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The impact of superficial injections of radiocolloids and dynamic lymphoscintigraphy on sentinel node identification in oral cavity cancer: a same-day protocol

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Aim **To evaluate the role of dynamic lymphoscintigraphy with a same-day protocol for sentinel node biopsy in oral cavity cancer.**

Methods **Twenty-two consecutive patients affected by cT1-2N0 squamous cell carcinoma of the oral cavity were enrolled between September 2001 and November 2005. After a local anaesthetic (10% lidocaine spray), a dose of 30–50 MBq of ^{99m}Tc human serum albumin nanocolloid, in ml saline, was injected superficially (1–2 mm subendothelial injection) into four points around the lesion. Dynamic lymphoscintigraphy was acquired immediately (256 × 256 matrix, 5 min pre-set time, LEGP collimator) in lateral and anterior projections. The imaging was prolonged until the lymph nodes of at least two neck levels were visualized (time required min). About 3 h later (same-day protocol) the patients had a radioguided sentinel node biopsy. Elective neck dissection was performed in the first 13 patients; whereas the last nine patients had elective neck dissection only if the sentinel node was positive. Sentinel nodes were dissected into 1 mm thick block sections and studied by haematoxylin & eosin staining and immunohistochemistry (anticytokeratin antibody).**

Results **The sentinel nodes were found on the 1st neck level in 13 cases, on the 2nd neck level in eight cases, and on the 3rd neck level in one case (100% sensitivity). The average number of sentinel nodes was 2.2 for each patient. The sentinel node was positive in eight patients (36%); with six of them having the sentinel node as the exclusive site of metastasis. No skip metastases were found in the 14 patients with negative sentinel node (100% specificity).**

Conclusion **Our preliminary data indicate that superficial injections of radiocolloid and dynamic lymphoscintigraphy provide a high success rate in sentinel node identification in oral cavity cancers. Dynamic lymphoscintigraphy helps in distinguishing sentinel node from second-tier lymph nodes. The same-day protocol is advisable in order to correctly identify the first sentinel node, avoiding multiple and unnecessary node biopsies, without reducing sensitivity.** ***Nucl Med Commun*** **29:318–322** © **2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: dynamic lymphoscintigraphy, oral cavity, same-day protocol, sentinel node, squamous cell carcinoma, superficial injection

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Introduction

Squamous cell carcinoma (SCC) is thought to arise from keratinizing or malpighian epithelial cells. Squamous epithelium consists of five to seven cell layers and is avascular, therefore non-irritated epithelium does not bleed. This is true for SCC *in situ* as well, because it does not break through the basement membrane; therefore, blood vessels are not exposed. Oral cavity SCC usually remains localized to the head and neck for months or years. Local tissue invasion is followed by metastasis to regional draining lymph nodes in the neck and distant lymphatic metastases tend to occur late. Haematogenous metastases are usually associated with large or persistent tumours and occur more commonly in immunocompromised patients [1].

The presence of lymph node metastases is the most important prognostic factor in head & neck (H&N) cancer, decreasing survival by 50%. Therefore, reliable staging of the neck is imperative to determine further management of the disease [1].

Management of the clinically negative neck in SCC of the oral cavity continues to be a topic of debate in

the literature. Incorrect clinical staging is, in fact, expected in approximately 20–30% of cases [1–5]. Many studies suggest that selective or conservative modified radical neck dissections are the 'gold standard' in most patients [3].

The sentinel node technique has the potential to decrease the number of neck dissections performed in clinically negative necks [4]. Sentinel node biopsy (SNB) is becoming established as an accurate method of staging lymph node involvement in melanoma and breast cancer and relies on the assumption that if the sentinel node is clear of metastases, the remaining nodes are clear too. However, SNB in the H&N region is a technically demanding procedure [5–8] because lymphatic drainage in this region is known to be particularly complex.

From the literature the incidence of occult metastatic disease detected with SNB has a mean value of 33.4% and the sensitivity is estimated to be around 94%. However, a poor concordance was observed between blue dye and lymphoscintigraphy [9,10].

The aim of our study was to evaluate the impact of dynamic lymphoscintigraphy, with a newly developed same-day protocol for SNB in early oral cavity cancer, to detect the sentinel node closest to the tumour site.

Materials and methods

Between December 2001 and November 2005, we enrolled 22 consecutive patients affected by cT1-2N0MO SCC of the oral cavity: 12 males, 10 females, average age 62.6 years (range, 28–80 years). Fourteen patients had SCC in the lateral margin of the tongue, four in the oral mucosa, and four had SCC found in mouth. Diagnosis was made by biopsy. Ultrasound (US) and computed tomography (CT) scan examinations were carried out on all patients and all of them were negative for the presence of neck involvement (N0). The interpretative criterion of US/CT was to consider a lymph node to be positive (N +) if its size was > 1.5 cm, had central necrosis or spherical shape or a marginal enhancement following intravenous administration of contrast. These nodes were excluded from the study (following the application of these criteria, 16 patients were excluded).

The lymphoscintigraphy was performed 3 h before surgery (same-day protocol). No specific preparation for the performance of lymphoscintigraphy was adopted, other than usual preoperative restrictions.

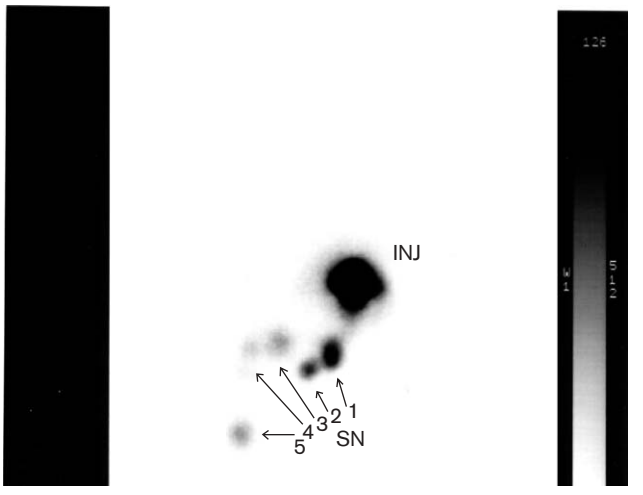
The patient's necklaces, dental prosthesis and all relevant metallic items were removed. The patient was imaged in a position to mirror that at surgery. A local anaesthetic was given (10% lidocaine spray) before injecting a dose of 30–50 MBq of ^{99m}Tc-HSA-nanocolloidal diluted in 0.4 ml of saline. We performed four superficial injections in the sub-epithelial stroma at four sites around the tumour. The needle was introduced for about 1–2 mm under the surface of the epithelium surrounding the tumour.

A mouthwash was used immediately following radiotracer injection to prevent pooling or swallowing of residual radioactivity by the patient. Dynamic and early static planar scans were acquired immediately after the injections by using a large-field-of-view gamma camera coupled with a parallel-hole collimator (General Electrics, Infinia SPECT H3000WY, USA) (256×256 matrix, 5 min pre-set time, Zoom 1.5, LEGP collimator) in lateral and anterior projections. The examination was prolonged as necessary to visualize at least two neck levels (Fig. 1).

The skin overlying the sentinel node was marked with a permanent marker pen. A radioguided SNB was performed 3 h after scintigraphy using a hand-held commercially collimated available gamma probe (Scintiprobe 100; Pol.hi.tech., Italy). Technical characteristics are as follows: linear probe with an external diameter of 14 mm, mounting a shielded NaI detector; energy range, 30–385 keV; gamma detecting efficiency of 99% for the ^{99m}Tc peak energy (140 keV); sensitivity threshold of 370 Bq (10 nCi) and spatial resolution of 5 mm at 2 mm distance.

The excised sentinel nodes were fixed in 10% neutral buffered formalin for 12–24 h. The whole lymph node was cut in 1–2 mm thick sections and embedded in paraffin.

Fig. 1



Lymphoscintigraphy in a patient with a squamous cell carcinoma of the tongue. The scan was acquired 30 min after injections and shows the point of injections (INJ), the sentinel nodes (1,2) and the 2nd tier lymph nodes (3,4,5).

From each paraffin block 10 sections were stained with H&E and by immunohistochemistry for cytokeratin (MNF116, diluted 1/50).

The study was approved by the Local Ethics Committee and was conducted according to government regulations. All patients gave written informed consent prior to entering the study.

Results

The first sentinel node was identified 5 min after injections in all cases: at the 1st neck level (NL) in 13 patients, 2nd neck level in eight patients, and 3rd neck level in one patient. In two patients bilateral lymph drainage was observed. We identified a total of 49 sentinel nodes (mean, 2.2 sentinel nodes per patient). Thus, sensitivity for the detection of sentinel node in our series was 100%.

A positive sentinel node (N +) was found in eight patients, and all were identified by step sectioning and routine H&E staining. In six out of the eight patients the sentinel node was the exclusive site of metastasis, and no other positive lymph nodes were found after END (Table 1). In the other two of eight patients with a positive sentinel node other positive lymph nodes were found at elective neck dissection. Of note, such positive lymph nodes were found at the same neck level of the positive sentinel node (this is a common finding also for other malignancies as breast cancer and melanoma); moreover, they were not detected at preoperative US and CT scan work-up, probably due to their small size (less than 1.5 cm maximum diameter). No skip metastasis was found in the 14 patients with negative sentinel node. Thus, specificity in our series was 100%. No relapse was observed during the subsequent follow-up of 10–35 months (mean, 23.2 months).

Discussion

The clinical role of sentinel node biopsy has been well established in breast cancer and melanoma patients, and more recently it has been proposed for other tumours such as SCC of the oral cavity. In a multi-institutional study [1], the sensitivity of sentinel node biopsy in SCC of the oral cavity has been estimated as 94%. However, it is 'technically challenging' and sensitivity decreases to 57% with less experienced operators; moreover, a longer learning curve is required than for breast cancer or melanoma patients [7,8].

The sole use of the blue dye without radiotracer is discouraged due to a very poor identification rate for sentinel nodes, depending on different features of the H&N lymphatic system [11].

The scanning electron micrograph showed that the lymphatic system consists of complex capillary networks, which collect the lymph in various organs and tissues. Lymphatic capillaries are abundant in the sub-epithelium stroma, have numerous anastomoses and are without

Table 1 **Results of pathologic examination of the sentinel nodes (SNs), arranged for neck level (NL), and other lymph nodes, in our study**

Patient number	Age (years)	Sex	Site of cancer	cTNM	Lat	SN – 1st NL	**SN + 1st NL**	SN – 2nd NL	**SN + 2nd NL**	SN – 3rd NL	**SN + 3rd NL**	OLN – END	**LN + END**	pTNM
1	78	M	Tongue	T2N0M0	L	0	0	1	0	0	**1**	52	**2**	**T2N2bM0**
2	28	F	Tongue	T1N0M0	L	0	0	1	0	0	0	32	0	T1N0M0
3	43	M	Tongue	T2N0M0	L	1	0	1	0	0	0	25	0	T2N0M0
4	59	F	Tongue–FOM	T2N0M0	L	1	0	0	0	0	0	57	0	T3N0M0
5	55	M	Buccal mucosa	T1N0M0	L	2	0	0	0	0	0	36	0	T1N0M0
6	63	F	Tongue	T1N0M0	R	0	0	1	0	0	0	57	0	T1N0M0
7	54	M	Tongue–FOM	T2N0M0	L	1	0	0	0	0	0	56	0	T2N0M0
8	52	M	Tongue	T2N0M0	L	0	0	4	0	0	0	77	0	T2N0M0
9	66	M	Tongue	T2N0M0	R	2	**1**	2	0	1	0	50	0	**T2N1M0**
10	45	M	Tongue	T2N0M0	R	1	0	0	**1**	0	0	83	0	**T3N1MO G2**
11	71	M	Tongue	T2N0M0	R	0	0	1	**1**	0	0	54	0	**T3N1MO G**
12	54	M	Tongue	T2N0M0	L	1	0	0	**1**	0	0	55	0	**T2N1M0 G3**
13	80	F	Tongue	T1N0M0	L	0	0	1	0	1	0	25	0	T1N0M0 G2
14	70	F	Tongue	T2N0M0	R	0	0	0	**1**	0	0	46	**3**	**T2N2b G2/3**
15	52	M	FOM	T1N0M0	L–R	0	0	1	**1**	0	0	106	0	**T1N1 G3**
16	78	F	Buccal mucosa	T1N0M0	R	3	0	1	0	0	0	3	0	T1NO G1/2
17	72	M	Tongue	T1N0M0	R	2	0	2	0	0	0	0	0	T1N0 G2
18	65	F	Buccal mucosa	T1N0M0	R	1	0	0	0	0	0	6	0	T1N0 G1
19	75	M	Tongue	T2N0M0	L	0	**1**	1	0	0	0	26	0	T1N1 G3
20	82	F	Palate gengiva	T1N0M0	L–R	3	0	1	0	0	0	4	0	**T1N0 G1**
21	72	F	Tongue	T1N0M0	L	0	0	0	0	2	0	0	0	T1N0
22	64	F	FOM	T1N0M0	M	1	0	0	0	0	0	1	0	T1NO G2–3

END, elective neck dissection; NL, neck level; OLN, other lymph node; SN, sentinel node; Information given in bold type refers to biopsy positive nodes.

valves. The tongue has blind lymph capillaries in the filliform papillae with the underlying plexus [2]. In the head and neck there are more than 300 lymph nodes (about one-fifth of the total body nodes) and an elaborate system of collecting vessels conducts the lymph from the capillaries to the large veins of the neck. In addition, the lymphatic vessels of the H&N have a greater number of valves (twice as many as in the rest of body) that are placed at shorter intervals than in those of the lower extremities [2]. The effect of gravity and the features of the H&N lymphatic system provide faster lymph drainage in the H&N lymphatic system than in other parts of the body. In some feline studies both radiocolloid and blue dye traversed the lymphatics rapidly, appearing in the H&N sentinel nodes in less than 5 min [12].

The method of radiotracer administration is a crucial step in the lymphoscintigraphy study. In our protocol, we performed four superficial peri-lesional injections of radiocolloid. The needle was introduced for 1–2 mm under the surface of the epithelium, where there is a high concentration of lymphatic capillaries: this provides a larger surface area for uptake, faster lymph drainage and better identification of sentinel nodes in a shorter period of time [10,13].

We found it preferable to perform superficial injections because adopting the deep injection method prolongs the time required for lymphoscintigraphy, degrades the quality of images, and increases the general background and liver activity. All these factors contribute to a reduced success rate in sentinel node identification [1,7–10].

Moreover, in our protocol, using nanocolloids with dynamic lymphoscintigraphy made it possible to rapidly distinguish the second-tier and third-tier lymph nodes located on distant levels in less than 30 min (Fig. 1), thus providing a high success rate in sentinel node identification in oral cavity cancers. These data are in agreement with another recently published experience using a lymphoscintigraphic technique similar to that of the present study [5]. Moreover, we recommend using a LEGP collimator, in order to increase the sensitivity of the procedure and allow the visualization of lymphatic drainage.

It is worth noting that in the two patients (numbers 1 and 14, see Table 1) in whom positive lymph nodes other than the sentinel node were found at elective neck dissection, such lymph nodes were found at the same neck level of the sentinel node. These lymph nodes were not detected at preoperative US and CT scan work-up, probably due to their small size (less then 1.5 cm maximum diameter).

The advantages of dynamic lymphoscintigraphy in early SCC of the oral cavity can be summarized as follows: (1) the visualization of the lymphatic tracks running from the tumour; (2) the rapid demonstration of the lymphatic drainage basins that are potential sites of metastatic disease; (3) the accurate determination of the number and location of sentinel nodes within those drainage basins, thus distinguishing the sentinel nodes from the second-tier and the third-tier lymph nodes, and (4) the ability to mark the location of any sentinel nodes for subsequent radioguided surgical dissection.

In our same-day protocol we decided to examine at least two sentinel nodes for each patient, in order to improve the sensitivity of the lymphoscintigraphic approach that we developed.

We have demonstrated high sensitivity of our protocol (100% sensitivity) in identifying occult lymph node metastases: a total of eight out of 22 patients were upstaged from cN0 to pN+. Our same-day protocol succeeded in detecting sentinel nodes almost always on the 1st neck level or 2nd neck level, therefore limiting the number of nodes examined and the extension of the approach without reducing sensitivity.

Conclusion

Our technique of superficial injections of radiopharmaceutical combined with dynamic lymphoscintigraphy achieved a high success rate in sentinel node identification in cases of early SCC of the oral cavity.

The sentinel nodes were commonly observed in all patients 5 min after the injections, in regional basins near to the primary tumour, at the 1st or 2nd neck levels. Using nanocolloids labelled with ^{99m}Tc as radiocolloids, we recommend an interval of about 3 h (same-day protocol) between injections and biopsy to identify the first sentinel node correctly, avoiding multiple and unnecessary node biopsies. We suggest examining at least two sentinel nodes for each patient to increase sensitivity of the procedure.

Appreciating the small number of our series, we await further prospective clinical studies and longer follow-up observational trials to establish whether this technique might become the standard of care in the management of early SCC of the oral cavity.

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