	94%**
Other	20	21	95%*	43	44	98%**
All	26	28	93%	59	61	97%

SLNB, sentinel lymph node biopsy; N, nodal metastasis in the neck; Sens., sensitivity; TN, true negative; NPV, negative predictive value
*: $p = 0.44$. sensitivity FOM compared with sensitivity other tumour sites
**: $p = 0.48$. NPV FOM compared with NPV other tumour sites

There were 51 patients staged as pT1 (59%) and 36 as pT2 (41%). Upstaging of the cN0 neck in pT2 lesions occurred significantly more frequently compared to pT1 lesions (47% vs. 18%; p = 0.003). The regional recurrence rates for pT1 and pT2 lesions were 1/51 (2%) and 4/36 (11%) respectively, however did not reach statistical significance (p = 0.15).

Survival analysis

Between SLNB negative and SLNB positive patients OS and DSS were similar because none of the patients died from other causes than the disease.

For all patients, OS and DFS were 92% and 85%, respectively. Stratification by N-stage resulted in OS and DFS of 100% and 84%, respectively for SLNB negative patients and 73% and 88% respectively, for SLNB positive patients. SLNB negative patients had statistically significant better OS (p = 0.002, Figure 1), whereas the difference in DFS was not statistically significant (p = 0.11).

For multiple comparisons in the Kaplan-Meier method a Bonferroni correction was applied. With respect to the type of metastasis, a significant lower overall survival rate was demonstrated for patients with macrometastases compared to SLNB negative

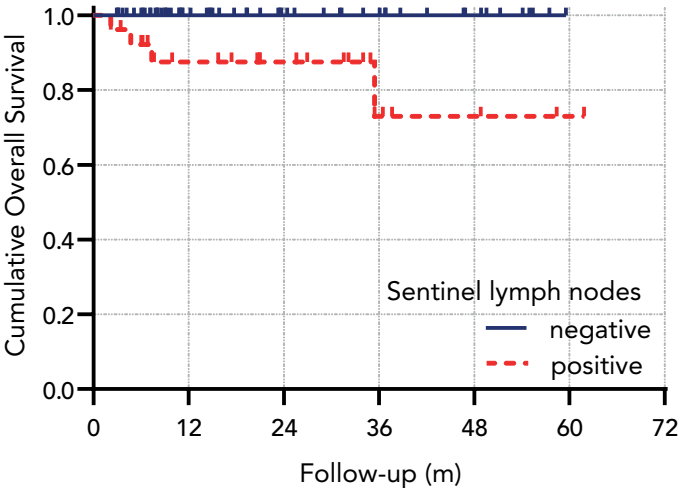

Figure 1. Overall survival in patients with SLNB negative and SLNB positive tumours (p = 0.002)

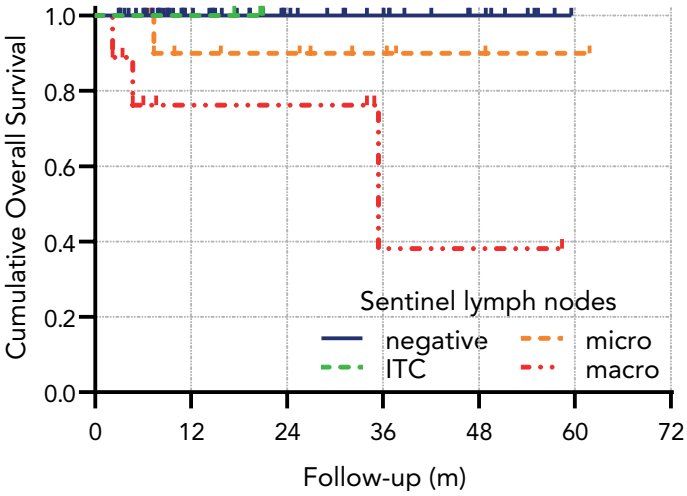

Figure 2. Overall survival according to different size of metastasis. The comparison between macrometastasis and SLNB negative patients is statistically significant (p = <0.005), whereas the other comparisons were not statistically significantly different.

patients (p = <0.005), whereas the difference in survival rate between patients with a micrometastasis and patients with a macrometastasis or SLNB negative patients was not statistically significantly different. However, these outcomes were partially due to the Bonferroni correction because the comparison between micrometastases and SLNB negative patients was no longer significant (p = 0.028) after this correction (p = 0.17). In addition, no statistical significant differences were observed between the ITC patient group and any other group, probably due to the low number of ITC patients (Figure 2).

DISCUSSION

In this study we retrospectively evaluated the diagnostic value of SLNB in 90 patients with early stage (T1-T2, cN0) oral cavity squamous cell carcinoma, with follow-up as reference standard. Sensitivity and negative predictive value were 93% and 97%, respectively. Regional recurrence rate of all patients was 6%. The false negative rate was 2/61 (3%), which is comparable to false negative rates in studies of Pezier et al. and Civantos et al. and is relatively low in comparison with other large SLNB trials with false negative rates of 6-9%.[14,20-22] However, most of these studies with higher false negative

rates have a longer follow-up allowing occult metastases to become clinically manifest. The regional recurrence rate in SLNB positive patients was 3/26 (12%). In 2 patients recurrence was found in operated levels, one patient with a well lateralized tumour developed regional metastasis contralaterally, so this metastasis would also have been missed by conventional ipsilateral END. This recurrence rate in SLNB positive patients is relatively high compared to Pezier et al. and Gurney et al. who reported rates of 6%.[14,28] However, a systematic review of 109 papers found regional recurrence rates of 13% in surgically treated early stage oral cancer.[29] Moreover, a recently published two-center review of 164 patients with pT1-T2 squamous cell carcinoma of the tongue and pathologically staged N0 by END reported a regional (with or without local) recurrence rate of 18%.[30]

In the cohort of patients with SPECT-CT as imaging technique (57/88 (65%)) none of the patients with a negative SLNB developed a regional recurrence in the follow-up, however the follow-up in this cohort is only 10 months. In contrast to the cohort without SPECT-CT, 2 patients with a negative SLNB developed regional metastasis, resulting in a false negative rate of 2/31 (7%). It should be mentioned that the follow-up in this cohort is 40 months. Because of the small number of patients and the difference in follow-up between the groups, evaluation of SPECT-CT in the present study is complicated and further research is mandatory.

As found in other studies, preoperative lymphoscintigraphy identified SLNs with high accuracy (98%) and SLNs were also found outside the expected drainage pathways, which is recognized as one of the benefits of SLNB.[21,31-33] Another benefit of SLNB is reducing the number of dissected lymph nodes for histopathological evaluation, compared to END. By selection of the nodes which are most representative for the nodal neck status thorough evaluation by SSS and IHC is possible, this in contrast to routine histopathological evaluation of a ND specimen, in which the large number of removed lymph nodes would make thorough evaluation too laborious. SSS and IHC are suitable for the detection of micrometastasis and ITC which probably would have been missed using routine histopathological evaluation. In the SLNB positive patients, 62% (16/26) was staged positive due to the presence of a micrometastasis or ITC. This high prevalence confirms the importance of these meticulous histopathological technique. In the 25 neck dissections, only in 5/9 (56%) patients with macrometastasis, additional positive lymph nodes were found in the ND specimen (Table 2). This is partly in contrast with Broglie et al., who found in 2 of 10 (20%) patients with ITCs and in 4 of 32 (13%) patients with micro- or macrometastases additional positive lymph

nodes in the neck dissection specimen.[34] Gurney et al. reported that the number and distribution of positive additional nodes in the neck, especially if outside the SLN basin, have an adverse impact on outcome.[28] Groups based on tumour deposit had not been made but they suggest that the presence of a single positive SLNB does not imply a poor prognosis. This may potentially lead to more specified management plans for different prognostic groups as already implemented with a validated nomogram in breast cancer.[35]

It may be that with SLNB tumour deposit can reliably be predicted, selecting the patients who truly benefit from an additional ND. Our preliminary data suggest that a wait-and-scan policy may be sufficient not only for patients with a negative SLNB, but also for patients with micrometastasis or ITC in the SLNB. However, to confirm this theory larger numbers of patients with longer follow-up are needed.

DFS (92%) and OS (85%) using SLNB after negative USgFNAC in this study were comparable with our wait and scan (USgFNAC) strategy as previously reported (94% and 82%, respectively). OS seems to be better for patients undergoing a ND because of a positive SLNB as compared to patients undergoing a delayed ND in a wait and scan strategy (88% and 64%, respectively).[17,35] Patients with nodal metastases had poorer outcome in overall survival analysis (Figure 1).

Broglie et al. was the first who demonstrated worse outcome in patients with higher metastatic tumour load in SN, after stratification according to Hermanek et al.[34,36] We also found lower overall survival rates in patients with higher metastatic tumour load in SNs (Figure 2). However, due to the low number of patients in some groups and the small number of events, statistical significance was not always reached.

In the present study in floor of mouth tumours a lower identification rate and poorer accuracy as compared to other oral cavity subsites were found, although not statistically significant as in the studies of Ross et al. and Alkureishi et al.[20,23] Despite the difficulty of pre- and peroperative localization and harvesting of SLNs in floor of mouth carcinomas due to the close proximity to the nodal basins and the primary tumour site, SLNB seems still reliable. In only 29% of the patients hotspots were identified in early lymphatic mapping probably due to slower lymphatic drainage in the floor of mouth. We believe that late lymphoscintigraphic imaging should be considered for these tumours to minimize the risk of false-negative results.[37] The late visualisation of a hotspot on lymphoscintigraphy in floor of mouth tumours may also

be due to the invisibility of the real SLN because of "shine-through" and consequently misinterpretation of a second echelon node as SLN. New technological developments using tracers for PET-CT and fluorescence imaging of SLNs may give an explanation for this observation in conventional lymphoscintigraphy.[38,39]

CONCLUSION

The data of this study demonstrate that SLNB alone is a safe and reliable staging procedure in the management of patients with T1-T2,cN0 oral squamous cell carcinoma and adequately selects patients for additional neck dissection or follow-up; sensitivity 93% and negative predictive value 97%. SLNB resulted in a 30% upstaging rate. Of those SLNB positive patients 62% were diagnosed with micrometastases or ITCs without any additional lymph node metastasis in the neck dissection specimen. Macrometastatic disease resulted in significantly lower OS compared to SLNB negative patients. The clinical relevance of micrometastatic disease and isolated tumour cells needs to be further investigated.

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Elective neck dissection or sentinel lymph node biopsy in early stage oral cavity cancer patients: the Dutch experience

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ABSTRACT

Background Sentinel lymph node biopsy (SLNB) has been introduced as diagnostic staging modality for detection of occult metastases in patients with early stage oral cancer. Comparisons regarding accuracy to the routinely used elective neck dissection (END) are lacking in literature.

Methods Retrospective, multicenter cohort study, including 390 patients staged by END and 488 by SLNB.

Results The overall sensitivity (84% vs. 81%, $p = 0.612$) and negative predictive value (NPV) (93%, $p = 1.000$) was comparable between END and SLNB patients. The END cohort contained more pT2 tumours (51%) compared to the SLNB cohort (23%) ($p < 0.001$). No differences were found for sensitivity and NPV between SLNB and END divided by pT stage. In floor of mouth (FOM) tumours, SLNB had a lower sensitivity (63% vs. 92%, $p = 0.006$) and NPV (90% vs. 97%, $p = 0.057$) compared to END. Higher disease specific survival (DSS) rates were found for pT1 SLNB patients compared to pT1 END patients (96% vs. 90%, $p = 0.048$).

Conclusion In absence of randomized clinical trials, this study provides the highest available evidence that in oral cancer, SLNB is as accurate as END to detect occult lymph node metastases, except for floor of mouth tumours.

INTRODUCTION

In early stage (cT1-2N0) oral squamous cell carcinoma (OSCC), 20-30% of the patients are diagnosed with occult metastases despite advances in imaging modalities.[1-4] Conventionally, early stage OSCC patients underwent either watchful waiting or an elective neck dissection (END) for neck staging.[5,6] A recent study showed favorable survival rates for patients who underwent END compared to watchful waiting irrespective of the infiltration depth.[7] However, in this study the incidence of occult lymph node metastases was up to 45%, suggesting a less accurate diagnostic work-up and a different target group which may hamper generalizability of their results.[8] Last decade, the sentinel lymph node biopsy (SLNB) was introduced in OSCC as a less invasive alternative with lower morbidity rates compared to an END.[9] In early stage OSCC the most recent meta-analysis reported a pooled sensitivity of 87% and a pooled negative predictive value of 94% for the SLNB procedure in detecting occult metastasis.[10]

Because of the low invasiveness and high accuracy rates, the SLNB procedure is implemented in many national head and neck guidelines. In the Netherlands, head and neck oncology care is centralized and the majority of these head and neck cancer institutions use SLNB for staging the clinically node negative neck in early stage OSCC patients.[11,13] Despite the implementation in guidelines, the END is still the staging strategy in early stage OSCC in the majority of medical centers worldwide, even in developed countries with well-organized health care.[14] Cramer et al. showed that SLNB is rarely utilized (<5 %) in suitable cases in the United States.[15]

Both the END and SLNB have clinically relevant limitations. A pivotal disadvantage of an END is the overtreatment of 70 to 80% of the patients with a more invasive procedure.[16] Several studies reported the differences in complication rates, postoperative morbidity and cost-effectiveness in favor of the SLNB compared to the END procedures.[17,22] Conversely, a significantly lower accuracy for SLNB in floor of mouth OSCC is reported which is caused by the "shine-through" phenomenon.[11,12,23,25] This refers to a situation in which the sentinel node is not identified when it is within the flare of radiation from the primary tumour site (Figure 1). The ultimately necessary comparison, e.g. a randomized clinical trial, between the staging accuracy of END versus SLNB is currently not available.

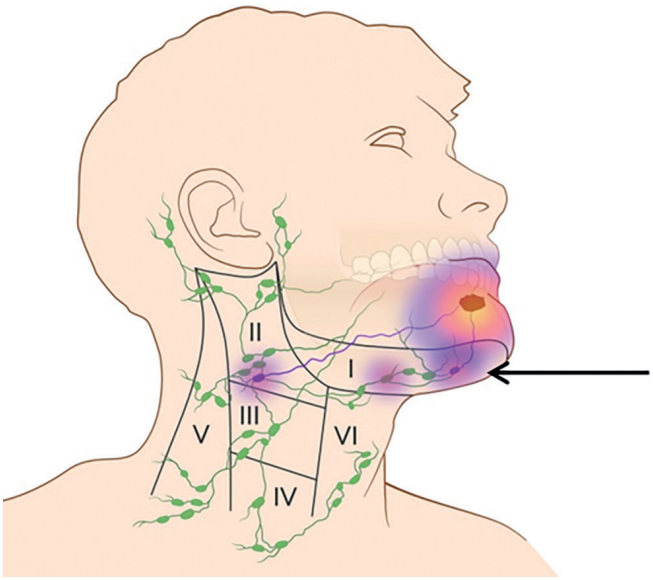

Figure 1. "Shine-through" phenomenon. Radiation flare of the primary tumour overshines the hotspot of sentinel lymph node in close proximity to the primary tumour (arrow).

This study presents the accuracy in staging of the cN0 neck and survival rates of either END and SLNB in two large retrospective cohorts. Patients of both cohorts are collected in the same dedicated head and neck centers.

MATERIALS AND METHODS

Patients were included from five Dutch head and neck centers that are now all performing SLNB as standard diagnostic modality for early stage OSCC. Patients were included if they were treated between 1990 and 2015 for the END cohort and between 2007 and 2018 for the SLNB cohort. The overlap in years of treatment (2007-2015) between END and SLNB is caused by the different time of introduction of the SLNB procedure in individual hospitals involved in this study. To keep the cohorts as homogeneous as possible, END patients were collected in the same centers and were treated before these centers introduced the SLNB. Consequently, the END cohort provided longer follow-up with respectively a median follow-up of 4.5 (IQR 2.5 to 7.3) years versus 2.2 (IQR 1.0 to 4.1) years ($p < 0.001$). Tumours are classified

according to the 7th TNM classification system. All clinical and histopathological data were retrospectively collected from the electronic patient files. Information regarding SLN location and pathological LN assessment in both END and SLNB are listed prospectively and standardised as part of the daily clinical practice in the participating centers. All patients were staged as cN0 by palpation and imaging (ultrasound, CT and/or MRI). In case of suspected lymph nodes, ultrasound-guided fine needle aspiration cytology was performed.

The SLNB procedure was performed according to the European Association of Nuclear Medicine/Sentinel European Node Trial joint practice guidelines and has been described extensively before by some of the participating institutions.[11,12,26] The procedure corresponds with the recommendations in the guidelines from the 2018 SLNB consensus conference held in London.[27,28] Shortly, the procedure consists at least of three modalities; preoperative visualization of SLNs (using peritumoural injections of [^{99m}Tc]Tc-nanocolloid) with planar dynamic and static lymphoscintigraphy, including SPECT-CT scanning, in a one or two day protocol. Intra-operative detection and extirpation of SLNs was performed using a handheld gamma probe. Harvested SLNs were assessed using step-serial-sectioning with haematoxylin and eosin staining and additional keratin staining.[26] Metastasis were classified as isolated tumour cells (ITC) (size ≤ 0.2 mm), micrometastasis (MiM) (size > 0.2 mm and ≤ 2 mm), or macrometastasis (MaM) (size > 2 mm) according to Hermanek et al.[29] SLNB negative patients had a watchful waiting strategy as reference, while SLNB positive patients were treated by neck dissection and/or radiotherapy on the neck. In case of a negative SLNB patients follow a strict follow-up regimen; in the first year, patients visit our outpatient clinic every 2 months, in the second year every 3 months, in the third year every 4 months and in the fourth and fifth year every 6 months.

The END cohort consisted of early stage OSCC patients with a (selective (level I-III/IV) or modified radical) neck dissection as part of the primary treatment. Data of these END patients was available in 4 of the participating centers. In these centers, END was the first choice over watchful waiting when tumour depth of invasion was estimated as above 4 mm.[5,6,30] In 2 centers, frozen sections of one or two clinically most suspicious lymph nodes were routinely assessed intra-operatively during END and in case of a detected metastasis, the END was converted to a therapeutic modified radical neck dissection (MRND). In case of negative fresh frozen material, only a level I-III END was performed with postoperative lymph node assessment using conventional haematoxylin and eosin staining on formalin-fixed paraffin-embedded tissue.

Ethical consideration

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of UMC Utrecht (no. 17/766). The Internal Review Board waived the requirement for the investigator to obtain a signed consent form for all subjects. All samples and data were handled according to General Data Protection Regulation.

Statistical analysis

Categorical data are given with N and percentage using the Fisher's exact test and Chi-square test to analyse differences. The Mann-Whitney U (skewed distribution) and Student's t-test (normally distributed) were used for continuous data and are given with respectively the median and interquartile range (IQR) and the mean with the standard deviations (SD). True positives (TP), true negatives (TN), and false negatives (FN) are defined as respectively pN+ (TP), pN0 without (TN) and pN0 with (FN) detection of a regional recurrence after primary treatment. The Kaplan-Meier method and log-rank test was performed for survival analysis (disease-specific survival (DSS) and regional recurrence free survival (RRFS)). Five-years DSS was defined as time from treatment till death or last clinical visit. Deaths caused by the early stage OSCC within five years after treatment were counted as event. Five years RRFS was defined as time from treatment till regional recurrence without local disease or last clinical visit. Regional recurrences without local disease and within five years after treatment were counted as event for the RRFS. Regional recurrences with presence of local recurrence or second primary tumours were excluded. A Bonferroni correction was used for log-rank tests for multiple testing and was defined by multiplying each p-value to the total number of comparisons. A value of $p < 0.05$ was considered statistically significant. All statistical tests were analysed using IBM SPSS Statistics 23 for Windows (Statistical Package for the Social Sciences, Inc., Chicago, IL, USA).

RESULTS

In total 390 (44%) END patients and 488 (56%) SLNB patients were used for analysis. Patient and tumour characteristics are given in Table 1. The END cohort contained a higher rate of pT2 tumours (51%) compared to the SLNB cohort (23%) ($p < 0.001$). The anatomical locations differed between the two groups, with more floor of mouth (FOM) tumours (34% vs. 27%) and less tongue tumours (50% vs. 62%) in the END cohort ($p = 0.007$). END treated patients were significantly more often treated with post-operative radiotherapy (34% vs. 11%, $p < 0.001$).

Table 1. Patient and tumour characteristics

Characteristic		SLNB		END		
		n	(%)	n	(%)	p-value
Total		488	(56)	390	(44)	
Gender	Female	237	(49)	178	(46)	0.377
	Male	250	(51)	212	(54)	
Age at treatment (years)	Median (IQR)	63	(55 to 69)	62	(53 to 70)	0.767
	Range	20 to 93		22 to 95		
cT (7th)	T1	335	(69)	136	(35)	<0.001
	T2	153	(31)	254	(65)	
pT (7th)	T1	371	(76)	184	(47)	<0.001
	T2	113	(23)	201	(51)	
	T3	4	(1)	3	(1)	
	T4	0	(0)	2	(1)	
pN	Negative	381	(78)	291	(75)	0.264
	Positive	107	(22)	99	(25)	
Metastasis size	ITC (< 0.2 mm)	15				
	Micro (0.2 to 2.0 mm)	31				
	Macro (> 2 mm)	61				
Postoperative RTx	Yes	52	(11)	131	(34)	<0.001
	No	436	(89)	259	(66)	
Location	Tongue	302	(62)	196	(50)	0.007
	FOM	131	(27)	133	(34)	
	Cheek / Buccal / Trigonum	34	(7)	35	(9)	
	Others	21	(4)	26	(7)	
END levels	I to III	NA		300	(77)	NA
	I to IV	NA		16	(4)	
	I to V	NA		74	(19)	
Follow-up	Time in years median (IQR)	2.2	(1.0 to 4.1)	4.5	(2.5 to 7.3)	<0.001
	Range (years)	0.0 to 9.7		0.0 to 20.8		
	Regional recurrences	25	(5)	19	(5)	1.000
	Deceased	52	(11)	140	(36)	<0.001
	Deceased by disease	18	(4)	45	(11)	<0.001

Abbreviations: END, elective neck dissection; SLNB, sentinel lymph node biopsy; ITC, isolated tumour cells; MiM, micrometastasis; MaM, macrometastasis, FOM, floor of mouth.

The overall sensitivity of detecting occult metastasis was comparable between the END and SLNB patients (84% vs. 81%, p = 0.612). Both groups had a similar negative predictive value (NPV) (93%, p = 1.000) (Table 2). Because of dissimilarity in pT staging, we separately analysed the accuracy for pT1 and pT2. In the SLNB cohort a trend towards a higher, though not significantly different, sensitivity was observed for pT2 tumours compared to pT1 tumours (88% vs. 76%, p = 0.075). In the END cohort, pT2 tumours also showed a higher sensitivity in comparison to pT1 tumours (90% vs. 70%, p = 0.010). NPVs did not differ significantly regarding pT stage within the groups. No significant differences were found for sensitivity and NPV between the SLNB and END when corrected for pT stage (Table 2).

Floor of mouth tumours

In total 131 (27%) of the SLNB and 133 (34%) of the END patients had a tumour located in the FOM. SLNB had a lower sensitivity (63% vs. 92%, p = 0.006) and NPV (90% vs. 97%, p = 0.057) compared to END. The SLNB had a higher (but not significantly) sensitivity (86% vs. 80%, p = 0.315) and NPV (95% vs. 92%, p = 0.250) compared to END for other (non-FOM) anatomical locations. When comparing FOM tumours with other non-FOM locations within the SLNB group, there was a lower sensitivity (63% vs. 86%, p = 0.008) and NPV (90% vs. 95%, p = 0.113). In contrast, within the END group at most a trend towards a higher sensitivity (92% vs. 80%, p = 0.114) and a higher NPV (97% vs. 92%, p = 0.130) was observed for FOM tumours compared to other anatomical locations. Of the 11 FOM patients with a false negative SLNB, 64% (7/11) had a regional recurrence in level I. In 3 FOM END patients, 1 patient (33%) had a regional recurrence in level I, the remaining patients had a regional recurrence in level II or higher.

Five years disease specific survival

The DSS was significantly longer for SLNB pT1 patients (96%) compared to END pT1 (90%, p = 0.008), SLNB pT2 (90%, p = 0.001) and END pT2 (86%, p < 0.001) patients. No significant differences in DSS were seen between the other groups. After the Bonferroni correction, the SLNB pT1 had still a significant longer DSS compared to the other groups: END pT1 (p = 0.048), SLNB pT2 (p = 0.006) and END pT2 (p < 0.001) (Figure 2A).

We furthermore analysed the differences between DSS of the END and SLNB groups divided by anatomical location (FOM vs. other locations, Figure 2B). SLNB staged patients with a FOM tumour had a longer DSS compared to END FOM patients (98% vs. 87%, p = 0.021). The other (non-FOM) SLNB patients had longer DSS compared

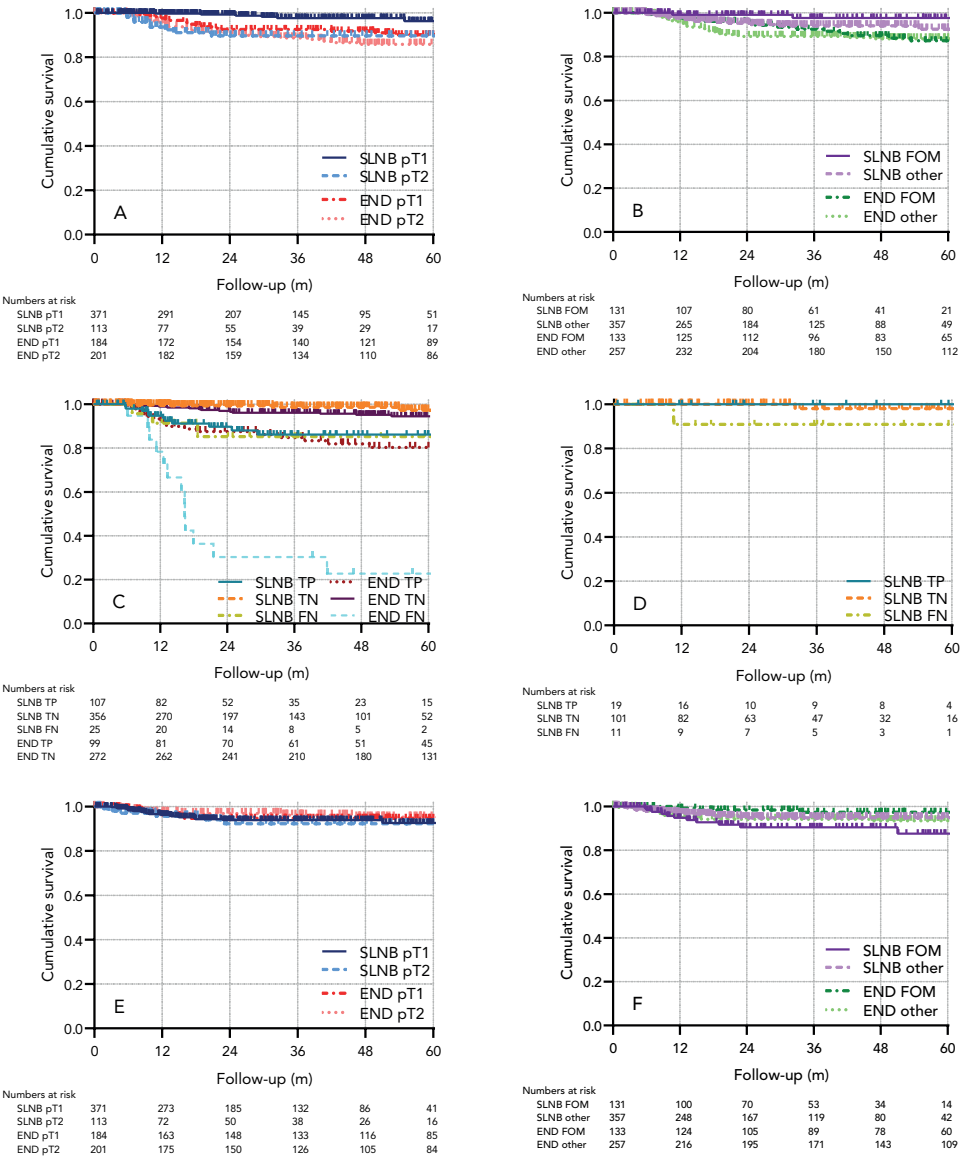

Figure 2. Survival analyses. Disease specific survival between END and SLNB patients divided by T stage (A) and by anatomical location (B). Because of the low number of pT3 (n = 8) and pT4 (n = 2) staged patients for each of the staging methods, these pT stages were excluded from the disease specific survival analysis divided by T stage (A). Disease specific survival analysis of the END and SLNB groups divided for true positives, true negatives and false negative patients (C). SLNB staged patients with a FOM tumour were also divided by true positives, true negatives and false negative patients (D). Regional recurrence free survival between END and SLNB patients divided by T stage (E), and by anatomical location (F).

Table 2. Sensitivity and negative predictive value

		SLNB		END		
		%	(95% CI)	%	(95% CI)	p-value
Overall	Sensitivity	81	(76 to 90)	84	(73 to 87)	0.612
	NPV	93	(90 to 95)	93	(91 to 95)	1.000
pT1*	Sensitivity	76	(51 to 84)	70	(65 to 85)	0.637
	NPV	94	(90 to 96)	94	(91 to 96)	1.000
pT2*	Sensitivity	88	(82 to 96)	90	(77 to 96)	0.776
	NPV	91	(88 to 97)	94	(83 to 96)	0.565
FOM**	Sensitivity	63	(79 to 98)	92	(44 to 80)	0.006
	NPV	90	(91 to 99)	97	(85 to 94)	0.057
Other locations**	Sensitivity	86	(70 to 88)	80	(78 to 92)	0.315
	NPV	95	(88 to 95)	92	(92 to 97)	0.250

Abbreviations: SLNB, sentinel lymph node biopsy, END, elective neck dissection ; FOM, floor of mouth; NPV, negative predictive value
* pT1 versus pT2 witin the SLNB group: sensitivity p = 0.075 and NPV p = 0.415
* pT1 versus pT2 witin the END group: sensitivity p = 0.010 and NPV p = 1.000
** FOM versus Other locations within the SLNB group: sensitivity p = 0.008 and NPV p = 0.113
** FOM versus Other locations within the END group: sensitivity p = 0.114 and NPV p = 0.130

to the END others (93% vs. 88%, p = 0.046). Only the difference between SLNB FOM compared to END others remains significant after the Bonferroni correction (p = 0.017).

In the DSS analysis with the END and SLNB groups divided for TP, TN and FN (Figure 2C), the SLNB TN had the longest DSS (97%) and was only not significantly different compared to the END TN (95%). END TN and SLNB TN had significant longer DSS compared to the other groups (Figure 2C). Regarding the FN cases, the END FN (23%) had the shortest DSS and was significant different compared to all other groups. The SLNB FN (85%) had a comparable DSS compared to the SLNB TP (86%). After the Bonferroni correction, the differences between SLNB FN (85%) and END TN (95%) and between SLNB TP (86%) with END TN (95%) were not significant anymore. The DSS analysis for SLNB staged patients with a FOM tumour and divided by TP, TN and FN (Figure 2D), showed the shortest DSS for SLNB FN patients (91%), however no significant differences were found between these three groups.

Five years regional recurrence free survival

The RRFS of END and SLNB patients divided by T stage showed no significant differences between both groups (Figure 2E). However, when comparing the RRFS for the END and SLNB groups divided by anatomical location (FOM vs. other anatomical locations), a significant shorter RRFS was observed for the SLNB FOM group as compared to the END FOM group (88% and 97%, p = 0.008, (p = 0.048 Bonferroni corrected) Figure 2F).

Additional metastasis in the MRND after a positive SLNB

A histopathologically positive SLNB was found in 107 of the 488 patients (22%). ITCs were detected as largest metastatic deposit in SLNB in 15 patients (14%), MiM in 31 patients (29%) and MaM in 61 patients (57%). A positive SLNB was followed by an additional (selective) neck dissection in 86% of the patients (92/107). Five patients refused any additional treatment (1x MiM, 4x MaM), and the remaining 10 patients received additional radiotherapy instead of a neck dissection. Radiotherapy was required for primary tumour control in 5 cases (2x ITC, 1x MiM, 2x MaM) and was therefore extended to the neck. In 4 cases (1x MiM and 3x MaM), radiotherapy was initiated because of 2 or more positive SLNs or extranodal extension, and in 1 patient (MiM) extensive surgery was not considered feasible due to major comorbidities. None of these 10 patients developed regional recurrences during follow-up.

Additional non-SLN metastases were found in the neck dissection specimen in 21% of the patients (19/92) with a therapeutic neck dissection after SNLB. Of these patients, the majority (17/19, 90%) had MaM in the SLN as largest tumour deposit. Patients had a very low risk of having additional metastases if the SLN contained ITCs (8%, 1/13) or MiM (4%, 1/27) compared to MaM (33%, 17/52) (Table 3, p = 0.005).

Disease specific survival per size of metastasis (ITC, MiM and MaM) did not reach significance, although a trend between ITC and MaM was observed (p = 0.091, Bonferonni corrected p = 0.182).

Table 3. Additional positive lymph nodes in complementary neck dissection specimen in case of positive SLNB

		Yes	No	Other	Total
SLNB	ITC	1	12	2	15
	MiM	1	26	4	31
	MaM	17	35	9	61
Total		19	73	15	107

DISCUSSION

This study evaluated the diagnostic value of END and SLNB for early stage OSCC in two large cohorts in the Netherlands. We found an overall sensitivity of 84% in the END cohort and 81% in the SLNB cohort with an NPV of 93% for both cohorts. The SLNB procedure performed worse only in floor of mouth (FOM) tumours while no significant differences between the two cohorts were found for all other anatomical locations. To date, this is the first study that compares END and SLNB procedures for cN0 neck staging in a setting with a numerously powered homogeneous group of patients.

The END cohort showed an overall sensitivity of 84% and a NPV of 93%, based on 19 patients developing regional metastases without evidence of a local recurrence or second primary tumour, resulting in a regional recurrence rate of 7% in the END group. This number is lower than reported by Ganly et al. and Mizrachi et al., who presented both regional recurrence rates up to 15% during follow-up after a negative END.[31,32] A recent meta-analysis showed 274 regional recurrences in 2577 early stage oral cavity cancer patients, treated with END (regional recurrence rate 10.6%).[33]

We reported an overall sensitivity of 81% in the SLNB cohort. A trend towards lower sensitivity rates for SLNB procedures can be observed over the last years.[10] In a meta-analysis of Liu et al., recent publications (2009-2016) showed a sensitivity of 86% compared to 92% for the group of early publications (2000-2008). A possible explanation for this trend could be that SLNB are currently performed in a "routine" instead of research setting, with learning curves of new SLNB performing surgeons included.[23] Moreover, all our patients are treated following the principle that in case of a negative SLNB a watchful waiting strategy is followed. In previous publications

with higher sensitivity rates, a complementary neck dissection was often performed as gold standard (or validation) for the SLNB procedure. It is however well-known that watchful waiting reveals very small metastases, where these will be missed in up to 15% with routine histopathological examination of a neck dissection specimen (erroneously classified as true-negative).[34,35]

Another explanation of our overall lower sensitivity in the SLNB cohort is a high number of false negatives in the group of patients with FOM tumours compared to the group of patients with non-FOM tumours (sensitivity 63% vs. 86%, p = 0.008). As shortly mentioned before in the introduction, lower accuracy rates of SLNB in FOM tumours have been published previously.[11,12,23-25] Regional recurrences were particularly located in level I (64%), supporting the theory of missed SLNs in this level by the "shine-through" phenomenon. Indirectly, this theory was supported by the data of our END cohort showing an excellent sensitivity of 92% in FOM tumours with only 1/3 patients (33%) showing a regional recurrence in level I. Our data suggest that SLNB in its current form is not reliable enough to detect occult metastases in FOM tumours, due to missed (positive) SLNs in level I. However, as shown in survival analyses, the inferior accuracy of SLNB in FOM tumours did not cause lower disease-specific survival (Figure 2B). This implies that salvage neck dissection was successful in most cases, although it should be noted that this may require more extensive surgery and postoperative radiotherapy.

Two additional techniques are recommended in the SLNB surgical guidelines of the consensus meeting in 2018 to overcome the lower sensitivity of the SLNB in FOM tumours.[27] One technique is a superselective level I neck dissection as described by Stoeckli et al.[25] The second option is the use of hybrid tracers with a fluorescence label.[36,37] Additionally, Agrawal et al. reported about the use of Tilmanocept, a novel 99m-Technetium tracer with high sensitivity and NPV for detection of occult metastases, also in case of FOM tumours.[38] Since this study confirmed a lower accuracy for the SNLB procedure in FOM tumours, we opt nationally to perform the superselective level I neck dissection as mentioned above.

Our study showed that in the majority of SLNB positive patients (57%) MaM were detected as largest tumour deposit, but undeniably a considerable number of cases had smaller deposits in the SLN (ITCs or MiM). Of 52 patients with MaM treated with a complementary neck dissection, 33% revealed additional (non-SLN) metastases. This number is in line with published literature.[39] As previously published, in only a

small number of cases with ITC and MiM additional non-SLN metastases were found in complementary neck dissection specimens.[39] Our study demonstrated in only 2 cases with ITC or MiM additional non SLN metastases (5%). Due to the limited number of studies in literature, it is difficult to determine the value of complementary neck dissections in case of ITCs or MiM. In addition, one has to consider that complementary neck dissection specimens are not examined as meticulously as SLNs. Currently, data is lacking to safely omit therapeutic neck dissections in case of ITC or MiM.

Disease specific survival of the false-negative cases of both cohorts addresses an important finding. SLNB FN patients had an almost equal DSS compared to the true positive patients. In contrast, in the END FN cohort a dramatic decreased survival was shown compared to the END TP patients. Our data clearly underlines the importance of correct staging using a minimal invasive method, given this inferior survival for FN patients, specifically in the END cohort.

One of the limitations of this study remains its retrospective design. In the END cohort a considerable number of the patients were diagnosed as clinically N0, based on potentially dated ultrasound, CT and MRI scanners and before widespread application of FDG-PET for staging purposes. There is also a significant difference in pT stage with more pT2 staged tumours in the END cohort. A possible explanation is that the majority of patients in the END cohort was selected based on depth of invasion (> 4 mm), inevitably resulting in less pT1 tumours. For that reason, we compared also the sensitivity and NPV between SLNB and END divided by pT stage and found no significant difference. Besides the diameter of the tumour reflected in pT stage in the 7th TNM classification, nowadays the 8th edition is used.[40,42] In the 8th edition, depth of invasion is newly incorporated for T stage and therefore our results could not directly be translated to the 8th TNM classification.[43,45] Another important difference between both groups is the prolonged follow-up in the END cohort. Although we expected (and identified) regional recurrences particularly in the first 2 years, a longer follow-up could result in more regional recurrences and/or disease specific deaths.

CONCLUSIONS

In conclusion, detection of lymph node metastases in oral cancer using sentinel lymph node biopsy is as accurate as elective neck dissection, except for floor of mouth tumours. SLNB showed higher disease specific survival rates as compared

to the END, also after stratifying for different pT stages or anatomical locations. As randomized controlled trials comparing the accuracy of SLNB with routinely used END are currently lacking, this retrospective cohort study provides the highest evidence of the effectiveness of SLNB in oral cancer.

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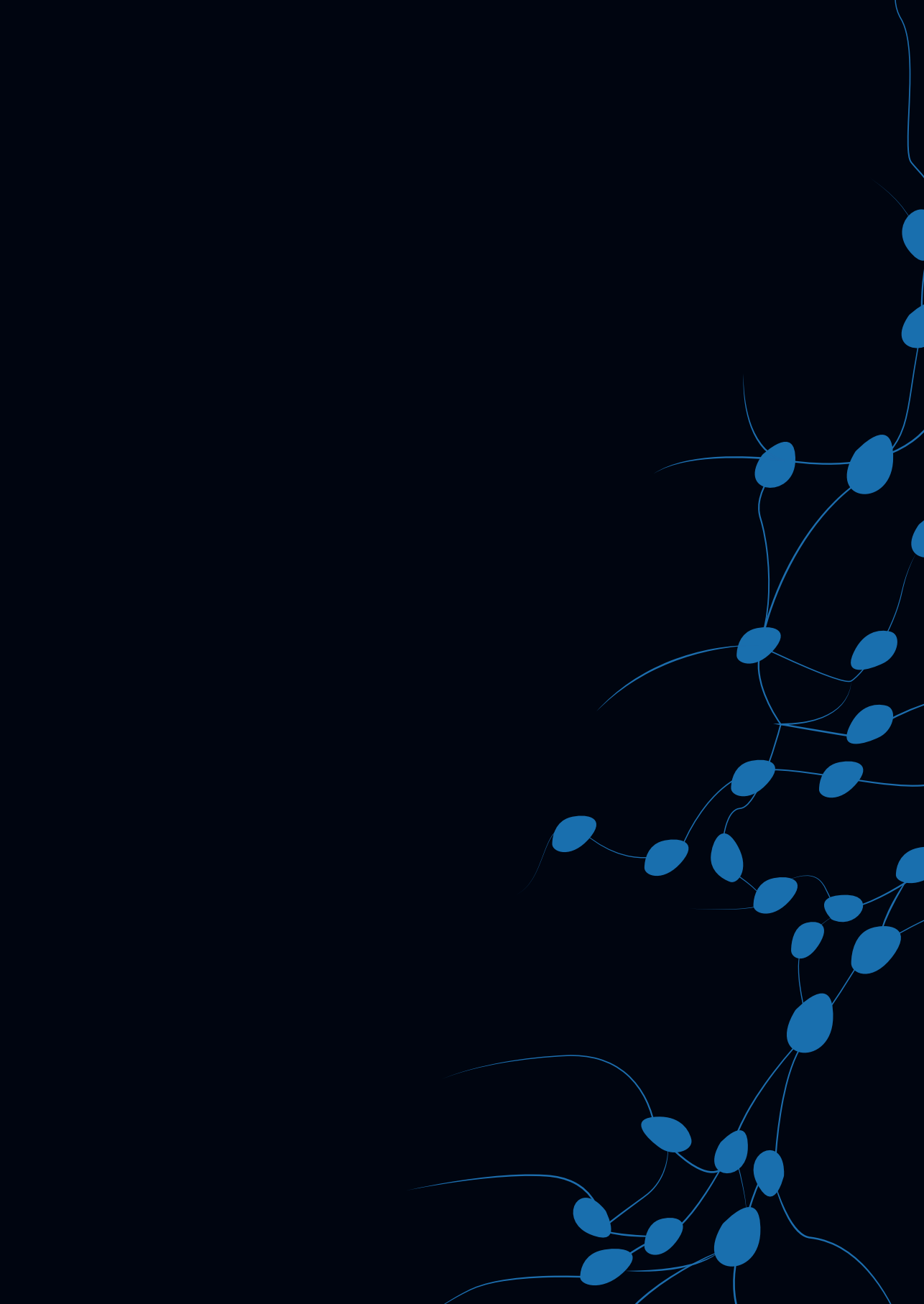

POTENTIAL APPLICATIONS

Additional non-sentinel lymph node metastases in early oral cancer patients with positive sentinel lymph nodes

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ABSTRACT

Objective To determine risk factors for additional non-sentinel lymph node metastases in neck dissection specimens of patients with early stage oral cancer and a positive sentinel lymph node biopsy (SLNB).

Methods A retrospective analysis of 36 previously untreated SLNB positive patients in our institution and investigation of currently available literature of positive SLNB patients in early stage oral cancer was done. Degree of metastatic involvement (classified as isolated tumour cells (ITC), micro- and macrometastasis) of the sentinel lymph node (SLN), the status of other SLNs and additional non-SLN metastases in neck dissection specimens were analyzed.

Results Of 27 studies, comprising 511 patients with positive SLNs, the pooled prevalence of non-SLN metastasis in patients with positive SLNs was 31%. Non-SLN metastases were detected (available from 9 studies) in 13%, 20% and 40% of patients with ITC, micro- and macrometastasis in the SLN, respectively. The probability of non-SLN metastasis seems to be higher in case of more than one positive SLN (29% vs. 24%), absence of negative SLNs (40% vs. 19%) and a positive SLN ratio of more than 50% (38% vs. 19%).

Conclusion Additional non-SLN metastases were found in 31% of neck dissections following positive SLNB. Presence of multiple positive SLNs, absence of negative SLNs and a positive SLN ratio of more than 50% may be predictive factors for non-SLN metastases. Classification of SLNs into ITC, micro- and macrometastasis in future SLNB studies is important to answer the question if treatment of the neck is always needed after positive SLNB.

INTRODUCTION

Sentinel lymph node biopsy (SLNB) has been introduced for the detection of occult lymph node metastasis in patients with early stage oral cancer. Observational trials (with only neck dissection after positive SLNB) have demonstrated that SLNB is a sensitive method in the detection of occult cervical lymph node metastases. A recent meta-analysis found a pooled sensitivity of 91% (95% CI 84-95%) and a negative predictive value ranging from 92% to 98% when follow-up was used as reference standard.[1] Long term follow-up studies showed that SLNB is a safe procedure.[2,3] Recently, we reported a sensitivity of 93% and a negative predictive value of 97% of SLNB in our first 90 early oral cancer patients.[4]

Metastatic tumour deposits can be categorized as isolated tumour cells (ITC), micrometastasis and macrometastasis. ITCs are generally defined as tumour deposits ≤ 0.2 mm (pN0i+), micro- (pN1mi) and macrometastases (pN1) as tumour deposits of 0.21-2.0 mm and > 2.0 mm, respectively. Additionally, for ITC more specific histopathological characteristics have been described: no contact with vessel or lymph sinus wall, no extravasation, no extravascular stromal reaction and no extravascular tumour cell proliferation.[5]

So far, the same strategy has been used in case of sentinel nodes with ITCs, micro- and macrometastases, which means a (selective) neck dissection. Broglie et al. found significantly higher hazard ratios in overall, disease specific and disease free survival for micrometastases and macrometastases, whereas ITCs were significant determinants for disease specific survival compared with SLN negative patients.[6]

A report of a European multicenter study on 109 oral squamous cell carcinoma patients with positive SLNB showed additional (non-SLN) metastases in 34.4% of the neck dissection specimens.[7]

The recent update of this trial demonstrated a statistically lower overall survival for micro- and macrometastases compared with ITC.[8] If a reliable nomogram to predict non-SLN metastases based on degree of metastatic tumour deposits in SLNs can be developed, SLNB might be a therapeutic rather than just a diagnostic procedure, i.e. avoiding subsequent tumour-negative neck dissections. The aim of the present retrospective study and literature review is to analyze risk factors for the presence of non-SLN metastases in SLNB positive early oral cancer patients.

MATERIAL AND METHODS

Retrospective study

From February 2007 until October 2014, 139 consecutive patients with cT1-2N0 squamous cell carcinomas of the oral cavity or oropharynx underwent transoral excision and SLNB. After approval of the Institutional Review Board and Ethics Committee, informed consent was obtained until SLNB was performed as standard procedure in our institution. SLNB was performed according to the EANM/SENT joint practice guidelines.[9] A detailed description of the procedure in our institution had been described previously.[4]

A positive SLNB was followed by (selective) neck dissection in 36/37 (97%) patients (one patient with ITC was treated by radiotherapy only, which was indicated for adverse histopathological findings of the primary tumour).

The neck dissection specimen was histopathologically examined for additional lymph node metastases using a routine procedure (no step-serial sectioning and immunohistochemistry). The presence and localisation (level) of additional lymph node metastasis were scored for each patient.

The numbers of tumour positive (1 vs. >1) and negative (0 vs. ≥1) SLNs and their ratio (≤50% vs. >50%) were scored for each patient.

Literature analysis

Studies included in recent meta-analyses were analyzed for data on the degree of metastatic involvement of SLN, the status of other SLNs and additional non-SLN metastases in neck dissection specimens following positive SLNB.[1,10] Additionally, references were explored to identify other relevant articles. If presented (or could be subtracted from the data provided) the rate of positive non-SLN were scored for ITC, micrometastasis, macrometastasis, number of positive SLNs (1 vs. >1), number of negative SLNs (0 vs. ≥1), and their ratio ratio (≤50% vs. >50%) per patient.
Due to low numbers no statistical analyses were performed.

RESULTS

Retrospective study

At least one histopathologically positive SLN was found in 36/139 (26%) of patients, yielding a total of 43 positive SLNs. One patient with a paramedian T1 tongue tumour was diagnosed with bilateral positive SLNs. In both neck sides the largest tumour deposit in the positive SLN, respectively ITC and macrometastasis, was separately investigated. The remaining patients with at least 2 positive SLNs had only unilateral metastasis and the largest tumour deposit was taken for evaluation and follow-up of the neck. Overall, we analyzed 36 patients with 37 SLN positive neck sides, subdivided into 7 necks with ITC, 14 with micro- and 16 with macrometastasis (Table 1 and 2).

Table 1. Data of demographic and tumour-related patient characteristics

		Status of SLNB	
Characteristic	Overall (%)	Negative (%)	Positive (%)
Patients, n (%)	139 (100%)	103 (74%)	36 (26%)
Gender, n (%)			
Male	71 (51%)	54 (52%)	17 (47%)
Female	68 (49%)	49 (48%)	19 (53%)
Median age (y) (range)	60 (27-86)	60 (27-85)	62 (29-86)
Tumour location, n (%)			
Tongue	86 (61%)	62 (60%)	24 (66%)
Floor of mouth	40 (29%)	31 (30%)	9 (25%)
Buccal mucosa	6 (4%)	6 (6%)	0
Inferior alveolar process	4 (3%)	2 (2%)	2 (6%)
Soft palate	3 (2%)	2 (2%)	1 (3%)
Clinical T stage, n (%)			
T1	97 (70%)	81 (79%)	16 (44%)
T2	42 (30%)	22 (21%)	20 (56%)
No of SLNs	328	285 (87%)	43 (13%)
Follow-up, (m) (range)			
Observation time (months)	36 (1-102)	36(1-102)	36 (1-98)

Abbreviations: SLNB, sentinel lymph node biopsy; SLNs, sentinel lymph nodes

Table 2. Prevalence of ITC, micrometastasis and macrometastasis in positive SLNs

Study	All	ITC	Micro	Macro
Barzan[11]	2*	0 (0%)	1 (50%)	1 (50%)
Mozillo[12]	4	0 (0%)	4 (100%)	0 (0%)
Stoeckli[13]	9	1 (11%)	5 (56%)	3 (33%)
Keski-Säntti[14]	2	0 (0%)	1 (50%)	1 (50%)
Bilde[15]	11	3 (27%)	6 (55%)	2 (18%)
Atula[16]	34	5 (15%)	14 (41%)	15 (44%)
Kovacs[17]	9	0 (0%)	3 (33%)	6 (67%)
Alkureishi[18]	42 **	0 (0%)	10 (24%)	32 (76%)
Burcia[19]	38	14 (37%)	15 (39%)	9 (24%)
Terada[20]	5	0 (0%)	3 (60%)	2 (40%)
Broglie[6]	42	10 (24%)	19 (45%)	13 (31%)
Present study	36	6 (16%)	14 (39%)	16 (44%)
Total	**234**	**39 (17%)**	**95 (41%)**	**100 (43%)**

Abbreviations: ITC, isolated tumour cells; micro, micrometastasis; macro, macrometastasis; SLN, sentinel lymph node
* only results of cNO early oral cancer
** definition of micrometastasis: only detected by step serial sectioning and/or immunohistochemistry

In none of the SLNs with ITC based on size, extravasation, extravascular stromal reaction or extravascular tumour cell proliferation were found, but all these SLNs had contact with lymph sinus wall.

In 6/36 (17%) patients who underwent a subsequent neck dissection additional lymph node metastases were found. All patients had T2 tumours and the SLN had contained a macrometastasis (Table 3).

Additional non-SLN metastases were found in level I (n=3), level III (n=6), level IV (n=1) and level V (n=1). In 1 patient non-SLN metastasis was restricted to the same level as the positive SLN, in 1 patient in adjacent and nonadjacent levels and in 4 patients non-SLN metastasis were only found in nonadjacent levels.

If >1 SLN was positive, 2/5 (40%) of the patients had additional neck metastases compared to 4/31 (13%) in patients with a single positive SLN. In 2/13 (15%) patients with solely positive SLN(s) additional non-SLN metastases were found (vs. 17% if synchronous presence of negative SLNs were present). If more positive than negative SLNs were present (>50% SLN positive) additional non-SLN metastases were found in 3/14 (21%) patients compared to 3/22 (14%) if a similar or higher number of negative than positive SLNs were found (Table 3).

Review of literature

Eleven studies had categorized the size of tumour deposits in SLNs.[6,11-20] Including the data from our study, ITC was present in 17% of 234 patients (range 0-37%), micrometastasis in 41% (19-100%) and macrometastasis in 43% (0-76%) (Table 3). Additional non-SLN metastases were mainly found in levels I, II and III and sometimes in level IV or V.[7,13,15,16,21,22] The pooled prevalence of non-SLN metastasis in patients with positive SLN(s) of the present study and 26 other studies was 31% (156/511).[6,7,11-17,21-37] The pooled probability of non-SLN metastasis in the present study and 8 other studies was 13% (4/32), 20% (11/55) and 40% (19/49) for ITC, micro- and macrometastases, respectively.[6,11,13,15-17,23,25] This probability was 26% (37/144) for micro- and macrometastases combined.

Including our results, a higher pooled prevalence for additional non-SLN metastases had been found when >1 positive SLNs were present (29% vs. 24%)[11,13,16,22-24,30], absence of negative SLNs (40% vs. 19%)[13,15,16,22-24,30,33,37] and in case of a positive SLN ratio of more than 50% (38% vs. 19%).[13,16,22-24,30,33] Results are shown in Table 3.

Table 3. Prevalence of non-SLN metastases in relation to size of the SLN metastasis, number of positive SLNs and ratio positive/negative SLNs

Study	All	ITC	Micro	Macro	1 pos SLN	> 1 pos SLN	0 neg SLN	≥ 1 neg SLN	≤ 50% SLN pos	> 50% SLN pos
Zitsch[23]	50% (2/4)		50% (2/4)		67% (2/3)	0% (0/1)	50% (1/2)	50% (1/2)	50% (1/2)	50% (1/2)
Taylor[24]	0% (0/4)				0% (0/3)	0% (0/1)	0% (0/1)	0% (0/3)	0% (0/2)	0% (0/2)
Barzan[11] $	0/% (0/2)		0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)
Civantos[26]	30% (3/10)	0% (0/2)	38% (3/8)							
Hoft[22]	25% (3/12)				33% (3/9)	0% (0/3)	50% (2/4)	13% (1/8)	13% (1/8)	50% (2/4)
Jeong[30]	33% (2/6)				20% (1/5)	100% (1/1)	50% (1/2)	25% (1/4)	25% (1/4)	50% (1/2)
Stoeckli[13] @	56% (5/9)	0% (0/1)	40% (2/5)	100% (3/3)	50% (3/6)	67% (2/3)	67% (4/6)	33% (1/3)	33% (1/3)	67% (4/6)
Stoeckli[13] #	21% (4/20)	0% (0/5)	11% (1/9)	50% (3/6)	14% (2/14)	33% (2/6)	20% (1/5)	20% (3/15)	15% (2/13)	29% (2/7)
Bilde[15]	9% (1/11)	33% (1/3)	0% (0/6)	0% (0/2)	11% (1/9)	0% (0/2)	0% (0/1)	10% (1/10)	11% (1/9)	0% (0/2)
Tartaglione[33]	25% (2/8)				25% (2/8)	0% (0/0)	100% (1/1)	14% (1/7)	14% (1/7)	100% (1/1)
Atula[16]	39% (13/33)	20% (1/5)	46% (6/13)	40% (6/15)	43% (6/14)	20% (1/5)*	50% (3/6)	31% (4/13) *	33% (4/12)	43% (3/7)
Kovacs[17]	11% (1/9)		0% (0/3)	17% (1/6)						
Broglie[6]	14% (6/42)	20% (2/10)	13% (4/32)							
Rigual[37]	75% (3/4)				50% (2/4)	0% (0/0)	100% (2/2)	50% (1/2)	50% (1/2)	100% (2/2)
Present study	17% (6/36)	0% (0/6)	0% (0/14)	38% (6/16)	13% (4/31)	40%(2/5)	15% (2/13)	17% (4/23)	14% (3/22)	21% (3/14)
Total	**24% (51/210)^**	**13% (4/32)**	**20% (11/55)**	**40% (19/49)**	**24% (26/107)**	**29% (8/28)**	**40% (17/44)**	**19% (18/91)**	**19% (16/85)**	38% (19/50)
			26% (37/144)							

Abbreviations: SLN, sentinel lymph node; ITC, isolated tumour cells; micro, micrometastasis; macro, macrometastases; pos, positive; neg, negative

** only for 5 patients specific data available*
@ only feasibility and validation phase
 # clinical application phase update by Broglie et al 2012
$ only cNO early oral cancer
^ Rate based on studies presented in Table 3. In general, the prevalence of non-SLN metastases in all 26 studies analyzed including our study was 31% (156/511).

DISCUSSION

Patients with positive SLNB undergo generally subsequent (completion) neck dissection because there is no reliable means of detecting or predicting non-SLN metastasis. SLNB is associated with significant less morbidity than elective neck dissection and identification of patients who do not benefit from subsequent neck dissection may decrease this morbidity even further.[38]

The prediction of presence of non-SLN metastasis in the neck after positive SLNB can theoretically be improved in two ways: dividing the tumour deposits in SLNs in subgroups or adding other predictive factors in a risk profile.

Combining the present study with our analysis of the literature we found an inverse relation between the size of tumour deposits in the SLN and the probability of a non-SLN: 13% for ITC, 20% for micrometastasis and 40% for macrometastasis. Since the prevalence of non-SLN metastasis in the neck dissection specimen following ITC in SLNs is substantial, in early stage oral cancer one can not refrain from neck dissection after any category of positive SLNB. When patients with a low risk of non-SLN metastasis can be identified, a wait and scan policy using USgFNAC may be justified.[39]

The commonly used definition of isolated tumour cells is based on size (0.2 mm or less) rather than designation of the metastatic tumour deposit. ITC is then considered to be a small micrometastasis "waiting to grow" (precursor of micrometastasis) with a risk these necks with SLNs containing ITC may also harbour micro- or macrometastases.[40] In the present study all ITCs based on size had the same morphologic features: no extravasation, extravascular stromal reaction or extravascular tumour cell proliferation, but all had contact with lymph sinus wall. Since these deposits seem to be real ITC this latter feature is debatable.

Review of the literature revealed that only a limited number of small studies classified SLN tumour deposits in ITC, micrometastasis and macrometastasis. The wide variety of rates of the different categories in our literature review may reflect the lack of uniformly used definitions.

Consequently these numbers are too low to perform reliable statistical analyses on the risk of non-SLN metastases in these different tumour deposits in SLNs. To explore if patients with ITC or micrometastasis in SLNs need a subsequent neck dissection it is

important that all future studies report SLN metastases in these categories. Only then the question if SLNB can be used as treatment, and not only as diagnostic procedure, in patients selected by the type of tumour deposit in SLNs can be answered.

In breast cancer SLNB is accepted as standard diagnostic technique for clinically node negative patients. Complete axillary lymph node dissection is generally recommended if the SLNB is positive. Non-SLN metastases are detected in 35 to 50% of SLN positive patients. Only some series report the prevalence of ITC and distinction between ITC and micrometastasis could be difficult.[42] The reported rate of micrometastasis as largest tumour deposit in SLN positive breast cancer patients varies considerably: from 24 to 93%.[43] In patients with tumour deposits in SLNs the prevalences of ITC, micrometastasis and macrometastasis is 7-16%, 16-32% and 58-78%, respectively. Non-SLN metastases are found in 0-13%, 12-27% and 48-50% in patients with ITC, micro- and macrometastases in SLNs, respectively.[44,49]

Different nomograms in predicting non-SLN metastases in breast cancer patients with a positive SLNB have been developed, usually including largest detected size of SLN metastasis and the proportion of involved SLNs among all removed SLNs.[43] The treatment strategy for micrometastasis in SLN is under debate. It has been suggested to refrain patients with ITC in their SLN from axillary lymph node dissection.[44-46] A recent review including 7,151 breast cancer patients with positive SLNB in whom an axillary lymph node dissection was omitted revealed an axillary recurrence rate of 0.7% (range 0-7.1%) for macrometastasis and 0.3% (range 0-3.4%) for micrometastasis and ITC. Unfortunately, micrometastasis and ITC could not be analyzed separately and details regarding adjuvant treatment were lacking in the majority of studies.[50] Since breast cancer patients are often treated with adjuvant systemic therapy these strategies can not easily be translated to early oral cancer patients who are usually treated with surgery as monotherapy.

A meta-analysis of predictive factors for non-SLN metastases in breast cancer patients with a positive SLN confirmed a high likelihood of non-SLN metastases for size of SLN metastasis of more than 2 mm (macrometastasis; odds ratio (OR) 4.22), extracapsular extension in the SLN (OR 4.10), one or less negative SLN (OR 2.66), more than one positive SLN (OR 2.60), tumour size > 2cm (OR 2.41), a ratio of positive SLN of more than 50% (OR 2.25) and lymphovascular invasion (OR 2.24).[51] Recently the same authors developed a risk score based on these parameters.[52]

In oral oncology, Gurney et al reported other predictive factors for the presence of non-SLN metastases in SLNB positive necks: tumour site (higher risk as the primary tumour was located at the posterior part of the oral cavity), increased stage (T2-4 stage at higher risk) and number of negative SLNs (lower risk in higher number of negative SLNs).[7] In the present study all patients with non-SLN metastases had T2 oral squamous cell carcinoma. Although tumour thickness or depth of invasion and molecular markers have predictive value for the presence of (occult) lymph node metastasis, their role in predicting the presence of non-SLN metastasis in oral cancer patients with a positive SLNB is not known yet.[53,54]

Our retrospective study suggests if both a positive SLN and a negative SLN are present the prevalence of non-SLN metastases seems nearly equal compared to patients with solely positive SLNs, in contrast to other studies (Table 3). Since distinguishing real SLNs from second echelon nodes may be difficult, it can be anticipated that (some of) these negative SLNs may be in fact second echelon nodes.[55] If more positive than negative SLNs are present the probability of non-SLN metastases seems to be higher, also in case of a ratio of positive SLNs of more than 50%. Due to the low number of cases statistical analysis could not be performed and more larger studies are needed to confirm these ideas.

A large multicenter study showed in 1/122 neck dissections following positive SLNBs of early oral cancer non-SLN metastases in levels other than I-III.[7] These non-SLN metastases had been found in 15% of the patients in the same level, in 17% in an adjacent level and in 2% in a nonadjacent level. In our retrospective study all non-SLN metastases were found in levels I-IV except one in level V. In this latter patient 2 positive SLNs and 5 additional non-SLNs were found. In 67% (4/6) of the patients non-SLNs were only found in nonadjacent levels. If future studies report on the level involved by non-SLN metastases more tailored (super)selective neck dissections may be defined.

Analysis of the literature, including our present study, showed that additional non-SLN metastases were found in 31% of neck dissections following positive SLNB. Selected by tumour deposit, these percentages were 13% for ITC, 20% for micro- and 40% for macrometastasis in SLNs. This prevalence may be underestimated since in most studies non-SLNs are examined using routine histopathological examination without step serial sectioning and immunohistochemistry. Studies on neck dissection specimens show that immunohistochemistry can reveal small metastases in 15% of the patients that remain undetected in routine H&E staining.[56]

Reporting other risk factors may be useful to develop a nomogram selecting SLNB positive patients for neck dissection and active surveillance or wait and scan follow-up. Presence of more than one positive SLN, absence of negative SLNs (besides a positive SLN) and a positive SLN ratio of more than 50% may be predictive factors for non-SLN metastasis in SLNB positive patients. To this point, there is no well argued reason to refrain from an additional neck dissection based on these risk factors or tumor size in the SLN. The presented data support the use of a selective neck dissection, when because of a SLNB positive neck an additional neck dissection is indicated.

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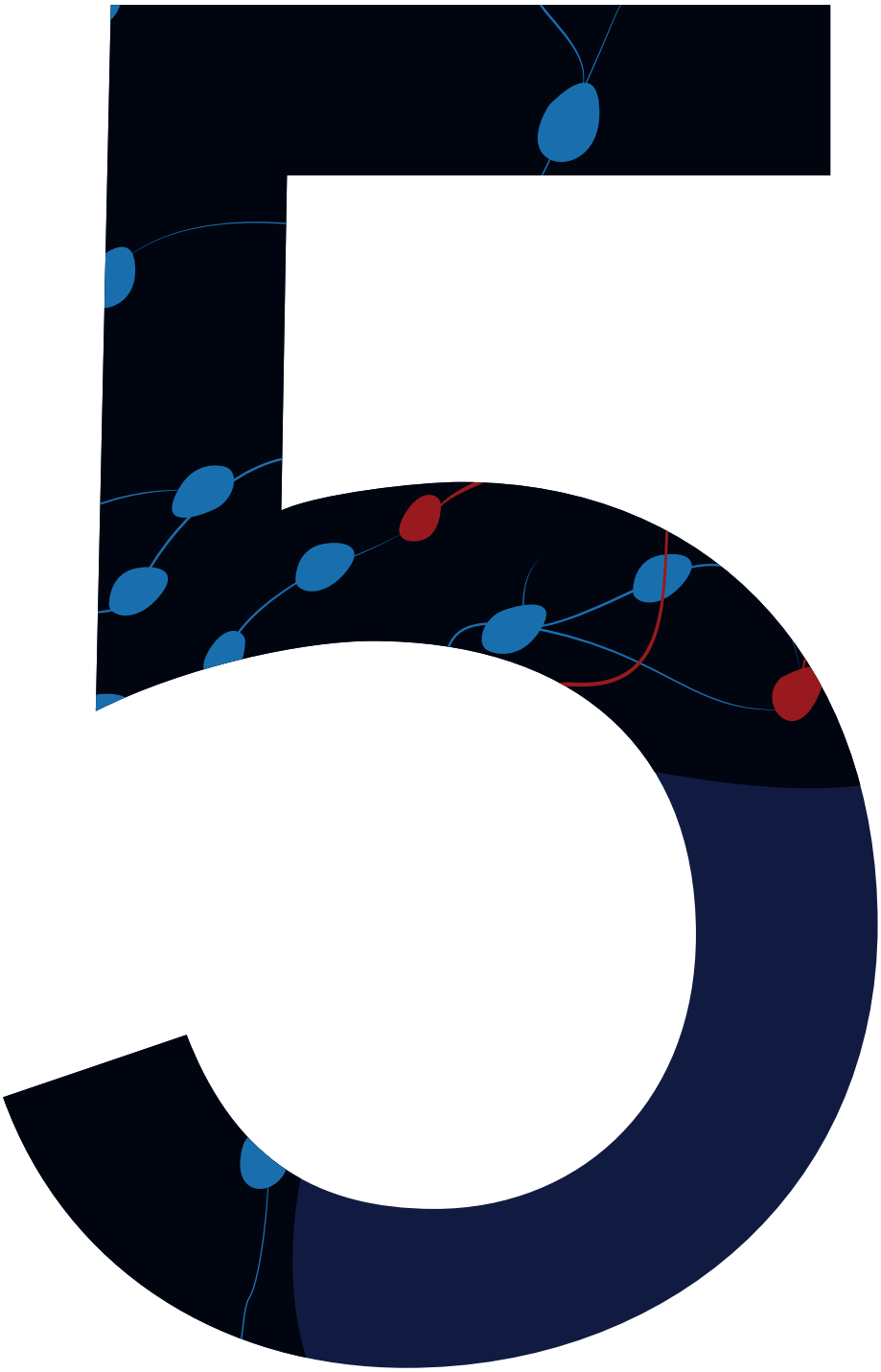

Depth of invasion in early stage oral cancer patients staged by sentinel node biopsy

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ABSTRACT

Background To investigate if depth of invasion (DOI) can predict occult nodal disease in patients with cT1-2N0 (7th TNM) oral squamous cell carcinoma (OSCC) staged by sentinel lymph node biopsy (SNLB).

Methods In 199 OSCC patients DOI measurements and SNLB were performed.

Results Metastases were found in 64 of 199 patients (32%). Of these 64 patients, the mean DOI was 6.6 mm compared to 4.7 mm in patients without metastases ($p = 0.003$). The ROC-curve showed an area under the curve of 0.65 with a most optimal cut-off point of 3.4mm DOI (sensitivity 83% and specificity 47%). Regional metastases were found in 15% of patients with DOI ≤ 3.4 mm.

Conclusion DOI seems to be a poor predictor for regional metastasis in patients with cT1-2N0 OSCC. Therefore, staging of the neck using SLNB in early stage oral cancer patients should also be performed in tumours with limited DOI and probably in T3 (8th TNM) OSCC ≤ 4cm diameter.

INTRODUCTION

In patients with oral squamous cell carcinoma (OSCC) presence of cervical metastases is regarded as the main prognostic factor.[1-4] More recently, sentinel lymph node biopsy (SLNB) in early stage oral cancer is gaining acceptance as a diagnostic staging method for occult regional metastasis. The most recent meta-analysis found a pooled sensitivity of 87% (95% CI 85-89%), a negative predictive value of 94% (95% CI: 93-95%) and an AUC of 0.98 (95% CI: 0.97-0.99).[5] The SLNB procedure detected occult metastases in around 30% of the patients, who will be additionally treated with a complementary (selective) neck dissection or radiotherapy.[6,7]

In case of elective neck dissection (END) as a histopathological staging method, depth of invasion (DOI) of the primary tumour is the most promising predictive factor for nodal metastases.[8-10] Huang et al. performed a meta-analysis and recommended END in case of tumour thickness of ≥4 mm.[8] However, most of their included studies reported on DOI and used the definition according to Moore et al. to measure "from a theoretical reconstructed normal mucosal line to the deepest extent of growth".[11]

The debate in literature is ongoing due to large variation in study groups, measurements techniques and cutoff values.[9] As reported in a recent large study, DOI was associated with a higher incidence of regional failure, but still has a poor sensitivity and specificity for nodal involvement.[12] Brockhoff et al. found different DOI cut-off values for different tumour locations determining a 20% or greater risk of having nodal metastases. They suggested to offer a neck dissection at >2 mm DOI in tongue tumours, 2-3 mm DOI in floor of mouth tumours and 3-4 mm DOI for the retromolar trigone and alveolus/hard palate tumours.[13]

Several studies have been conducted to identify the best predictor for occult nodal disease in patients with early stage oral cancer.[4,14-23] In most of studies, DOI turns out to be the best histopathological predictor for regional metastases.[4,14-19,22] This is also reflected in the new 8th TNM classification in which DOI is now incorporated as histopathological determinant for clinical and pathological T staging.[24-26]

SLNB allows us to histopathologically examine the lymph nodes with the highest risk of containing metastases more precise than routine examination of all lymph nodes in END.[27] In SLNB-negative patients a watchful waiting strategy of the neck renders the opportunity for micrometastasis, which can easily missed by routine histopathological examination of a neck dissection specimen, to become clinical manifest.[28] Therefore,

SLNB can serve as a more accurate reference standard than END for the evaluation of tests predicting the presence of lymph node metastases.

The aim of this study was to assess if DOI of the primary tumour can predict occult nodal disease in patients with a cT1-2N0 (according to 7th AJCC classification) OSCC who underwent SLNB.

MATERIALS AND METHODS

In two Dutch head and neck centers 199 patients were prospectively enrolled between 2007 and 2016. All patients had early stage oral cancer, a clinically negative neck (cT1-T2N0), underwent SLNB as staging method and were treated by means of transoral excision of the primary tumour.

Institutional approval was obtained. Written informed consent was not deemed necessary according to national medical ethical guidelines due to the retrospective nature of the study.

The SLNB procedure was performed according to the EANM/SENT joint practice guidelines as has been previously described.[6,27,29]

The sentinel lymph nodes were histopathologically examined by two experienced head and neck pathologists (SMW and EB). The SLNs were stained with hematoxylin-eosin (H&E) and cytokeratin AE1/3 at step-serial sectioning levels of 150μm. At least 6 levels were investigated. Every sectioning level was also examined with additional keratin immunohistochemistry (IHC) and positive IHC slices were compared to H&E slices to confirm metastases.

For this study, patients with regional metastases during follow-up in case of a negative SLNB (false-negatives), were considered as patients positive for metastases.
Depth of invasion of the primary tumour was measured by use of digital microscopic imaging or ocular micrometer. According to the 8th American Joint Committee on Cancer (AJCC) TNM classification, DOI was considered to be the actual mass present beneath the basement membrane, or in case of ulceration or exophytic lesions the theoretical reconstruction of the basement membrane (Figure 1).[24]

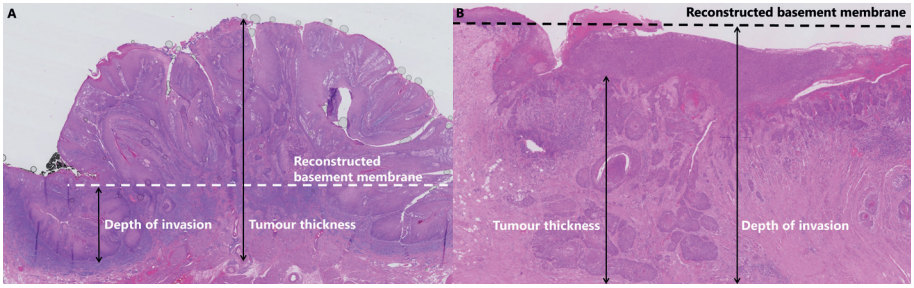


Figure 1. Measuring depth of invasion from the deepest point of invasion – reconstructed basement membrane line in exophytic tumour (A) and ulcerative tumour (B)

Statistical analysis

The Chi-square test and Fisher exact test were used to compare categorical variables. DOI was correlated to nodal status with univariate logistic regression analysis. The receiver operating characteristic (ROC) curve was used to identify a possible cut-off value whereof DOI could serve as optimal predictor for regional metastases (and could act as deciding point performing a "watchful waiting" strategy or SLNB). All statistical analysis was carried out using SPSS 21 for Windows (IBM, Chicago, IL) in cooperation with a statistician.

8th American Joint Committee on Cancer TNM classification

The recent introduction of the 8th AJCC TNM classification system needs special attention since it specifically describes DOI as parameter in staging.[24] The impact of using this system is described.[25,26]

Tumours were staged according to both classifications and incidence of metastases according to T-stage are presented. The 8th TNM classification is also used to see if a better distinction between T stages in overall survival, disease specific survival and isolated regional disease free survival could be made compared to the 7th TNM classification.

RESULTS

In this cohort of 199 cT1-T2N0 patients at least one positive SLN was found in 52 (26%) patients. In another 12 patients with a (false) negative SLNB, regional metastases were encountered during follow-up, which resulted in 64 (32%) patients with regional metastases. In these 64 cases, mean DOI was 6.6 mm (95% CI 5.48-7.68) compared to 4.7 mm (95% CI 4.17-5.21) in patients without regional metastasis (p = 0.003). Patient characteristics are listed in Table 1.

In univariate logistic regression analysis an odds ratio of 1.15 (95% CI 1.05 – 1.26) had been found for increasing DOI per 1 mm with a P-value of 0.002. The ROC-curve

Table 1. Data of demographic and tumour-related patient characteristics

		Histopathological status of the neck	
Characteristics	Overall (%)	Negative (%)	Positive (%)
Patients, No (%)	199 (100%)	135 (68%)	64 (32%)
Gender, No (%)			
Male	100 (50%)	66 (66%)	34 (34%)
Female	99 (50%)	69 (70%)	30 (30%)
Median age (y) (range)	63 (27-87)	64 (27-87)	63 (29-86)
Tumour location, No (%)			
Tongue	121 (61%)	80 (66%)	41 (34%)
Floor of mouth	53 (27%)	38 (72%)	15 (28%)
Buccal mucosa	16 (8%)	11 (69%)	5 (31%)
Inferior alveolar process	5 (3%)	3 (60%)	2 (40%)
Other	4 (2%)	3 (75%)	1 (25%)
Clinical T classification, No (%)*	132 (66%)	103 (78%)	29 (22%)
T1	67 (34%)	32 (48%)	35 (52%)
T2			
Depth of invasion, (mm) (95%CI)	5.3 (4.77-5.81)	4.7 (4.17-5.21)	6.6 (5.48-7.68)
Follow-up, (months) (range)			
Observation time	19 (1-104)	20 (1-104)	17 (1-104)

*T classification according to 7th AJCC classification

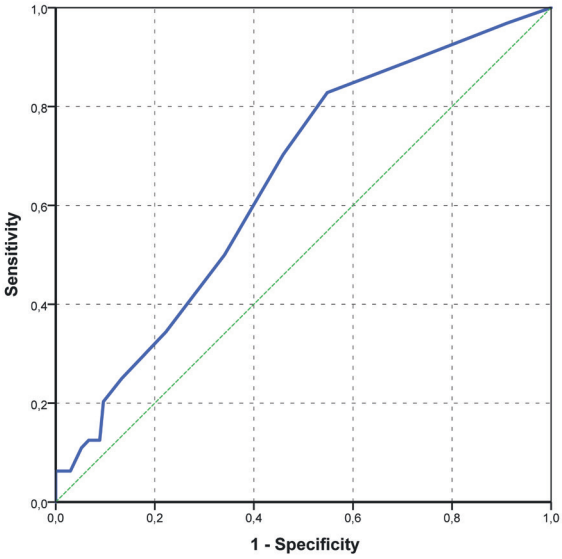
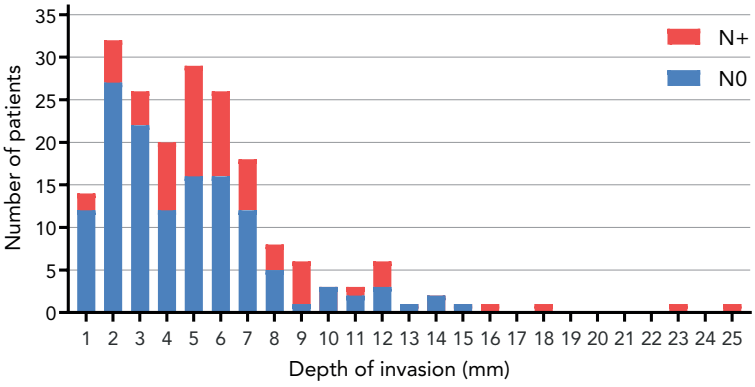


Figure 2. ROC-curve for prediction of presence of lymph node metastasis by depth of invasion, area under the curve of 0.65



N+	2	5	4	8	13	10	6	3	5	0	1	3	0	0	0	1	0	1	0	0	0	0	1	0	1
N0	12	27	22	12	16	16	12	5	1	3	2	3	1	2	1	0	0	0	0	0	0	0	0	0	0

Figure 3. Distribution of nodal metastases per mm depth of invasion

(Figure 2) showed an area under the curve of 0.65 with a most optimal cut-off point on a DOI of 3.4 mm (sensitivity 83%, specificity 47%) (Table 2). Of all patients with tumours ≤ 3.4 mm DOI, still 15% (11/74) had regional metastases, which is illustrated in Figure 3.

8th American Joint Committee on Cancer TNM classification

The change in T-classification (due to the influence of DOI as classification parameter) by using the new TNM staging system (8th edition) is listed in Table 3. In total, 49 pT1 tumours (TNM7) are classified as pT2 (TNM8) and 15 pT1 tumours (TNM7) are classified as pT3 tumours (TNM8).

Table 2. Numbers for different cut-off values

DOI (mm)	Sensitivity	Specificity
1	97	9
2	89	29
3	83	45
4	70	54
5	50	66
6	34	78
7	25	87
8	20	90
9	13	91
10	13	93

Table 3. Shift in T stages according to 8th TNM classification

T stage	7th TNM	8th TNM	Upstaging
pT1	152 (76%)	88 (44%)	-64 (- 42%)
pT2	44 (22%)	92 (46%)	+48 (+109%)
pT3	3 (2%)	19 (10%)	+16 (+533%)
Total	199 (100%)	199 (100%)	

No statistical significant difference between pT1 and pT2 tumours was found for isolated regional disease free survival (Figure 4), disease specific survival and overall survival using either the 7th or 8th edition of TNM classification. Because of the small numbers of pT3 tumours, no statistical analysis was performed comparing pT3 with pT1 and/or pT2 tumours.

Incidence of occult lymph node metastases according to pT classification was analyzed for both classifications and is listed in Table 4, showing in the 8th classification a decreased incidence in all T classifications, particularly for pT3 tumours.

Table 4. Incidence of occult lymph node metastases by T classification

T classification	7th TNM	8th TNM
pT1	40/152 (26%)	17/88 (19%)
pT2	21/44 (48%)	38/92 (41%)
pT3	3/3 (100%)	9/19 (47%)
Total	64/199 (32%)	64/199 (32%)

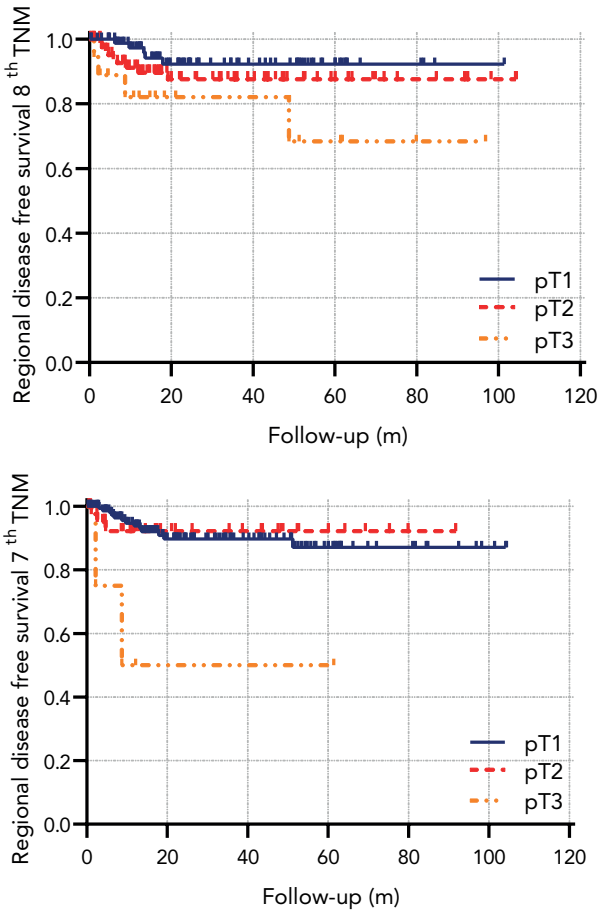


Figure 4. Isolated regional disease free survival analysis with 7th and 8th TNM classification respectively. Comparison between pT1 vs. pT2 in 8th TNM classification did not reach statistical significance (P = 0.231).

DISCUSSION

Based on our results DOI could be considered as predictor for SLN metastases in early stage oral cancer. However, it should be clear that with an AUC of 0.65 in our ROC-analysis the evidence for using it in clinical practice is at least questionable. Fifteen percent off all patients below the cut-off value of 3.4 mm had metastases, which makes it in our opinion reasonable to stage every patient with SLNB, regardless of DOI of the primary tumour.

With this study we could identify metastases with use of the meticulous work-up of sentinel lymph nodes by using step-serial sectioning and additional keratin immunohistochemical staining, or in case of a negative SLNB by regular follow-up. When comparing our results to the published literature some considerations have to be made.

Firstly, it is essential to realize that the majority of published data about DOI and cervical lymph node metastases referred to END (or watchful waiting) as the golden standard.[4,12,14,15,17,19,22,30,36] To our knowledge, in only 3 articles SLNB or SLNB-assisted neck dissections were used as a staging tool.[18,20,37] The routine histopathological work-up of the END is less meticulous and hence presumably less accurate. Indeed, micrometastases remain undetected in up to 13% of routinely processed ENDs.[38,39] Using the SLNB protocol, the presence of metastasis can be determined more precisely within the lymph node with the highest risk (the SLN). Because of the "watchful waiting" strategy in case of a negative SLNB, isolated tumour cells and micrometastases can develop into a clinically detectable metastasis during follow-up. Therefore, in our opinion, SLNB is a more accurate reference standard for staging the clinical negative neck than END.

Secondly, many studies have been published regarding this topic applying different definitions of depth of invasion, infiltration depth and tumour thickness. Originally described by Moore et al. DOI and tumour thickness are not the same.[11] They performed a new measurement from an imaginary mucosal line (also defined in their article as a theoretical reconstruction of a basement membrane) besides the measurement of Breslow and they found a better correlation between survival and DOI by using this new line. This topic was later discussed in detail in the meta-analysis of Pentenero et al. resulting in the recommendation to measure from the (theoretical reconstructed) basement membrane, which is also the recommendation of the AJCC.[9,24] It is essential to realize that measuring from the basement membrane is theoretically not the same as

measuring from the mucosal line, which is mostly described as method for measuring the DOI. However, this is more for theoretical than practical purposes assuming the small thickness of healthy epithelium, so still reliable comparisons between both measurements could be made.

Although both meta-analyses conclude that DOI correlates with regional lymph node involvement, they did mention different study groups, measurement techniques and cut-off values, which hamper good comparison between these studies.[8,9] Both studies found a wide range for cut-off values of 1.5-10 mm, with a most optimal cut-off value of 4 mm in the meta-analysis of Huang et al.[8] A recent large study of 469 patients, which was published after both meta-analyses, used also a cut-off value of 4 mm to show an association between DOI and nodal involvement, though with poor sensitivity and specificity.[12] The optimal cut-off value found in our study (3.4 mm) is close to this value. However, still 15% of our patients below this 3.4 mm cut-off value showed regional metastases. Therefore, in our opinion SLNB should be offered to all patients, also those with limited DOI tumours (Figure 3). Other studies using a ROC analysis to determine this optimal cut-off value found comparable values, i.e. 4 mm and 4.59 mm.[5,19] The study of Goerkem et al. using this analysis did not found an optimal cut-off value.[20] That study and our present one are the only studies that use SLNB-alone as reference standard. In 78 patients Goerkem et al. found an average DOI of 6.45 mm, with an area under the curve of 0.54 in the ROC analysis, concluding that DOI (and separately also tumour thickness) should not be used for assessment of elective treatment of the neck. Moreover, they suggested that SLNB should be used in all early stage oral cavity carcinomas with a cN0 neck.[20]

In another study, by Alkureishi et al., with SLNB (and SLNB-assisted neck dissection) as reference standard a considerable heterogeneity in study groups has to be taken into account when comparing the results with the present study. In this study patients with cT3-T4 tumours and oropharyngeal tumours were included as well.[37] They analyzed a cohort of 172 patients of whom 134 patients had oral tumours with a mean DOI of 7.3 mm. Patients underwent SLNB alone or SLNB-assisted neck dissection, however the number of cases in both groups is unfortunately not reported. This may be important because histopathological examination of a neck dissection specimen is a suboptimal reference standard as compared to watchful waiting. They found nodal metastases in 41% of patients and demonstrated that in both oral and oropharyngeal cancer tumour depth reached a stronger correlation with nodal metastases than T-classification. The most optimal cut-off value for oral cavity cancer alone in their cohort was 4 mm

(sensitivity 83% and specificity 47%). Despite their higher mean DOI a comparable optimal cut-off value with comparable sensitivity and specificity rates has been found for oral cavity tumours only. They concluded that it is hard to predict which patients are at high-risk for occult metastases based on a single tumour depth measurement.
In the article of Bilde et al. DOI (and tumour thickness) were significantly associated with cervical lymph node metastases with a cut-off value of 4 mm, however no statistical analyses were presented for substantiation of this cut-off value.[18] In addition, all patients were treated with SLNB-assisted neck dissection and the median tumour depth was 3.5 mm which makes an appropriate comparison with other studies difficult. During the last years DOI of the primary tumour is recognized to be of increasing value with respect to regional metastases and survival. A large international study demonstrated that using DOI with intervals of 5 mm improves discrimination in outcome.[40] This is also reflected in the 8th TNM staging system by Amin et al. in which DOI, together with diameter of the tumour, classifies for T classification.[25] With respect to our data, a large shift in pT classification was observed by using this new classification which is in agreement with other studies.[40,43] Interestingly, also the incidence numbers altered in the 8th classification (Table 4). In the pT3 (8th TNM classification) group, 47% of the patients showed regional metastases. In our opinion, these data suggest that SLNB could be helpful in patients with pT3cN0 OSCC ≤ 4 cm diameter, selecting more than half of them to avoid an unnecessary elective neck dissection.

Interestingly, with survival analyses for the 8th TNM classification slightly (but statistically not significant) better distinction was only observed in isolated regional disease free survival, while we expected to distinguish better in all survival analyses. Why we did not reach a evident correlation is hard to explain. Evidently, only early stage oral cancers were included. Possibly, pooling all T classifications, (T1-T4) the new classification generally provides a better distinction compared to the 7th classification in our group of patients. These data have to be investigated in future research. In addition, this cohort is obviously smaller and with a shorter follow-up in contrast to the previous analyses on which this new classification was based.[25,40] However, also Dirven et al. did not find a satisfying discrimination between pT1 and pT2 with respect to survival analyses in the 8th classification, although a comparison with the 7th classification was not established.[42]

Reliable clinical application of the TNM-8 staging system is challenging. Most articles are based on specimen driven DOI measurements, while for pretreatment decision making DOI has to be clinically assessed. Lydiatt et al. describe that clinical

examination of DOI requires careful palpation and attention to detail, supplemented by radiographic assessment.[26]

Recently, a meta-analysis found a high correlation (r = 0.88) between intraoral ultrasono-graphy and histopathological thickness measurements.[44] Furthermore, Alsaffar et al. described a good correlation between clinical assessment, MRI and pathology, particularly in thicker tumours.[45] It should be clear that with the introduction of the 8th classification system, further research in preoperative measurements of DOI is required.

In conclusion, depth of invasion seems to be a poor predictor for regional metastasis in patients with cT1-2N0 OSCC. Therefore, staging of the neck using SLNB in early stage oral cancer patients should also be performed in tumours with limited depth of invasion and probably in T3 (8th TNM) OSCC ≤ 4 cm diameter.

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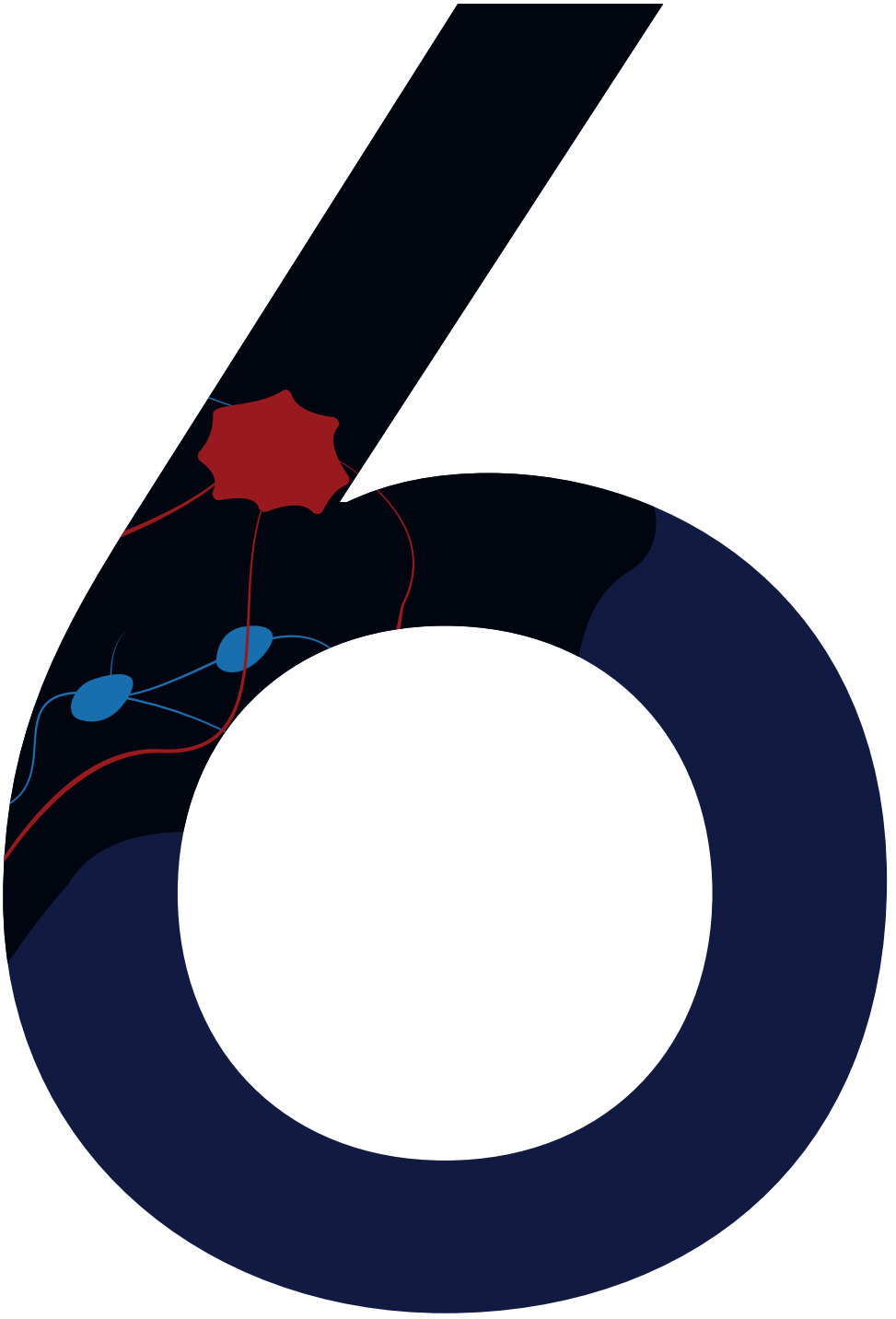

High rate of unexpected lymphatic drainage patterns and high accuracy of sentinel lymph node biopsy in oral cancer after previous neck treatment

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ABSTRACT

Rationale This study evaluates the lymphatic drainage patterns and determines the accuracy of the sentinel lymph node biopsy (SLNB) in patients diagnosed with a cT1-2N0 OSCC and a history of neck surgery or radiotherapy in three Dutch head and neck centers.

Materials and Methods Retrospective analysis of 53 cT1-2N0 OSCC patients,who underwent SLNB between 2007 and 2016, after a history of neck surgery or radiotherapy. Ten patients had previous treatment of the neck only contralateral from the current tumour. These ten patients were not used for the analysis of lymphatic drainage patterns. The 43 patients with previous ipsilateral or bilateral treatment of the neck had a history of ipsilateral SLN extirpation (n=9; 21%), neck dissection (n=16; 37%), radiotherapy (n=10; 23%), or combined neck dissection and radiotherapy (n=8; 19%).

Results SLNs were detected in 45 patients, resulting in an identification rate of 85% (45/53). Three patients (7%) had at least one positive SLN. One patient (1/45; 2%) was diagnosed with regional recurrence during the follow-up after a negative SLNB (sensitivity 75%, negative predictive value 98%). The first SLN was detected in level I-III in 58% of the patients, unexpected drainage patterns were observed in 30% (first SLN level IV 9% and level V 5% and contralateral neck in well-lateralized tumours 16%). In 12% no lymphatic drainage pattern was visible.

Conclusions SLNB seems to be a reliable procedure for neck staging of cT1-2N0 OSCC patients with a previously treated neck. SLNB determines the individual lymphatic drainage patterns, enabling visualization of unexpected drainage pattern variability in 30% of these patients.

INTRODUCTION

Presence of lymphatic metastases in the neck is consistently observed as main prognostic factor in patients with oral squamous cell carcinoma (OSCC).[1-3] Sentinel lymph node biopsy (SLNB) proved to be reliable as diagnostic staging modality for detection of occult lymph node metastases: in a large recent meta-analysis a pooled sensitivity of 87% (95% CI 85-89%), a negative predictive value of 94% (95% CI 93%-95%) and an AUC of 0.98 (95% CI 0.97-0.99) were found.[4] These meta-analysis results are based on patients with primary OSCC and a previously untreated neck. Despite the relatively common local recurrences and second primary tumours in head and neck cancer, only one study of Flach et al. reported about the accuracy of the SLNB in 22 patients with a previously treated neck.[5]

It is well known that patients with OSCC suffer a high risk for local recurrences (10-30%) and an annual risk of 3-4% for developing second primary tumours.[3,6-8] Previous treatment of the neck most likely alters lymphatic drainage patterns. Current evidence about the drainage patterns in previously treated OSCC patients using SLNB is limited to a study by Flach et al. (n=22) and a feasibility study by Pitman et al. (n=5).[5,9] Experience of alteration in lymphatic drainage patterns after previous treatment has also bene reported in breast cancer and melanoma.[10-14] While gaining more and more experience with SLNB in our institutions during the last years, SLNB has been used increasingly as staging method in patients with a previously treated neck. Moreover, SLNB is valuable in assessment of the individual lymphatic drainage patterns, compensating for potential variabilities as a result of previous treatment which were reported in 67% of the cases by Flach et al.[5]

However, since the study of SLNB in OSCC patients with a previously treated neck consisted of only 22 patients, more research had to be performed to confirm the findings of that study.[5] The aim of this study was to assess the accuracy of SLNB and secondly, to evaluate the lymphatic drainage patterns in a consecutive cohort of cT1-2N0 patients with a previously treated neck in three Dutch head and neck cancer centers.

METHODS

In three Dutch head and neck centers 53 patients diagnosed between 2007 and 2016 met the inclusion criteria and were retrospectively analyzed. Patients with early stage local recurrent disease or second (or even third) primary squamous cell carcinoma of the oral cavity or oropharynx with a clinically negative neck and surgical resection of the tumour combined with SLNB staging of the neck were included (cT1-2N0, following the 7th TNM staging classification, Table 1). In their history, all patients had received prior treatment of the neck with SLNB, neck dissection, (chemo)radiotherapy or a combination of these modalities (supplementary data 1). Twelve patients were previously included in the study by Flach et al, their follow-up was updated.[5]

The SLNB procedure was described extensively before.[15,16] Briefly, patients received preoperatively injections with [^{99}mTc]Tc-nanocolloid. followed by dynamic and static lymphoscintigraphy and SPECT/CT scanning one day before surgery, intra-operatively gamma probe detection and postoperative step serial sectioning of the sentinel lymph node with additional immunohistochemical keratin staining.

As visualized in our study design (Figure 1) all 53 patients were used for analysis regarding the accuracy of the procedure and 43 patients were included for the drainage pattern analysis. Earlier studies showed the potential of bilateral drainage patterns in well-lateralized patients. Because of this potential bilateral drainage also 10 patients were included with a history of only contralateral treatment of the neck (their first tumour was contralateral of the second) whom might affect the SLNB accuracy.[16,18] In OSCC lymphatic drainage is at least expected in level I – III at the ipsilateral side of the neck.[17] With the second aim to detect unexpected drainage patterns, only 43 patients with previous treatment of the ipsilateral side of the neck were used for lymphatic drainage pattern analysis.

In this study, definition of lateralization of the neck is related to the site of the local recurrence or second primary tumour.

Ethical consideration

Due to the retrospective design no approval was required from the hospital research ethics board of our centers according to the Dutch ethical regulations. SLNB was part of the standard management of these patients and patient information regarding clinical and pathological characteristics and follow-up was retrospectively collected from electronic patient files.

Table 1. Patient characteristics. Ipsilateral and contralateral side of the neck is related to the side of the local recurrence or the second primary.

Characteristics	No.	(%)
Total number of patients	53	(100)
Gender		
Male	29	(55)
Female	24	(45)
Age y mean (SD)	65	(55 - 75)
(range)		(44 - 88)
pT status (7th TNM)		
1	44	(83)
2	9	(17)
Tumour locations		
Tongue	31	(58)
FOM	9	(17)
Buccal mucosa	5	(9)
Inferior alveolar process	4	(8)
Other	4	(8)
Previous treatment or surgery ipsilateral neck		
No	10	(19)
RT alone	8	(15)
ND alone	16	(30)
ND + RT	8	(15)
CRT	2	(4)
SLNB	9	(17)
Previous treatment or surgery contralateral neck		
No	25	(47)
RT alone	9	(17)
ND alone	6	(11)
ND + RT	6	(11)
CRT	2	(4)
SLNB	5	(9)
Follow-up		
Follow-up time months, median (IQR)	26	(13 - 42)
Regional recurrence	1	(1)
Death	13	(25)
Death of local recurrence or second primary	4	(8)

Abbreviations: FOM, floor of mouth; RT, radiotherapy; ND, neck dissection; CRT, chemoradiation; SLNB, sentinel lymph node biopsy

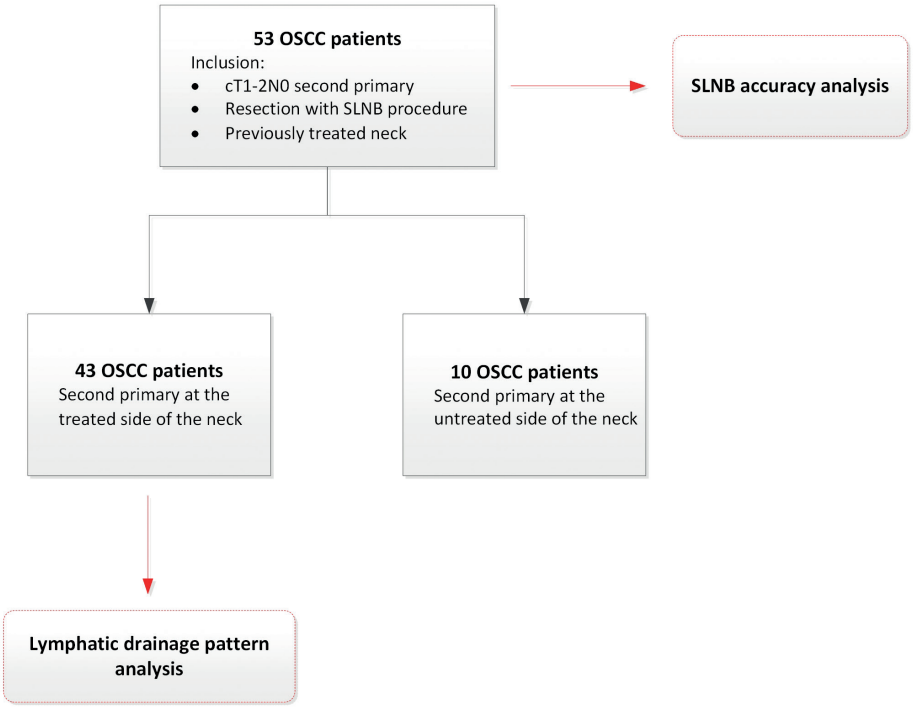


Figure 1. Study design

All 53 patients were used for the SLNB accuracy analysis, only the 43 patients with a history of neck treatment at the ipsi- or bilateral side were used for the analysis of altered lymphatic drainage patterns.

RESULTS

The data of 53 patients, 29 male (55%) and 24 female (45%) were used for analysis. Mean age was 65 years. Tongue was the most affected tumour location (59%), followed by floor of mouth. Forty–four patients (83%) were diagnosed with a pathologically T1 tumour and 9 patients (17%) with a T2 tumour. These and other characteristics are summarized in Table 1. Characteristics per patient are given in supplementary data 1.

SLNB accuracy

Fifty-three patients were used for the SLNB accuracy analysis. Neck dissection, with or without postoperative radiotherapy, was seen most as previous treatment in both the ipsilateral and contralateral neck compared to the local recurrence or second primary side (Table 1). Thirteen patients (25%) died during follow-up of which four (8%) died as a result of the local recurrence or second primary tumour in the oral cavity (disease specific death: median 26 months, IQR 13 – 42 months).

No SLNs were visualized by lymphoscintigraphy in 7 of these 53 patients resulting in an 87% imaging detection rate. In one patient no SLNs were detected intraoperatively, despite preoperative visualization. In two patients with bilateral drainage on lymphoscintigraphy the SLNs were not detected in one neck side intraoperatively, but were harvested in the other side of the neck, resulting in a surgical detection rate of 93% (43/46, supplementary data 1). In total, at least one SLN was harvested in 85% of the patients (45/53). Three patients had a positive SLN, respectively in the ipsilateral neck with a history of a SLNB, in the ipsilateral neck without a history of pretreatment and in the ipsilateral neck with a history of chemoradiation therapy. In the first two patients, no additional metastases were detected after harvesting respectively 21 and 17 lymph nodes in the completed neck dissection specimens. Because of the history of chemoradiation and the metastasis size (ITC), the last patient received watchful waiting instead of a neck dissection. These 3 patients did not show regional disease during follow-up.

One patient (2%) was diagnosed with regional recurrence without local disease in level II at the ipsilateral side of the neck after 7 months of follow-up. This patient had a second primary tumour located in the buccal mucosa and only negative SLNs were found in level I at the contralateral side. This patient was previously treated with a MRND at the ipsilateral side of the neck for the first primary tumour, followed by postoperative chemoradiation at both sides of the neck. This patient was still alive after 19 months of follow-up after the regional recurrence was surgically removed and postoperatively irradiated.

One regional recurrence resulted in a 75% sensitivity with a 95% CI of 22% – 98% (3 of 4 true positive) and 98% NPV with a 95% CI of 88% – 100% (42 of 43 true negative) of the SLNB in patients with a previously treated neck.

If we restrict the accuracy analysis to patients with a history of neck dissection and/or radiotherapy in the ipsilateral neck, one out of 34 patients showed a positive SLN and one patient showed regional recurrence after a negative SLNB, resulting in a 50% sensitivity (1 of 2 true positive) with a 95% CI of 3%-97% and a NPV of 97% (32 of 33 true negative) with a 95% CI of 82%-100%.

Lymphatic drainage patterns

In 38 of the 43 patients with a second primary or local recurrence at the previously treated neck side SLNs were detected, resulting in an 88% identification rate. The five patients without detectable SLNs had in common a history of radiotherapy of the neck (supplementary data 1). Since lymphatic drainage is expected generally in levels I-III for OSCC, in 30% (13/43) patients unexpected drainage was found. Of these 13 patients, four patients showed SLNs located ipsilaterally in level IV as closest located SLN, in two patients this closest location was ipsilaterally in level V. Seven patients had only SLNs located contralateral from the side of the well-lateralized local recurrence or second primary tumour (supplementary data 1). Besides a lower identification rate, unexpected drainage was more common in patients with a history of neck irradiation compared to patients with a history of a SLNB and comparable to patients with a previous neck dissection, respectively 40% versus 11% and 38%. However the highest unexpected drainage was found after a history of neck dissection combined with postoperative radiotherapy (88%). Localization of harvested SLNs per patient and per different prior treatment are given in supplementary data 2. Some SLNs were found in earlier dissected neck levels. For example, eight of the 13 patients with a history of a selective supraomohyoid neck dissection had SLNs located in level I – III, also three of the seven patients with a history of a MRND had SLNs located in level II – IV (supplementary data 2).

If we restrict the drainage pattern analysis to patients with a history of treatment of the ipsilateral neck, unexpected drainage patterns were found in 12 (35%) of the 34 patients and no drainage to any side of the neck was found in 5 patients (12%).

DISCUSSION

This study demonstrates that SLNB in a previously treated neck can be performed with a high accuracy (sensitivity 75%, NPV 98%). In this study unexpected lymphatic drainage patterns were found in 30% of the patients and no drainage was found in 12% of the patients.

SLNB in early stage OSCC has been frequently described in literature during the last decade with high sensitivity rates and negative predictive values.[4] SLNB was initially implemented in our institutions for patients with primary OSCC without previous treatment of the neck. However, after gaining more experience with SLNB, this staging technique was also extended to patients with a previously treated neck.[5] As a result of the previous treatment, lymphatic drainage patterns could be disrupted resulting in aberrant drainage patterns compared to primary OSCC. Lack of knowledge about these aberrant drainage patterns resulted in missing a standard neck staging and standard elective neck dissection in previously treated patients. Flach et al. showed in a study of 22 patients that the SLNB could be useful in previously treated patients with a high sensitivity and negative predictive value for neck staging and especially for assessment of the individual lymphatic drainage patterns after previous treatment.[5]
As mentioned in the introduction, only one feasibility study and the above mentioned study of Flach et al. are published for SLNB in patients with a pretreated neck.[5,9] However, interesting studies in a variety of tumour types have been published regarding SLNB in recurrent or second primary tumours. In a recent meta-analysis of aberrant lymphatic drainage in recurrent breast cancer an 59.6% intraoperatively SLN identification rate was found.[10] The authors concluded that SLNB in these patients avoided unnecessary axillary lymph node dissection and provide targeted localized surgery.[10] Similarly, in recurrent vulvar cancer the SLNB procedure seemed feasible, although the authors stated that the procedure appears technically more challenging compared to initial surgery. In a cohort of 27 patients, SLNs were found in two groins at unpredicted localizations and four lateral tumours showed bilateral SLNs.[18] Beasley et al. reported about the feasibility of SLNB in recurrent melanoma (107 patients) and also found in 24% of the patients additional sites of SLNs compared to the first SLNB procedure.[19]

Although it is difficult to compare different tumour types, a trend towards a lower identification rate of SLNs compared to untreated patients was observed in present and all above mentioned studies. The most common explanation is the damage of

lymphatic pathways due to prior treatment and a more difficult technical procedure to harvest SLNs in previously treated nodal basins. In untreated OSCC identification rates of 97-98% have been reported, while in this study a rate of 85% was found.[15,16,20,21] All patients without harvested SLNs had radiotherapy in history, sometimes combined with surgery. This lower identification rate was not observed in patients with a prior SLNB procedure, possibly reflecting that SLNB ensures less damage to lymphatic vessels compared to radiotherapy. Furthermore, despite the lower identification rate in previously treated patients no lower NPV of the SLNB for neck staging was found in this study. This might indicate that lymphatic drainage patterns in these patients are not only aberrant, but may even be absent. Nonetheless, this study included only three patients with positive SLNs and one patient with a regional recurrence after a negative SLNB procedure. Due to the low number of SLN positive patients and regional recurrences, it might be prematurely to conclude that SLNB is a reliable procedure in previously treated patients. This is also reflected in a sensitivity rate with a wide 95% CI. However, the high NPV of 98% with a 95% CI of 88%-100% strongly suggest that SLNB is a promising procedure for these pretreated patients, but its reliability needs further investigation.

Although surgery of the lymphatic drainage patterns is part of the SLNB procedure, the procedure is strictly not part of the treatment but belongs to the diagnostic modalities for neck staging. Therefore subanalysis of patients with a history of neck treatment (neck dissection and/or radiotherapy) are presented in the results regarding the accuracy of the SLNB procedure and lymphatic drainage patterns. These figures indicate that in OSCC patients who had undergone more extensive treatment of the neck (i.e. neck dissection and/or radiotherapy) lymphatic drainage follow more frequently an unexpected pattern or was absent (35% vs. 30%). Due to the low number of lymph node metastases (2 and 3) the sensitivity of SLNB (50% and 75%) could not sensibly be compared.

Unexpected drainage pathways are generally reported in all tumour types, including our study. These findings strengthen the value of SLNB in assessing the individual lymphatic drainage pattern. In patients who received already prior treatment (e.g. radiotherapy) it is perhaps even more important to select the actual lymph nodes at risk for metastasis, considering the fact that treatment options are limited due to their prior therapy. In this study an overall unexpected drainage pattern was found in 30% of the patients, which was most frequently found after prior radiotherapy (40%) and especially when this was preceded by a neck dissection (88%). In early stage OSCC

patients with an untreated neck unexpected drainage patterns were reported in up to 16% in a large multicenter trial.[22]

Even though it is well possible to determine individual drainage patterns with the SLNB, one of the disadvantages is to perform an additional neck dissection during a second surgical procedure in case of a positive SLNB procedure. Although improvements, a recent review concluded that still no other modality (e.g., ultrasound, CT, MRI and PET-CT) is accurate enough to detect occult metastasis preoperatively in a clinically negative neck reliably.[23] Moreover, posttreatment effects and the high rate of unexpected drainage in pretreated patients might affect the sensitivity of these modalities in detecting occult metastasis.

A limitation of the accuracy analysis is the low number of metastasis and regional recurrences in our cohort. A possible explanation for these low numbers compared to untreated patients (with an often reported risk of nodal metastases of approximately 25-30%) could be our close follow-up scheme after treatment of their first tumour. Patients in follow-up are potentially earlier diagnosed with recurrent or second primary OSCC, which might cause a relatively high number of early T1 tumours in this cohort. Despite these limitations, this study showed that metastasis appear in early stage local recurrences and second primary tumours. Currently, no guidelines about neck treatment are available for cT1-2N0 OSCC patients with a previously treated neck. In untreated OSCC prognosis was better after an elective neck dissection (of the standard lymph node levels at risk for metastasis) compared to a 'wait and see' policy.[24] Because of the aberrant drainage patterns, we advocate to use the SLNB also in patients with early stage second primaries or local recurrences to select patients who might benefit from treatment of the neck. However, more extensive research is needed to confirm that this strategy actually improves the prognosis of these patients.

CONCLUSION

SLNB seems to be a reliable procedure for neck staging of cT1-2N0 OSCC patients with a previously treated neck. Moreover, SLNB determines the individual lymphatic drainage patterns, enabling visualization of drainage pattern variability in 30% of these patients.

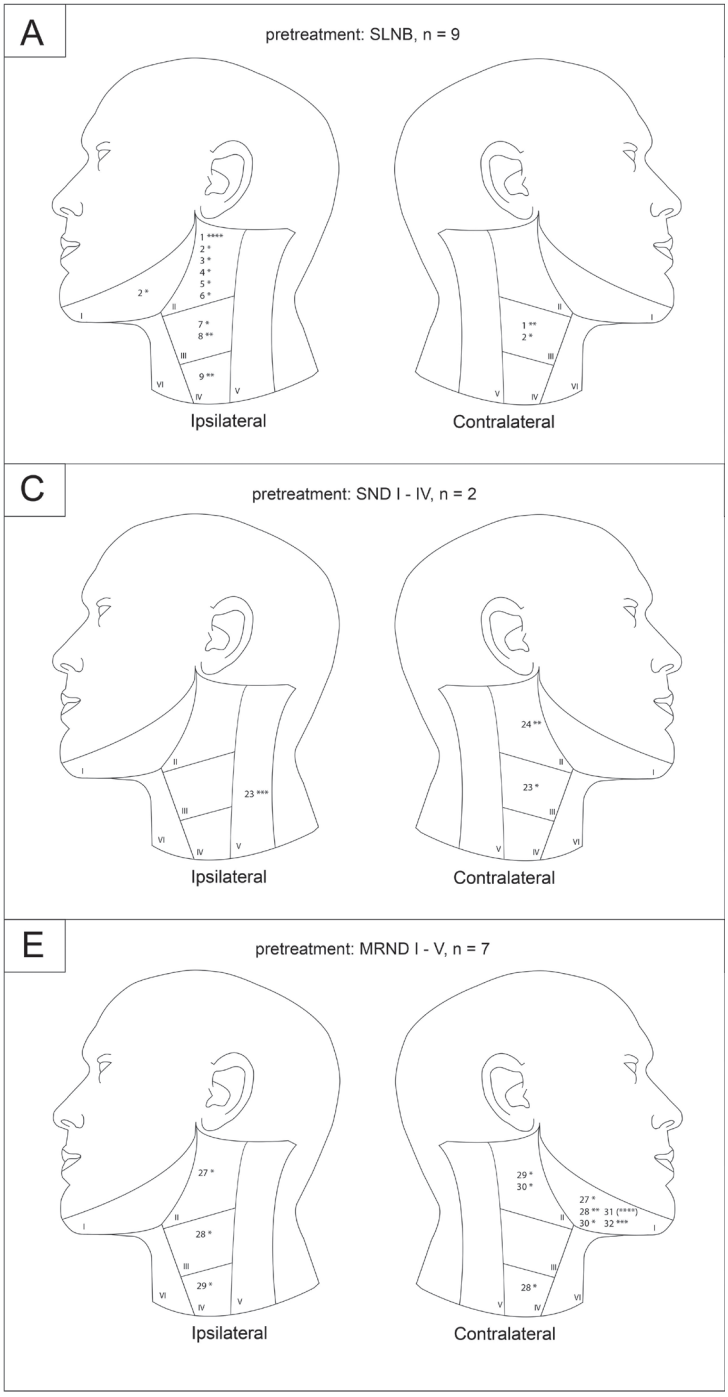
Supplementary data 1

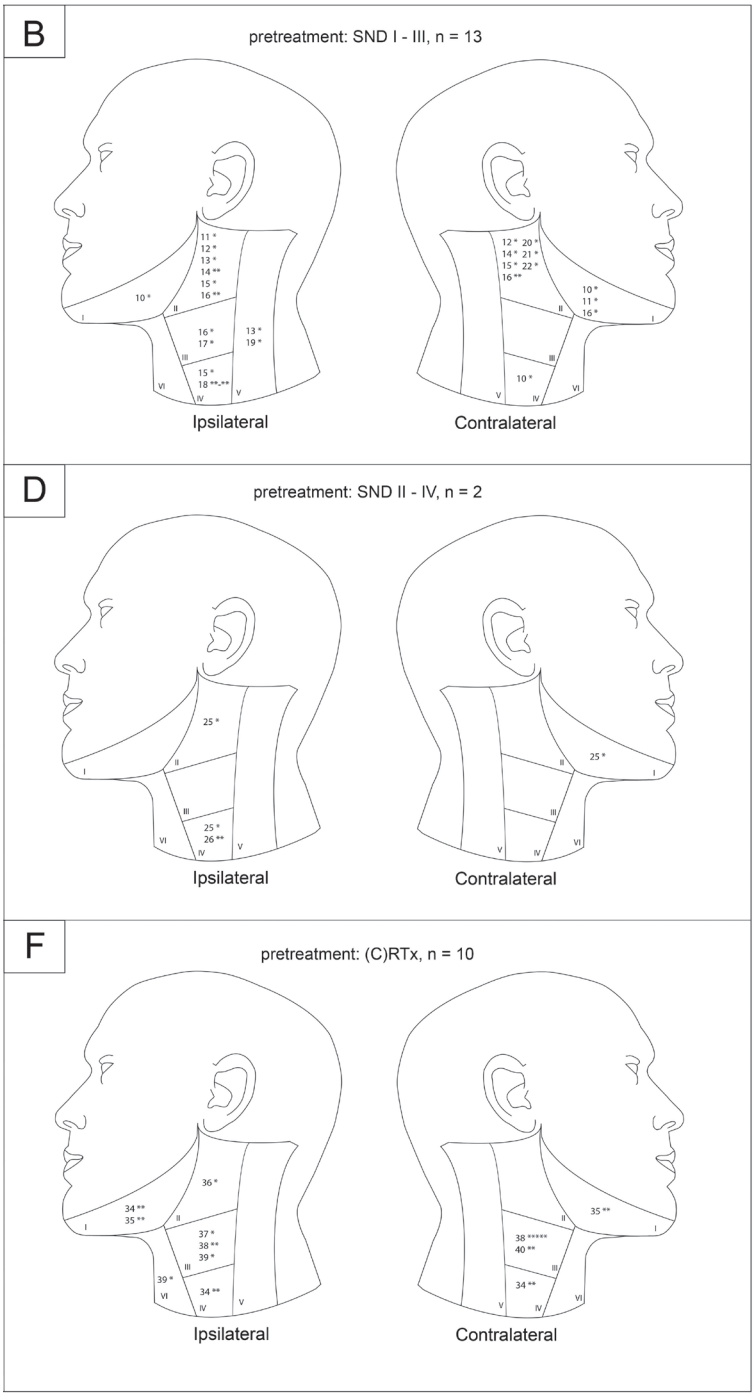
Abbreviations: 7th, 7th TNM classification; RT, radiotherapy; CRT, chemoradiotherapy; ND, neck dissection; MRND, modified radical neck dissection; SLNB, sentinel lymph node biopsy; FoM, floor of mouth; MFH, Malignant Fibrous Histiocytoma; CIS, Carcinoma in situ.

Number	Sex	Age	cT	cN	pT	pN	Tumor		History	History	Lymphatic	SLNB	Head neck
							location	Side	ipsilateral	contralateral	drainage patterns	positive	oncology history
1	M	67	1	0	1	0	Tongue	L	SLNB	No treatment	Ipsi and contralateral	No	T1N0 Tongue
2	F	61	1	0	1	0	Floor of mouth	M	SLNB	No treatment	Ipsi and contralateral	No	T1N0 FoM
3	F	71	1	0	1	0	Tongue	R	SLNB	SLNB	Ipsilateral	No	TT2N0 Tongue
4	F	49	1	0	1	0	Tongue	L	SLNB	No treatment	Ipsilateral	No	T1N0 FoM
5	F	44	1	0	1	0	Tongue	R	SLNB	No treatment	Ipsilateral	No	T1N0 Tongue
6	M	55	1	0	1	1	Tongue	R	SLNB	MRND	Ipsilateral	Yes, micro	T1N1 FoM
7	F	71	1	0	1	0	Floor of mouth	R	SLNB	SLNB	Ipsilateral	No	T1N0 FoM
8	F	59	1	0	1	0	Tongue	L	SLNB	SLNB	Ipsilateral	No	T2N1 Tongue
9	M	73	1	0	1	0	Tongue	L	SLNB	No treatment	Ipsilateral	No	T1N0 Tongue
10	M	59	1	0	2	0	Tongue	L	Selective ND I-III	No treatment	Ipsi and contralateral	No	(1) T1N0 Buccal mucosa, (2) T1N0 FoM
11	M	50	1	0	1	0	Tongue	L	Selective ND I-III	No treatment	Ipsi and contralateral	No	T1N0 Tongue
12	F	88	2	0	2	0	Hard palate	M	Selective ND I-III	No treatment	Ipsi and contralateral	No	T4N0 Superior alveolar process
13	F	49	1	0	1	0	Tongue	L	Selective ND I-III	No treatment	Ipsilateral	No	T1N2b Tongue
14	F	82	1	0	1	0	Buccal mucosa	L	Selective ND I-III	No treatment	Ipsi and contralateral	No	(1) T2N0 Inferior alveolar process, (2) T1Nx Buccal mucosa
15	M	75	1	0	1	0	Buccal mucosa	L	Selective ND I-III	No treatment	Ipsi and contralateral	No	T2N0 Buccal mucosa
16	F	76	1	0	2	0	Inferior alveolar process	L	Selective ND I-III	No treatment	Ipsi and contralateral	No	T1N0 Inferior alveolar process
17	M	74	1	0	1	0	Tongue	L	Selective ND I-III	No treatment	Ipsilateral	No	T1N0 Tongue
18	M	71	1	0	1	0	Tongue	R	Selective ND I-III	No treatment	Ipsi and contralateral	No	T1N0 Tongue
19	F	51	1	0	1	0	Buccal mucosa	R	Selective ND I-III	Selective ND I-III	Ipsilateral	No	(1) T4aN0 Buccal mocusa, (2) T1N0 Tongue
20	M	78	1	0	2	0	Tongue	R	Selective ND I-III	No treatment	Contralateral	No	T1N0 Tongue
21	M	72	1	0	2	0	Floor of mouth	L	Selective ND I-III + RT	No treatment	Contralateral	No	(1) Retromolar trigone, (2) T2N0 Buccal mucosa
22	F	63	2	0	1	0	Tongue	R	Selective ND I-III + RT	No treatment	Contralateral	No	T2N1 Tongue
23	F	51	2	0	2	0	Tongue	L	Selective ND I-IV	No treatment	Ipsi and contralateral	No	T2N1 Tongue
24	F	68	1	0	1	0	Tongue	L	Selective ND I-IV	No treatment	Contralateral	No	T1N2b Tongue
25	M	51	1	0	1	0	Tongue	L	Selective ND II-IV	Selective II-IV	Ipsi and contralateral	No	(1) MFH grade 2, (2) T2N2b Hypopharynx

26	M	57	1	0	1	0	Floor of mouth	R	Selective ND II-IV	MRND	Ipsilateral	No	T2N0 Tonsil
27	F	73	2	0	1	0	Inferior alveolar process	L	MRND	No treatment	Ipsi and contralateral	No	Tongue
28	M	60	1	0	1	0	Tongue	L	MRND + RT	No treatment	Ipsi and contralateral	No	T2N0 Tongue
29	F	59	1	0	1	0	Tongue	R	MRND + RT	RT alone	Ipsi and contralateral	No	T2N2b Tongue
30	M	68	1	0	1	0	Tongue	R	MRND + RT	No treatment	Contralateral	No	T2N2b Hypopharynx
31	F	67	2	0	2	0	Buccal mucosa	R	MRND + CRT	CRT	Contralateral	No	(1) T1N0 Buccal mucosa, (2) T2N0 Inferior alveolar process
32	M	71	2	0	1	0	Tongue	R	MRND + RT	Selective ND I-IV + RT	Contralateral	No	Larynx
33	M	66	1	0	1	0	Tongue	L	MRND + RT	Selective ND I-IV + RT	No	NA	T4N1 Larynx
34	M	58	1	0	1	0	Floor of mouth	L	RT alone	RT alone	Ipsi and contralateral	No	T1N1 Supraglottic larynx
35	F	69	1	0	1	0	Floor of mouth	R	RT alone	RT alone	Ipsi and contralateral	No	T2N0 Supraglotic larynx
36	F	60	1	0	1	0	Tongue	L	RT alone	RT alone	Ipsilateral	No	T2N0 Glottic larynx
37	M	74	1	0	1	0	Tongue	R	RT alone	RT alone	Ipsilateral	No	T4N0 Larynx
38	F	62	1	0	1	0	Uvula	L	RT alone	RT alone	Ipsi and contralateral	No	T3N0 Uvula
39	M	80	1	0	1	1	Tongue	R	CRT	No treatment	Ipsilateral	Yes, itc	(1) T4N1 Tongue, (2) T1Nx Tongue
40	F	60	1	0	1	0	Tongue	R	RT alone	No treatment	Contralateral	No	T1N0 Oropharynx
41	M	69	1	0	1	0	Floor of mouth	P	RT alone	RT alone	No	NA	T2N1 Tongue
42	M	58	1	0	1	0	Buccal mucosa	R	RT alone	No treatment	No	NA	Myxofibrosarcoma maxillary sinus
43	M	57	1	0	1	0	Floor of mouth	R	CRT	CRT	No	NA	(1) T3N0 Supraglottic larynx, (2) T1N0 FoM
44	M	76	1	0	1	0	Tongue	R	No treatment	MRND	Ipsilateral	No	T1N0 Tongue
45	M	61	2	0	1	1	Floor of mouth	L	No treatment	RT alone	Ipsi and contralateral	Yes, macro	T4aN1 Larynx
46	M	70	2	0	2	0	Retromolar trigone	L	No treatment	RT alone	Ipsilateral	No	(1) T2N0 Soft palate, (2) CIS FoM
47	M	81	1	0	1	0	Tongue	R	No treatment	SLNB	Ipsilateral	No	T2N0 Tongue
48	M	49	1	0	1	0	Tongue	L	No treatment	SLNB	Ipsilateral	No	T1N0 Tongue
49	F	66	1	0	1	0	Tongue	L	No treatment	MRND + RT	No	NA	T2N2b Tongue
50	M	57	1	0	1	0	Tongue	L	No treatment	MRND + RT	No	NA	T2N0 Tongue
51	M	52	1	0	1	0	Pharyngeal arch	R	No treatment	MRND + RT	Ipsilateral	No	T2N3 Tonsil
52	F	60	1	0	1	0	Inferior alveolar process	L	No treatment	MRND	Contralateral	No	T1N1 Inferior alveolar process
53	F	71	1	0	2	0	Inferior alveolar process	R	No treatment	Selective ND I-III + RT	No	NA	T2N0 Tongue

Supplementary data 2




B
pretreatment: SND I - III, n = 13
11 *
12 *
13 *
14 **
15 *
16 **
10 *
16 *
17 *
13 *
19 *
15 *
18 **-**
12 * 20 *
14 * 21 *
15 * 22 *
16 **
10 *
11 *
16 *
10 *
Ipsilateral
Contralateral
D
pretreatment: SND II - IV, n = 2
25 *
25 *
26 **
25 *
Ipsilateral
Contralateral
F
pretreatment: (C)RTx, n = 10
36 *
34 **
35 **
37 *
38 **
39 *
39 *
34 **
35 **
38 *****
40 **
34 **
Ipsilateral
Contralateral

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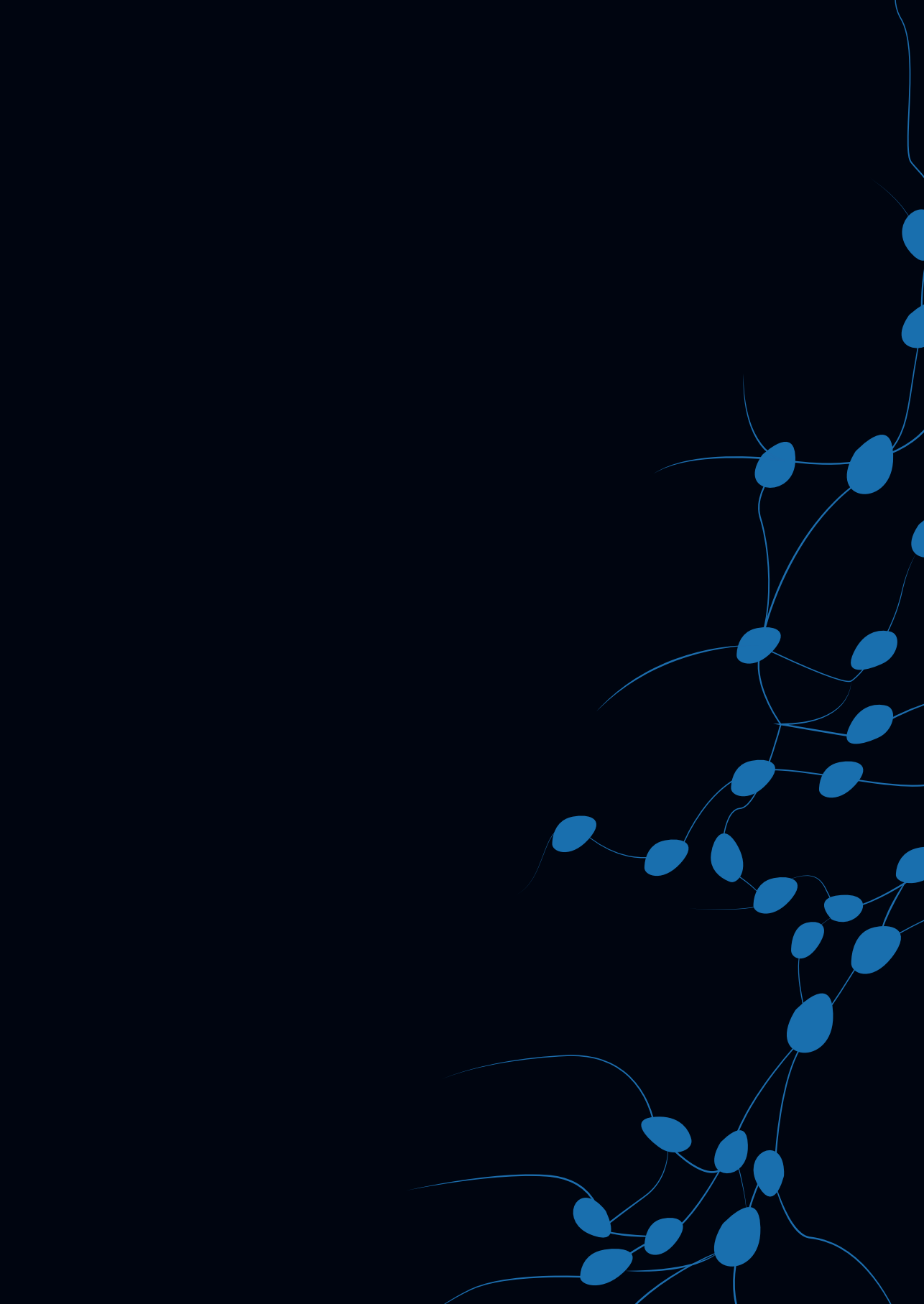
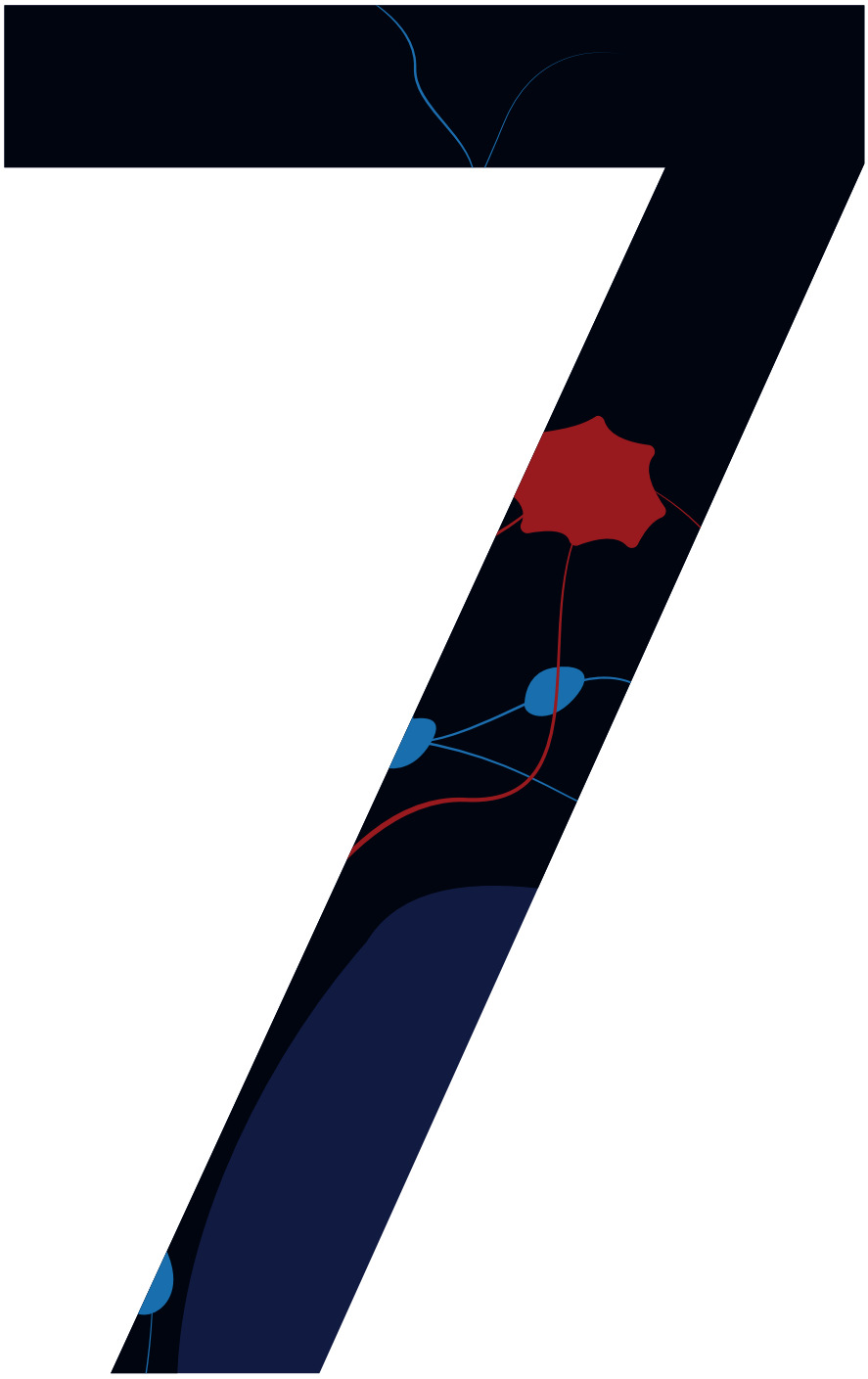



IMPROVEMENTS



The added value of SPECT-CT for identification of sentinel lymph nodes in early stage oral cancer

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ABSTRACT

Purpose To assess the role of Single Photon Emission Computed Tomography with Computed Tomography (SPECT-CT) for the identification of sentinel lymph nodes (SLNs) in patients with early stage (T1-T2) oral cancer and a clinically negative neck (cN0).

Methods In addition to planar lymphoscintigraphy, SPECT-CT was performed in 66 consecutive patients with early stage oral cancer and a clinically negative neck. The addition of SPECT-CT to planar images was retrospectively analyzed for the number of additional SLNs, more precise localization of SLNs and importance of anatomical information by a team consisting of a nuclear physician, surgeon and investigator.

Results Identification rate for both imaging modalities combined was 98% (65/66). SPECT-CT identified 15 additional SLNs in 14 patients (22%). In 2/15 (13%) of these additional SLNs the only metastasis was found, resulting in an upstaging rate of 3% (2/65). In 20% of the patients with at least one positive SLN the only positive SLN was detected due to the addition of SPECT-CT. SPECT-CT was considered to add important anatomical information in 2 patients (3%). In 5/65 (8%) of the patients initially scored SLNs on planar lymphoscintigrams were scored as non-SLNs when SPECT-CT was added. There were 4 false negative SLN biopsy procedures in this cohort.

Conclusions The addition of SPECT-CT to planar lymphoscintigraphy is recommended for the identification of more (positive) SLNs and better topographical orientation for surgery in sentinel lymph node biopsy for early stage oral cancer.

INTRODUCTION

Sentinel lymph node biopsy (SLNB) in early stage oral cancer is increasingly accepted as standard of care for staging of occult lymph node metastasis. Trials in which only neck dissection is performed after positive SLNB have demonstrated that SLNB is a sensitive method in the detection of occult cervical lymph node metastases. A pooled sensitivity of 91% (95% CI 84-95%) and a negative predictive value of 92-98% were found in a meta-analysis, however some lower sensitivity rates had been reported in recent large studies.[1-3] In most studies the procedure had a lower accuracy in patients with floor of mouth tumours, probably due to the "shine-through" phenomenon; the large injection spot of the primary tumour overshines the eventual sentinel lymph nodes (SLNs) in level I.

Visualization of SLNs is routinely carried out with dynamic and static planar lymphoscintigraphy using a ^{99m}Tc-labelled colloidal tracer frequently combined with a blue dye intraoperatively. In our institute Single Photon Emission Computed Tomography with Computed Tomography (SPECT-CT) is routinely performed. After introduction of the SPECT-CT for SLNB in oral cancer in 2003 by Even-Sapir et al. most studies conclude that SPECT-CT enhances useful information in localization of the SLNs and provides additional SLNs as described in the review of Haerle et al. [4,5] Studies of SPECT-CT in SLNB which included different locations of primary tumours found especially advantages for tumours with close proximity to the SLN and complex lymphatic regions which is the case in the head and neck region.[6]

The aim of this present study is to determine the added value of SPECT-CT to the planar dynamic and static lymphoscintigraphic images in patients with early stage oral cancer.

MATERIAL AND METHODS

From June 2011 until January 2014, 66 consecutive patients with early stage oral cancer and a clinically negative neck (cT1-T2, N0) were retrospectively analyzed. During this period SLNB was performed as standard procedure in our institution therefore written informed consent was not obtained. All patients underwent transoral excision and SLNB. The SLNB was performed according to the EANM/SENT joint practice guidelines.[7] In this article we describe only the imaging part of the procedure in our institution in detail, as the entire procedure had been previously described.[8]

All patients underwent the procedure in a 2-day protocol with peritumoural injections of ^{99m}Tc labelled nanocolloidal albumin (Nanocoll; GE Healthcare, The Netherlands) in 4 quadrants at the closest proximity of the primary tumour. The injections had a volume of 0.1-0.2 mL each and the median dose of injected radioactivity was 102 MBq (range 91-111). To avoid spillage of the radiocolloid, the patients will be required to perform a mouthwash immediately after injection. No side effects due to the colloidal injections had been observed.

Planar and SPECT images were acquired with a SPECT-CT gamma camera (Siemens, Erlangen, Germany). Planar lymphoscintigraphy started directly after injection of the tracer. Planar images were acquired in dynamic mode (128x128 matrix, 20 frames of 1 min) in anteriorposterior projection and static mode (256x256 matrix, during 2 min) in anteriorposterior and lateral projections. In addition to the planar imaging, SPECT-CT scans had been routinely performed in all patients without changing the patient's position. SPECT (matrix 128x128, non circular, 32 steps, 40 seconds per step, slice thickness 4.8 mm) took 24 minutes, CT (40 mAs, 130 kV, slice thickness 1.5 mm) took approximately 5 minutes.

The SPECT images were reconstructed by filtered back projection (FBP: Generalized Hanning, cut-off 0.90, alpha 0.5, no attenuation correction) and iterative reconstruction (Iterative Flash3D with CT attenuation correction (CTAC): 6 iterations, 8 subsets, Gaussian filter 12). The CT study was reconstructed with 5 mm slice thickness (Kernel B08s) and in soft tissue setting with 2 mm slice thickness (Kernel B30s). Reconstructions were obtained in transversal, sagittal and coronal planes.

Subsequently the identified SLNs were anatomically categorized according to the levels of the neck as proposed by the Committee for Head and Neck Surgery and Oncology of the American Academy of Otolaryngology – Head and Neck Surgery (AAO-HNS).[9] The SLNs were marked on the patient's skin using a ^{57}Cobalt marker and confirmed using a handheld gamma probe (Europrobe II; Eurorad, Strasbourg, France). In this retrospective analysis we focused on the additional value of the SPECT-CT imaging on the number of SLNs, their localization and the additional value of better topographical orientation preoperatively. Exclusion of initially considered SLNs on planar imaging due to SPECT-CT was also considered clinically relevant.

A clear visible and rapidly appearing lymph node was considered to be a SLN according to the definition of Morton.[10] Less visible lymph nodes (especially in presence of a clear

SLN) were considered second or third echelon and had not been marked on the skin. In this study, all images were evaluated by a team consisting of a nuclear physician, a head and neck surgeon and an investigator. The team had to reach consensus in every patient. All team members had experience with at least 20 patients with SLNB imaging and early stage oral cancer. First the planar lymphoscintigraphic images alone were interpreted, thereafter the team compared the planar imaging with the SPECT-CT and the potential additional value had been assessed. Additional hotspots on SPECT-CT that received direct drainage from the primary tumour were considered as SLNs, while level or neck side was not relevant for being an SLN. The additional hot spots found on SPECT-CT were considered also as SLNs if the intensity of uptake in the additional lymph node was at least as hot as considered SLNs on planar lymphoscintigraphy. If the additional hotspots on SPECT-CT were more proximal to the primary tumour compared with other considered SLNs on planar lymphoscintigraphy they were also scored as SLNs. Additional caudal hotspots with low uptake, not increasing in time, were considered to be second-echelon lymph nodes. A caudal focus with a clearly visible connecting lymphatic vessel from a cranial SLN was also considered a second-echelon lymph node.[11] There was no limit on number of SLNs.

A calculation how many SPECT-CT scans are needed to find 1 additional SLN was also performed, a so called "number needed to SPECT-CT". This calculation is a variation on the well-known number needed to treat, which is the inverse of the absolute risk reduction. This number needed to SPECT-CT will be calculated by 100/percentage of patients with (positive) additional SLNs on SPECT-CT.

Anatomical information by SPECT-CT was considered to be important if the head and neck surgeon in the scoring team would probably make a different (or more accurate) surgical approach based on the additional information.

If regional disease during follow-up occurred after a negative SLNB, the procedure was considered as false negative.

RESULTS

In this cohort of 66 patients the identification rate of SLNs was 98% (65/66). In 1 patient no SLN could be identified on either planar lymphoscintigraphy or SPECT-CT, however this patient showed no metastasis in the untreated neck during regular follow-up for almost 5 years. In 22% (14/65) of the patients, 15 additional SLNs could be identified due to SPECT-CT imaging. The additional SLNs related to other identified SLNs had been found in the same (2 SLNs), adjacent (6 SLNs) and non-adjacent (4 SLNs) levels or in the other neck side (3 SLNs). One of these additional SLNs could not be found intraoperatively. In the remaining 14 SLNs metastasis were present in 2 SLNs (13%). At least one positive SLN was found in 10 patients and in 2 of these patients (20%) the positive SLN had been identified due to the addition of SPECT-CT. These 2 metastases (one micrometastasis in level III ipsilateral (T1 floor of mouth tumour) and one macrometastasis in level II ipsilateral (T2 tongue tumour)) were the only SLNs containing metastasis in the neck, resulting in an upstaging rate of 3% (2/65 patients) (Figure 1).

Five (100/22%) SPECT-CT scans are needed to identify 1 additional SLN compared with planar lymphoscintigraphy. This "number needed to SPECT-CT" is 34 (100/2.9%) for identification of 1 additional SLN containing metastasis.

In contrast to these additional SLNs, in 8% (5/65) of the patients a hot spot was considered to be a SLN based on planar lymphoscintigraphy but was not after SPECT-CT (e.g. injection spot rather than SLN in 4 patients). In 28% (18/65) of the patients the anatomic levels of the SLNs on lymphoscintigraphic imaging had been changed with help of the SPECT-CT imaging. In 1 patient SPECT-CT identified 1 additional SLN, but also 1 considered SLN on planar lymphoscintigraphy could be scored as non-SLN. This results in a full concordance rate according to number and level of SLNs between planar lymphoscintigraphy and SPECT-CT imaging of 54% (35/65).
With respect to the location of the primary tumour SPECT-CT identified more additional SLNs in patients with floor of mouth tumours compared with tumours of the tongue (42% vs. 13%, $P = 0.07$). In both tumour subsites 1 additional SLN showed metastasis and in each of these two tumour subsites SLNB was considered as false negative in 2 patients (Table 1).

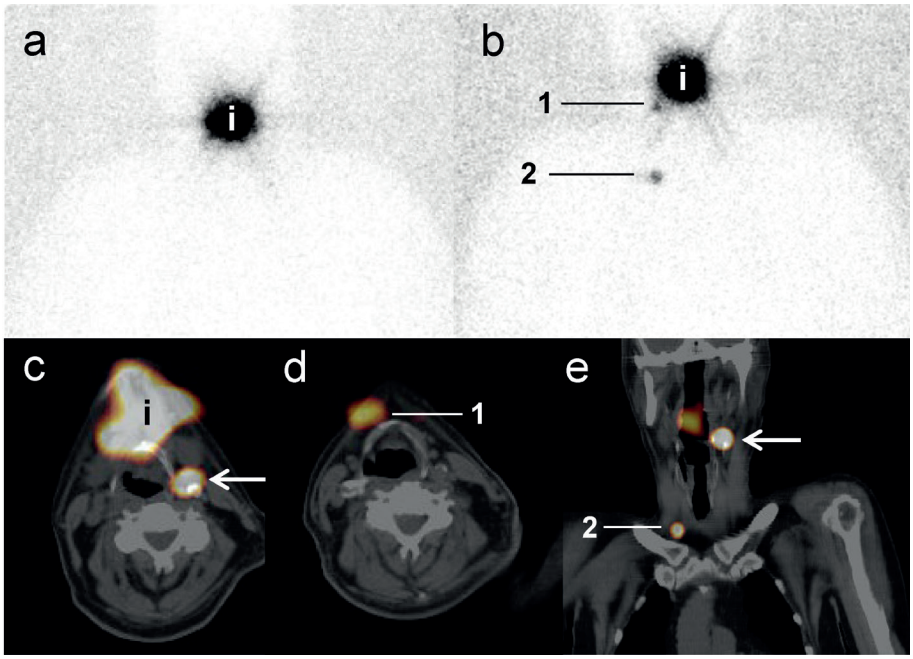

Figure 1. SPECT-CT shows additional SLN level II on the left side (arrow). Patient with a clinically T2N0 tongue tumour on the left side. (a) Planar lymphoscintigraphy showed directly post injection the injection spot (i) but no SLNs, 1 hour post injection (b) 2 hotspots, judged as SLN level IA right (1) and second echelon lymph node in level IV right (2). (c, d, e) SPECT-CT showed an additional hotspot (arrow), considered as SLN level II on the left side. Due to the high amount of uptake in level IV right on SPECT-CT (2), exploration with the gamma probe was performed during surgery. During surgery 3 SLNs had been identified (level IA right, level IV right and level II left), all hot, not blue. The SLN level II left contained a macrometastasis. A complementary neck dissection (selective I-IV) had been performed without additional metastasis on histopathological examination. No evidence of disease during follow-up of 32 months was observed.

Table 1. Additional SLNs due to SPECT-CT imaging according to tumour localization

Location	All patients	Patients with additional SLNs	Positive additional SLNs	False negatives
Tongue	39	5 (13%)	1	2
Floor of mouth	19	8 (42%)	1	2
Buccal mucosa	4	1 (25%)	0	0
Other	3	0	0	0
Total	65	14 (22%)	2	4

Abbreviations: SLNs, sentinel lymph nodes

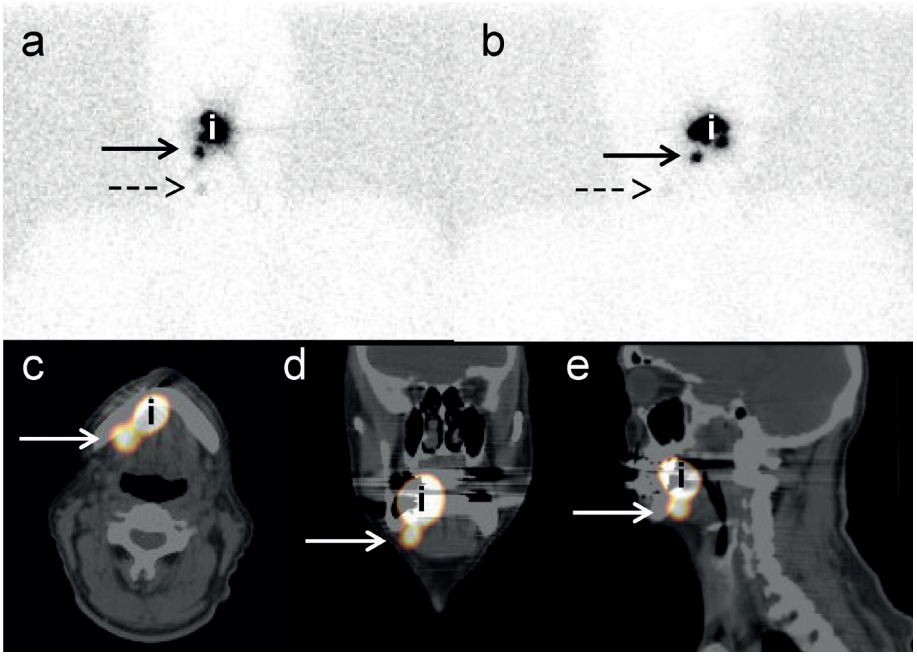

Figure 2. Example of better topographical orientation. Patient with a clinically T2N0 floor of mouth tumour on the right side. (a) On the planar lymphoscintigraphy the hotspot is clearly visible (arrow), considered to be a sentinel lymph node in level I. Also a less visible hot spot was observed (dashed arrow), considered to be a second echelon node. (b) Lateral projection of the planar lymphoscintigraphy on the right side of the neck with the same intense hotspot in level I (arrow) and very weak uptake in the considered second echelon node (dashed arrow). (c, d, e) SPECT-CT shows a hotspot just behind the mandible (white arrow) with close relationship to the injection spot of the primary tumour (i) and was actually considered as a sublingual node.

DISCUSSION

In this study of 66 patients with early stage oral cancer, we retrospectively evaluated the additional value of SPECT-CT compared to the conventional planar lymphoscintigraphy for the detection of SLNs. To our knowledge, this is the largest single-center study investigating the additional value of SPECT-CT in oral cancer. In a multidisciplinary setting both imaging modalities were separately investigated and in 22% of the patients additional SLNs were found on SPECT-CT imaging. In 20% of the SLN-positive patients the positive SLN had been identified only with SPECT-CT. These additional positive SLNs result in an upstaging rate to a positive neck of 3% in the total cohort, in other words we have to make 34 SPECT-CT scans to identify 1 additional positive SLN. These results are more or less comparable with some previously reported studies[11-14], but some other studies report higher rates.[15,16] A study with barely no additional SLNs due to SPECT-CT had also been reported.[17] It is hard to find a reasonable explanation for these differences in almost comparable patient groups and comparable imaging modalities.[5] One reason may be the difference in imaging protocols throughout Europe with respect to the amount of injected radioactivity and the time of injection related to the surgical procedure (same- or 2-day protocols).[18] Another explanation can be the practice variation in defining SLNs on planar lymphoscintigraphy as shown by Flach et al.[19] In order to perform consistent lymphoscintigraphic evaluation, defining the SLNB concept is essential. There are many definitions of the SLN and many articles discuss the subject. The definition of Morton et al. which says 'a sentinel node is the first draining lymph node on the direct lymphatic drainage pathway from the primary tumour site' best reflects the stepwise spread of cancer through the lymphatic system.[10] However, this is a theoretical concept and does not always aid the clinician in interpreting a lymphoscintigraphic scan as an individual situation, because it is regularly not so clear-cut as this theory. Describing how to interpret lymphoscintigraphic imaging with a view to identify foci (hot spots) as SLN in a simple and straightforward way is not easy. In a study on interobserver agreement many experienced observers correctly considered SLNs as the lymph nodes directly draining from the injection site, and/or single radioactive nodes in a basin, whereas other important criteria as uptake intensity, time of appearance, relevance of neck side and level were rated differently. Interobserver agreement can be influenced by a number of factors. If a single focus is visualized there will be no disagreement. However, in a complex nodal basin as the neck area, several foci are often visible. This harbours an increased risk of not identifying the correct SLN and/or misinterpretation of second echelon nodes as SLNs.[20] In view of the literature it seems that despite the additional information, SPECT-CT is not able yet to solve the problem of difficult interpretation of SLNs.

The study of Haerle et al. showed all their additional SLNs in the same or adjacent levels as hotspots detected by planar lymphoscintigraphy alone and they suggest that even necks without hotpots should be explored with the gamma probe intraoperatively, based on the fact that the gamma probe identified SLNs in patients without hotpots on imaging.[12] In contrast to their study we found 7 additional SLNs in a non-adjacent level or even in the other neck side compared to planar lymphoscintigraphy. However, we still found the (dynamic) planar lymphoscintigraphy of additional value in differentiating SLNs and second echelon nodes, especially using the criterion of rapidly emerging hot spots. Therefore we recommend a combination of planar static and dynamic imaging followed by SPECT-CT as the currently best imaging procedure for SLNB.

We hypothesized that we could find additional SLNs due to SPECT-CT especially in patients with SLNs in close proximity of the primary tumour as is the case for SLNs in level I with a primary tumour in the floor of mouth. Indeed in 5 patients additional SLNs had been identified in level I, however 4 of these patients had a tongue tumour and only 1 a floor of mouth tumour. In addition, in 4 patients (2 tongue tumours, 2 floor of mouth tumours) a hot spot considered to be a SLN could be identified as injection spot rather than SLN in level I by SPECT-CT. We found a trend for more additional SLNs in floor of mouth tumours compared with tongue tumours, also resulting in a lower number needed to SPECT-CT (not presented).

Despite our experience with SLNB in oral cancer, we report a relatively high number of false negative patients in this study. In this small cohort of 19 floor of mouth tumours 2 false negatives were present, compared to 2 false negatives in 39 tongue tumours. In 1 patient with a left-sided floor of mouth tumour the initially found SLN was located in level I on the right side, then this patient returned with a metastasis in level I on the left side 6 months after SLNB, which had been probably missed on the planar lymphoscintigraphy and SPECT-CT. The other false negative patient with a floor of mouth tumour had a regional metastasis in level III 13 months after SLNB. Both patients are alive with no evidence of disease for more than 2.5 years. Both patients with a tongue tumour and false negative SLNB had regional metastasis in level II ipsilateral, approximately 1 year after SLNB. One patient is alive with no evidence of disease for 3 years, one patient had been lost to follow-up.

In our opinion SPECT-CT did not solve the problems of the lower accuracy in patients with floor of mouth tumours, despite the higher number of additional identified SLNs due to SPECT-CT. The finding that additional SLNs were mainly found in other

levels than level I, suggests that the "shine-through" phenomenon remains the most common problem in floor of mouth tumours. Other new technologies and procedures, e.g. superselective neck dissection of level I, [^{99m}Tc]Tc-Tilmanocept, fluorescence-guided SLNB and PET/CT lymphoscintigraphy with ^{89}Zr-nanocolloidal, seem promising to improve the accuracy of the SLNB in floor of mouth tumours.[20-24]

It should be clear that SPECT-CT allows better anatomical information for the surgeon preoperatively in all cases. In 2 patients our team had determined this information of evident importance. We described a sublingual node on SPECT-CT, which had been scored as level I on planar lymphoscintigraphy (Figure 2). Sieira-Gil et al. had also found sublingual SLNs by SPECT-CT (2 cases), which had not been detected by planar lymphoscintigraphy.[14] Due to the better topographical orientation the anatomical levels of the SLNs had been changed in 28% of the patients and better delineation against surrounding tissues could be done. Nevertheless it remains still difficult to determine the extent to which SPECT-CT influences the surgical approach related to planar lymphoscintigraphy particularly due to the use of the handheld gamma probe just before incision. To get more insight in this additional value the surgical procedure should be planned blinded to the results of the SPECT-CT and replanned after revealing the SPECT-CT. Our study suggests that SPECT-CT is helpful preoperatively and probably because of the better anatomical orientation surgery could be performed more safely than with planar lymphoscintigraphy alone.

We report a relatively low concordance rate of 54% between planar lymphoscintigraphy and SPECT-CT in comparison to the concordance rate of 81% of Haerle et al.[12] However, they only report a concordance rate according to number of hotpots on both imaging modalities, where we also include changes of anatomical levels of the hotspots in this rate.

Nowadays, SLNB for early stage oral cancer is gaining more acceptance worldwide and had recently been included in many guidelines. In the beginning SLNB had been reported to be safe with planar lymphoscintigraphy alone, but in general all studies published in the last 5 years had performed SLNB with SPECT-CT in addition to planar imaging despite of only moderate evidence in reported literature so far.

We conclude that SPECT-CT after static and dynamic planar lymphoscintigraphic imaging has the potential to detect more (22%) SLNs than planar lymphoscintigraphy alone, especially in patients with floor of mouth tumours, resulting in an upstaging

rate of 3% in all patients. In 20% of the patients with at least one positive SLN the only positive SLN was detected due to the addition of SPECT-CT. Moreover, SPECT-CT provides better topographical orientation for the surgeon preoperatively. We recommend the addition of SPECT-CT in SLNB for patients with early stage oral cancer, however other improvements are still mandatory to increase the accuracy of this procedure.

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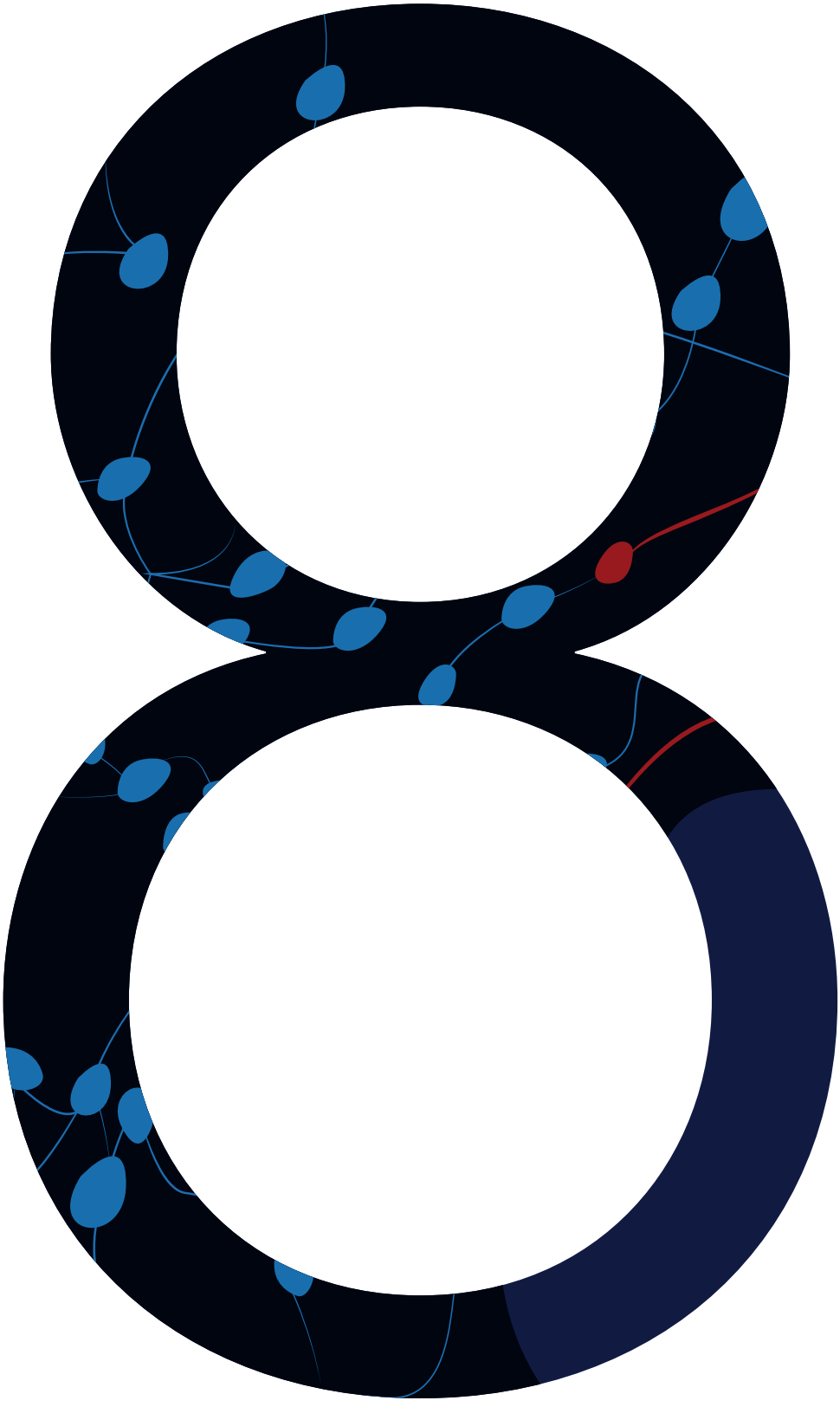

Sentinel lymph node detection in oral cancer: a within-patient comparison between [^{99m}Tc]Tc-tilmanocept and [^{99m}Tc]Tc-nanocolloid

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ABSTRACT

Purpose Sentinel lymph node (SLN) biopsy has proven to reliably stage the clinically negative neck in early-stage oral squamous cell carcinoma (OSCC). [^{99m}Tc]Tc-tilmanocept may be of benefit in OSCC with complex lymphatic drainage patterns and close spatial relation to SLNs.

Methods A prospective within-patient evaluation study was designed to compare [^{99m}Tc]Tc-tilmanocept with [^{99m}Tc]Tc-nanocolloid for SLN detection. A total of 20 patients with early-stage OSCC were included, who underwent lymphoscintigraphy with both tracers. Both lymphoscintigraphic images of each patient were evaluated for SLN detection and radiotracer distribution at 2-4 hours post injection.

Results The injection site's remaining radioactivity was significantly lower for [^{99m}Tc]Tc-tilmanocept (29.9%), compared to [^{99m}Tc]Tc-nanocolloid (60.9%; $p<0.001$). Radioactive uptake in SLNs was significantly lower for [^{99m}Tc]Tc-tilmanocept (1.95%) compared to [^{99m}Tc]Tc-nanocolloid (3.16%; $p = 0.010$). No significant difference was seen in SLN to injection site ratio in radioactivity between [^{99m}Tc]Tc-tilmanocept (0.066) and [^{99m}Tc]Tc-nanocolloid (0.054; $p = 0.232$). A median of 3.0 and 2.5 SLNs were identified with [^{99m}Tc]Tc-tilmanocept and [^{99m}Tc]Tc-nanocolloid, respectively ($p = 0.297$). Radioactive uptake in higher echelon nodes was not significantly different between [^{99m}Tc]Tc-tilmanocept (0.57%) and [^{99m}Tc]Tc-nanocolloid (0.86%) ($p = 0.052$). A median of 2.0 and 2.5 higher echelon nodes were identified with [^{99m}Tc]Tc-tilmanocept and [^{99m}Tc]Tc-nanocolloid, respectively ($p = 0.083$).

Conclusion [^{99m}Tc]Tc-tilmanocept had a higher injection site clearance, but at the same time a lower uptake in the SLN, resulting in an SLN to injection site ratio, which was not significantly different from [^{99m}Tc]Tc-nanocolloid. The relatively low radioactive uptake in SLNs of [^{99m}Tc]Tc-tilmanocept may limit intraoperative detection of SLNs, but can be overcome by a higher injection dose.

INTRODUCTION

The sentinel lymph node biopsy (SLNB) procedure is a diagnostic staging method that is applied in a variety of tumour types, including oral squamous cell carcinoma (OSCC). The procedure aims to identify the first draining lymph nodes, the 'sentinel lymph nodes' (SLN), which are most likely to harbour metastases. The histopathological status of the SLN should reflect the histopathological status of the rest of the nodal basin, and additional treatment of the nodal basin (e.g. surgery or radiotherapy) should only be performed in case of metastatic involvement of the SLN. So far, the routine procedure consists of preoperative peritumoural injection of a 99m-technetium ([^{99m}Tc])-labelled colloid followed by dynamic and static lymphoscintigraphy using planar and single photon emission computed tomography (SPECT) imaging.[1-3] Intraoperative detection is possible using a portable gamma probe.

It has been demonstrated that by using this approach, the SLNB procedure reliably stages the clinically negative neck (cN0) in early stage OSCC with a sensitivity of 87% and a negative predictive value of 94% in the most recent meta-analysis.[4] However, one of the most frequently mentioned difficulties of this procedure occurs when the injection site around the primary tumour produces a large hotspot on lymphoscintigraphy, possibly hiding SLN(s) in close proximity of the primary tumour, usually referred as "shine-through" phenomenon (Figure 1).

This phenomenon is particularly evident in floor of mouth tumours and multiple studies demonstrated a (significantly) lower accuracy of the SLNB procedure in floor of mouth tumours compared to other tumour locations in the oral cavity.[5-8] Some authors even advocate adding a superselective level I resection in these cases.[9] Secondly, on lymphoscintigraphy it is often difficult to differentiate hotspots between SLNs and second echelon nodes.[10] As a result, second echelon lymph nodes may erroneously be considered as SLNs, resulting in an unnecessary extension of the surgical procedure.
A new radioactive agent, [^{99m}Tc]Tc-tilmanocept (Lymphoseek ®, Navidea Biopharmaceuticals, Inc.), has been specifically designed for SLN identification and is registered for this purpose in both the USA and Europe. [^{99m}Tc]Tc-tilmanocept is a small sized receptor targeted (CD206) sentinel lymph node detection agent (Figure 2).[11] Due to its proposed rapid clearance from the injection site, rapid uptake and high retention within the SLN, as well as low uptake by the remaining (higher echelon) lymph nodes, [^{99m}Tc]Tc-tilmanocept may particularly be of benefit in floor of mouth tumours and other head and neck tumours with complex drainage patterns and close spatial relation to

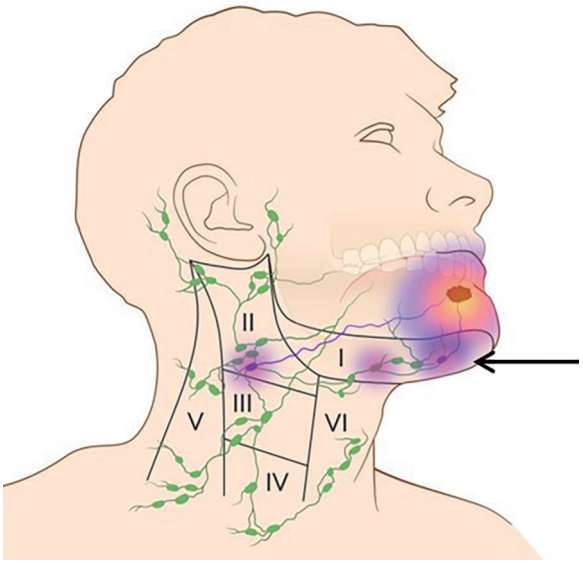

Figure 1. "Shine-through" phenomenon. Radiation flare of the primary tumour overshines the hotspot of sentinel lymph node in close proximity to the primary tumour (arrow)

the SLN.[12,13] A multicentre validation study using [^{99m}Tc]Tc-tilmanocept for SLNB in head and neck squamous cell carcinoma showed an SLN identification rate of 97.6%, a false negative rate of 2.56% and a negative predictive value of 97.8%.[14] Of note, these high figures were also obtained in floor of mouth cancers, which strengthened the idea that [^{99m}Tc]Tc-tilmanocept may diminish the "Shine-through"effect and improve the SLN detection rate for this subsite.

In Europe [^{99m}Tc]Tc-nanocolloid is the most frequently used radiocolloid for SLN mapping. So far, there are no studies performed comparing head to head [^{99m}Tc]Tc-tilmanocept with [^{99m}Tc]Tc-nanocolloid.

The aim of the present study is to investigate the injection site clearance and uptake in SLN(s) of [^{99m}Tc]Tc-tilmanocept in comparison with a standard [^{99m}Tc]Tc-nanocolloid by means of lymphoscintigraphy in early stage oral cancer patients.

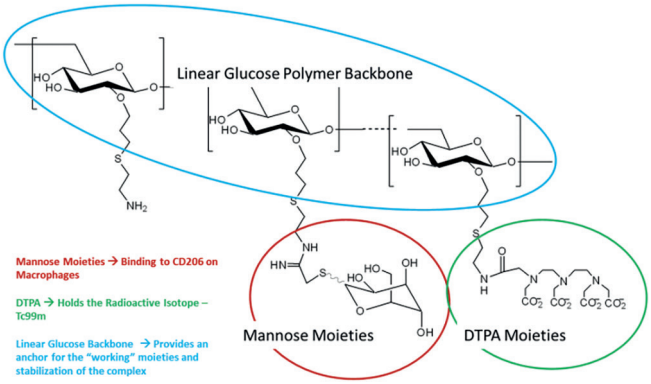

Figure 2. [^{99m}Tc]Tc-tilmanocept (Lymphoseek) structure and functional elements

MATERIAL AND METHODS

A monocenter prospective within-patient evaluation study was designed in order to compare [^{99m}Tc]Tc-tilmanocept with our routinely used [^{99m}Tc]Tc-nanocolloid tracer, in terms of SLN visualization, injection site clearance and uptake in SLN(s). This study was approved by the medical ethical review board of the University Medical Center Utrecht (NL58099.041.17).

All patients had an early-stage cT1-2N0M0 OSCC (TNM Staging AJCC UICC 8th Edition). Clinical nodal staging was confirmed by at least ultrasound and, in case of suspicious lymph nodes, ultrasound guided fine-needle aspiration cytology. In most cases MRI was conducted as well, as part of clinical staging.
Patients with a history of neck dissection, neck irradiation or gross injury to the neck, that would hamper surgical dissection of SLNs, were excluded from this study. Besides, patients with a history of head and neck malignancies in the last 5 years were excluded as well.

This study consisted of 2 groups containing 10 patients each (Figure 3). In the first group (cohort 1), 50µg of [^{99m}Tc]Tc-labelled tilmanocept (74 MBq in 0.4mL) was prepared according to manufacturer's instructions. All tracers were administered in 4 peritumoural injections of 0.1mL, followed by lymphoscintigraphy. Four to 11 days later, these 10 patients subsequently underwent a [^{99m}Tc]Tc-nanocolloid (routine dose

120 MBq) lymphoscintigraphy. After the first cohort, interim analysis was carried out before continuing with the second cohort.

In cohort 2, tracers were administered in opposite order; first 74 MBq [^{99m}Tc]Tc-nanocolloid, followed by 74 MBq [^{99m}Tc]Tc-tilmanocept. In both cohorts the same imaging protocol was applied.

In an effort to administer both tracers at the same injection spots, photographic images were made of the peritumoural injections with consent of patients. Following injection of the second radio-agent, the same imaging protocol was applied. Patients reported their pain scores during the injection procedure for both tracers using the Numeric Pain Rating Scale (NPRS).[15]

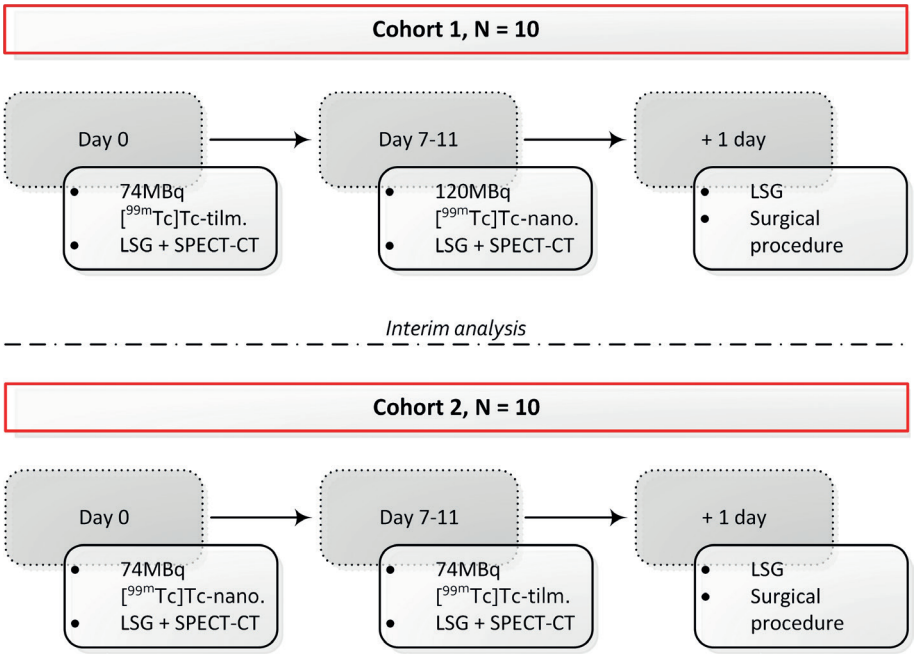

Figure 3. Study design

Abbreviations; [^{99m}Tc]Tc-tilm., [^{99m}Tc]Tc-tilmanocept; [^{99m}Tc]Tc-nano., [^{99m}Tc]Tc-nanocolloid; LSG, lymphoscintigraphy

Imaging protocol

Directly post injection planar images were acquired in dynamic mode (128 × 128 matrix, 20 frames of 1 min) in anterior-posterior projection followed by static mode (256 × 256 matrix, during 4 min) in anterior-posterior and lateral projections (30min and 2h post-injection), on a Siemens Symbia T16 SPECT/CT scanner, using 'low- and medium energy' (LME) collimators to limit septal penetration (reducing "shine-through").[16] In addition to the planar imaging 2h post-injection, SPECT-CT scans were acquired on a 128 × 128 matrix (pixel spacing, 3.9 × 3.9 mm), with 128 angles, 20 s per projection, over a non-circular 360° orbit (CT: 110 kV, 40 mAs eff., 16 x 1.2 mm). SPECT images were reconstructed using clinical reconstruction software (Siemens Flash3D), with attenuation- and scatter correction (6 iterations, 8 subsets, 5 mm Gaussian filter). Additionally, quantitative SPECT reconstructions were generated using the Utrecht Monte Carlo System (UMCS), a dedicated SPECT reconstructor which includes Monte Carlo modelling of scatter and collimator-detector interactions.[17,18] During lymphoscintigraphy, a source with known radioactivity was scanned in the same frame as the patient, acting as a verification of quantitative accuracy.

Intraoperative detection and histology

Intraoperative detection of SLN(s) was performed using a portable gamma probe, according to standard protocol.[3] The last injected radio-agent was leading to identify SLNs during surgery. In the present study no superselective neck dissection of the preglandular triangle of level I was performed in floor of mouth tumours. All harvested nodes were histologically examined for metastasis using step serial sectioning (intervals of 150 µm) with haematoxylin-eosin and pan-cytokeratin antibody (AE 1/3) staining at each level.

Evaluation of images

Paired images of both tracers were evaluated regarding similarity of depicted draining lymph node basins, the number and location of SLNs and their histopathology. Furthermore, the amount of radioactivity that resided in the injection site, SLNs, higher echelon nodes, and reference source were measured from quantitative SPECT-CT images, acquired 2h post-injection.

Volumes of Interest (VOIs) around the injection site, SLNs and the reference source were automatically defined using in-house developed software, adopting a local peak finding algorithm and watershed segmentation (Figure 4A).[19] The VOIs were manually validated with 3D segmentation software ITK-SNAP (Figure 4B).[20]

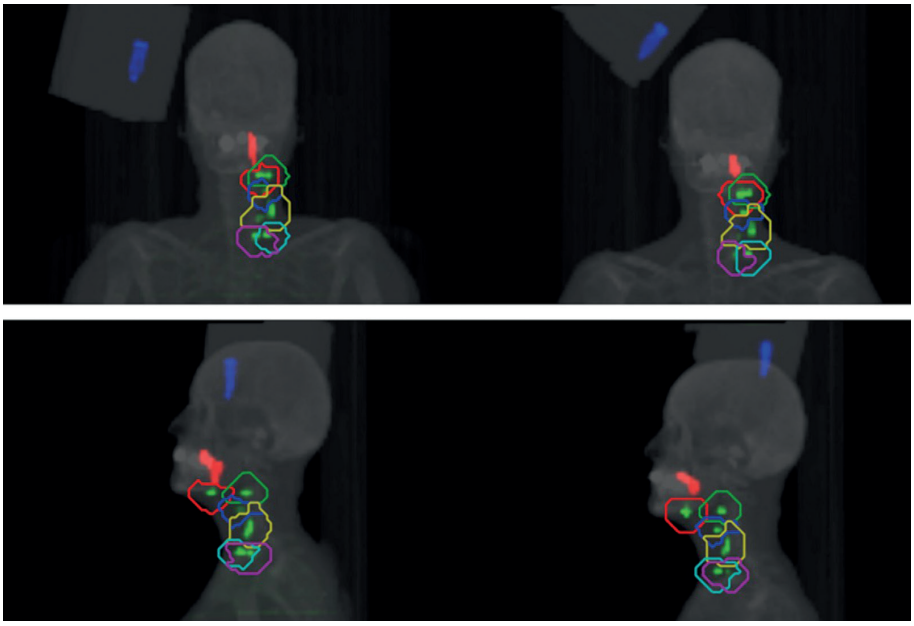

Figure 4A. Algorithmic defined VOIs for all hotspots within the scanned area for both [^{99m}Tc]Tc-tilmanocept and [^{99m}Tc]Tc-nanocolloid
Summed intensity projections of SPECT reconstructions of the same patient, injected with either [^{99m}Tc]Tc-tilmanocept (left) and [^{99m}Tc]Tc-nanocolloid (right).
Injection site: Red hotspot
Reference source: Blue hotspot
'Hot' lymph nodes: Green hotspots with coloured VOIs

All quantitative results of VOI measurements are presented as percentages of the amount of injected radioactivity. The remaining radioactivity outside of the VOIs but within field of view of the SPECT acquisition, was regarded to be 99m-Technetium located outside the (S)LNs, injection site or reference source and is further addressed as background radioactivity. Since the measured cumulative background radioactivity is strongly dependent on the volume of the patient within the field of view of SPECT acquisition, the background activity is also presented in terms of standardized uptake value (SUV), analogous to PET (i.e. average measured activity concentration in background, divided by the average activity concentration in the entire patient, based on body mass).

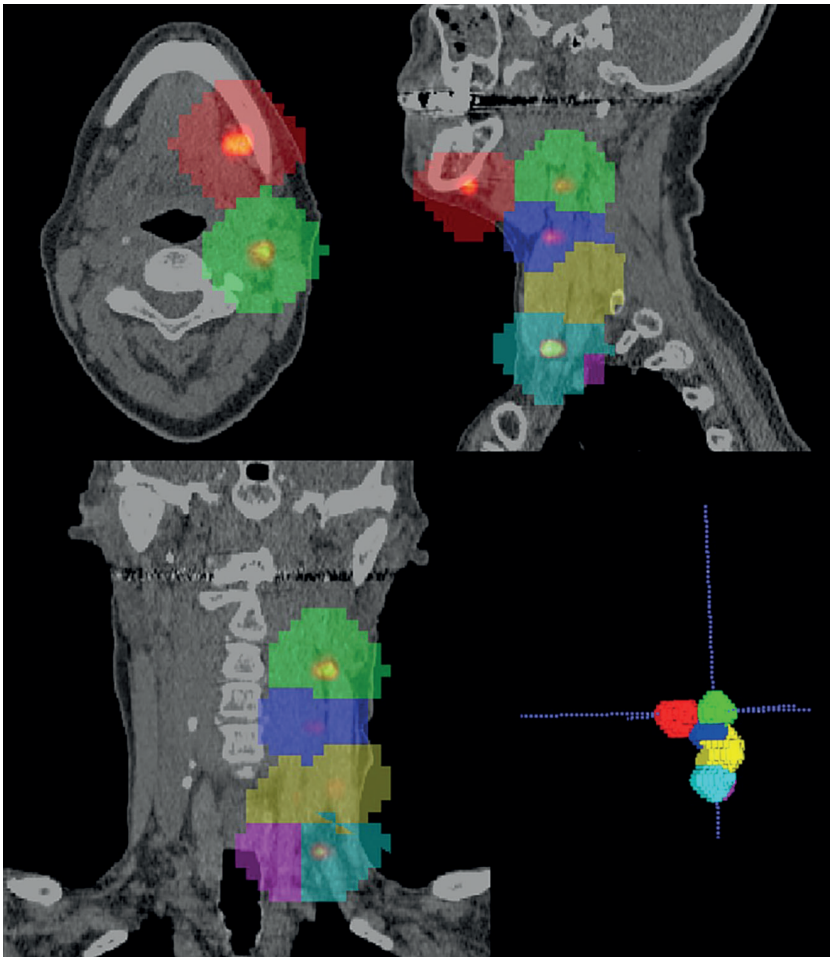

Figure 4B. Verification of VOIs containing 'hot' lymph nodes using 3D segmentation software (ITK-SNAP)
Sentinel lymph nodes: Red and green VOI
Higher echelon nodes: Blue, yellow, turquoise and purple VOI

For qualitative evaluation of [^{99m}Tc]Tc-nanocolloid and [^{99m}Tc]Tc-tilmanocept lymphoscintigraphy, images of each subject for both tracers were blinded and scored by 2 head and neck surgeons and 2 nuclear medicine physicians. Per image, every hotspot was classified as SLN using a 3-point scale (yes, potential, no). Afterwards, every "potential" scored SLN was eventually dichotomized into 'yes' or 'no' by the observers, based on their advice to surgically harvest the concerning lymph node. Besides, all observers rated the difficulty for reviewing the images (i.e. easy, moderate, hard). Inter-observer variability regarding the selected SLNs between observers was assessed.

Ultimately, data from qualitative analyses were matched with quantitative results of corresponding VOIs and correlated with intra-operative and pathological findings of the harvested (S)LNs.

Statistical analyses

All data was analyzed with professional statistics software (IBM SPSS Statistics Version 25.0). Data is expressed as mean ± SD for parametric continuous variables and as median for nonparametric continuous variables. Number of cases and percentages are presented as categorical variables. All quantitative results of VOI measurements are presented as percentages of the amount of injected radioactivity.

To compare the amount of radioactivity in the injection site, SLNs, higher echelon nodes and background between [^{99m}Tc]Tc-tilmanocept and [^{99m}Tc]Tc-nanocolloid, paired Samples T-tests were applied for parametric variables, while Wilcoxon Signed Rank tests were applied for nonparametric variables. To compare the "SLN to injection site ratio" in radioactivity between [^{99m}Tc]Tc-nanocolloid and [^{99m}Tc]Tc-tilmanocept, a Wilcoxon Signed Rank test was applied.
To determine inter-observer variability regarding selected SLNs between observers for both [^{99m}Tc]Tc-nanocolloid and [^{99m}Tc]Tc-tilmanocept lymphoscintigraphic images, Fleiss Kappa statistics were applied [21]. Finally, to compare the rated difficulty for reviewing [^{99m}Tc]Tc-nanocolloid and [^{99m}Tc]Tc-tilmanocept lymphoscintigraphic images, McNemar tests were applied.

A p-value of < 0.05 was regarded as statistically significant.

RESULTS

Characteristics of the 20 patients and tumours are listed in Table 1. The oral tongue was the most affected tumour location. In 5 (25%) cases the floor of mouth was involved. In total, 49 SLNs were harvested (median 2), of which 12 (24%) showed metastasis. These 12 positive SLNs were harvested from 7 patients, making 35% (7/20) of our study population positive for lymphatic metastasis. Distribution of hotspots and SLNs per tracer per patient is given in supplementary data 1.

Table 1. Patient characteristics

Characteristics	Overall (%)
Patients, n (%)	20 (100%)
Gender, n (%)	
Male	13 (65%)
Female	7 (35%)
Median age (y) (range)	63 (39-77)
Tumour location, n (%)	
Tongue	14 (70%)
Floor of mouth	5 (25%)
Lower gum	1 (5%)
Clinical T stage, n (%)*	
T1	9 (45%)
T2	11 (55%)
Pathology primary tumour	
Diameter (mm) (range)	19 (6-44)
Depth of invasion (mm) (range)	6 (1-13)
Pathology sentinel lymph nodes	
Negative	37 (76%)
Positive	12 (24%)
Median harvested SLNs (range)	2 (1-5)
Number of SLN-positive patients	7 (35%)

*T stage according to 8th AJCC TNM classification

Quantitative analyses (Table 2)

The radioactivity remaining in the injection site was significantly lower for [^{99m}Tc]Tc-tilmanocept (29.9%; SD±7.6), compared to [^{99m}Tc]Tc-nanocolloid (60.9%; SD±16.1) ($p < 0.001$).

The radioactive uptake in SLNs was significantly lower for [^{99m}Tc]Tc-tilmanocept compared to [^{99m}Tc]Tc-nanocolloid (1.95% vs. 3.16% respectively, $p = 0.010$). The SLN to injection site ratio between [^{99m}Tc]Tc-tilmanocept (0.066) and [^{99m}Tc]Tc-nanocolloid (0.054) was not statistically different ($p = 0.232$).

In 20 patients, a median of 3.0 and 2.5 SLNs were identified with [^{99m}Tc]Tc-tilmanocept and [^{99m} Tc]Tc-nanocolloid respectively ($p = 0.297$).

The number of higher echelon nodes did not differ significantly between both tracers with a median of 2.0 in the [^{99m}Tc]Tc-tilmanocept cohort and 2.5 in the [^{99m}Tc]Tc-nanocolloid group ($p = 0.083$). [^{99m}Tc]Tc-tilmanocept showed less radioactive uptake in higher echelon nodes in comparison with the [^{99m}Tc]Tc-nanocolloid group, although not statistically significant (0.57% vs 0.86% respectively, $p = 0.052$).

[^{99m}Tc]Tc-tilmanocept showed a higher background radioactivity in comparison with [^{99m}Tc]Tc-nanocolloid (2.23% vs 0.41% in field of view of the SPECT, $p < 0.001$. SUV: 0.132 vs. 0.018, $p < 0.001$).

A median pain score (NPRS) of 3.0 (range 0 – 8) was reported for [^{99m}Tc]Tc-tilmanocept compared to 2.0 (range 0 – 8) for [^{99m}Tc]Tc-nanocolloid ($p = 0.041$).

Qualitative analyses

Interobserver agreement regarding selection of SLNs with a 3-point scale using Fleiss Kappa statistics showed substantial agreement for both [^{99m}Tc]Tc-tilmanocept and [^{99m}Tc]Tc-nanocolloid ($\kappa = 0.677$ [95% CI 0.619-0.735] vs. $\kappa = 0.725$ [95% CI 0.668-0.782] respectively, not significantly different). When dichotomizing, both tracers reached excellent agreement with an equal Fleiss Kappa ($\kappa = 0.885$ [95% CI 0.804-0.966] for [^{99m}Tc]Tc-tilmanocept and $\kappa = 0.885$ [95% CI 0.806-0.963] for [^{99m}Tc]Tc-nanocolloid).

[^{99m}Tc]Tc-tilmanocept scans were categorized scored as easy (6x), moderate (10x) and hard (4x), whereas [^{99m}Tc]Tc-nanocolloid was ranked as easy (6x), moderate (9x) and hard (5x) (McNemar test, $p = 0.80$).

No serious adverse events or allergic reactions were reported in our study population.

Table 2. Quantitative analyses

	[^{99m}Tc]Tc-tilmanocept	[^{99m}Tc]Tc-nanocolloid	P value
Radioactivity remaining in injection site	29.9%; SD±7.6 (range 17.10 – 43.95)	60.9%; SD±16.1 (range 30.26 – 89.58)	<0.001
Uptake in SLNs	1.95%; IQR±2.6 (range 0.21 – 6.80)	3.16%; IQR±3.9 (range 0.04 – 11.90)	0.010
SLN to injection site ratio	0.066; IQR±0.1 (range 0.001 – 0.20)	0.054; IQR±0.07 (range 0.001 – 0.22)	0.232
Number of SLNs	3.0; IQR±2 (range 0 – 4)	2.5; IQR±1 (range 1 – 5)	0.297
Number of higher echelon nodes	2.0; IQR±2 (range 0 – 5)	2.5; IQR±3 (range 0 – 6)	0.083
Uptake in higher echelon nodes	0.57%: IQR±1.64 (range 0.001 – 7.15)	0.86%: IQR±2.17 (range 0.001 – 6.95)	0.052
Background activity	2.23%; IQR±2.01 (range 0.93 – 5.76)	0.41%; IQR±0.96 (range 0.01 – 1.55)	<0.001
Pain score (NPRS)	3.0; IQR±3 (range 0 – 8)	2.0; IQR±4 (range 0 – 8)	0.041

Abbreviations; SD; standard deviation, IQR; interquartile range, SLN; sentinel lymph node

DISCUSSION

The present study is the first within-patient evaluation comparing [^{99m}Tc]Tc-tilmanocept to [^{99m}Tc]Tc-nanocolloid. We showed a significantly higher injection site clearance for [^{99m}Tc]Tc-tilmanocept but also a significantly lower uptake in the SLN in comparison with [^{99m}Tc]Tc-nanocolloid. No significant difference was seen in SLN to injection site ratio. There was an excellent interobserver agreement for both [^{99m}Tc]Tc-tilmanocept and [^{99m}Tc]Tc-nanocolloid. Thereby, difficulty of scans interpretation was equal for both tracers.

Currently, there are no other within-patient evaluation studies comparing [^{99m}Tc]Tc-tilmanocept to another radioactive tracer. Only one RCT so far, has been published by Unkart et al., who presented a trial of 57 breast cancer patients comparing [^{99m}Tc]Tc-tilmanocept with [^{99m}Tc]Tc-sulfur colloid regarding pain after injection of both tracers.[22]

They showed a higher pain sensation in the first 3 minutes after injection of [^{99m}Tc]Tc-sulfur colloid compared to [^{99m}Tc]Tc-tilmanocept. In contrast, in our study a higher pain score was found for [^{99m}Tc]Tc-tilmanocept as compared to [^{99m}Tc]Tc-nanocolloid, regardless whether [^{99m}Tc]Tc-tilmanocept was injected as first or second tracer. However our study size is small and the clinical relevance of a difference of 1 point (median 2.0 vs. 3.0) is questionable.

Additionally, Unkart et al. found no statistical differences in breast cancer patients concerning number of hotspots, number of removed SLNs, time to surgical removal or number of blue nodes for [^{99m}Tc]Tc-Tilmanocept compared to [^{99m}Tc]Tc-sulfur colloid.[23] However, this study was not especially designed for analysing differences regarding SLN identification. Randomizing patients for either the one or the other tracer did not clearly clarify discrepancies between both tracers with respect to drainage patterns due to a high variability in lymphatic drainage per patient, especially in complex lymphatic regions. Therefore, it is our opinion that a within-patient study design is superior to reveal characteristics regarding lymphatic drainage patterns of both tracers.

As already mentioned in the introduction, [^{99m}Tc]Tc-tilmanocept was specifically designed for SLN identification, providing characteristics that could be of potential value in complex lymphatic regions, as is the case in OSCC. Our data clearly underlines its theoretical effect of a more rapid clearance of the radioactivity from the injection site due to its smaller molecular size. This may benefit SLN detection, particularly in situations with close spatial relation between injection site and SLNs, which is especially the case in floor of mouth tumours. Using [^{99m}Tc]Tc-tilmanocept, Agrawal et al. supported this theory with an impressively low false-negative rate of 2.56% for SLNB in OSCC, which was also found in FOM tumours.[14] In that study however, a complementary neck dissection in the same session was performed as validation method (reference standard) for the SLNB procedure. However, micrometastases remain undetected in up to 15% of routinely processed neck dissection specimens.[24,25] Therefore, in case of a negative SLNB, a wait-and-scan approach should be considered as the best gold-standard.[26] As a consequence, further studies with long term follow-up are needed to investigate the efficacy of [^{99m}Tc]Tc-tilmanocept for detection of occult metastases.

In our study, a higher percentage of radioactivity in background was seen for [^{99m}Tc]Tc-tilmanocept compared to [^{99m}Tc]Tc-nanocolloid. One possible explanation could be the smaller molecular diameter of 7 nanometers, which enhances diffusion into

lymphatic channels as well as blood capillaries. As stated by Ellner et al., [^{99m}Tc]Tc-tilmanocept showed a percentage of injected dose below 2.6% for liver, kidney, bladder and head.[27] Although the background radioactivity for [^{99m}Tc]Tc-tilmanocept was still marginal (2.23%; SUV 0.132), it explains the residual distribution of [^{99m}Tc]Tc-tilmanocept in the presence of a lower radioactivity residing in both the injection site, as well as in the lymph nodes.

One of our study limitations is the difference in amount of radioactivity between both tracers in the first 10 patients: 74 MBq [^{99m}Tc]Tc-tilmanocept vs. 120 MBq [^{99m}Tc]Tc-nanocolloid respectively. [^{99m}Tc]Tc-tilmanocept was approved by FDA (Food and Drug Administration) and EMA (European Medicines Agency) for identification of SLNs using 74 MBq in a two-day protocol. In our institution SLNB is routinely performed with 120 MBq [^{99m}Tc]Tc-nanocolloid. Because the first 10 patients were surgically treated based on [^{99m}Tc]Tc-nanocolloid, they received this routinely used amount of radioactivity to safely perform SLNB. This difference was corrected during quantitative analysis by correlating measured radioactivity in the VOIs to the radioactive dose injected. In the second 10 patients, [^{99m}Tc]Tc-tilmanocept was leading for SLNB procedure and therefore the amount of radioactivity could be equalized for both tracers (74 MBq). Another limitation is the impossibility of comparing hotspots at different time points post injection. Due to the impossibility of performing attenuation correction on planar lymphoscintigraphy, we unfortunately could not reliably compare SLN visualization at different time points due to different imaging modalities. Intensity of hotspots could easily be under- or overestimated based on physiological structures in near surroundings (e.g. mandible). On planar lymphoscintigraphy only anterior-posterior or oblique images could be used. This impedes us from differentiating and analyzing hotspots located in the same plane. Therefore, we opted to perform only quantitative analysis based on SPECT-CT.

In some patients for whom [^{99m}Tc]Tc-tilmanocept was leading to identify SLNs during surgery, it proved challenging to accurately locate SLNs due to a scarce of activity on the second day, which was considered a drawback by the surgeon. This may be due to the relatively low radioactive uptake in SLNs of [^{99m}Tc]Tc-tilmanocept that was seen in our population. As the injected activity was lower than used in [^{99m}Tc]Tc-nanocolloid SLNB (74 vs. 120 MBq) with also lower uptake in SLNs (3.16% vs. 1.95%) this resulted in less activity in SLNs in SLNB with [^{99m}Tc]Tc-tilmanocept, on average 1.4 MBq vs. 3.8 MBq at time of SLN scintigraphy. Vidal-Sicart et al. faced similar challenges during intraoperative localisation of SLNs using [^{99m}Tc]Tc-tilmanocept, which can probably be overcome by a higher injection dose of [^{99m}Tc]Tc-tilmanocept.[13]

In conclusion, our results suggest that [^{99m}Tc]Tc-tilmanocept had a higher injection site clearance, but at the same time a lower uptake in the SLN, resulting in an SLN to injection site ratio, which was not significantly different from [^{99m}Tc]Tc-nanocolloid. The relatively low radioactive uptake in SLNs of [^{99m}Tc]Tc-tilmanocept may limit intraoperative detection of SLNs, but might be overcome by a higher injection dose.

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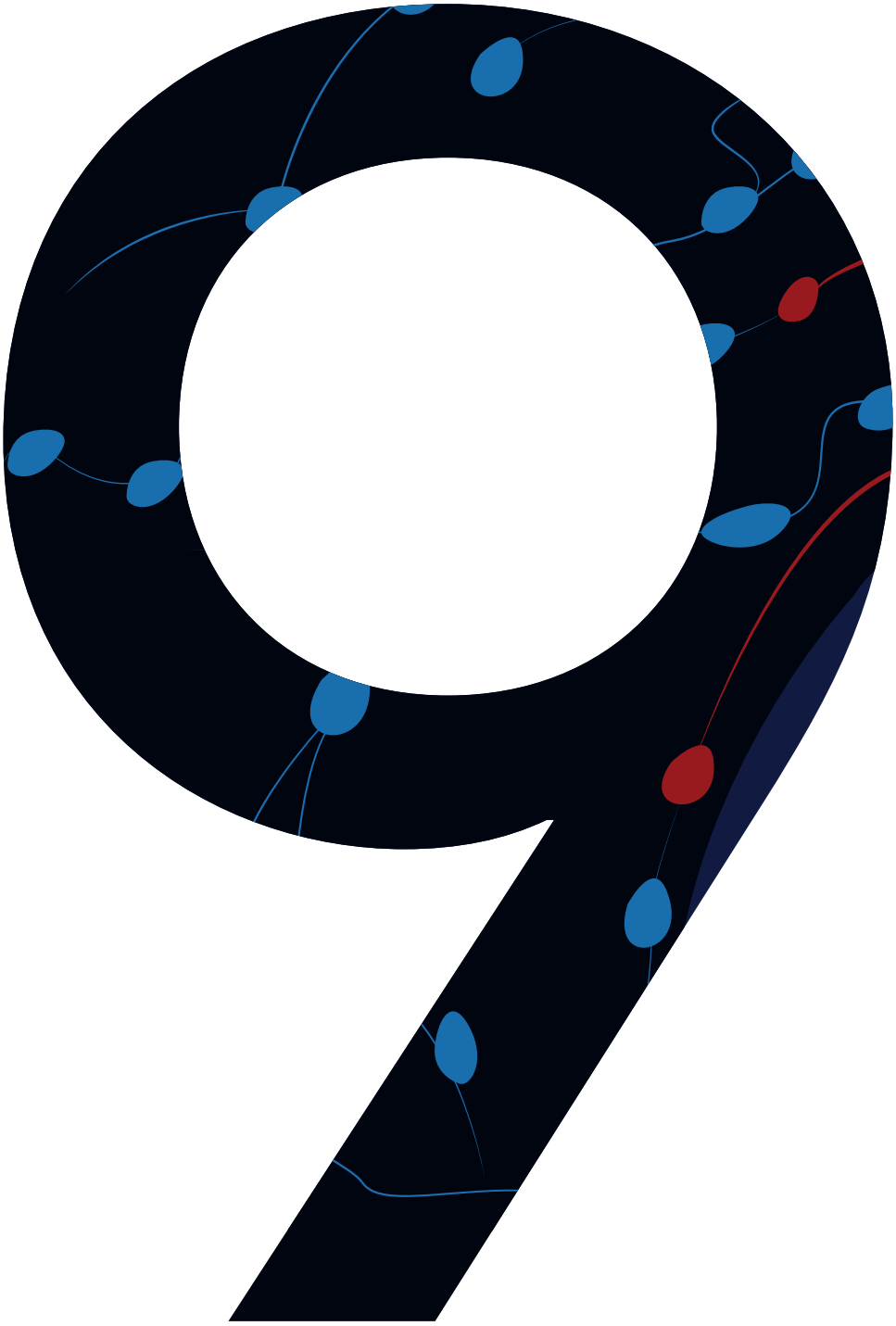

Summary, general discussion and future perspectives

Nederlandse samenvatting

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Dankwoord

SUMMARY, GENERAL DISCUSSION AND FUTURE PERSPECTIVES

This thesis addresses one of the most important challenges in HNSCC, i.e. staging of the clinically node negative neck. As stated in the introduction of this thesis, physical examination of the neck as well as conventional imaging techniques (computerized tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and ultrasonography (US)) are not reliably able to detect occult lymphatic metastases.[1] Due to all clinical and radiological limitations, a reasonable part of patients had a substantial risk of having occult metastases. Specified for early stage oral cavity cancer, this risk is estimated about 30%.[2-4] A dilemma exists how to treat these patients. One could treat all patients prophylactically with an elective neck dissection (with surgical overtreatment in up to 70%), or to leave the neck untreated and pursue patients in a stringent "watchful waiting" policy. Because lymphatic metastases are a major prognostic factor in patients with HNSCC, improvements are highly necessary.[5,6] The sentinel lymph node biopsy (SLNB) procedure has been introduced as a diagnostic staging procedure in a variety of tumour types, including head and neck cancer during the last decade. The SLNB procedure aims to identify the first draining lymph node (sentinel lymph node (SLN)), which is most likely to harbour metastases based on an orderly pattern of lymphatic drainage within a nodal basin. Therefore, a SLN should always contain metastatic disease in case of lymphogenic spread. Shortly, the SLNB procedure consists of three steps; preoperative identification of SLNs based on lymphoscintigraphic imaging, surgical harvesting of radioactive lymph nodes, and extensive histopathological examination of the harvested SLNs (***chapter 1***).

In this thesis the value, potential applications and improvements of SLNB in early stage oral cancer patients are described.

In 2007 SLNB was used for the first time in the Netherlands as diagnostic imaging modality in a patient having early stage oral cavity cancer. Since then, a consecutive cohort was collected to determine the diagnostic efficacy of SLNB. In ***chapter 2***, the results of 90 patients are presented. In this study patients were treated following the principle that only in case of a SLN containing metastasis (positive SLNB) an additional complementary neck dissection was performed. All negative SLNBs were followed up by physical examination and ultrasound-guided fine-needle aspiration cytology every 3 months during the first year of follow-up. In 87 of 90 patients (97%) it was possible to harvest at least 1 SLN. In 30% (26/87) of the patients a positive SLNB was found. Of

the 61 SLNB negative patients, 2 patients (3%) developed regional metastasis during follow-up (median follow-up 18 months). This resulted in a sensitivity of 93% and a negative predictive value of 97%. In this cohort, the sensitivity of SLNB in patients with a tumour located in the floor of mouth (FOM) seemed lower, although not statistically significant (86% vs. 95%, p = 0.44). Furthermore, this study showed that the majority of SLNB positive patients had isolated tumour cells (ITC) or micrometastases as largest tumour deposit in the SLN. We concluded that SLNB alone is a safe and reliable staging procedure. Also we stated that the clinical relevance of micrometastatic disease needs to be further investigated. As a result of this and other studies, SLNB was nationwide adopted and implemented in our guidelines.[7,8] A non-significant difference in accuracy regarding location of the primary tumour was in line with 2 other studies published so far.[9,10] The most common theory for this lower accuracy in FOM tumours is the "shine-through" phenomenon. Due to the short distance between the primary tumour and the SLN(s) in level I, the large peritumoural injection spot will overshine the uptake in potential SLN(s). As a consequence, these SLN(s) may not be visualized and thus not be identified, presumably resulting in lower accuracy in FOM tumours.

In the most recent meta-analysis comprising 66 studies (3566 patients) a pooled sensitivity of 87% and a pooled negative predictive value of 94% was found.[11] Due to the overall predominantly good performance, SLNB was quickly adopted during the last decade as diagnostic staging method. Compared to the routinely performed END, its minimally invasive design combined with a high sensitivity for detecting occult metastases offers the possibility to reduce the number of surgically overtreated patients. In order to reliably compare accuracy rates of END and SLNB, we performed a nationwide multicenter study of 5 Dutch head and neck centers. In ***chapter 3*** we retrospectively analyzed 390 patients staged by END and 488 by SLNB. The overall sensitivity of detecting occult metastasis was comparable between END and SLNB (84% vs. 81%, p = 0.612). Both groups had a similar NPV as well (93%, p = 1.000). Although there is dissimilarity in pT staging between both cohorts (more pT1 in SLNB cohort), also when corrected for pT stage, no significant differences between both cohorts were found. Due to the above mentioned "shine-through" phenomenon in FOM tumours, we separately analyzed these tumours. SLNB showed a statistical significant lower sensitivity for FOM tumours compared to END FOM tumours (63% vs. 92%, p = 0.006). Also, SLNB FOM tumours showed a significant lower sensitivity compared to non-FOM tumours in the SLNB cohort (63% vs. 86%, p = 0.008). Supporting the "shine-trough" phenomenon, the majority of regional metastasis during follow-up were found in level I. In general we concluded that SLNB is as accurate as END for

detection of occult lymphatic metastases, except for FOM tumours. It is remarkably clear that in this study the overall accuracy of SLNB had decreased compared to the study presented in chapter 2. As also stated in a meta-analysis of 2017, there is a trend visible that more recent publications showing lower accuracy rates compared to early reports.[11] A multicenter design with slightly different in hospital protocols, a more "routine" instead of research setting and inclusion of learning curves of new SLNB performing surgeons could be possible explanations for this trend. A prolonged follow-up will also increase the chance of developing regional metastases and thus lowering the accuracy. Despite this lower accuracy for SLNB, it must be realized that END is not doing better. In addition, END resulted also in more morbidity (e.g. shoulder dysfunction and nerve injury).[12] One of the limitations of this study remains its retrospective design. To compare both strategies as decently as possible, a multicenter (and possibly multinational) randomized controlled trial must be performed. So far, as randomized controlled trials comparing the accuracy of SLNB with routinely used END are lacking, this retrospective cohort study provides the highest evidence of the effectiveness of SLNB in oral cancer.

The SLNB cohort in our study is so far the largest cohort worldwide in literature and is showing that SLNB in FOM tumours is significantly doing worse. In other words, SLNB in its current practice is not accurately detecting occult metastases in FOM tumours. Nowadays, a variety of research is performed to solve this problem. One possible solution could be the use of a higher resolution imaging (positron emission tomography (PET)) using a PET-tracer.[13] Although it was shown that PET-CT had a superior preoperative distinction between SLNs and second echelon nodes compared to routinely used SPECT-CT, intraoperative detection using a PET-probe diminished its clinical applicability.[14] In an attempt to avoid intraoperative use of a PET-probe, the combination of a PET-tracer with a very short half-life, e.g. gallium-68 for preoperative imaging and a technetium-99m (^{99m}Tc)tracer for intraoperative SLN detection can be used. This approach is currently under investigation. Other approaches with higher resolution imaging using nonradioactive tracers include magnetic resonance (MR) lymphography and CT lymphography.[15] During a feasibility study in oral cavity cancer, SLNs were detected in all 26 patients with MR lymphography, most often with visualization of lymph node vessels as well (81%).[16] They concluded that MR lymphography is a safe and reliable imaging technique for preoperative identification of SLNs. Another possibility for detection of SLNs on MR lymphography is when using superparamagnetic iron oxide (SPIO) particles. These particles act as contrast agent and iron deposition is seen within sinuses and macrophages. These nodes

can preoperatively be visualized on MRI and during surgery by using a handheld magnetometer. In patients with breast cancer, identification of SLNs using SPIO particles was not inferior to the standard technique.[17] With respect to CT lymphography, a validation study in oral cavity cancer showed a detection rate of 90.3%, a sensitivity of 80.0%, and a negative predictive value of 95.8%.[18] Later on, they presented a combination of preoperative CT lymphography and intraoperative use of indocyanine green fluorescence with a detection rate of 89%.[19] Although these new inventive techniques using nonradioactive isotopes are promising, further studies are needed to evaluate both MR- and CT lymphography techniques in a larger cohort of patients. Not only improvements with imaging techniques can contribute to solve the problem of the "shine-through" phenomenon, also in the field of radioactive tracers potential improvements are investigated. The routine procedure consists of preoperative peritumoural injection of a ^{99m}Tc-labelled colloid followed by lymphoscintigraphy. In Europe, [^{99m}Tc]Tc-nanocolloid is the most frequently used radiocolloid for SLN mapping. As relatively new radioactive agent, [^{99m}Tc]Tc-tilmanocept (Lymphoseek ®) has been specifically designed for SLN identification.[20] [^{99m}Tc]Tc-tilmanocept is a small sized receptor targeted (CD206) SLN detection agent. Its smaller size in combination with binding on macrophages in lymph nodes may be of added value by proposing a rapid clearance from the injection site, rapid uptake and high retention within the SLN, as well as low uptake by the remaining (higher echelon) lymph nodes. In particular, these characteristics may be of benefit in FOM tumours with close spatial relationship between the primary tumour and the SLN. A validation study using [^{99m}Tc]Tc-tilmanocept showed an SLN identification rate of 97.6%, a false negative rate of 2.56% and a negative predictive value of 97.8%.[21] These high figures were also obtained in FOM carcinomas. Two other additional techniques are recommended in the SLNB surgical consensus guidelines of 2018.[22] Firstly, fluorescent tracers may also be helpful for identification of nodes close to the injection site.[23,24] One disadvantage is the limited depth of penetration of the fluorescent signal (approximately 1 centimeter), only allowing SLNs on the surface to be visualized. However, in combination with a radioactive isotope (e.g. indocyanine-green-[^{99m}Tc]Tc-nanocolloid), fluorescence could be of added value. In 30 patients with a total of 94 SLNs, 11 SLNs (12%) were only identified using fluorescence with near-infrared imaging. Of note, 10 of these SLNs were located in level I (and thus in close proximity with the primary tumour). Secondly, the technique of Stoeckli et al. is mentioned in the surgical consensus guidelines.[25] This technique consists of routinely investigating level I nodes with a gamma probe after submental and preglandular fat pad mobilization or dissection through a submandibular incision. They showed that 50% of the SLNs in level I were only detected intraoperatively. We believe that when

performing SLNB in patients with a FOM tumour, this last mentioned technique of exploring level I should be implemented in routine treatment of these patients until the accuracy of SLNB in FOM tumours is improved to a level comparable to other oral subsites.

In our SLNB cohort, 107 patients showed at least one histopathological positive SLN, followed by a complementary neck dissection in 92 patients. According to the classification of Hermanek et al. metastases are categorized based on size into isolated tumour cells (≤ 0.2 mm), micrometastasis (> 0.2 mm and ≤ 2 mm) or macrometastasis (> 2 mm).[26] Of our SLNB positive patients, 14% had ITCs, 29% micrometastases and 57% macrometastases. The complementary neck dissection showed additional non-SLN metastases in 1 patient with ITC, 1 patient with micrometastases and 17 patients with macrometastases. No significant differences were observed regarding disease specific survival categorized per size of metastases. In ***chapter 4*** we presented an overview of 27 studies comprising 511 SLNB positive patients in order to determine risk factors for additional non-SLN metastases in complementary neck dissection specimens. Additional non-SLN metastases were found in 31% of the neck dissection specimens. Regarding size of the tumour deposit in SLNB, non-SLN metastases were detected (available in 9 studies) in 13%, 20% and 40% of patients with ITC, micro- and macrometastasis in the SLN, respectively. These results are somewhat different from our abovementioned nationwide multicenter study. So far, due to the limited number of studies in literature, it is difficult to determine the value of complementary neck dissections in case of ITCs or micrometastasis. In addition, one has to consider that complementary neck dissection specimens are not examined as meticulously as SLNs. Studies on neck dissection specimens show that immunohistochemistry can reveal small metastases in 15% of the patients that remain undetected in routine haematoxylin and eosin (H&E) staining.[27] To explore if patients with ITC or micrometastasis in SLNs need a subsequent neck dissection it is important that all future studies report SLN metastases in these categories. Only then the question can be answered if SLNB can be used as treatment, and not only as diagnostic procedure, in patients selected by the type of tumour deposit in SLNs. Currently, data is lacking to safely omit therapeutic neck dissections in case of ITC or micrometastasis.

Besides size of tumour deposit in the SLNB, other factors may also contribute to the risk of having non-SLN metastases. Reporting other risk factors could be useful to develop a nomogram selecting SLNB positive patients for a complementary neck dissection or wait and scan follow-up. In our study, the probability of non-SLN metastasis seems to

be higher in case of more than one positive SLN (29% vs. 24%), absence of negative SLNs (40% vs. 19%) and a positive SLN ratio of more than 50% (38% vs. 19%). If a reliable nomogram to predict non-SLN metastases can be developed, in a subset of patients SLNB might be a therapeutic rather than just a diagnostic procedure by avoiding subsequent tumour-negative neck dissections. Multiple studies showed that axillary lymph node dissection following positive SLNB could be avoided in the majority of T1-2,cN0 breast cancer patients without compromising locoregional recurrence-free, disease-free and overall survival.[28,29] In breast cancer, several nomograms have been developed and validated to calculate the risk of additional non-SLN metastasis in patients with a positive SLNB.[30-32] In general, extranodal extension, tumour size, SLNB metastasis size, positive SLN ratio > 50% and lymphovascular invasion are related with the presence of non-SLN metastasis. While these nomograms are promising one have to consider the different approach of treatment between breast cancer and oral cavity malignancies. Since breast cancer patients are often treated with (neo)adjuvant systemic therapy, these nomograms could not easily be translated to oral cancer patients who are usually treated with surgery alone. Further research is mandatory to explore our above mentioned features in order to develop a nomogram for oral cavity cancer as well.

Relevant in the earlier noticed dilemma of performing END or a watchful waiting policy is the higher risk of having nodal metastases with an increasing depth of invasion (DOI) of the primary tumour. DOI is the most promising predictive factor for nodal metastases using END as histopathological staging method.[33-35] Generally, END was recommended in case of ≥4 mm tumour thickness and/or depth of invasion. These definitions are often used interchangeably in literature, although nowadays the definition of Moore et al. to measure "from a theoretical reconstructed normal mucosal line to the deepest extent of growth" is widely adopted.[36] As stated above with END a high number of metastases (up to 15%) remain undetected using routine H&E staining. In SLNB negative patients a watchful waiting strategy during follow-up renders the opportunity for ITC and micrometastases (missed by routine H&E examination of a neck dissection specimen) to become clinical manifest. Therefore, we believe that SLNB can serve as a more accurate reference standard than END for evaluation of tests predicting presence of nodal metastases. In ***chapter 5*** we aimed to assess if DOI can predict occult nodal disease in 199 early stage oral cancer patients who underwent SLNB. In 64 patients with regional metastases (positive SLNB and false negative SLNB), mean DOI was 6.6 mm compared to 4.7 mm in patients without regional metastasis ($p = 0.003$). The receiver operating characteristic (ROC) curve showed an area under

the curve of 0.65 having a most optimal cut-off point on a DOI of 3.4 mm (sensitivity 83%, specificity 47%). To note, of all patients with tumours ≤ 3.4 mm DOI, still 15% (11/74) had regional metastases. Based on these results, in our opinion SLNB should be offered to stage every oral squamous cell carcinoma patient without the need to enter the neck for resection or reconstruction, regardless of DOI. This is in line with the only other article in literature using SLNB-alone as reference standard presenting an area under the curve of 0.54.[37] They suggested also to use SLNB in all early stage oral cavity carcinomas with a cN0 neck.

During last years, research into the value of DOI of the primary tumour regarding regional metastasis and survival has expanded.[38] A large international study showed improved discrimination in outcome using DOI by intervals of 5 mm.[39] This is also reflected in the new TNM classification system (8th edition) in which DOI is incorporated as histopathological determinant for clinical and pathological T staging.[40,41] In this new classification, DOI of > 5 mm was used for upstaging from T1 to T2 and DOI > 10 mm for upstaging to T3. Upstaging rates vary in the order of 12 to 37% (without upstaging due to different pN staging).[42-46] Most often, upstaging resulted in a better (but not always significant) discrimination between pT stages. We classified our patients according to the 7th and 8th TNM classification systems, consequently resulting in a large shift towards higher pT stages in the new edition. In survival analyses though, using the 8th TNM classification resulted only in a slightly (not significant) better distinction for regional disease free survival compared to the 7th edition. Overall survival and disease specific survival did not differ between both editions at all. A possible explanation for this discrepancy between our cohort and the study on which the new classification was based could be the evidently smaller tumour size and shorter follow-up of our population. Thereby, it should be addressed here too, that neck dissection specimens were used as reference for having regional metastasis (with the previously explained potential risk of missed metastasis). So far, no other studies were published regarding the comparison of survival analyses between the 7th and 8th edition in early stage oral cancer patients who underwent SLNB.

The implementation of DOI as determinant not only for histopathological T staging but also clinical T staging is challenging. Most articles are based on specimen driven DOI measurements, while for pretreatment decision making DOI has to be clinically assessed. Lydiatt et al. describe that clinical examination of DOI requires careful palpation, supplemented by radiographic assessment.[41] A meta-analysis found a high correlation between intraoral ultrasonography and histopathological thickness

measurements in tongue tumours (r=0.88). On average, they showed a minimal and clinically acceptable overestimation of 0.5 mm on ultrasonography. Other imaging modalities, as MRI or CT-scans appeared to measure less accurate in particularly thin tumours. These findings were confirmed by a more recent meta-analysis, again showing higher correlation between histopathological thickness and ultrasonography compared to MRI (r=0.96 vs. 0.88).[47] Given these better results, its easier applicability and lower costs intraoral ultrasonography could be the approach of choice determining clinical DOI.

In the last part of this thesis we would like to focus on new possibilities using SLNB and also on ways to actually improve the procedure itself. All SLNB literature showing high sensitivity and negative predictive value rates are based on patients with primary early stage oral cancer and a previously untreated neck (e.g. no neck dissection and/or (chemo)radiotherapy). Previously treatment of the neck may disrupt lymphatics and most likely therefore alters lymphatic drainage patterns. Despite the relatively common local recurrences and second primary tumours in oral squamous cell carcinoma, only one study reported about the accuracy of SLNB in 22 patients with a previously treated neck.[48] In that study, patients with ipsi- or bilateral neck treatment had a SLN detection rate of 83% and unexpected lymphatic drainage patterns were observed in 67% of the patients. Due to the increased experience using SLNB in general, during the last years patients with a previously treated neck are staged by SLNB as well. In a collaboration of three Dutch head and neck centers we retrospectively analyzed the accuracy of SLNB in 53 patients with a previously treated neck and evaluated the lymphatic drainage patterns in 43 patients in this cohort (***chapter 6***). We presented an imaging detection rate of 87% and a surgical detection rate of 93%. This is relatively low compared to previously untreated patients with identification rates of 97-98%.[7,10,49] Interestingly, all patients without identified SLNs had radiotherapy in history, sometimes combined with surgery. In contrary, patients with a prior SLNB had no lower identification rate, suggesting that SLNB ensures less damage to lymphatic vessels compared to radiotherapy. In this study, three patients showed a positive SLNB and only one patient showed regional recurrence during follow-up (false negative SLNB), resulting in a 75% sensitivity and 98% negative predictive value. The low number of regional metastases could be potentially explained by a close follow-up scheme after treatment of their first primary tumour, also reflected by a high number of pT1 tumours in this study. Due to only 4 patients with regional metastases, it might be prematurely to conclude that SLNB is reliable in pretreated patients. However, a negative predictive value of 98% suggest that SLNB is at least promising and further research is necessary to determine its reliability.

Assuming that lymphatic drainage is expected normally in levels I-III for oral cavity cancer, in 30% (13/43) of the patients unexpected drainage was found. Besides the described study of Flach et al., no other studies are published for SLNB in early stage oral cavity cancer with a previously treated neck.[48] Correlating to patients with an untreated neck, unexpected drainage patterns were reported in 16% of patients in a large multicenter trial.[50] However, in a variety of other tumour types SLNB has been described in recurrent or second primary tumours. In recurrent breast cancer lower identification rates were observed and aberrant lymphatic drainage were seen in up to 40%.[51,52] Extra-axillary lymphatic drainage and drainage to the contralateral axilla was significantly more observed in patients previously treated with axillary lymph node dissection ALND compared to prior SLNB. Both studies concluded that repeat SLNB is feasible and should replace routine ALND as standard axillary restaging procedure in recurrent disease. Similarly in recurrent vulvar cancer and melanoma unpredicted drainage patterns were found compared to previously untreated patients.[53,54] These findings strengthen the value of SLNB in assessing the individual lymphatic drainage pattern.

Identifying individual lymphatic drainage patterns was exactly the idea of Cabañas when performing SLNB in the first patients in 1977.[55] In the first decades SLNB identification was done with planar static and dynamic lymphoscintigraphy only.[56] Visualization of SLNs could also be performed by single photon emission computed tomography with computed tomography (SPECT-CT). SPECT-CT was introduced in 2003 for SLNB in oral cancer.[57] As described in a review SPECT-CT provides useful information in localization of SLNs and showed additional SLNs compared to planar dynamic and static lymphoscintigraphy.[58] To determine the added value of SPECT-CT we analyzed 66 patients with early stage oral cavity cancer (***chapter 7***). According to the definition of Morton et al. a clear visible and rapidly appearing lymph node was considered to be a SLN.[59] In one patient no SLN could be identified on both imaging modalities (identification rate 98%). In 22% of the patients (14/65), 15 additional SLNs could be identified due to SPECT-CT. In two of these additional SLNs metastasis were found, resulting in an upstaging rate due to SPECT-CT of 3% (2/65). A positive SLNB was found in 10 patients and in of two of these patients (20%) the positive SLN was identified due to the addition of SPECT-CT. We found that five SPECT-CT scans are needed to identify one additional SLN. For identification of one positive SLN this number is 34. In contrast to add SLNs, SPECT-CT also diminished in five patients the number of SLNs based on planar lymphoscintigraphy (e.g. injection spot rather than SLN in four patients). In comparison with our results both lower, comparable and higher

rates are published in literature.[58] A possible explanation for these differences may be the variability in imaging protocols, amount of injected radioactivity and the time of injection related to surgery (same- or 2-day protocol).[50] Moreover, defining SLNs on lymphoscintigraphy could be difficult. Interobserver variability of lymphoscintigraphic interpretation shows moderate agreement among 16 observers.[60] Studies of SPECT-CT in SLNB which included different locations of primary tumours found especially advantages for tumours with close proximity to the SLN and complex lymphatic regions which is the case in the head and neck region.[61] Therefore, we hypothesized that SPECT-CT could be of value especially in patients with FOM tumours, hopefully reducing the "shine-through" phenomenon as previously explained. With respect to the location of the primary tumour SPECT-CT identified more additional SLNs in patients with floor of mouth tumours compared with tumours of the tongue (42% vs. 13%, $p = 0.07$). However, these additional SLNs were not necessarily identified in level I. In our opinion SPECT-CT did not solve the problem of "shine-through" as described in FOM tumours, resulting in a persistent lower accuracy for FOM tumours.

As second objective, we determined the additional value of SPECT-CT regarding topographical orientation and anatomical information. SPECT-CT was supposed to be important if the surgeon would probably make a different (or more accurate) surgical approach based on the additional information. Important additional anatomical information of the SLNs preoperatively due to SPECT-CT imaging was observed in 3% of the patients (2/65). Anatomical levels of SLNs were changed in 28% of the patients by SPECT-CT. Obviously, in the rest of the patients a better topographical orientation for the surgeon had been provided by SPECT-CT compared with planar imaging. Although we had the impression that SLNB could also be successfully performed with planar lymphoscintigraphy only, our study suggests that SPECT-CT is helpful preoperatively. The EANM practical guidelines declared SPECT-CT (if available) as mandatory providing accurate localization and depth evaluation of SLNs.[62] As limitation so far, SPECT-CT identifies only SLNs preoperatively. Freehand SPECT-CT is an innovative technique guiding the surgeon intraoperatively to the exact location of SLNs. It enables 3D reality displays based on data acquisition by a freehand scan. Nowadays, research showed the feasibility of the freehand SPECT-CT.[63,64] Besides good accuracy it provides helpful information facilitating SLNB in a quarter of the cases. Freehand SPECT-CT seems promising so far, although still some comparable difficulties as with the conventional gamma probe are observed (i.e. "shine-through" phenomenon).[63]

Altogether, the "shine-through" phenomenon remains the most important limitation of SLNB in oral cavity cancer. A wide variety of possible solutions are reviewed and discussed above. In ***chapter 8*** we focused on the previously described newly designed radioactive agent, [^{99m}Tc]Tc-tilmanocept (Lymphoseek ®). Due to its proposed rapid clearance from the injection site, rapid uptake and high retention within the SLN, as well as low uptake by the remaining (higher echelon) lymph nodes, [^{99m}Tc]Tc-tilmanocept may particularly be of benefit in floor of mouth tumours. In Europe [^{99m}Tc]Tc-nanocolloid is the most frequently used radiocolloid for SLN mapping. So far, there are no studies performed comparing head to head [^{99m}Tc]Tc-tilmanocept with [^{99m}Tc]Tc-labelled nanocolloid. We designed a prospective within-patient evaluation study to investigate injection site clearance and uptake in SLNs in 20 patients. The injection site's remaining radioactivity was, as was expected, significantly lower for [^{99m}Tc]Tc-tilmanocept (29.9%; SD±7.6), compared to [^{99m}Tc]Tc-nanocolloid (60.9%; SD±16.1) ($p<0.001$). The radioactive uptake in SLNs was significantly higher for [^{99m}Tc]Tc-nanocolloid compared to [^{99m}Tc]Tc-tilmanocept (3.16% vs. 1.95% respectively, $p = 0.01$). There were no differences regarding number of SLNs, number of second echelon nodes or SLN to injection site ratio. There was an excellent interobserver agreement for both agents. Currently, there are no other within-patient evaluation studies comparing [^{99m}Tc]Tc-tilmanocept to other radioactive agents. Unkart et al. presented a randomized controlled trial of 52 breast cancer patients showing no differences concerning number of hotspots, number of SLNs and time to surgical removal for [^{99m}Tc]Tc-tilmanocept compared to [^{99m}Tc]Tc-sulfur colloid.[65] However, randomizing patients for either the one or the other tracer did not clearly clarify discrepancies between both tracers with respect to drainage patterns due to a high variability in lymphatic drainage per patient, especially in complex lymphatic regions like the neck. Therefore, it is our considered opinion that a within-patient study design is superior to reveal characteristics regarding lymphatic drainage patterns of both tracers. We propose that further research is mandatory to investigate the efficacy of [^{99m}Tc]Tc-tilmanocept for detection of occult metastasis in early stage oral cancer patients.

CONCLUDING REMARKS

This thesis concludes that SLNB is a reliable method for detection of occult metastases in patients with early stage oral cavity cancer. SLNB renders a personalized assessment of the complex lymphatics in the head and neck region and could therefore also be used in patients who are previously treated in the neck. Despite the overall good performance, room for improvement still exists. In the near future research should focus on improvements to increase the accuracy of SLNB in patients with FOM tumours. The frequently mentioned phenomenon of "shine-through" limit the applicability of SLNB in these patients. This thesis investigated the value of SPECT-CT and the use of a new radioactive tracer ([^{99m}Tc]Tc-tilmanocept) to reduce this phenomenon. So far, we had to conclude that both techniques are promising, although the technique of exploring level I should be implemented in routine treatment of these patients until the accuracy of SLNB in FOM tumours is improved to a level comparable to other oral subsites.

During the last decade, SLNB in head and neck cancer is gaining more and more attention worldwide and is therefore increasingly used as diagnostic method. This thesis provides evidence for its applicability and suitability in oral cancer patients. Furthermore, this thesis addresses important unmet needs that should be further explored in the near future.

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NEDERLANDSE SAMENVATTING

Dit proefschrift beschrijft één van de belangrijkste uitdagingen binnen de hoofd-hals oncologie; de stadiëring van de klinisch negatieve hals bij vroeg-stadium mondholtecarcinomen. Lichamelijk onderzoek van de hals, alsmede conventionele beeldvormende technieken zoals CT, MRI, PET en echo zijn niet betrouwbaar genoeg in het detecteren van occulte lymfekliermetastasering. Dit houdt in dat een aanzienlijk deel van de patiënten risico heeft op metastasen. Voor het vroeg-stadium mondholtecarcinoom wordt dit risico geschat op 30%. Hoe deze patiënten moeten worden behandeld is een terugkerend dilemma. Enerzijds kunnen de lymfeklieren profylactisch worden verwijderd door middel van een electieve halsklierdissectie (HKD), waarbij 70% van de patiënten dus wordt "overbehandeld". Anderzijds kan de hals ongemoeid worden gelaten en worden patiënten vervolgd met een "watchful waiting" beleid. Gedurende de eerste jaren van follow-up kan er dan een therapeutische halsklierdissectie worden verricht indien zich lymfekliermetastasen voordoen. Dit dilemma ontstaat met name als de hals niet geëxploreerd hoeft te worden bij het verwijderen van de primaire tumor, zoals het geval is bij kleine (vroeg-stadium) mondholtecarcinomen. Aangezien lymfekliermetastasering een belangrijke prognostische factor is bij patiënten met hoofd-hals kanker, zijn verbeteringen voor de detectie van deze metastasen hard nodig.

De schildwachtklier (SWK) procedure is tegenwoordig bij een verscheidenheid aan oncologische ziektebeelden ingevoerd als diagnostisch onderzoek voor de bepaling van lymfekliermetastasering. Dit is ook het geval bij het vroeg-stadium mondholtecarcinoom. De SWK-procedure heeft als doel betrouwbaar onderscheid te maken tussen patiënten die baat hebben bij het uitvoeren van een halsklierdissectie of bij wie een afwachtend beleid kan worden gevolgd. Het SWK-concept gaat ervan uit dat een tumor in eerste instantie draineert op één of enkele lymfeklieren, de SWK(en), en dat de eerste lymfekliermetastase altijd in deze SWK(en) uitgroeit. In het kort bestaat de procedure uit 3 stappen; preoperatieve identificatie van SWK(en) door middel van lymfoscintigrafische beeldvorming, het chirurgisch oogsten van SWK(en), en nauwkeurige histopathologische beoordeling van de geoogste klieren (***hoofdstuk 1***).

In dit proefschrift worden achtereenvolgens de waarde, potentiële toepassingen en verbeterpunten van de SWK-procedure bij het vroeg-stadium mondholtecarcinoom besproken.

In 2007 werd in Nederland voor het eerst gebruik gemaakt van de SWK-procedure voor de detectie van lymfekliermetastasering bij een patiënt met een mondholtecarcinoom. Sindsdien is een opeenvolgend cohort bijgehouden om de accuratesse en de toepasbaarheid van de SWK-procedure te bepalen. In ***hoofdstuk 2*** tonen wij de resultaten van 90 patiënten. In deze studie werd alleen een complementerende halsklierdissectie uitgevoerd indien de SWK metastasering toonde. Het was mogelijk om bij 87 van de 90 patiënten (97%) ten minste één SWK te oogsten. In 30% van de patiënten (26/87) toonde de SWK metastasering. Van de 61 SWK-negatieve patiënten ontwikkelden 2 patiënten (3%) gedurende de follow-up toch een lymfekliermetastase (fout-negatieve SWK-procedure). Dit resulteerde in een sensitiviteit van 93% en een negatief voorspellende waarde van 97%. In dit cohort leek er sprake te zijn van een verminderde sensitiviteit bij patiënten met een tumor gelegen in de mondbodem, al was dit niet significant verschillend (86% vs. 95%, p = 0.44). Verder bleek dat bij de meerderheid van de patiënten met een metastase in de SWK, dit berustte op geïsoleerde tumorcellen of micrometastasen. Wij concludeerden dat de SWK-procedure een veilige en betrouwbare methode is om de klinisch negatieve hals te stadiëren. Tevens stelden wij dat de klinische relevantie van micrometastasen in de SWK(en) verder dient te worden onderzocht. Nadien werd de SWK-procedure ook opgenomen in nationale richtlijnen voor de behandeling van het vroeg-stadium mondholtecarcinoom.

Een verminderde accuratesse van de SWK-procedure bij het mondbodemcarcinoom wordt ook in andere studies gerapporteerd. Theoretisch wordt dit toegeschreven aan het zogenoemde "shine-through" fenomeen. Door de korte afstand tussen de primaire tumor en de SWK in level I bestaat de kans dat de peritumorale injectieplaats de uptake van radioactiviteit in de lymfeklieren overstraalt.

Een meta-analyse van 66 studies beschrijft een sensitiviteit van 87% en een negatief voorspellende waarde van 94% voor de SWK-procedure bij het mondholtecarcinoom. Door deze goede accuratesse werd de SWK-procedure de laatste jaren snel geïmplementeerd als diagnosticum. Het minimaal invasieve concept geeft de mogelijkheid het aantal chirurgisch overbehandelde patiënten (door middel van een electieve HKD) te reduceren. Om de accuratesse daadwerkelijk te vergelijken met de electieve HKD, werd een multicenter onderzoek verricht met 5 Nederlandse hoofd-hals centra. In ***hoofdstuk 3*** beschrijven wij de resultaten van het retrospectieve onderzoek van 390 patiënten gestadieerd door middel van een electieve HKD en 488 patiënten met de SWK-procedure. De sensitiviteit van het detecteren van occulte

lymfekliermetastasering was vergelijkbaar tussen beide cohorten (84% vs. 81%, p = 0.612). Beide groepen hadden dezelfde negatief voorspellende waarde (93%, p = 1.000). Hoewel er ongelijkheid is tussen beide groepen ten aanzien van pT1 tumoren (meer pT1 in de SWK groep), zijn er eveneens geen verschillen tussen beide cohorten als voor pT stadiëring gecorrigeerd wordt. Wel toonde de SWK-procedure een statistisch significant lagere sensitiviteit dan de electieve HKD voor patiënten met een tumor in de mondbodem (63% vs. 92%, p = 0.006). Ook binnen het SWK-cohort werd een lager sensitiviteit gevonden voor mondbodemcarcinomen ten opzichte van niet-mondbodemcarcinomen (63% vs. 86%, p = 0.008). Ter ondersteuning van het "shine-through" fenomeen werd het merendeel van deze lymfekliermetastasen tijdens follow-up gevonden in level I. In het algemeen concludeerden wij dat de SWK-procedure even nauwkeurig is als de electieve HKD voor detectie van occulte lymfekliermetastasen, behalve voor mondbodemcarcinomen.

In ons SWK-cohort werden bij 107 patiënten lymfekliermetastasen in de SWK aangetroffen. In 92 van deze patiënten werd een aanvullende HKD verricht. Metastasen werden geclassificeerd op basis van grootte; geïsoleerde tumorcellen (ITC) (≤ 0.2 mm), micrometastasen (> 0.2 mm en ≤ 2 mm)en macrometastasen (> 2 mm). Van onze patiënten had 14% ITC, 29% micrometastasen en 57% macrometastasen. In het aanvullende halsklierdissectiepreparaat werden niet-SWK metastasen aangetroffen bij 1 patiënt met ITC, bij 1 patiënt met micrometastasen en in 17 patiënten met macrometastasen. In ***hoofdstuk 4*** presenteerden wij een overzicht van 27 onderzoeken met 511 SWK-positieve patiënten om risicofactoren te bepalen voor het hebben van niet-SWK metastasen in complementerende halsklierdissectiepreparaten. Niet-SWK metastasen werden aangetroffen bij 31% van de patiënten. Deze metastasen werden aangetroffen in 13% van de patiënten met ITC, 20% van de patiënten met micrometastasen en in 40% van de patiënten met macrometastasen in de SWK. Of patiënten met ITC en micrometastasen een aanvullende HKD nodig hebben is op basis van deze studie lastig in te schatten. Op de vraag of de SWK-procedure bij sommige patiënten wellicht niet alleen als diagnosticum maar ook als daadwerkelijke behandeling kan fungeren, dient verder onderzoek verricht te worden. Momenteel is er onvoldoende bewijs om een aanvullende HKD veilig achterwege te laten bij ITC of micrometastasen in de SWK.

Naast de hierboven genoemde grootte van de metastase in de SWK, kunnen ook andere factoren bijdragen aan het risico op niet-SWK metastasen. Het rapporteren van andere risicofactoren kan nuttig zijn voor de ontwikkeling van een nomogram,

waarbij SWK-positieve patiënten kunnen worden geselecteerd voor een aanvullende behandeling of "watchful waiting". In ons onderzoek lijkt de kans op niet-SWK metastasen hoger te zijn in het geval van meer dan één positieve SWK (29% vs. 24%), afwezigheid van een negatieve SWK (40% vs. 19%) en een positieve SWK-ratio van meer dan 50% (38% vs. 19%). Als er een betrouwbaar nomogram kan worden ontwikkeld om niet-SWK metastasen te voorspellen, kan de SWK-procedure bij een subgroep van patiënten een therapeutische en niet enkel diagnostische procedure zijn door het vermijden van tumor-negatieve halsklierdissecties.

In het eerder genoemde dilemma van het ofwel uitvoeren van een electieve HKD dan wel "watchful waiting" is invasiediepte van de primaire tumor van belang. Invasiediepte is de belangrijkste voorspeller van lymfekliermetastasen. Over het algemeen werd in het verleden een electieve HKD verricht indien de invasiediepte ≥4 mm bedroeg. Het is hierbij wel relevant dat bij routinematig histopathologische beoordeling van het dissectiepreparaat metastasen onopgemerkt kunnen blijven (tot 15%). Door de uitgebreidere histopathologische analyse van de SWK alsmede de follow-up bij een negatieve SWK (waarbij kleine metastasen de kans hebben om manifest te worden), kan de SWK-procedure als meer accurate referentiestandaard dienen dan de electieve HKD voor de evaluatie van testen die metastasen voorspellen. In ***hoofdstuk 5*** onderzochten wij of invasiediepte van de primaire tumor occulte lymfekliermetastasering kan voorspellen in 199 patiënten. In 64 patiënten werd lymfekliermetastasering aangetroffen en in deze groep was de gemiddelde invasiediepte hoger dan bij patiënten zonder metastasen (6.6 mm vs. 4.7 mm, p = 0.003). De "receiver operating characteristic (ROC)" curve toonde een waarde onder de curve van 0.65, met een optimaal afkappunt bij 3.4 mm invasiediepte (sensitiviteit 83%, specificiteit 47%). Van alle patiënten met tumoren ≤ 3.4 mm had echter nog steeds 15% (11/74) regionale metastasen. Naar onze mening zou op basis van deze resultaten de SWK-procedure aan elke patiënt met een vroeg-stadium mondholtecarcinoom moeten worden aangeboden teneinde de hals te stadiëren, ongeacht de invasiediepte van de primaire tumor.

De mogelijk predictieve waarde van invasiediepte komt ook tot uiting in het nieuwe TNM-classificatiesysteem (8^{e} editie), waarin invasiediepte wordt opgenomen als histopathologische determinant voor klinische en pathologische T-stadiëring. In de nieuwe classificatie wordt een invasiediepte > 5 mm gebruikt voor "upstaging" van T1 naar T2 en bij een invasiediepte van > 10 mm "upstaging" naar T3. Wij classificeerden onze patiënten volgens zowel het 7^{e} als het 8^{e} TNM classificatiesysteem, wat resulteerde

in een grote verschuiving naar hogere T stadia. Bij overlevingsanalyses resulteerde het gebruik van de 8e TNM-classificatie echter slechts in een iets (niet significant) beter onderscheid voor regionale ziektevrije overleving in vergelijking met de 7e editie. De totale overleving en ziektespecifieke overleving verschilden helemaal niet tussen beide edities.

In de laatste hoofdstukken van dit proefschrift wordt de focus gelegde op nieuwe mogelijkheden van het gebruik van de SWK-procedure en op verbeteringen van de huidige procedure. Alle literatuur richt zich vooralsnog op patiënten met een niet eerder behandelde hals. Mede gezien de relatief vaak voorkomende lokale recidieven en tweede primaire tumoren bij orale plaveiselcelcarcinomen, hebben wij gekeken of ook bij patiënten met een reeds behandelde hals de SWK-procedure van toegevoegde waarde is. In samenwerking met drie Nederlandse hoofd-halscentra hebben wij retrospectief de nauwkeurigheid van de SWK-procedure geanalyseerd bij 53 patiënten (***hoofdstuk 6***). Op beeldvorming kon bij 87% van de patiënten tenminste 1 SWK worden gedetecteerd, en peroperatief kon in deze groep bij 93% daadwerkelijk een SWK worden geoogst. Deze getallen zijn iets lager in vergelijking met patiënten die niet eerder in de hals zijn behandeld. Vermeldenswaard is dat bij alle patiënten bij wie geen SWK kon worden geïdentificeerd radiotherapie op de hals was gegeven in het verleden, soms in combinatie met een operatie. Patiënten met een SWK-procedure in de voorgeschiedenis hadden echter geen lagere detectiewaarden, wat suggereert dat de SWK-procedure voor minder schade aan de lymfevaten zorgt. Aangenomen dat lymfedrainage normaliter wordt verwacht in halslevels I-III bij mondholtecarcinomen, zagen wij bij 30% (13/43) van de patiënten onverwachte drainage. Dit is aanmerkelijk hoger dan bij patiënten met een niet eerder behandelde hals. Deze bevindingen versterken de waarde van de SWK-procedure om individuele lymfedrainagepatronen te identificeren. In deze studie hadden drie patiënten een positieve SWK en slechts één patiënt vertoonde een regionaal recidief tijdens follow-up, resulterend in een sensitiviteit van 75% en een negatief voorspellende waarde van 98%. Het lage aantal regionale metastasen kan mogelijk worden verklaard door de nauwkeurige follow-up na behandeling van hun eerste primaire tumor. Vanwege dit lage aantal patiënten met regionale metastasen, kan het prematuur zijn om te concluderen dat de SWK-procedure betrouwbaar is bij patiënten met een behandeling van de hals in het verleden. Een negatief voorspellende waarde van 98% suggereert echter dat de procedure op zijn minst veelbelovend is en verder onderzoek rechtvaardigt.

In de eerste decennia (vanaf 1977) werd SWK-identificatie alleen gedaan met planair statische en dynamische lymfoscintigrafie. In 2003 werd SPECT-CT voor het eerst geïntroduceerd voor identificatie van de SWK bij het mondholtecarcinoom. Om de toegevoegde waarde van SPECT-CT te bepalen, analyseerden wij 66 patiënten waarbij de planaire lymfoscintigrafie werd vergeleken met SPECT-CT (***hoofdstuk 7***). In één patiënt kon op beide beeldvormende technieken geen SWK worden gevonden (identificatie ratio 98%). In 22% (14/65) van de patiënten toonde de SPECT-CT in totaal 15 extra SWKen. In twee van deze additionele SWKen werd een metastase gevonden, met als gevolg dat bij 3% van de patiënten regionale metastasen werden aangetroffen door SPECT-CT. In totaal werden bij 10 patiënten metastasen in de SWK gevonden en bij twee van deze patiënten (20%) werd de positieve SWK geïdentificeerd door SPECT-CT. We ontdekten dat vijf SPECT-CT scans nodig zijn om één extra SWK te identificeren. Om één extra positieve SWK te tonen, moeten er 34 SPECT-CT scans worden gemaakt. In tegenstelling tot het vinden van additionele SWKen, verminderde SPECT-CT ook bij vijf patiënten het aantal SWKen ten opzichte van planaire lymfoscintigrafie (bijvoorbeeld injectieplaats in plaats van SWK).

Daarnaast werd beoordeeld of er sprake was van toegevoegde waarde met de SPECT-CT ten aanzien van preoperatieve topografische oriëntatie en anatomische informatie. SPECT-CT werd belangrijk geacht als de chirurg op basis van de aanvullende informatie waarschijnlijk een andere (of meer nauwkeurige) chirurgische benadering zou kiezen. Dit werd waargenomen bij 3% van de patiënten (2/65). Levels waarin de SWKen zich bevonden werden bij 28% van de patiënten aangepast op basis van SPECT-CT. Vanzelfsprekend verschaft SPECT-CT bij de rest van de patiënten een betere topografische oriëntatie voor de chirurg, maar waren wij van mening dat de procedure ook veilig had kunnen worden uitgevoerd met alleen planaire lymfoscintigrafie. Onze studie concludeerde dan ook dat SPECT-CT additionele (en soms ook metastase bevattende) SWKen identificeert en dat het gebruik van SPECT-CT nuttig kan zijn voor het preoperatief plannen van de chirurgische benadering.

In ***hoofdstuk 8*** van dit proefschrift onderzochten wij een nieuwe radioactieve tracer, [^{99m}Tc]Tc-tilmanocept (Lymphoseek ®). Deze tracer is specifiek ontwikkeld voor de detectie van SWKen. [^{99m}Tc]Tc-tilmanocept heeft een kleinere partikelgrootte dan het routinematig gebruikte [^{99m}Tc]Tc-nanocolloid en bindt specifiek aan de CD206 receptor in macrofagen. Dit zou moeten resulteren in een snellere klaring bij de injectieplaats, een hoge retentie van de tracer in de SWK en minimale drainage richting tweede echelon klieren. Deze karakteristieken zouden met name nuttig

zijn bij het verminderen van het eerder benoemde "shine-through" fenomeen bij mondbodemcarcinomen. Prospectief onderzochten wij zowel [^{99m}Tc]Tc-tilmanocept als [^{99m}Tc]Tc-nanocolloid bij 20 patiënten met een vroeg-stadium mondholtecarcinoom. Elke patiënt kreeg beide tracers toegediend om lymfedrainagepatronen te vergelijken tussen beide tracers in dezelfde patiënt. Uitgangspunten waren hierbij de klaring van de tracer bij de injectieplaats en de uptake van de tracer in de SWK. De resterende radioactiviteit op de injectieplaats was significant lager voor [^{99m}Tc]Tc-tilmanocept (29%; SD±7.6),vergeleken met [^{99m}Tc]Tc-nanocolloid (60.9%; SD±16.1) ($p<0.001$). De radioactieve opname in de SWKen was significant hoger voor [^{99m}Tc]Tc-nanocolloid vergeleken met [^{99m}Tc]Tc-tilmanocept (respectievelijk 3.16% vs. 1.95%, $p = 0.01$). Er waren geen verschillen met betrekking tot het aantal SWKen, het aantal tweede echelon klieren of de verhouding tussen SWK en injectieplaats. Er was een uitstekende interobserver-overeenkomst voor beide tracers. Concluderend stelden wij dat [^{99m}Tc]Tc-tilmanocept een lagere resterende radioactiviteit bij de injectieplaats liet zien, maar tegelijkertijd een verminderde uptake in de SWK. Dit resulteert dan ook in een niet significant verschillende verhouding SWK tot injectieplaats met de routinematig gebruikte [^{99m}Tc]Tc-nanocolloid. Verder onderzoek is noodzakelijk om de daadwerkelijke werkzaamheid van [^{99m}Tc]Tc-tilmanocept te onderzoeken in patiënten met mondholtecarcinomen.

CONCLUSIE

Dit proefschrift concludeert dat de SWK-procedure een betrouwbare methode is voor het detecteren van occulte lymfekliermetastasen bij patiënten met een vroeg-stadium mondholtecarcinoom. De SWK-procedure biedt bij elke patiënt een individueel lymfedrainagepatroon en kan ook worden toegepast bij patiënten die al eerder in de hals zijn behandeld. Ondanks de goede accuratesse is er nog steeds ruimte voor verbetering. In de nabije toekomst zou het onderzoek zich moeten concentreren om de procedure te verbeteren bij patiënten met een tumor in de mondbodem. Het veelbesproken "shine-through" fenomeen beperkt de toepasbaarheid van de SWK-procedure bij deze patiënten. Dit proefschrift onderzocht onder meer de waarde van SPECT-CT en het gebruik van een nieuwe radioactieve tracer ([^{99m}Tc]Tc-tilmanocept) om dit fenomeen te verminderen. Tot dusverre moet echter worden geconcludeerd dat beide technieken weliswaar veelbelovend zijn, maar niet probleemoplossend. De techniek waarbij peroperatief routinematig level I wordt geëxploreerd dient derhalve te moeten worden geïmplementeerd totdat de nauwkeurigheid van de SWK-procedure

is verbeterd tot een niveau dat vergelijkbaar is met dat van andere tumorlocaties in de mondholte.
De SWK-procedure bij het vroeg-stadium mondholtecarcinoom krijgt de laatste jaren wereldwijd steeds meer aandacht en wordt steeds vaker ingezet als diagnostische methode. Dit proefschrift levert bewijs voor de toepasbaarheid en geschiktheid van deze procedure. Daarnaast stelt dit proefschrift enkele tekortkomingen vast die in de nabije toekomst verder dienen te worden onderzocht.

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Contralateral regional recurrence in lateralized early-stage oral cancer: sentinel lymph node biopsy versus elective neck dissection
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ABOUT THE AUTHOR

Inne den Toom was born on the 24th of February 1991 in Rotterdam, the Netherlands. After graduating from secondary school (Erasmiaans Gymnasium) in 2009, Inne made a slightly controversial move as "Rotterdammer" to Amsterdam for starting his medical school at the Vrije Universiteit of Amsterdam. At the end of his bachelor (2012), his research career started at the department of Otolaryngology / Head and Neck Surgery under supervision of dr. D.A. Heuveling and prof. dr. R. de Bree. Here, the foundation has been laid for this thesis. Chasing his surgical dream, Inne performed a senior internship at the department of Surgery (mentor prof. dr. G. Kazemier), after which he obtained his medical degree in August 2015.

To continue his ongoing research, Inne followed prof. dr. R. de Bree towards the UMC Utrecht and started as MD/PhD-student at the department of Head and Neck Surgical Oncology. He focused on improvements and national implementation of the sentinel lymph node biopsy procedure for oral squamous cell carcinoma. He supervised medical students during their scientific internships, participated in several national board committees and was awarded twice as best oral presentation at large international congresses.

In December 2018, he started as surgical resident not in training at the Noordwest Ziekenhuisgroep Alkmaar under supervision of dr. W.H. Schreurs and dr. K.J. Ponsen. Since July 2020, Inne started as surgical resident in training at the Franciscus Gasthuis & Vlietland under supervision of dr. T.M.A.L. Klem.

9

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