SENTINEL NODE BIOPSY IN SQUAMOUS CELL CARCINOMA OF THE ORAL CAVITY

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Head and Neck Squamous Cell Carcinoma (SCC) spreads via lymphatics to the regional draining lymph nodes in the neck. Since the presence of lymph node metastases is the most important prognostic factor in Head and Neck (H&N) cancer, decreasing survival by 50%, reliable staging of the neck in this disease is imperative to determine further management. [14]

Data from Literature show an incidence of occult metastases in oral cavity tumours ranging from 12% to 50% (mean 33%) . Traditional imaging techniques (UlS, CT, MRI) are not able to obtain a detailed staging of N0 neck, having a specificity of 75% to 92% [14]. So, for most Authors [7, 23] the gold standard in these cases is to treat the patients with elective neck dissection and routinelly pathological examination of the surgical specimen. This policy means that up to 60-70 % of patients with N0 neck have unnecessary operations, with its associated morbidity and without the assurance to reach a 100% of reliable staging tool, because of conventional pathologic evaluation.

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 SN is clear of metastases, the remainder of nodes are clear too.

In the late 1980s, Donald L. Morton proposed the innovative concept of “lymphatic mapping with sentinel lymph node biopsy” for melanoma using a Blue Dye [9]. This is injected into the tissue at the sight of the primary tumor; than it passes along the lymphatic channels that drain this tumor. The first node to turn blue (the “Sentinel node”) is the node most likely to contain any cancer cells migrating from the primary tumor to the drainage basin.
SNB is also an accurate method of staging axillary lymph node involvement in breast cancer. 953 patients treated from 1996 to 2000, with negative sentinel nodes not submitted to axillary dissection, were followed-up. The 5 year overall survival rate was 98 %. [18,19]

The more recent definition of Sentinel Node by BNMS Guidelines (October 2004) is as follows: "Sentinel Node (SN) is any lymph node which receives lymphatic drainage directly from a tumour. Second-tier and third-tier nodes receive drainage in a later phase. SNs may not be the hottest nodes at the time of imaging; SNs may not be the ones closest to the tumour; they may be more than one SN”.

Dynamic scintigraphy and intraoperative blue dye mapping have made that clear. Sometimes there are two lymphatic channels originating in the region of the primary tumor and running to two different lymph nodes.[9]

It is important, for an accurate lymphatic mapping, to know the anatomical distribution of lymphatic system. In H&N there are more than 300 lymph-nodes (about 1:5 of the total body nodes). The lymphatic system consists of complex capillary networks which collect the lymph in various organs and tissues [6]. Lymphatic capillaries are abundant in the dermis; they have numerous anastomoses and are without valves. In the epidermis and subcutaneous tissue there are not lymphatic capillaries. The tongue has the blind lymph capillaries in the filiform papillae with the underlying plexus. An elaborate system of collecting vessels conducts the lymph from the capillaries to the large veins of the neck. In the lymphatic vessels of H&N the valves are more numerous and they are placed at shorter intervals than in those of the lower extremity.

Lymphoscintigraphy depicted collateral patterns of lymphatic drainage and these patterns are frequently different from classical anatomical models such as Sappey’s lines.

The technique of SNB in Oral Cavity Tumours involves the combination of mapping the main lymph node fields of the neck by Lymphoscintigraphy with Radioguided SNB and the Blue Dye technique.

**Lymphoscintigraphy with radioguided SNB**

There is no special preparation for the lymphoscintigraphy, other than preoperative restrictions. The patient’s necklaces, dental prosthesis and all relevant metallic items should be removed. The position during the exam should mirror that of the patient at surgery. The Lymphoscintigraphy is performed in the 24–hr period prior to surgery [13].

The radiopharmaceutical favoured in Europe is 99mTc-HSA Nanocolloid. It has an optimal particle size (<80nm). The colloidal isotopes are phagocytosed by the macrophages within the
lymph node, keeping the tracer in the draining node. Other radiopharmaceuticals in use are: 99mTc-Albu-Res and 99mTc-Sulfur Colloid.

A local anaesthetic (10% lidocaine spray) is given before injection. A small volume, about 0.4 mL, and a low dose between 30 and 50 MBq is injected superficially, in subepithelial stroma, at four sites around the tumour. A mouthwash is used immediately following injection to prevent pooling or swallowing of residual radioactivity by the patient.

Superficial injections are advised, because in the sub-epithelium stroma there is a high rate of lymphatic capillaries: this provides a larger surface area for uptaking, a faster lymph drainage and a better identification of SN in shorter time. This determines an highest success rate in SN identification. [13, 14, 20] When deep injection is performed the time of the lymphoscintigraphy is longer, the quality of image is poor, the blood accumulation rate of tracer and background are increased, and the success rate in SN identification is lower. The image acquisition protocol commenced immediately after injection using Gamma Camera with LEGP or High Sensitivity collimator (Pixel matrix 256x256) in Lateral and/or Anterior views. Dynamic and early Static images are acquired immediately after injection up to 30 minutes. The skin overlying the SN should be marked with an indelible marker pen. Aims of Dynamic and Early Static Lymphoscintigraphy are:

- to demonstrate which lymphatic drainage basins are potential sites of metastatic disease;
- to determine the number and location of SNs within those drainage basins;
- to mark the location of any SNs for subsequent radioguided surgical dissection;
- to try to distinguish SNs from second-tier and third-tier lymph nodes. [Fig.1]

Detection of SN is performed through the intact skin to confirm the lymphoscintigraphic localization of SN marked on the skin surface with dark dats.

The lymphatic basin is searched by hand held gamma probe (Neoprobe – Ethicon) for SNB during surgical procedure and at the end of it, on the surgical specimen and on lymphnodes selectively excised [Fig.2]. The activity counts of the SN should be at least three times than count of the background activity.

**The Blue Node Technique**

Blue dye may provide additional information in the localization of SNs. At the commencement of surgery, 0.25 mL of Patent Blue V dye, diluted 1:3 in water, will be injected around the tumour.

In H&N both Blue-dye and Radiocolloid traverses the lymphatics quickly, appearing in the SN in less than 5 minutes, but the radiocolloid remains in the SN longer than Blue Dye. [2]
The use of blue dye remains controversial and some Authors do not use it [16,20]. Disadvantages are blue staining of the healthy tissue around the tumor border, that could make the resection more difficult. Low incidence of anaphylactic reactions is also described.

The Pathologic Protocol
The SN is fixed in 10% neutral buffered formalin for 12-24 hours. The whole lymphnode is bisected through the hilum or through the long axis of the node. If the thickness of the halves is more than 2mm the slices are further trimmed to provide additional 2 mm thick blocks. If there is no tumour on initial histological examination step-serial sections will be prepared at an additional six levels in the block at approximately 150 micron intervals. One H&E stained section will be prepared at each level. If the nodes still appear histologically negative, an immediately adjacent section from each level will be examined by immunohistochemistry using the multi-cytokeratin antibody (AE1/AE3)

According to Canniesburn trial protocol all cases are classified by a pathology code as follows:
- 1 Tumour positive on first H&E examination.
- 2 Initially tumour negative, but tumour positive on examination of H&E
  of step serial sections.
- 3 Negative at stages 1 and 2 but positive by immunohistochemistry.
- 4 Cytokeratin positivity not showing the features of viable tumour cells.
- 5 Negative at all stages.
- 6 Negative on first H&E examination. Further examination not performed
  since other sentinel nodes contained viable tumour either on H&E or
  immunohistochemistry

SNB in Oral cavity tumours
The first reported case of a patients with neck metastases successfully identified by SNB in H&N-SCC was by Alex and Krag (1996) in a supraglottic cancer [1]. Many other studies have been published in the following years [Tab I.], but the most important collection of data on SN in oral cavity tumours is from the multicenter trial protocol adopted in the First International Conference on SNB in Mucosal H&N Cancer held in Canniesburn Glasgow, in June 2001 [13]. Twenty-two Centers contributed to the protocol with 316 cN0 neck included. SN were identified in 301 necks (95%); 76 necks were positive (25%) on detailed histopathological examination of SN, while 225 were negative (8 of them had disease within the neck specimen). The overall
sensitivity of the procedure was 90% (range: 57-94% depending on the experience of different centres).

Most of these preliminary studies were based on SNB identification in cases undergoing neck dissection to test the procedure. Nowadays SNB alone is used to stage the clinically N0 neck.

At the present time, the incidence of occult metastases detected by SNB is about 37% (range 12.5% – 55%) and the sensitivity of the procedure could be stated up to 95%, confirming that there is a role for SNB for staging the clinically N0 neck [3,4,5,8,10,12,14,15,16,22].

Critical observations concerning the routinely clinical use of SNB protocol are based on the elevated number of lymph-nodes detected as SNs, and on their localisation in distant levels of the neck (level III, IV). The mean number of SNs detected is more than 2.5 in several studies (3, 4,5,8,15,16). Furthermore, SNs have been detected outside level I and II in many cases: 25% (12), 30% (13) and 50% (15) with, sometimes, SN in level IV and V.

These data limit the practical application of the protocol, because in these cases it seems to be easier and safer to perform a selective neck dissection.

To limit these problems, according to our experience [17,20, 21], we want to stress the following key points:

A) detailed and careful examination of Neck imaging to stage NO is recommended: US evaluation is operator dependent and not universally available, so it is better to perform CT scan or MRI. Exclusion criteria are a maximum diameter of the node > 1.5 cm, marginal enhancement following i.v. administration of medium contrast; central necrosis, spherical form, unsharp or not definable countour.

B) In lymphoscintigraphic examination it is really important to do 4 perilesional superficial subepithelial injections, not deeply, because of lack of lymphatic capillaries. The time of analysis is not more than 30 minutes, sufficient to visualize at least two neck levels. According to our experience same day protocol is more useful.

C) SNB in floor of the mouth tumors is more difficult, because SN may be too close to the primary injection site to be easily discernible by gamma probe. Correct lymphoscintigraphic technique, the use of blue dye, and a careful exploration of level I is recommended. In these cases removal of primary T first, to eliminate the background radioactivity at the primary site, can aid in subsequent identification of hot nodes close to the primary. [Fig. 3 ]
D) Radioguided surgery is mandatory. The activity counts of the SN selectively excised is measured by gamma probe and should be at least three times the count of the background activity.

E) The learning curve expects an experience of minimum 10 procedure. The first step is SNB + elective neck dissection to examine all the specimen and test the procedure in your hands and in your Institution. SNB procedure need a strict cooperation between ENT, Nuclear Medicine and Histopathology Equipes.

F) Follow up of these patients must be accurate: every 3 months up to three years and every 6 months up to 5 years, with clinical and imaging investigation for patients with SN negative. A positive SN will lead to a neck dissection within 28 days.

Our protocol plans lymphoscintigraphic exams 2-3 hours before radioguided surgery without using blue dye. We studied 13 patients, mostly T1-T2 oral cavity tumours, with SN and elective neck dissection and 6 patients only with SNB. SN was detected in all cases by lymphoscintigraphy and radioguided biopsy. SN was found in level I in 11/19 cases (58%) and in level II in 15/19 cases (79%). In all cases SNs were in level I and/or Level II, while in level I-II only, in 79% of cases. In 3 cases (16%) SN was found in level III and only in one case in level V. Eight out of nineteen SNs were positive (42%), and all of them were identified by step serial sectioning on routine H&E staining. Elective neck dissection in these positive cases revealed some more positive lymphnodes in two cases out of 475 lymphnodes examined. Eleven cases were SN negative. In the preliminary part of our experience, the 8 SNB negative cases were submitted to neck dissection: no one had positive lymph nodes out of 375 neck nodes examined. No one, till now, had neck recurrence. The mean follow up of these patients group is 24.9 month (range 12-36 months). The mean number of SNs dissected was 2.5 for each patient.

Our data confirm that the radiolocalisation of SN in patients affected by SCC of the oral cavity allows to identify the lymphatic pathways for each tumor site, and to detect the SN almost always in level I or II, at least in two levels, so limiting the number of lymphnodes to be examined and the extension of the surgical approach.

Of course, a larger data base from multicentre trials would confirm the validity of this approach and the next goal is to avoid in selected patients unnecessary and invasive radical neck dissections.
REFERENCES


<table>
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<tr>
<th>Authors</th>
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TAB I: Some reports from Literature. Data from different studies are not homogeneous by TNM staging (T1-T4) and by the incidence of occult metastases detected (8%-61%) and the rate of SN identification.
**Fig. 1** SCC 1/3 right anterior of the tongue. The scan shows the points of injection (INJ) the SNs (1,2) at 1st neck level and the second-tier and third-tier lymph nodes (3,4,5).

**Fig. 2** The lymphatic basin is searched by hand held gamma probe (*Neoprobe – Ethicon*) for SNB during surgical procedure (left) and at the end of it (right), on the surgical specimen and on lymphnodes selectively excised.
Fig. 3: SCC of the floor of the mouth. The scan shows the points of injection (INJ) and the SNs at neck level I and II. The first SN appears close to the site of T, but clearly distinguishable.