Role of intra-operative sentinel lymph node biopsy in oral cavity and oropharynx squamous cell carcinoma: preliminary data

Il ruolo della biopsia intraoperatoria del LinfonodoSentinella in pazienti affetti da carcinoma squamocellulare del cavo orale ed orofaringeo: dati preliminari della nostra esperienza

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Key words / Parole chiave
- Oral cavity tumours • Oropharynx tumours • Surgical treatment • Neck dissection • Lymphoscintigraphy • Sentinel lymph node
- Carcinoma del cavo orale • Carcinoma dell’orofaringe • Trattamento chirurgico • Svuotamento laterocervicale • Linfoscintigrafia • Linfonodo sentinella

Summary / Riassunto
In patients with squamous cell carcinoma of the oral cavity and oropharynx, the presence of latero-cervical lymph node metastases was found to be the most important of the better known prognostic factors. Still, today, the most reliable technique by which to detect the presence of lymph node metastases is surgery aimed at the dissection of the latero-cervical space; albeit, this surgical procedure has been shown to be an over-treatment in a large percentage of patients presenting squamous cell carcinoma, clinically, radiologically and histologically negative, at neck level. The technique of intra-operative biopsy of sentinel lymph node, routinely used in the staging and treatment of tumours with elective lymphatic involvement such as carcinoma of the breast and malignant cutaneous melanoma, has progressively caught the attention of head and neck surgeons in the most important referral centers in the world, and, indeed, its role has been hypothesised in the treatment of patients with squamous cell carcinoma of the oral cavity and oropharynx with clinically N0 neck. Preliminary results are reported, concerning the use of this intra-operative sentinel lymph node biopsy technique with double tracer in patients presenting squamous cell carcinoma originating in the mucosa of the upper air-digestive tract, clinically and radiologically free from disease at latero-cervical level.

Introduction
Squamous cell carcinoma (SCC) of the oral cavity and oropharynx spreads through the lymphatic pathway to the latero-cervical lymph node stations of the neck, and it has been hypothesised that this spread occurs through emboli 1. Since the presence of lymph node metastases is the most important prognostic factor, of those currently known, in patients with SCC of the head and neck 2, scrupulous staging of the neck is mandatory in determining the need for further treatment. The physical and imaging examinations (nuclear magnetic resonance (NMR); computed tomography scan (CT), ultrasonography (US)) have not been found to be completely reliable in establishing lymph node involvement 3, 5, bearing in mind that about 30% of patients with a clinically negative neck (cN0), actually present occult metastases in the material collected following neck dissection 6. Today, the only reliable way to determine
the presence of latero-cervical metastases is invasive surgery such as more or less extended neck dissection. A constantly increasing number of head and neck surgeons are reluctant to carry out these procedures in patients with SCC, in a clinically early stage, on account of the associated morbidity. Furthermore, the attitude of “wait and see” has been shown to be accompanied by a large percentage of metastases occurring after the primary surgical procedure of the tumour and, on the other hand, to carry out neck dissection in all cases might be considered as “over-treatment” in a large patient population. Since prognosis is more favourable in a disease spreading to the latero-cervical lymph nodes, in the early stage, than in the more advanced stage, treatment of patients with clinically N0 neck is, still today, one of the more controversial issues in the treatment of SCC of the oral cavity and oropharynx. In malignant melanoma and breast cancer, a similar debate has emerged concerning the possibility of studying and treating regional lymph nodes, and in this field, the technique of sentinel lymph node (SLN) biopsy has progressively gained popularity. Using the double tracer method, a supravital staining (Patent Blue V dye or Isosulfan Blue dye) and a radiolabelled tracer (99mTc nanocolloid sulphate) are injected in sequence around the site of the tumour (or around the site of the previous excision). The lymph vessels are stained blue and can thus be mapped to the first lymph node filter. Use of pre-operative static and/or dynamic lymphoscintigraphy makes localisation of the radioactive lymph nodes easier, some of which may not be stained despite housing microscopic nests of neoplastic cells. In the case of malignant melanoma, patients with no clinically appreciable regional lymph nodes and a SLN free from metastases have a low risk of developing metastases outside the regional lymph centre. Besides becoming routine procedure in staging and treatment of malignant melanoma and breast cancer in the earlier stages, intra-operative biopsy of the SLN has been successfully used also in other neoplasias such as penile cancer, vulvar cancer, colon, and Merkel cell carcinoma. Last but not least, this technique, over the last few years, has played an increasingly important role in establishing the presence of lymph node metastases in patients presenting SCC of the upper air-digestive tract. Recent reports in the literature, as well as ongoing studies, suggest that this technique should be routinely employed in the management of the clinically and radiologically negative neck (cN0), even if the method employed is not without certain technical difficulties.

Preliminary results are reported of the study performed at the Maxillo-Facial Surgery Section ENT-Dentistry-Ophthalmologic and Cervico-Facial Dept. of the University of Parma (Italy), which consists in application of this technique of biopsy study of SLN with the double tracer method in patients presenting SCC of the oral cavity and oropharynx with a clinically and radiologically negative neck (cN0).

**Patients and methods**

A total of 10 patients (7 male, 3 female; mean age of 55.5 years (range: 34-74)) were enrolled in the study between October 2000 and September 2001 at the Maxillo-Facial Surgery Section ENT-Dentistry-Ophthalmologic and Cervico-Facial Dept. of the University of Parma (Italy). All presented SCC of the oral cavity and oropharynx with a varying degree of keratinisation (staging: G1, G2, G3), the neck being clinically and radiologically negative (cN0). Recent or previous surgical treatment and/or radiotherapy, on the head or neck, were considered exclusion criteria for the study since distribution of the lymphatic drainage pathway, following these forms of treatment, does not allow a reliable intra-operative search of SLNs. As far as concerns the characteristics of the neoplasias, these were carcinomas of various sizes (T1 in 2 patients, T2 in 3 patients, T3 in 5 patients) and sites (tongue edge in 5 patients, base of tongue in 1 patient, pharyngeal tonsil in 1 patient, retromolar trigonus in 2 patients, median anterior part of mouth floor in 2 patients). All patients were submitted to a selective neck dissection procedure of the supraomohyoid type (homolateral at the neoplasia site in 8 patients, while in 2 cases presenting a median tumour, dissection was performed on both sides of the neck) and then observed at follow-up for a mean period of 6 months (range: 2-12).

**Sentinel lymph node biopsy using double tracer technique**

Biopsy of SLN, with the double tracer method, is carried out in 2 steps: pre- and post-operative phases, followed by anatomo-pathological evaluation of the SLNs detected and those isolated from the material collected from selective neck dissection. It is worthwhile, before describing these various phases, to briefly outline the drugs and equipment used. Radiolabelled tracer In keeping with the pharmacokinetic aspects, a 99mTc labelled sulphate nanocolloid was used (Human filtrated albumin; diameter of the particles <0.2 µm), able to effectively concentrate in the lymphatic afferent, passing through in a very short time, and remaining in the regional lymph nodes in a sufficient amount to be able to be detectable, before and during surgery (approximately 24 hours after the injection procedure). SLNs, detectable by means of pre-operative lymphoscintigraphy and intra-operative gamma-camera evaluation,
LYMPHOSCINTIGRAPHY: A STANDARD DOSE OF RADIO-LABELLED TRACER

Procedure

All patients underwent static and/or dynamic lymphoscintigraphy. Operating times for incision of the neck are established by the pharmacokinetic characteristics of the tracer: an incision performed too early (<5 minutes) would mean that staining of the regional lymph nodes would be lacking due to interruption of lymphatic afferents, before these could have come into contact with the dye; vice versa, excessively delaying incision (>20 minutes) would allow the dye to reach the regional lymph centre, thus staining an excessive number of lymph nodes or, alternatively, with even longer times, to be “washed out” due to the extreme rapidity of the lymphatic flow at cervical level, without visualisation of lymph nodes. Lymph nodes identified by means of supravital tracer are referred to, by convention, as “blue”, on account of their staining.

Static and/or dynamic lymphoscintigraphy: performed at the Nuclear Medicine Unit of Parma Hospital, enables a first identification of SLNs according to the radioactivity levels emitted by previously radio-labelled lymph nodes. At 10 and 60 minutes (control) after the injection procedure, it is possible to record, on static and/or dynamic images, the levels of radioemission detected in the head-neck area.

Gamma-camera (γ-Probe)

This instrument comprises 3 main components: a detector crystal (cadmium-telluride) able to detect even low amounts of energy emitted as γ-rays by radioactive decay of 99mTc; a collimator, protecting crystal from risks of incandescence; a transformer, which allows conversion of the energy levels revealed in acoustical signals, and visible on display (counts).

Pre-operative phase

Informed consent to the investigation was obtained from all patients. At 18–20 hours before the surgical procedure all patients underwent static and/or dynamic lymphoscintigraphy: a standard dose of radioactive tracer (99mTc nanocolloid sulphate, 700–750 μCi in 1 ml of physiological solution with 0.9% NaCl) was injected into the infiltration points of the radio-labelled tracer, prior to neck incision. Thereafter, the cervical flap was incised and lifted, and then the operating field was explored, directly and by means of a gamma-probe. The SLNs detected were isolated from the excised material, identified with silk suture, and sent to the Institute of Pathology in Parma for histological evaluation.

Results

Results are outlined in Tables I and II:

- Intra-operative investigations enabled SLNs to be identified in all patients, for a total of 25 (mean: 2.5 SLNs/patient);
- in 2 patients with median SCC, SLNs have been identified bilaterally;
- the level of the lymph nodes involved varied considerably (Table II) in relationship to the site of the neoplasia;
- of the 24 SLNs examined, 8 were positive, of which 2 positive in 2 patients;
- in the 6 patients with positive SLNs, no other metastatic lymph nodes were identified, among those isolated from the latero-cervical material dissected;
- in the 2 patients with median tumour, no bilaterally positive SLNs were identified (only 1 positive SLN, in one of them);
- in 1 patient with positive SLN, the presence of a paucicellular neoplastic embolus was detected in the peri-lymph node lymphatic afferent vessel (metastasis in transit);
- of the 24 SLNs detected, 20 were “hot-blue”, 4

Intra-operative phase

Once general anaesthesia was induced, a standard dose of supravital radiotracer (Patent Blue V dye, 1 ml) was injected, using a syringe with a fixed needle, into the infiltration points of the radio-labelled tracer, prior to neck incision. Thereafter, the cervical flap was incised and lifted, and then the operating field was explored, directly and by means of a gamma-probe. The SLNs detected were isolated from the excised material, identified with silk suture, and sent to the Institute of Pathology in Parma for histological evaluation.

Anatomo-pathological evaluation

The SLNs, sent to the Institute of Pathology in Parma, separately from the latero-cervical material dissected, were fixed in 10% formalin neutral buffer solution, divided into 2 halves along the hylus (or major axis when the hylus could not be identified) and cut in serial thick (2 mm) and thin (150 μm) sections. Sections were processed in series by means of routine staining i.e., haematoxylin-eosin (H&E), and with the immuno-histochemical technique (anti-cytokeratin pool antibodies AE1/AE3).

The lymph nodes isolated from the latero-cervical material dissected were fixed in 10% formalin neutral buffer solution, divided into 2 halves along the hylus (or major axis when the hylus could not be identified) and prepared in serial thick (2 mm) and thin (150 μm) sections. Sections were processed in series by means of routine staining i.e., H&E.
only “hot”;
– all positive SLNs were “hot-blue”;
– the radio-labelled tracer was found to be more reliable than the surpavital tracer in the search for SLNs.

**Discussion**

The term “sentinel lymph node” was introduced, for the first time, in 1977, by R.M. Cabanas, the American urologist \(^{21}\), who hypothesised that the penile SCC metastasised, via the lymphatic pathway, always at the level of the same lymph node (adjacent to the superficial epigastric vein), biopsy of which would have provided an appropriate evaluation of the pathologic stage of the entire inguinal lymph centre. However, the presence of false negatives, revealed in later research, led to this idea being abandoned. Only 15 years later, in 1992, did D.L. Morton, in studies on early stages of malignant melanoma \(^{10}\), define SLN as the first lymph node draining the primary neoplasia: he realised that its localisation was not predefined, but depended upon the site of neoplasia itself and upon individual variability of the lymphatic drainage pathways. He, therefore, proposed a lymphatic mapping technique based upon the use of a surpavital tracer which, injected into the subcutaneous peri-tumoural site, would stain the lymphatic vessels and regional lymph nodes draining the melanoma; later, the presence of neoplastic cells within this tissue, having been confirmed by biopsy studies, would have confirmed the need to surgically treat the entire lymph centre; if this were not the case, the latter would have been considered metastasis-free, and thus spared. This technique was immediately found to be very accurate in the staging of malignant melanoma, in the early stages, to the extent that, a retrospective study, performed by Morton 7 years later \(^{22}\), showed that the false negative rate varied be-

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**Table I.** Data from the 10 patients studied.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Site of neoplasia</th>
<th>Clinical stage of tumour</th>
<th>Clinical stage of lymph nodes</th>
<th>Pathological stage of lymph nodes</th>
<th>SLNs detected</th>
<th>Hot-blue SLNs detected</th>
<th>Level of SLNs predicted by lymphoscintigraphy</th>
<th>Positive LNs No.</th>
<th>Positive hot-blue SLNs No.</th>
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<tbody>
<tr>
<td>1</td>
<td>OT</td>
<td>T2</td>
<td>cN0</td>
<td>pN1</td>
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<td>1</td>
<td>LEV II, LEV I</td>
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<td>1</td>
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<tr>
<td>2</td>
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<td>T3</td>
<td>cN0</td>
<td>pN0</td>
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<td>3</td>
<td>LEV I, LEV II Facial</td>
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<td>None</td>
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<tr>
<td>3</td>
<td>TB</td>
<td>T3</td>
<td>cN0</td>
<td>pV1</td>
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<td>2</td>
<td>LEV II, LEV I</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>RMT</td>
<td>T2</td>
<td>cN0</td>
<td>pN2b</td>
<td>2</td>
<td>2</td>
<td>LEV II, LEV I</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Aof-OT</td>
<td>T3</td>
<td>cN0</td>
<td>pV1</td>
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<td>4</td>
<td>LEV II right (LEV II ln)</td>
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<td>1</td>
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<tr>
<td>6</td>
<td>PT</td>
<td>T3</td>
<td>cN0</td>
<td>pN1</td>
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<td>LEV II, LEV III (LEV II ln)</td>
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<td>1</td>
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<td>cN0</td>
<td>pN0</td>
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<td>LEV III left (LEV II ln)</td>
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<td>None</td>
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<td>cN0</td>
<td>pN0</td>
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<td>LEV I, LEV II</td>
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<td>None</td>
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<tr>
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<td>T1</td>
<td>cN0</td>
<td>pN1</td>
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<td>1</td>
<td>LEV I</td>
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</tr>
<tr>
<td>10</td>
<td>OT</td>
<td>T2</td>
<td>cN0</td>
<td>pN0</td>
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<td>2</td>
<td>LEV I, LEV II</td>
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</tr>
</tbody>
</table>

**Table II.** Variability in levels of involved lymph nodes related to site of primary neoplasia.

<table>
<thead>
<tr>
<th>Site of neoplasia</th>
<th>SLNs detected</th>
<th>Levels of lymph nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Aof-OT</td>
<td>2</td>
<td>Ib</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>III</td>
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<td></td>
<td>1</td>
<td>IV</td>
</tr>
<tr>
<td>5 OT</td>
<td>2</td>
<td>Ia</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Ib</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>II</td>
</tr>
<tr>
<td>1 RMT</td>
<td>1</td>
<td>Ib</td>
</tr>
<tr>
<td>1 PT</td>
<td>1</td>
<td>II</td>
</tr>
<tr>
<td>1 TB</td>
<td>1</td>
<td>II</td>
</tr>
</tbody>
</table>

OT: oral tongue; TB: tongue base; RMT: retromolar trigone; PT: pharyngeal tonsil; Aof-OT: anterior oral floor-oral tongue; LEV: lateral epitrochlear vein; LN: lymph node.
between 0% and 2%, in the various series. Similar results were reported by Giuliano et al., who described its use in the staging of breast cancer in the early stages, in a study on a large number of patients, published in 1994. These Authors identified SLNs in 114/174 patients (65.5%), and in 109 of these (95.6%), biopsy findings provided an accurate pathological stage of the ascellar lymph centre. Albeit, already since those early reports, an increasing number of surgeons have stressed the possibility of technical-procedural difficulties, concerning the intra-operative lymphatic mapping method, to the extent that the possibility of successfully identifying the SLNs was directly correlated with the skill of the surgeon. Great progress was made in 1993, the year in which Alex et al. proposed a new method for lymphatic mapping based upon peri-tumoural infiltration of a radio-labelled tracer, and intra-operative investigation of the SLN with the gamma-camera (γ-Probe) able to detect radio-emission centres; this was used, in that same year, by Krag et al. and by Alex himself, the first to propose SLN biopsy for staging of breast cancer in the early stages, instead of traditional surgical treatments which were both invasive and mutilating for the patients. In a series of 22 patients, the Authors detected SLNs in 18 (82%), with a high predictive value of the pathological stage of the ascellar lymph centre. Later, the technique was further improved with the use of pre-operative investigation by means of static and/or dynamic lymphoscintigraphy. Biopsy of SLN with the radio-labelled tracer method, especially if associated with use of supravital tracer (biopsy of the SLN with the double tracer method), offered the possibility of identifying a large number of SLNs, whilst, at the same time, not only making the technique easier to perform but ensuring that it was applied correctly.

The accuracy and progressive improvements in the methods have led to their elective use in the staging of malignant melanoma and breast cancer in the early stages, as well as its experimental use in other surgical fields, such as gastroenterology, endocrinology, gynaecology, as well as head-neck. As far as concerns the latter, the first data appeared relatively recently, with reports by Koch et al. and Pitman et al. in 1998, followed by those of Shoaib et al. in 1999; Alex et al., Zitsch et al., Mamelle, Chiesa et al. and Pitman in 2000; Dunne et al., Mozzillo et al., Shoaib et al. and Taylor et al. in 2001; and, finally, Werner et al. in 2002. Differences in the case series as well as the limited number of patients in these various reports do not allow any definitive results to be advanced. Albeit various oncology centres world-wide are actively engaged in experimental studies, at present, and already very promising results are emerging, including also those reported herein. Data from these studies were presented at the “1st International Conference on Sentinel Node Biopsy in Mucosal Head and Neck Cancer” held in Glasgow (Scotland, UK) in June 2001. Aim of the present study was to evaluate the possible application of this SLN biopsy technique in the management of patients presenting SCC of the oral cavity and oropharynx. With the combined use of pre-operative lympho-scintigraphy, intra-operative investigation of radio-labelled lymph nodes, intra-operative mapping of lymph nodes with supravital tracer, the present results (one or more SLNs detected in all 10 patients: a total of 25 SLNs, with a mean of 2.5 SLNs/patient; 8 SLNs positive for metastasis of squamous cell carcinoma in 6 patients, of which 2 in 2 patients; no other metastasis detected in lymph nodes extracted from residual material collected upon dissection; in 100% of cases, SLNs reflected the state of health of the entire latero-cervical lymph centre) suggest that this technique may, in selected patients, have a role in the identification of micrometastatic involves at latero-cervical level. This factor is of great prognostic importance, bearing in mind that this finding involved 35% of cases in neck clinically and radiologically negative. At present, it is generally held that micro-metastases, in the form of “neoplastic cell nests” within the lymph node parenchyma (typically subcapsular), or in the form of neoplastic emboli “in transit” in different lymphatic vessels, may spread, in sequence, along the various lymph node levels, and/or by spreading simultaneously along the lymphatic pathway, either main or secondary; this would explain the presence of “skip metastases” found in the dissection material in approximately 16% of patients with SCC of the oral cavity and oropharynx, whatever the T stage, provided that the condition is, indeed, N0 and M0. These metastases would appear to spread via the lymphatic pathway, by passing one or more regional lymph centres of drainage, remaining, in some cases, in only one site, in distal or unusual lymphatic stations. The method of only a supravital tracer has been shown to be unreliable, not only in the detection of SLNs in the neck of all patients, but also in the identification of micro-metastatic SLNs. In our limited experience, some SLNs, only partially stained blue, were first identified by means of radioactivity, thus confirming the greater reliability of the radio-labelled tracer vs the supravital tracer. As far as concerns the identification of radioactive lymph nodes, the main difficulty may be due to the close proximity of the SLNs to the site of tumour infiltration, since the radioactivity emitted by the latter tends to cover that of the SLNs possibly present in the immediate vicinity. In our study, pre-operative localization of “hot” SLNs was performed with the aid of a signaliser with a radioactive tip (99mTc) with which to establish the exact site in the scintigraphic display, prior to collecting static
images of the patient, whereas intra-operative localization did not give rise to any difficulty. In this respect, it should not be forgotten that some Authors have suggested the use, both in the pre- and intra-operative phase, of lead diskettes to shield radiation emitted by the tumour and surrounding tissues. Various radio-labelled colloids exist for use in the identification of SLNs: we have used a sulphate nanocolloid, labelled with $^{99m}$Tc, which was found to be versatile since it is rapidly drained from the lymphatic vessels, and, furthermore, remains, for several hours, in the first lymph centre of drainage. Lymphoscintigraphy was performed on the day before surgery since, in the early cases, we were not aware of the time necessary for localization of draining lymph nodes. It is important to make sure, during injection of the radiotracer, that this is not accidentally swallowed, since, if this were to become localized in the pharynx and oesophagus, it would be impossible to correctly interpret the scintigraphic findings. To avoid this possibility, patients were asked to rinse the oral cavity with a mouthwash, at the end of the injection procedure. As far as concerns use of the supravital tracer, infiltration should be carried out prior to neck incision, since there is less risk of interrupting the lymphatic vessels leading to LNS.

Spreading of the stain, particularly on surgical gloves or the skin of the neck, could lead to difficulty in the intra-operative mapping; it is, therefore, advisable to use a syringe with a fixed needle as well as a change of gloves prior to proceeding with the incision of the neck. In all these patients with clinically and radiologically negative neck, at least one SLN was identified, and each of these correctly revealed the patho-
logic stage of the latero-cervical lymph centre; it is, thus, possible to hypothesise its routine use in the staging of SCC of the oral cavity and oropharynx with clinically and radiologically negative neck, as in

Fig. 3. Cervical flap is lifted and neck fascias are opened, the 'blue' SLN is visible.

Fig. 4. Intra-operative examination with gamma-camera confirms the presence of a 'hot-blue' SLN.

Fig. 5. Intra-operative radiometric findings.

Fig. 6. Left: Detail of cortical layer of a lymph node with extra-lymph node lymphatic vessel inside which neoplastic cells with abundant eosinophilic cytoplasm are present. Right: at immunohistochemistry investigation, these cells show intense cytoplasmic positivity for cytokeratins. This reactivity demonstrates their epithelial origin. Bar: 50 µ. Magnification: x40.
breast cancer and malignant melanoma in the early stages. The times necessary to reach this goal are related to the setting up of a standard method in the various referral Centres throughout the world: to this end, a multicentre protocol has been set up to collect results of our research (Cannesburn Sentinel Node Biopsy Multicenter Trial, Glasgow, Scotland, UK) aimed at evaluating, on a large scale, the results of the application of this technique.

Case report

Patient: a 34-year-old male presenting SCC localised at the level of the right lingual margin (stage T2 N0 M0) (Fig. 1). Static lymphoscintigraphy was performed in the pre-operative phase by means of injection of a radio-labelled tracer ($^{99m}$Tc sulphate formed in the pre-operative phase by means of injection procedure, revealed the presence of 2 “hot”-shaped nodes in the neck, one located at the level of the right lingual margin (stage T2 N0 M0) (Fig. 1). Static lymphoscintigraphy was performed in the pre-operative phase by means of injection of a radio-labelled tracer ($^{99m}$Tc sulphate nanocolloid 700-750 µCi in 1 ml of physiological solution 0.9% NaCl) in the peri-tumoural site, using a syringe with a fixed needle. Lymphoscintigraphic findings, at 10 and 60 minutes (control) after the injection procedure, revealed the presence of 2 “hot”-shaped nodes in the neck, one located at the level of the right lingual margin (stage T2 N0 M0) (Fig. 1).

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