

# Sentinel lymph nodes in oropharyngeal and oral carcinomas

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## ABSTRACT

**Background:** Oropharyngeal and oral N0 squamous-cell carcinomas are upstaged to pN+ in nearly 35% of cases. Nevertheless, micrometastases can be missed by routine pathological examination. Sentinel lymph node (SLN) histology including immunohistochemistry may improve the sensitivity and specificity of node staging. The objectives of this study were to describe a technique for identifying and examining SLNs and to report preliminary results.

**Materials and method:** Twenty consecutive patients were included prospectively. Lymphoscintigraphy was performed after injecting Nanocis® on D0. A handheld gamma probe was used for sentinel node identification during surgery. Sentinel nodes were examined by standard histology and immunohistochemistry. Neck dissection was performed routinely.

**Results:** SLNs were identified in 19 patients. In the 4 patients with micrometastases, in-depth node studies led to pN upstaging from pN0 to pN2 in 1 patient and from pN1 to pN2 in 3 patients. No false negatives were recorded.

**Conclusion:** The technique described in this study may improve node staging. A multicenter study is needed to confirm these preliminary findings and to evaluate outcomes according to node stage.

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**Keywords:** Micrometastasis, Sentinel lymph node, Squamous cell carcinoma, N0, Immunohistochemistry.

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### INTRODUCTION

The prognosis of squamous cell carcinoma of the oropharynx and oral cavity classified N0 by preoperative investigations depends on the final pathological stage (pN) [1], the main factor being the number of positive nodes [2]. Among patients with N0 cancer, 28% to 36% have histological lymph node involvement [1]. The usual strategy is routine dissection of the most commonly affected levels, which are levels I, II, and III for cancers of the oropharynx and oral cavity [3]. When multiple nodes are involved, radiation therapy is required [4], whereas patients with pN0 disease do not need adjunctive therapy. Dissection provides better control of neck disease than does monitoring followed by salvage surgery if needed [5]. Thus, neck dissection plays both a diagnostic and a therapeutic role.

Nevertheless, two obstacles to neck dissection exist. First, understaging may occur when positive nodes are not harvested, particularly at level IV [6], or because routine histological examination of hematein-eosin stained sections may miss micro- and macrometastases [5]. Thus, a 10% node recurrence rate has been reported among patients classified pN0 after neck dissection [7 - 8]. Understaging reduces the likelihood of disease control and hinders evaluations of treatment strategies.

Second, nearly two-thirds of patients who undergo neck dissection are pN0. Thus, many patients are unnecessarily subjected to the intraoperative risks and morbidity associated with neck dissection [9].

Sentinel lymph node (SLN) detection seeks to overcome these obstacles [10 - 11]. A map of the lymphatic drainage in the region harboring the tumor is obtained in order to identify the first lymph nodes to which cancer cells are likely to spread. SLN detection does not identify node metastases: the SLNs must be removed and subjected to histological studies. The number of involved nodes is determined, and the involvement is categorized as isolated cancer cells <0.2 mm, micro-metastases <2 mm, macrometastases >2 mm, or capsular breach [12]. SLN detection decreases the risk of missing an involved lymph node located at an unusual site and supplies a representative sample of the entire node population for in-depth histological studies.

The objectives of this study were to describe a technique for localizing and examining SLNs in patients with squamous cell carcinoma of the oropharynx or oral cavity and to report the preliminary results obtained with this technique.

### PATIENTS AND METHODS

The study was conducted prospectively at the otorhinolaryngology department of the Montpellier Teaching Hospital, Montpellier, France, over a 20-month period (October 2002 to June 2004). The research project was approved by the hospital's clinical research department. Informed consent was not required, given that the strategy evaluated in the study had been approved by an international consensus conference panel [13], so that sentinel lymph node detection was not experimental. Consent to computerized handling of study data was obtained from each patient, and the study was registered with the National Commission for Freedom and Computerized Data Processing.

#### Inclusion criteria

There were:

- age older than 18 years;
- primary squamous cell carcinoma of the oropharynx or oral cavity documented by a biopsy with a histological study performed within the last month;
- tumor accessible to peritumoral injection;
- operable tumor as assessed by TNM stage, tumor location, and patient's general health status;
- negative history for previous treatment for head and neck cancer;
- no previous participation in the study;
- routine otorhinolaryngological panendoscopy showing no evidence of a synchronous second primary and clearly visualizing the primary, performed within the last 21 days;
- N0 preoperative stage, defined as absence of nodes felt during physical examination (regardless of size of the primary) or visualized by computed tomography (CT). Both the staging physical examination and the staging CT had to be performed within 21 days of study inclusion. Palpation of the neck was done by the study otorhinolaryngologist. CT was performed with injection of a contrast agent and was considered negative for nodal involvement when nodes were less than 1 cm in diameter (1.5 cm for the subdiaphragmatic nodes), oval, homogeneous, and free of contrast enhancement, evidence of perinodal spread (increased density of fat or vascular adhesions), and clustering (>3 nodes). Doubtful cases were resolved by discussion with the senior radiologist at the radiology department of the Gui de Chauliac Teaching Hospital. We excluded patients with active tumors at other sites or contraindications to scintigraphy (pregnancy or

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known allergy or intolerance to injected substances, most notably technetium-99).

### Patients

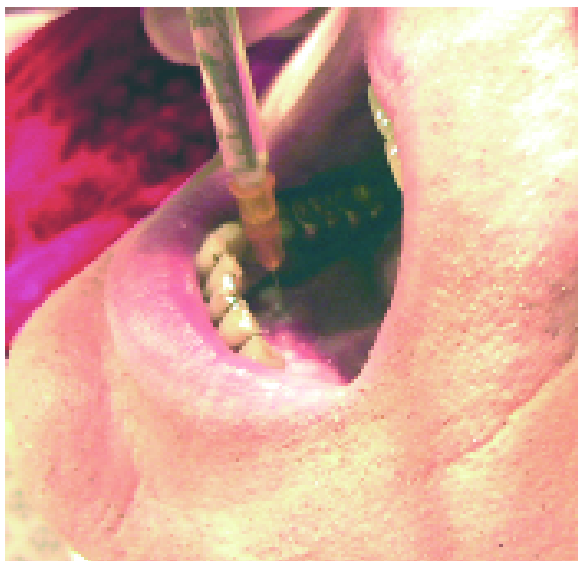
We included the 20 consecutive patients who met our inclusion and exclusion criteria during the study period. There were 15 men and 5 women with a mean age of 50 years. The primary was located in the oral cavity in 16 patients and in the oropharynx in 4 patients. Tumor stage was T1 in 12 patients, T2 in 7 patients, and T4 in 1 patient.

### Sentinel lymph node detection

Lymphatic mapping was achieved by lymphoscintigraphy preoperatively and using a portable gamma detector intraoperatively. On the morning of the surgical procedure, the patient was taken to the nuclear medicine department where the otorhinolaryngologist injected a radioactive tracer (technetium-99m colloidal rhenium sulfide, Nanocis® (CIS BIO International, Schering SA, Gif sur Yvette, France) by the intraoral approach after local anesthesia with 1% xylocaine. Three to four injections were given at the edge of the tumor in regions where the mucosa seemed normal (Figure 1). The total dose was 33 mBq in 0.9 ml. Lymphatic drainage was studied using a Picker two-head gamma camera with low-energy high-sensitivity collimators (Axis™, Picker, Cleveland, OH, USA). The acquisition matrix was

### Figure 1: Peritumoral tracer injection after local xylocaine anesthesia without adrenalin.

*The tracer was Nanocis® 0.9 mCi (33 mBq).*

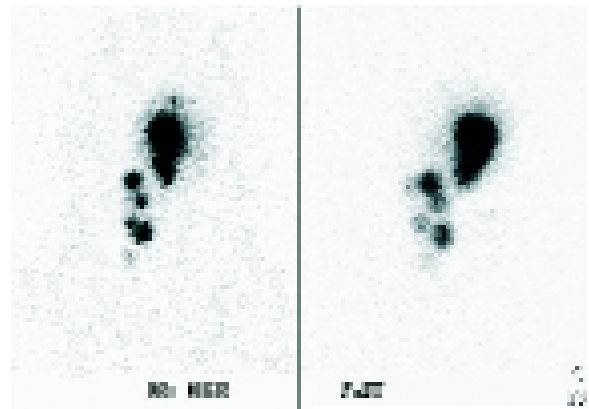


64x64 for dynamic images and 256x256 for the static image.

The patient was placed in the supine position with a pad under the shoulders to replicate the operative position. Anteroposterior and lateral images were acquired over a 3-minute period 30 to 60 minutes after the injection (Figure 2).

### Figure 2: Anteroposterior and lateral static lymphoscintigraphy images 30 minutes postinjection:

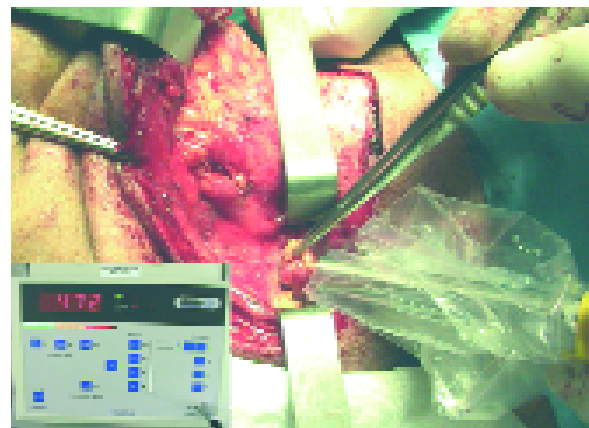
*four sentinel lymph nodes are visible.*



SLNs were identified and the overlying skin was marked. Surgery was started 2 hours after the injection. In 16 of the 20 patients, tumor removal was the first step. A conventional neck incision was used. SLNs were identified using a gamma detector (Gamma Sup®, Clerad-ARIES®, Clermont-Ferrand, FRANCE) equipped with a high-resolution collimator whose tip was encased in a disposable sterile sheath (Figure 3).

### Figure 3: Intraoperative identification of one of the sentinel lymph nodes.

*Inset: gamma detector control panel.*



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SLNs were selectively removed with the surrounding cellular tissue, taking care not to breach the capsule. Each removed node was placed outside the operative field and checked using the gamma detector to confirm the radioactivity reading. The removal site and other neck sites were checked for absence of meaningful radioactivity. The fresh SLN or SLNs were sent in a specially labeled container to the study pathologist for histological studies. Standard neck dissection was performed and the surgical procedure was completed using conventional methods. Level I, II, III, and IV nodes were removed routinely. Neck dissection specimens and tumor specimens were sent to the pathology laboratory separately for histological studies. Dissection specimens were routinely checked for absence of meaningful radioactivity.

### Pathology techniques

The fresh SLN(s) arrived at the pathology laboratory within 10 minutes of removal. The nodes were cut for cytology imprint, which were stained with hematein-eosin. The SLN(s) were then frozen in liquid nitrogen at -80 °C. Step-serial sections were cut for a morphological study with immunohistochemistry; in addition, sections for reverse transcriptase-polymerase chain reaction were obtained for another study. Two 5- $\mu$ m slices were cut at 250- $\mu$ m intervals, one for hematein-eosin staining and one for immunohistochemistry. For instance, for a 1-cm node, 2x40 slices were obtained. Primary anticytokeratin antibodies (KL1) were used for immunohistochemistry [13]. SLN involvement was reported according to the TNM classification established by the UICC in January 2003, in which the degree of node involvement is derived from the system developed by Hermanek et al. [12] (Table I). Nodes in the neck dissection specimens

**Table I: Classification of node involvement according to Hermanek et al. [12].**

Ic	Isolated tumor cells: groups of tumor cells less than 0.2 millimeters in diameter
Mi	Micrometastases: proliferation within the node of tumor tissue measuring no more than 2 mm in diameter
Ma	Macrometastases: proliferation within the node of tumor tissue measuring more than 2 mm in diameter

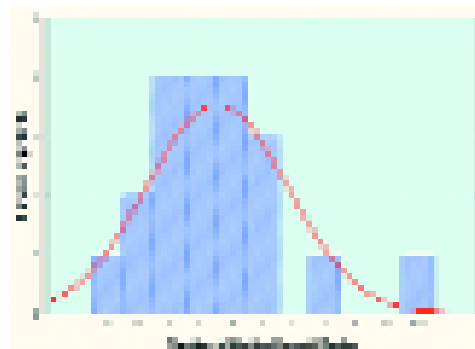
were examined using conventional methods: paraffin embedding, one section from each node less than 1 cm in diameter, and hematein-eosin staining.

## RESULTS

### Detection of sentinel lymph nodes

One or more SLNs were identified in 19 (95%) of the 20 patients. Mean ( $\pm$ SD) time to identification was 1h07 $\pm$ 0.37 during lymphoscintigraphy and 2h12 $\pm$ 0.45 during surgery. The mean number of SLNs per patient was 3.15 $\pm$ 2.00 by lymphoscintigraphy and 3.5 $\pm$ 2.6 during surgery (Figure 4).

**Figure 4: Distribution of the number of sentinel lymph nodes per patient (mean, 3.5).**



The patient with no identifiable SLN had a T1 tumor in the anterior floor of the mouth. Three injections were given, and the total dose (22 mBq) was lower than in the other patients. Time to identification of radioactive nodes was 45 minutes by lymphoscintigraphy and 3h15 during surgery. Table II shows the levels where SLNs were identified. Three patients had SLNs in level IV (Figures 5 A, B, and C). The primaries in these three patients were a T1 in the right anterolateral and middle parts of the floor of the mouth, a

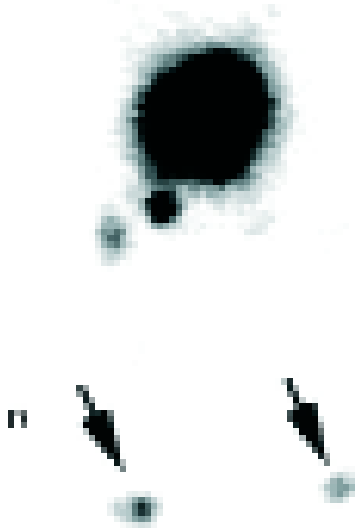
**Table II: Levels containing sentinel lymph nodes (SLNs) (n=71).**

Location of SLNs	IA	IB	IIA	IIB	III	IV
% (n=71)	8	15	27	6	34	10

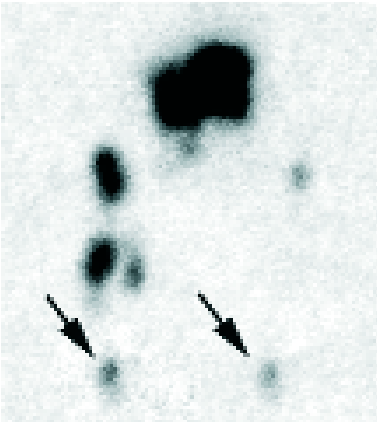
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**Figure 5: Lymphoscintigraphy.**

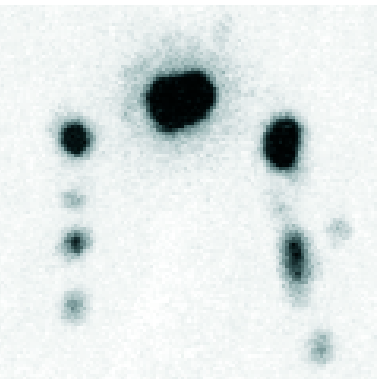
**A:** *pT1pN0 of the anterior floor;*



**B:** *pT1pN2bmi(sn) of the right outer edge of the mobile tongue. Micrometastasis in the sentinel lymph node from level IV. Note that drainage is bilateral in this patient whose lesion does not reach the midline.*



**C:** *T2 pN0 of the soft palate upstaged to pN2c mi (sn).*

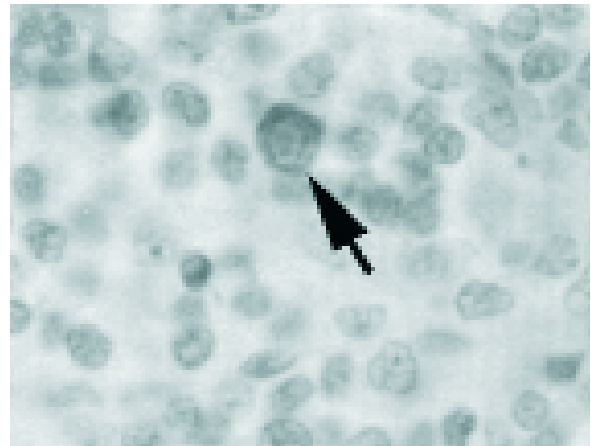


T1 in the left edge of the mobile tongue, and a T2 in the uvula and soft palate. In 1 of these patients, a micrometastasis was found in the level IV SLN, as well as in a level IIa SLN.

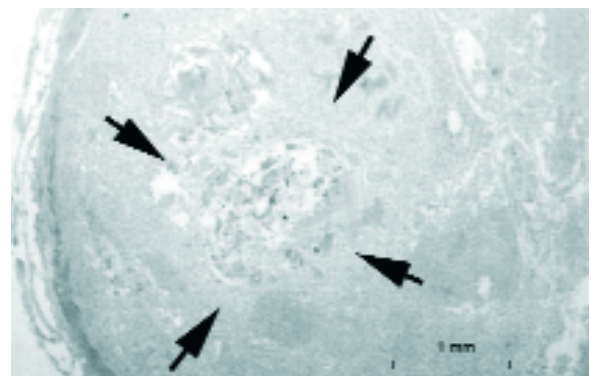
### Pathology findings

Of the 71 SLNs, 8 showed micrometastases and 3 macrometastases. In a patient classified pT2pN0 by conventional histology, immunohistochemistry on step-serial sections revealed isolated tumor cells and micrometastases in two bilateral SLNs (Figures 6 and 7). The stage was changed to pT2pN2b mi (sn), ("sn" for sentinel node). In three other patients, the stage by conventional histology was pN1 but immunohistochemistry revealed additional micrometastases at other sites: 2 of these patients were upstaged to pN2b mi (sn) and the remaining patient to pN2a R+mi (sn) (the capsule violation was identified by conventional histology). There were no false-negatives (negative SLNs and positive neck dissection nodes).

**Figure 6: Isolated tumor cell by immunohistochemistry (KL1).**



**Figure 7: Micrometastasis by immunohistochemistry (KL1).**



### Adjuvant treatment

Adjuvant radiation therapy was given to 5 patients. In 1 of these patients, radiation therapy was required for a T4 tumor invading the bony palate; this patient was pN0 (sn). Two other patients required radiation therapy for multiple nodal metastases in the neck dissection nodes; they also had micro- and macrometastases in the SLNs. In the two remaining patients, the need for radiation therapy was identified by the SLN detection method used in this study: one patient was upstaged from pN0 to pN2b mi (sn) and another from pN1 to pN2a mi (sn).

### DISCUSSION

This preliminary study established the feasibility of SLN detection in patients with accessible oral cavity and oropharyngeal squamous cell carcinomas [14]. Histology and immunohistochemistry on a few nodes seems to improve the sensitivity of micrometastasis detection. Several similar studies have been published recently [11]. However, a number of technical points remain open to question regarding both node detection and histological methods.

We chose Nanocis® because the compound is synthetic and the particles small enough to ensure rapid diffusion (mean diameter, 15-50 nm). Tracer kinetics studies showed very early uptake with identification of 62% of SLNs within the first minute. In our study, the detection rate was 19/20 after 60 minutes.

We performed lymphoscintigraphy on the day of surgery. In earlier studies, tracer injection on the day before surgery was found to carry a risk of errors due to wash-out of the tracer from the first nodes and rapid diffusion to nodes lower down in the drainage chain [16,17]. In addition, because technetium has a short-half-life, injection on the day before surgery requires use of a higher dose than injection on the same day. The only patient with no detectable SLN in our study had a tumor in the anterior floor of the mouth, in keeping with a report by Ross et al. [18]. A likely explanation is the close proximity between the injection site and the first node with tumors in this location. Coupling lymphoscintigraphy with single-photon emission computed tomography has been reported to improve the preoperative spatial resolution of sentinel lymph node detection [19] in a preliminary study of 13 patients. We have no experience with this technique.

Lymphoscintigraphy showed SLNs at level IV in 3

patients, including 1 patient with SLN micrometastases. Although this last finding did not modify the stage, it minimized the amount of residual disease left in the neck. Immunohistochemistry seems to improve the sensitivity and specificity of the diagnosis of node involvement. The results led to upstaging in 4 patients in our study. As reported for breast cancer and melanoma, the node involvement rate is closely dependent on the method used to detect node disease in patients with head and neck cancer. Thus, the proportion of micro- and macrometastases missed by conventional histology ranged from 2% to 9% in one study [12] but reached 30% in another [20]. Frozen-section histology has been recommended [21]. However, this method misses nearly 45% of micrometastases [22] and places a heavy technical and medicolegal burden on pathologists. An effective tool for intraoperative SLN detection is urgently needed. Molecular biology techniques are receiving attention in this respect [23].

### CONCLUSION

SLN localization improves pN staging of N0 necks. This technique reliably identifies micrometastases, provided step-serial sectioning and immunohistochemistry are used. Nevertheless, multicenter studies comparing outcomes in patients with isolated cancer cells versus micrometastases are needed to determine the impact of these node disease patterns and the potential benefits of anti-cancer treatment.

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