GUIDELINES

Joint practice guidelines for radionuclide lymphoscintigraphy for sentinel node localization in oral/oropharyngeal squamous cell carcinoma

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Abstract Involvement of the cervical lymph nodes is the most important prognostic factor for patients with oral/oropharyngeal squamous cell carcinoma (OSCC), and the decision whether to electively treat patients with clinically negative necks remains a controversial topic. Sentinel node biopsy (SNB) provides a minimally invasive method of determining the disease status of the cervical node basin, without the need for a formal neck dissection. This technique potentially improves the accuracy of histological nodal staging and avoids over-treating three-quarters of this patient population, minimizing associated morbidity. The technique has been validated for patients with OSCC, and larger-scale studies are in progress to determine its exact role in the management of this patient population. This article was designed to outline the current best practice guidelines for the provision of SNB in patients with early-stage OSCC, and to provide a framework for the currently evolving recommendations for its use. These guidelines were prepared by a multidisciplinary surgical/nuclear medicine/pathology expert panel under the joint auspices of the European Association of Nuclear Medicine (EANM) Oncology Committee and the Sentinel European Node Trial Committee.

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Abbreviations

SNB	Sentinel node biopsy
OSCC	Oral/Oropharyngeal squamous cell carcinoma
EANM	European Association of Nuclear Medicine
SENT	European Sentinel Node Trial group
END	Elective neck dissection
SLN	Sentinel lymph node
cN0	Clinical node-negative
pN0	Pathological node-negative
cN+	Clinical node-positive
pN+	Pathological node-positive
Tc-99m	Technetium-99
LSG	Lymphoscintigraphy
CT	Computed tomography
MRI	Magnetic resonance imaging
USg-FNAC	Ultrasound-guided fine-needle aspiration cytology
FDG-PET	18F-fluoro-deoxyglucose positron emission tomography
PET/CT	FDG-PET with low dose CT
MBq	Mega-becquerel
mSv	MilliSievert
µSv	MicroSievert
µGy	MicroGray
keV	kilo-electron volt
SPECT	Single photon emission computed tomography

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IV	Intravenous
ICRP	International Commission on Radiological Protection
57Co	Cobalt-57
153Gd	Gadolinium-153
cps	counts per second
QA	Quality assurance
QC	Quality control
UICC	Union Internationale Contre le Cancer (International Union Against Cancer)
H&E	Haematoxylin-eosin stain
MM	Micrometastasis
ITC	Isolated/individual tumour cells
RT-PCR	Reverse-transcriptase polymerase chain reaction

Introduction

Oral/oropharyngeal squamous cell cancer (OSCC) is one of the most common cancers worldwide, accounting for more than 274,000 new cases annually [1]. Three-quarters of affected people are in the developing world, while in developed countries, OSCC is the eighth most prevalent form of cancer. Determining the presence or absence of nodal metastasis is of paramount importance for staging, treatment planning and prognosis. The incidence of occult metastases in patients with clinically node-negative OSCC is high, with many series reporting rates greater than 30% [2–5]. Cervical lymph node involvement is the most important prognostic factor for patients with OSCC [5–7].

Elective treatment of the clinically-negative neck remains a controversial topic. Over the last two decades much work has been undertaken to find reliable predictors of occult metastases, of which tumour depth appears to be the best available [8–11]. However, the predictive capacity of tumour depth and other primary tumour characteristics is still insufficient to negate the need for surgical staging of the cervical node basin [12, 13].

Elective neck dissection (END) is the current gold-standard staging procedure for the clinically node-negative neck, providing valuable prognostic information regarding nodal status and simultaneously treating those patients found to be pathologiaclly node-positive. Previously, ENDs invariably took the form of a modified radical neck dissection; however, there is increasing evidence that selective neck dissection is as efficacious as comprehensive neck dissection in treating the negative neck [2, 14–20]. The shift toward more conservative surgical procedures has occurred primarily in the last two decades, facilitated by the work undertaken by Lindberg [21], Byers et al. [22] and Shah et al. [3] to describe the common patterns of lymphatic drainage. Knowledge of these patterns

has allowed the extent of neck dissections to be progressively limited to those nodal levels at highest risk, and sentinel node biopsy (SNB) represents an extension of this philosophy.

The aim of this review is to provide evidence-based guidelines for the use of SNB as a staging tool in patients with early OSCC, presenting the best available evidence at the time of writing. The existing literature was reviewed, utilizing electronic techniques (Medline, Best evidence, the Cochrane Library, Dare) and hand searching techniques. Where little or no data existed from randomized controlled prospective trials, emphasis was given to data from large series or reports from recognized experts in the field. It is recognized that higher-level evidence from future studies may modify the recommendations made in these guidelines.

Definition of a sentinel node

The sentinel node concept states that the spread of a tumour is embolic in nature, via the lymphatics to the first echelon lymph node(s) encountered in the regional draining basin. These represent the lymph nodes most likely to harbour occult metastases, and are designated the sentinel lymph nodes (SLN). Excisional biopsy and pathological evaluation of the SLNs therefore allows prediction of the disease status of the remaining cervical lymph node basin, potentially avoiding the need for a neck dissection. SLNs need not be those closest to the tumour, and there may be multiple SLNs [23]. With the application of early dynamic lymphoscintigraphy (LSG), lymphatic channels are usually visualized and nodes on a direct drainage pathway may be distinguished. The practical approach may include a combination of available detection techniques.

Lymphatic mapping and SNB were first reported in 1977 by Cabanas for penile cancer [24]. In 1992, Morton et al. [23] were the first to describe the use of intradermal isosulphan blue dye injection for lymphatic mapping and SLN localization in patients with malignant melanoma. The following year, Alex et al. [25] described a peritumoral intradermal injection of radioactive tracer (^{99m}Tc sulphur colloid), followed by imaging and intraoperative gamma probe radiolocalization of SLNs. The sentinel node concept has since been extensively studied and validated for patients with cutaneous melanoma [26] and breast cancer [27], and studies to date have indicated a high level of accuracy in patients with OSCC [28, 29].

Clinical indications

Inclusion criteria

The most important inclusion criterion for SNB is a clinically negative neck, as defined by physical examina-

tion and clinical imaging by CT, contrast-enhanced MRI, ultrasound-guided fine-needle aspiration cytology and/or ^{18}F-fluorodeoxyglucose PET with or without low-dose CT [30, 31]. There remains considerable debate as to the preferred imaging modality, and to date none has the ability to detect small or micrometastatic tumour deposits, but all techniques improve on the sensitivity of palpation alone, and are therefore recommended prior to SNB. Recently, the high specificity of PET has been highlighted as an important means of avoiding unnecessary neck dissections [30]. Gross lymphatic involvement can lead to distortion of the normal architecture, leading to aberrant drainage patterns and biopsy of false SLNs [32]. SNB is therefore contraindicated to stage clinically positive necks.

Following the first report of SNB for OSCC [33], the technique has undergone extensive validation against the gold-standard END, for tumours located in the oral cavity and accessible subsites of the oropharynx [29, 34]. It has been demonstrated to be an accurate means of staging the clinically negative neck, and more recently the potential prognostic value of SNB for these tumour sites has also been highlighted [35]. While SNB has been successfully reported for tumours in other locations such as the hypopharynx and supraglottic larynx [36], there remain significant technical barriers, and SNB for these sites should therefore still be considered investigational. Poor access to these sites requires general anaesthesia and endoscopic guidance for radiotracer injection, precluding the use of preoperative LSG, while the close proximity of the primary tumour to the first-echelon lymph nodes can potentially obscure the true location of SLNs. Additionally, advanced stage at presentation is common for these hidden tumours, precluding the use of SNB or indeed any surgical intervention.

There is an existing consensus that SNB for OSCC should be restricted to early tumours staged T1/2 [37–39]. Larger tumours are difficult to completely surround with the tracer injection, tend to drain to multiple lymphatic basins, and in most patients require a neck dissection for access to the primary tumour or defect reconstruction. Inclusion of T3/4 tumours in study protocols can lead to variability in the accuracy of the technique [37].

The first and most frequent indication for SNB is to stage the ipsilateral clinically node-negative neck in patients with a unilateral primary tumour. A second indication is for assessment of bilateral clinically node-negative necks in primary tumours close to, or crossing, the midline. The third indication is for assessment of the contralateral clinically node-negative neck in primary tumours close to the midline with an ipsilateral clinically node-positive neck, in order to decide whether these patients need bilateral neck dissections, or an ipsilateral neck dissection and contralateral SNB only. Patients should also be fit enough preoperatively to withstand a neck dissection.

Patients who have received prior radiation or surgical treatment to the neck are routinely excluded from SNB protocols, since the previous intervention can distort the normal lymphatic pathways and give rise to unexpected patterns of metastasis. It is possible that lymphatic mapping and SNB may yield potentially useful information in these patients. Similarly, patients with small recurrent or second primary tumours may also benefit from lymphatic mapping to guide surgical intervention. However, these applications of the SNB technique, whilst clinically attractive, remain largely unexplored.

Exclusion criteria

In pregnant women, the urgency and the necessity for staging the neck should be discussed. LSG is specifically contraindicated in the pelvis of pregnant women, but no such recommendations are currently available for the head and neck. As discussed in the section "Dosimetry – patient", the risk of fetal damage is negligible during routine SNB procedures. However, SNB protocols should be modified in pregnant patients to minimize the risks of radiation exposure and blue-dye injections. For example, the use of a 1-day protocol allows a lower injected radiation dose, and the additional radiation associated with SPECT-CT imaging may not be warranted in the pregnant patient. SNB can be performed in lactating women, but it is advised that breastfeeding be discontinued following the procedure. OSCC is rare in children, though each case should be treated individually. In the UK, Nanocolloid is approved for use in children, though licensing varies between countries. The potential benefits of SNB are not as well delineated in the paediatric population and, in practice, most SNB trial protocols generally exclude these patients from participation. Off-label use of radiopharmaceuticals should be considered with caution and respect to an individual risk-benefit analysis. Other relative contraindications include a known allergy to albumin colloid, and primary tumour treatment by external beam radiotherapy.

In summary, SNB is currently indicated for cT1/2, clinically node-negative oral and selected oropharyngeal SCC, where it may be considered a valid alternative to END. Other head and neck sites, histologies and clinical situations remain under investigation.

Radiopharmaceuticals

Introduction

A variety of colloidal and soluble tracers have been used over the years for lymph studies. It is believed that radiocolloids are taken up by macrophages in lymph nodes

whereas the transit of macromolecules through lymph nodes is delayed simply because of their large molecular size [40].

Choice of radiopharmaceutical

The main radiopharmaceutical used in European studies of SLN localization in oral cancers is ^{99m}Tc-labelled human serum albumin colloid (Nanocoll, GE Healthcare). Nanocoll has a particle size range of 5–80 nm, with a reported mean size of 8–30 nm [40]. Although in theory a larger particle such as Albures (GE Healthcare) or Sentiscint (Medi-Radiopharma) may be preferred for tumours in the floor of the mouth or anterior tongue where the lymphatic densities are high [37], Nanocoll performs satisfactorily in all tumour types studied. Nanocoll migrates to the SLN within minutes, yet prolonged retention allows surgery to take place the following day.

Other radiocolloids which have been used include ^{99m}Tc-rhenium sulphide colloid (Nanocis, IBA), which has been shown to have a mean particle size of 23–25 nm [41], and ^{99m}Tc-sulphide colloid. Standard preparations of ^{99m}Tc-sulphide colloid result in a wide range of particle sizes, so the product is often filtered through a 100- or 200-nm membrane filter to obtain a smaller and more uniform size distribution. While there are currently no clinical studies comparing different radiopharmaceuticals, investigators have described satisfactory results with each of the available colloids [42, 43].

Preparation and quality control

Nanocoll is labelled by incubation with ^{99m}Tc-pertechnetate at room temperature for 30 min [44]. Radiochemical purity can be checked by thin layer chromatography and the labelling efficiency should be >95%. The EANM guidelines on current good radiopharmacy practice (cGRPP, www.eanm.org) recommend that the labelling efficiency be checked on each preparation. The stated expiry is 6 h after preparation, although extended stability has been demonstrated [45].

Drug interactions and adverse effects

No interactions of drugs with radiocolloids are expected following local intradermal or subcutaneous administration. Adverse effects are rare and mild following interstitial administration of radiocolloids, although allergic reactions have been reported with Nanocoll [46, 47] and the blue dyes used at surgery [48]. The incidence of allergic reactions is too low to quantify, but appropriate emergency medicines should be kept available during the procedure.

Summary

[99m]Tc-labelled Nanocoll is easy to prepare and supply, and has suitable properties for SLN localization in oral cancers, with rapid migration to the SLN and prolonged retention.

Dosimetry – patient

General remarks

Presently available dosimetric data are derived from the breast cancer SNB literature, where the absorbed doses to patients are determined to be low; therefore, the radiation risk associated with this procedure is low. While no specific OSCC data exist, the radiopharmaceuticals and administered activity are identical, leading to the assumption that OSCC SNB is a safe procedure from the radiation protection point of view.

Patient exposure

The estimated local radiation dose varies greatly, depending on the administered dose and time to surgery. As mentioned in the section "Injection", there is little consensus on the optimal administered dose and timing of surgery relative to the radiocolloid injection. Most centres perform LSG within 24 h of surgery, but recommendations for administered activity range from 15 MBq (for a same-day procedure) to 120 MBq (for a 2-day procedure) in a total injection volume of 0.4–1.0 ml. The aim is to achieve an activity of at least 10 MBq at the time of surgery [49, 50]. Current EANM guidelines for SNB in breast cancer recommend a mean value for the effective dose of 0.048 mSv [51]. While other authors have reported doses approximately tenfold higher [52], these remain low compared with other nuclear medicine procedures. Extensive calculations performed at the Memorial Sloan Kettering Cancer Center have confirmed the safety of SNB by reporting an effective dose around 0.2 mSv [53].

Fetal exposure

The maximum value for fetal absorbed dose has been calculated to be 0.013 mSv following injection of 18.5 MBq [53]. This dose is equivalent to that received by the mother from 1 day of natural background radiation in the USA [52], and is orders of magnitude below the 100–200 mSv threshold for deterministic effects (malformation, growth retardation, neurodevelopmental abnormalities) [54]. Current consensus is that noncancer health effects are not detectable below 50 mSv [53], while congenital malformations occur above 200 mSv. With respect to

childhood cancer induction (stochastic effect), ICRP (International Commission on Radiological Protection) reports a threshold of 10 mSv for a 40% risk increase [54].

In summary, the advantages of SNB for OSCC outweigh the potential risks of the absorbed radiation dose, and this is also true for pregnant patients. While SNB is not contra-indicated in pregnant patients, it is preferable to use a same-day protocol, enabling a lower injected dose [50].

Lactating women

The current recommendation is that nursing mothers should suspend breast-feeding for 24 h following radiopharmaceutical injection, during which time the general anaesthetic agent and radiocolloid will be excreted in the breast milk [51].

Dosimetry – staff

Staff in operating room

Studies in breast and melanoma patients have determined the mean whole-body dose received by surgical staff to be <1μSv per operation [52, 55–57], with a maximum dose to the surgeon of <2μSv. The absorbed doses are further minimized when SNB is performed at 24 h after injection [58]. Monitoring of operating room personnel for occupational exposure during the procedure is therefore unnecessary, and additional shielding is not required. While the pregnant surgeon or scrub nurse requires specific consideration, radiation exposure from participation in fewer than 100 SNB operations during gestation will remain below the recommended limits for pregnant women [57].

Staff in pathology department

Radiation exposure to pathology staff is very low, and should not require badge monitoring. Even personnel performing unusually high numbers of procedures receive radiation doses well below established limits for members of the general public [59].

Radiation safety precautions

Labelling specimens as radioactive for transportation to the laboratory is not required, since the surface dose rate is <5μGy/h [60].

Radioactive clinical waste

Surgical instruments and pathology slides appear to stay at background radiation levels, while measurable contamination of absorptive surgical sponges and other materials used

in proximity to the injection site is observed [61]. It is advisable to monitor these materials for contamination, and contaminated materials should be held for an appropriate period of decay-in-storage before disposal [52, 59].

Injection

The lymphatic anatomy within the oral cavity and oropharynx is extremely complicated and varies significantly between subsites, emphasizing the need for precise injection technique [39, 62, 63].

Patient preparation

No special preparation is needed. Patients should be fully informed about the procedure, including discussion of potential problems such as bleeding and discomfort, before consent can be obtained.

Syringe, activity and volume

Tuberculin syringes with minimal dead space are recommended; otherwise 0.1 ml of air may be drawn into the syringe behind the radiocolloid to ensure complete administration. A 25- or 27-gauge needle should be used. The total activity to be injected may vary, depending on the size and location of the primary tumour. As described in the section "Dosimetry – patient", there is currently little consensus as to the optimum activity for injection [39, 64, 65], and this varies considerably from 15 to 120 MBq between studies. The total injected activity should be adjusted according to the timing of LSG with respect to surgery. Higher doses are required for a 2-day protocol, in order to ensure the activity exceeds 10 MBq at the time of surgery [50]. Small volumes of 0.1–0.2 ml per aliquot are recommended to minimize contamination due to the resistance of the tongue tissue. Contamination can be avoided by placement of a sheet over the injected region and a gauze swab over the needle puncture site before withdrawal. Following injection, the skin/mucosa should be checked for contamination.

Injection site and depth, and number of injections

Tracer should be injected at 0.1–0.5 cm from the tumour or scar margin. The number of aliquots to be injected varies (two to four) according to the size and location of the lesion. The tracer should be administered on each side of the tumour/scar keeping as a reference the orientation of the surgical scar. For lesions in sites with abundant soft tissue (i.e. soft palate or floor of the mouth) four separate submucosal injections must be given around the lesion (at

3, 6, 9 and 12 o'clock). For lesions located in muscle (i.e. tongue), injections should be performed according to the depth of the lesion. Ideally, the operating surgeon should be present for the injections to ensure consistency with injection of blue dye if used. Following injection, bleeding may be controlled with a gauze swab, and the patient should be asked to use a mouth rinse to minimize pooling of the radiotracer in the oral cavity [34].

Imaging

Introduction

LSG uses a gamma camera to assess the drainage of injected radiotracer via the lymphatic capillaries to the larger collector lymphatics until it either passes through, or is retained within, the regional lymph nodes [66]. Accurate preoperative localization and cutaneous marking of the SLNs correlates well with the precision of the surgical procedure [67, 68].

Cameras and camera settings (quality control)

A gamma camera with a large field of view equipped with a high- or ultrahigh-resolution low-energy collimator should be used, with a 10–20% window centred on the 140-keV energy peak of ^{99m}Tc. A two-headed camera allows simultaneous dynamic acquisition in the anterior and a lateral projection allowing more time for the static images and SPECT [69]. Quality control for the gamma camera should be routinely performed and should follow published protocols [70].

Image acquisition

Dynamic acquisition for 20 to 30 min (20 s per frame, with a 128×128 matrix [69] or 256×256 matrix [71]) starting *immediately after radiotracer injection* will show the drainage pattern and help to differentiate between SLNs, which can appear very early following injection, and second-echelon lymph nodes. Two (or three if a three-headed camera is used) simultaneous images in the anterior and lateral projections is recommended.

Static images in the anterior and lateral projections from one or both sides (and oblique as needed) are acquired (300 s, with a 256×256 matrix) to localize the nodes in three dimensions. If hot nodes are not clearly depicted, static images can be repeated at, for example, 2 h, 4–6 h or even just before surgery. The patient is imaged in the supine position with head up. A small flat pillow under the neck may help to fix the head and neck area.

SPECT imaging may improve the identification of SLNs, especially close to the injection site. Lesion detectability is increased by attenuation and scatter correction, which is easily accomplished with hybrid SPECT/CT devices [69, 72]. The increased anatomical detail provided with CT improves localization of SLNs to the anatomical neck level [69]. SPECT acquisition parameters can be 128×128 matrix, 180° in the anterior L-mode rotation, 3° angle step with 20–25 s per projection [73] or as 60 steps per head, 30 s each, and slice thickness 4.42 mm [69].

CT acquisition parameters differ depending on the CT system used. To date, most reports on SPECT/CT for SNB in oral cancer have used a low-end slow CT scanner (GE Hawkeye) with acquisition performed over 220° using 16 s for each transaxial slice, with a fixed tube current of 2.5 mA, 140 kV, and slice thickness 10 mm [69, 73]. With fast high-end CT scanners providing higher-quality scans, either a low dose or a higher dose of CT can be used. Low dose can be, for example, 20 ms per slice, slice thickness/increment 3/3 mm, and 120 kV. If a diagnostic CT scan is required, intravenous contrast agent can also be used. If CT images are used for attenuation correction, inspection of both uncorrected and attenuation-corrected SPECT images is recommended, to avoid overlooking contrast agent-induced artefacts on the latter.

A number of studies have shown advantages of adding SPECT/CT to planar imaging, including identification of missed SLNs, exclusion of ambiguous SLNs, and/or better anatomical localization in 30–47% of patients [69, 73]. However, it has been suggested that meticulous oblique planar imaging can visualize the additional SLNs seen with SPECT imaging, and this may represent an adequate alternative [43]. Furthermore, a number of investigators have reported no advantage to SPECT imaging with respect to the number and location of visualized hot-spots [74, 75]. The true role of SPECT imaging in OSCC SNB has yet to be determined. If used, SPECT/CT should not be a substitute for meticulous planar imaging technique.

The location of SLNs harvested during surgery does not always correlate perfectly with the preoperative imaging [69], though higher quality CT images can allow visualization of individual SLNs of <1 cm, leading to improved preoperative and intraoperative SLN localization [76].

Body contouring

To facilitate topographic localization, a ^{57}Co flood source (or, if available, a ^{153}Gd source) can be used for simultaneous transmission imaging in each projection. Since there is a risk of missing faint nodes when using a transmission source, it has been suggested that the scan be repeated without a transmission source [77]. Alternatively, a radioac-

tive point source may be used to outline the patient's contour while recording the scan.

Image interpretation

On dynamic imaging, SLNs are identified as one or more foci to which lymphatic drainage passes [78], and may be multiple, in one or several areas of the neck, ipsilateral and/ or contralateral to the primary tumour. Imaging should begin immediately, since SLNs can be seen in the first minute after injection [79]. Foci appearing only on later images are also labelled as SLNs, and most appear within 1 to 3 h [43, 80].

According to some reports, SPECT/CT may identify a median of one additional SLN compared with planar imaging. In addition, SLNs located very close to the primary tumour may be detected by SPECT/CT but not with the gamma probe during surgery [69]. While the benefits of SPECT/CT have not been universally accepted [74, 75], both planar and SPECT images demonstrate good or excellent inter- and intraobserver agreement for evaluation of SLNs, with kappa values of 68–89% [43].

Nonvisualization

SLNs are usually detected 15–60 min after radiotracer injection. Failure to detect SLNs may be related to incorrect injection technique or close proximity of SLNs to the injection site (e.g. floor of mouth tumours). In addition, metastatic deposits may block lymphatic drainage causing nonvisualization of SLNs [32, 81]. Repeat injection and imaging may be considered; however, proceeding to neck dissection is preferred in order to avoid a false-negative SLN.

Aberrant nodes and in-transit SLNs

Individual lymphatic mapping by LSG is a major advantage of SNB [81, 82], demonstrating occasional unexpected drainage to, for example, level IV or contralateral metastases from well-lateralized tumours [43, 64, 83–89]. In addition, LSG has been reported to detect "in-transit" lymph nodes: SLNs lying between the primary tumour and the regional lymph basin [90]. These have been described in the context of malignant melanoma, but to date there have been no reports of in-transit SLNs in OSCC.

Report and display

Introduction

There are two main indications for careful report and display of the results from LSG: (1) unambiguous guidance

for surgical biopsy, and (2) a comprehensive dataset for ongoing/future studies [37, 43].

Report

The type of radiocolloid, lot number, volume injected, and effective dose should be recorded, along with the initials and title of the nuclear medicine physician or surgeon performing the injection. The type of camera used and imaging technique should be described in detail [69]:

1. Start time for dynamic imaging.
2. Timing and location of the first echelon node(s) to appear.
3. Timing and number of anterior, posterior and oblique recordings.
4. Timing and location of any additional (second echelon) nodes. These should be clearly differentiated from the first echelon nodes.
5. If CT or hybrid imaging is used, the manufacturer, software and protocol should be described in detail. The number and location of nodes recorded by these modalities should be described and compared to planar recordings. If the results of the tomographic images differ from the planar recordings, this should be clearly stated.

Shine-through from the primary tumour or opposite side should be described and marked clearly on the images. Increased absorption in the thyroid gland can be seen due to unstable colloid solution as a result of a colloid production error [91], and this may lead to difficulties in interpreting the LSG images. Artefacts may also occur due to cutaneous contamination at the time of injection [92]. Rarely, a widened lymphatic capillary may form a "colloid lake"; however, the associated hot-spot will invariably disappear during subsequent imaging, in contrast with true SLNs.

Display

LSG findings should be summarized by the nuclear medicine physician, providing a clear, unambiguous report for preoperative consultation. In addition, hard-copy or digital copies of the LSG images should be available to the surgeon, both prior to and during surgery [37, 43].

Skin marking

First echelon nodes should be marked on the skin using an indelible marker of one colour, guided by gamma camera and hand-held gamma probe [93]. Second echelon nodes should be marked with a different colour, and clearly differentiated.

Use of dye

Introduction

The use of blue dye in head and neck mucosal cancer SLN surgery is optional. However, when used it is a useful adjunct to aid SLN localization and harvest. Blue dye cannot be used alone to perform OSCC SNB, but can be used in addition to radiolocalization with preoperative LSG and intraoperative gamma probe use [34].

Following injection, blue dye drains to the SLNs via the same lymphatic pathways as radiocolloid, staining the channels, which can then be followed to the first-echelon nodes. Direct visualization and dissection of these channels is a natural process for the head and neck surgeon.

Rarely, nonradioactive blue nodes may contain metastases in the absence of a tumour-positive radioactive node; two such SLNs were reported in a series of 40 patients undergoing SNB with both radiocolloid and blue dye injection [63]. The hand-held gamma probe is more sensitive for the detection of SLNs, and not all radioactive nodes will also appear blue [28]. However, blue dye may aid performance of SNB, in terms of both technical success of the procedure and identification of subclinical nodal metastasis.

Contraindications and special precautions

Blue dye is contraindicated in children, pregnant women, lactating women and those who have a history of allergy to the blue dye or any of the products. It can, however, be used in all mucosal SLN procedures, for any malignant process for which the procedure has been deemed suitable, including OSCC.

Blue dyes

In the UK and Europe, the blue dye used is Patent Blue V (Laboratoire Guerbet, Aulnay-Sous-Bois, France; Fig. 1) which comes in 2-ml vials at a concentration of 2.5%. Outside Europe, the use of other agents such as Isosulphan Blue (Lymphazurin) is more common. Gloves should be worn to avoid staining, and a gauze swab used to prevent dye spillage where possible. The dye will rarely mask the edge of the tumour and if this is a concern, the tumour edge can be marked prior to injection with staples or diathermy marks.

Timing of injection

Patent Blue V dye is injected at the time of surgery, under general anaesthesia. It takes approximately 10–15 min for a significant amount of dye to travel from the injection site to the SLN and this is the approximate time it takes to scrub,

Fig. 1 Patent Blue V dye

prepare the patient, make the initial excision and begin to explore the neck. The patient and anaesthetist should be informed that the dye will be excreted in the urine, and the urine will remain discoloured for approximately 2 days.

Injection technique

One vial of dye is injected slowly into the tissues surrounding and deep to the tumour, to minimize leakage from ulcerated tumours. Gauze swabs may be used to protect normal tissue and mop up excess dye. The number of injections is usually between two and four, but is as many as is necessary to completely surround the tumour on its deep and lateral surfaces. Occasionally it may be necessary to grab the tongue with forceps or a retracting suture during the injection. The injection site should not be massaged, in order to maintain oncological safety of the procedure.

Adverse effects

Anaphylaxis and allergic reactions, while rare, are a possibility [94], and clinicians should be mindful of this during the injection. In the event of a reaction, the injection should be discontinued and appropriate resuscitation performed. A decision should be made as to whether to continue or abandon the procedure based on clinical findings and discussion between the surgeon and anaesthetist. The mucosa is stained after injection, but the dye tends not to diffuse further than the margins of excision of the tumour and it has not been the authors' (T.S.) experience that dye interferes with pulse oximetry or pathological interpretation of the excised tumour specimen [34].

In summary, injection of blue dye is a useful adjunct to gamma probe localization of the SLN. It is an optional procedure, but one that offers significant advantages for OSCC SNB.

Gamma probe

Introduction

There are a wide variety of available gamma probes with individual feature sets, each requiring specific training and information. In many countries, effective competency training is required as part of the regulations governing the use of radioactivity.

Probe components

The gamma probe is a radiation detector, providing a count rate from gamma rays. The hand-held probe contains the radiation detector, either a crystal or a solid-state device, with surrounding metal shielding and collimation to give a restricted field of view (Fig. 2). It is connected to a power supply and an analyser unit which receives electrical signals from the radiation detector. The analyser and hand-held probe together form the probe system, which may be powered by mains connection or battery. The analyser provides a response related to the detected count rate, usually by audible pitch or volume variation and by a visual display as a dial or digital count rate (counts per second, cps). The probe technology is described in a number of reference books [95–97].

Probe size and shape

Probes typically have outer dimensions of 12–15 mm, with smaller probes producing problems related to the smaller—less-sensitive—detector, and less adequate shielding of the
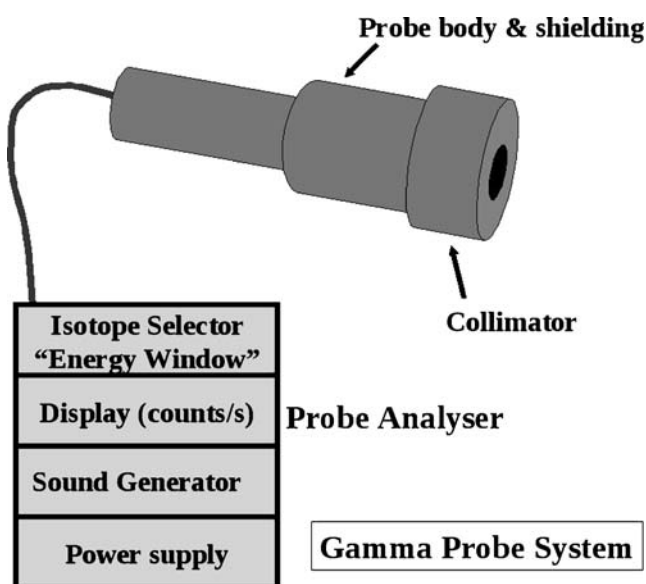

Fig. 2 Components of the gamma probe

probe housing from gamma rays. Probe tips may be angled relative to the handle. This may be viewed as an advantage for minimal access surgery, or a disadvantage according to the preference of the surgeon.

Probe performance

The probe performance is described in terms of its spatial resolution and its count sensitivity [98–100]. Spatial resolution indicates how spread out the signal is from a point source; Sensitivity is the number of counts per second for a given strength of source. At a typical node depth of 30 mm, a point source node will appear to be about 25 mm wide due to the imperfect spatial resolution of the probe, and resolution worsens with increasing distance. Many nodes contain well below 1% of the injected activity, and with the 6-h half-life of ^{99m}Tc the activity in a given node can be low, particularly if the surgery is delayed after injection of the radiopharmaceutical. A probe should be able to achieve sensitivity in the range 650–900 cps/MBq of ^{99m}Tc for a 3-cm deep node. For a 3-cm deep node with 1% uptake from a 40-MBq injection of radiocolloid, with surgery 2 h after injection, the surgeon will see a count rate of about 220 cps. The detected count rate falls rapidly with deeper nodes, and if this arises with lower percentage uptake and a longer delay to surgery there may be a much lower count rate and more difficult localization. With experience, localization at low count rates is possible, but with greater variability, longer search time and less confidence than at higher count rates.

The probe also picks up counts from sources that are not directly in front of the probe; gamma rays can penetrate through the side of the probe, and scattered gamma rays can enter the detector. Adequate shielding of the probe is therefore important, especially in OSCC due to close proximity of the injection site. The rejection of scatter is achieved by having a probe with a good "energy resolution", and with a narrow window.

Probe controls

The probe analyser has a number of settings affecting practical performance of the probe, and therefore ease of SLN localization. These include the following.

Energy window setting For ^{99m}Tc, the probe should be set to a fixed energy level of 140 keV, but the "width" setting is variable. The wider the window, the higher the sensitivity but more scatter is detected. This is especially problematic close to the injection site, and the "high sensitivity" (wider window) setting is therefore most useful for low uptake nodes remote from the injection site.

Collimation Collimators may be removable, allowing great gain in sensitivity while sacrificing spatial resolution. Removal of the collimation can aid localization of low-uptake nodes remote from the injection site.

Additional shielding Direct penetration of gamma rays through the side of the probe may be reduced by the use of lead plates to shield the injection site.

Integration time Some systems allow averaging of the signal over time, reducing signal variability. Integration times of more than one second must be used with caution, since the user may be misled by the delay between the probe position and the corresponding sound signal.

Count range The probe produces an audible change in pitch between a minimum and maximum counts per second, e.g. 100–1,000 cps. Counts outwith the set range will all produce the same (low or high) pitch, necessitating adjustment. Inappropriate range setting can lead to failure of localization. Some probe systems can automatically adjust the pitch range for the detected counts, though this can be confusing when trying to get a sense of the absolute count at any point.

Care of the probe and quality assurance

All radiation detectors must be checked and managed within a quality assurance programme. Surgeons are advised to work closely with their nuclear medicine colleagues and medical physicists in setting up quality control procedures. Recommendations for testing are:

- On purchase, tests of performance are advised to give a reference value for sensitivity, energy resolution and spatial resolution, and to form a baseline for day-to-day checks
- Before each use, a basic check of function and performance with determination of count rate sensitivity to a long-lived radioactive source and its energy spectrum.
- Visual inspection for damage, particularly cables and connectors. All users must be advised that the probe detector is easily damaged by dropping.
- In the operating room, aiming the probe at the injection site can demonstrate that the probe is functioning; however, this is not a substitute for quality control checks since even a 50% loss in sensitivity would not have any effect on the general response to the injection site.

Sterility

The probe is placed into a sterile sheath, though this makes the probe tip larger. The skin surface may be scanned before sheathing, in which case the probe must be decontaminated by wiping with 70% alcohol or another supplier-recommended agent. When removing a sheath, care must be taken not to accidentally take off any removable collimator, since these are costly to replace.

Common sources of error or problems

1. Dropping the probe will usually cause it to stop functioning, so staff should be made aware of its fragility, and quality assurance performed before each operating list. A spare probe can easily be interchanged. Damage to the cable and connectors is avoided by careful handling, aided by staff training.
2. Failure to replace a removable collimator considerably reduces spatial resolution.
3. An incorrect energy window setting may be caused by selecting the wrong isotope, or if the quality assurance procedures are performed with a ^{57}Co isotope and the procedure specifies that the energy window is set to ^{57}Co. To avoid this, perform all quality assurance procedures on the ^{99m}Tc window, unless the window is unusually narrow, in which case attention must be paid to resetting the window after quality assurance.
4. Good support from radiation experts in the nuclear medicine or medical physics department can be invaluable, particularly for routine quality assurance, optimization and purchase advice on performance. Awareness of the major pitfalls, both technical and patient-related, is essential.

Surgical technique and gamma probe detection

The following remarks are valid provided that: (a) preoperative LSG is carried out, and (b) no cervical cutaneous flap will be raised.

Procedure

At the time of LSG, SLNs are marked on the skin surface under scintigraphic guidance of a ^{57}Co-labelled pen-marker (held 90° to the skin surface) and controlled transcutaneously by the nuclear medicine physician with a collimated, hand-held gamma probe. Marking the skin with the head and neck in a position as similar as possible to the positioning during surgery may facilitate harvesting of the SLN.

Following radiotracer injection and LSG, the patient undergoes general anaesthesia and preparation for operation. Optionally, blue dye may be injected at this time. Transoral excision of the primary tumour is performed

either before or after SNB. Prior excision reduces the problem of "shine-through" from the injection site, but potentially limits the usefulness of blue dye due to rapid transit times through the lymphatics from the injection sites.

In the operating room, the gamma probe is covered with sterile latex and applied transcutaneously to confirm the accuracy of the skin markings, which may have changed due to changes in patient positioning between LSG and surgery [101]. The theoretically optimal search pattern is to start closest to the injection site, with the probe perpendicular to the skin, using a raster pattern of parallel lines 2 cm apart at right angles to the direction of the injection site. A rise in activity is then confirmed by scanning in the other direction. Scanning should be no faster than a few centimetres per second. However, excessively slow scanning can lead to loss of information from the change in pitch as the probe passes over a hot node. The drop in counts as the probe is angulated whilst over a hot-spot can confirm location.

Location

The LSG images and skin markings guide the site of incision, which is placed along the relaxed skin tension lines and positioned to facilitate excision of the scar should subsequent neck dissection be required. SLNs are reached using one or more small incisions, and removed from levels I–V according to the classification of Robbins et al. [102]. Subplatysma skin flaps are not routinely raised for biopsy-only procedures. The gamma probe is introduced into the space along the plane of dissection and angled in various directions to guide the surgeon to the SLN, which is then excised. SLNs in the jugular chain are found close to the internal jugular vein and those in level I will usually be found in the submandibular triangle. If blue dye is used, blue-stained lymphatics may be followed to the SLNs, which may be hot, blue, or both. The anatomical location of the SLNs should be noted, as should their colour and radioactivity ex vivo in the operating theatre because both blue dye and radioactivity will dissipate before the pathological examination. In order to avoid potential confusion, surgeons and histopathologists should agree beforehand on the exact nomenclature used for labelling SLNs and drainage basins. Following excision of SLNs, repeated readings are taken of the excision bed to ensure that there are no adjacent hot nodes that also need to be removed [36].

Selection of nodes

In OSCC, multiple SLNs are usually present, with reported mean numbers of 1.3 to 4 (range 1–11) [36]. Preoperative LSG may underestimate the number of SLNs, especially when multiple SLNs are in close proximity [87, 103]. However, the numerous other advantages of preoperative LSG counter the suggestion that it may safely be omitted from the procedure [87]. Careful consideration of inactive lymph nodes in the immediate vicinity of SLNs is imperative. Although non-sentinel nodes should not be excised, there may be a scenario where closely adjacent nodes are almost completely excised while dissecting out a SLN. Although this is uncommon, the non-sentinel node thus labelled may be sent for pathological examination [104].

While preoperative imaging should detect the majority of grossly involved nodes, clinical staging remains imperfect [105]. Any suspicious lymph nodes observed during SNB must be excised, even in the absence of radioactivity, since gross lymphatic involvement may block the flow of radiotracer to these nodes (see also the section "Measured radioactivity").

SLNs are ranked according to their respective tracer uptake ex vivo, with the SLN with the highest activity named SLN1, the second highest activity SLN2, and so forth. This does not mean that SLN2 is dependent on SLN1, as metastases may be found in any of the multiple SLNs independently [63, 106–108].

Close spatial relation

The problems of "shine-through", whereby the high radioactivity levels from the injection site are detected from behind the tissue of interest, and "scatter", in which the direction of radioactivity from the injection site is changed by the tissues and detected by the probe [36], are most prevalent in the submandibular and submental areas of the neck. For floor-of-mouth tumours, where the distance between primary site and SLN is smallest, this creates technical difficulty and results in lower SLN identification rates (86%, vs 96% for other OSCC subsites) [28, 109, 110]. Careful positioning of the gamma probe, judicious use of malleable lead shields and excision of the primary tumour before SLN localization may all help to minimize these effects [34]. Another option to improve identification in level I is to perform some initial dissection below the level of the marginal mandibular nerve, transecting the tissues down to the level of the mylohyoid muscle. In this manner, the lymph nodes are mobilized away from the oral cavity, and the gamma probe placed into the newly created tunnel and directed inferiorly away from the injection site [111].

Activity counting

Following excision, SLN radioactivity is confirmed ex vivo using the gamma probe, and must be above background activity to be classified as hot [43]. The SLN should be

placed on a surface away from the patient, or on the upturned probe tip (facing the ceiling). Anatomic location and radioactivity levels (counts per second) are recorded for all excised nodes. All radioactive nodes should be considered SLNs because, while there exists some confusion over the exact definition of a SLN [106], it is best to err on the side of patient safety. The lymphatic basin should be rechecked for reduced radioactivity after SLN excision [112]. Bed counts in the neck after removal of SLN almost never exceed 8–10 cps (with the head of the probe slightly turned away from the injection site).

Risk

SNB is not without risks, and injuries to the facial and spinal accessory nerves are possible through minimal exposure. Although complication rates of less than 1% are reported, the risk of injury to these nerves via minimal-access incisions is theoretically higher during SNB than during neck dissection. Similarly, neck dissection following positive SNB represents re-exploration in a recently operated field, and carries with it higher risks of nerve or vessel damage. This reinforces the need for minimal tissue injury during the initial SNB procedure [111].

Experience

It is clear that experience is needed before a surgeon starts performing SNB, as it carries a steep learning curve. This has led to the recommendation of completion of at least ten SNB-assisted ENDs before the procedure is performed alone [38]. In addition, it is necessary for practitioners of SNB to understand the theoretical aspects including handling of radioactivity and optimal use of gamma probes.

Measured radioactivity

The role of measured counts per second during gamma probe detection is unclear. Because of the narrow anatomic relationships, defining a lower cut-off point for SLNs is practically impossible. It has been suggested that the number of harvested SLNs may be limited to the three nodes with the highest absolute counts per second [87, 113] or the highest ratio of ex vivo node:background activity (as for early studies in melanoma) [25, 114–117], in order to reduce surgical morbidity.

For correct staging, at least the three nodes with the highest activity should be excised as SLNs [104, 113] and all positive SLNs are detected within the first five nodes of highest activity in each patient [87, 104]. The detection of more than five SLNs is very rare, with three-quarters of patients having three or fewer SLNs. For safety's sake, all radioactive nodes should be excised [69, 104].

In a study investigating the role of radioactivity in SNB, Kovacs et al. [104] found no significant difference in absolute counts per second between positive and negative SLNs (medians of 157 cps and 235 cps, respectively), and that the positive SLN need not have the highest tracer accumulation (range 13–4,716 cps) [36, 104]. Activity in SLNs was not found to correlate with the administered dose, and the highest activity was found in level II. The authors reported that there was one SLN in each patient with a significantly higher count rate than the remaining active nodes, and this node could be found in all levels.

Following SLN excision, the remaining lymphatic basin is searched for residual radioactive nodes by means of an in-situ survey measurement. A count-rate less than one-tenth that of the excised node with the lowest radioactivity is considered indicative that all SLNs have been identified and removed. In some centres, lymph nodes with count rates of less than one-tenth that of the "hottest" excised node are not removed. This practice is based on the results of the large Sunbelt Melanoma trial, demonstrating a low failure rate of 2% [118]. However, no similar data have yet been reported for SCC.

The time from injection has also been found not to affect the relative counts between SLNs [119], or if it does, it affects the results only in cases of very large time spans of >14 hours [120], depending on the half-life of the tracer used. It is important, however, that the time span between injection and surgery is consistent for a given study population.

As a result, the absolute radioactivity counts are less important than relative levels between the excised nodes in the context of SNB. Similarly, absolute radioactivity counts cannot be compared between centres due to differences in protocols.

Pathology evaluation of SLN

In OSCC, SNB has been examined only in the context of relatively small observational cohort studies and the pathology protocols have typically been designed to detect micrometastases and isolated tumour cells with high sensitivity. At present, the significance of finding micro-metastases and isolated tumour cells is unknown in OSCC. The grade of evidence is currently at level III, as described in the SIGN methodology for clinical guidelines [121]. Several large-scale validation studies (ACOSOG and the University of Miami [111], European Sentinel Node Trial group [122], the Danish national group [81] and the Brazilian head and neck group [123]) are currently underway.

Table 1 UICC classification of micrometastases and isolated tumour cells

Definition	Criteria
Metastasis	>2 mm
Micrometastasis	≥0.2 mm and ≤2 mm
Isolated tumour cells	<0.2 mm
	Single cells, small clusters
	No stromal reaction
	No contact with vessel wall

Histological definitions

The present UICC definitions are shown in Table 1 [31]. Whilst these definitions have been largely accepted, little information is available regarding the evidence on which they are based. The relationships between these definitions and TNM coding are shown in Table 2.

One element which is clearly subjective in nature is the assessment of cell viability, as the significance of individual cytokeratin positive "nonviable cells" is difficult to establish. Various terms have been employed for these cells, including "mummified cells" or thanatosomes [124]. The present authors' practice (K.H., P.S.) is to include such elements in the morphological description, with careful correlation to the adjacent H&E-stained section. Features which may be useful include the lack of a nucleus, but the biological potential of these cells is as yet unknown.

In addition, the presence of cells in the lymphatic plexus should always be recorded. Whilst their significance is unknown, it appears that they do not represent extracapsular extension of tumour.

Protocol

A well-defined, written, standard operating procedure should be established between the surgical team and the reporting pathology laboratory. This should include how the specimen should be delivered to the laboratory, outline the supporting documentation which accompanies it, and include appropriate elements of radiological protection practice. Other important elements may include agreed turnaround times and the manner in which the results are to be reported. An overview of the proposed pathological evaluation protocol is presented in Fig. 3.

Gross sectioning

The node or nodal basin should be fixed in 10% neutral buffered formalin (or equivalent) for 24 hours, as per standard laboratory practice. The nodes are described macroscopically, including dimensions, and excess fat is carefully trimmed away. Nodes less than 2 mm (longest dimension) should be processed whole, while nodes 2–5 mm should be cut through the hilum or longest pole to pole diameter, and both halves processed en face. Nodes greater than 5 mm should be cut into 2-mm slices longest pole to pole, with processing of all slices en face.

Step sectioning

A routine H&E section is prepared, and metastatic disease reported if present. If negative, six exact serial sections are mounted, and separately numbered 1–6. Next, 150 μm of material is discarded, or retained for research, before a further six numbered serial sections are mounted. This pattern is continued throughout the entire block. All

Table 2 Comparison of UICC and TNM classifications

UICC		TNM
Generic	SLN could not be assessed	pNX (sn)
	No SLN metastasis	pN0 (sn)
	SLN metastasis	pN1 (sn)
SLNs with micrometastasis only	Single ipsilateral node with micrometastasis	pN1 (sn)(mi)
	Multiple ipsilateral nodes with micrometastasis	pN2 (sn)(mi)
SLNs with isolated tumour cells	No sentinel lymph node metastasis histologically, negative morphological findings for isolated tumour cells	pN0 (i−)(sn)
	No sentinel lymph node metastasis histologically, positive morphological findings for isolated tumour cells	pN0 (i+)(sn)
	No sentinel lymph node metastasis histologically, negative nonmorphological findings[a] for isolated tumour cells	pN0 (mol−)(sn)
	No sentinel lymph node metastasis histologically, positive non-morphological findings[a] for isolated tumour cells	pN0 (mol+)(sn)

[a] Nonmorphological techniques such as PCR or flow cytometry.

Fig. 3 Pathology evaluation of SLNs
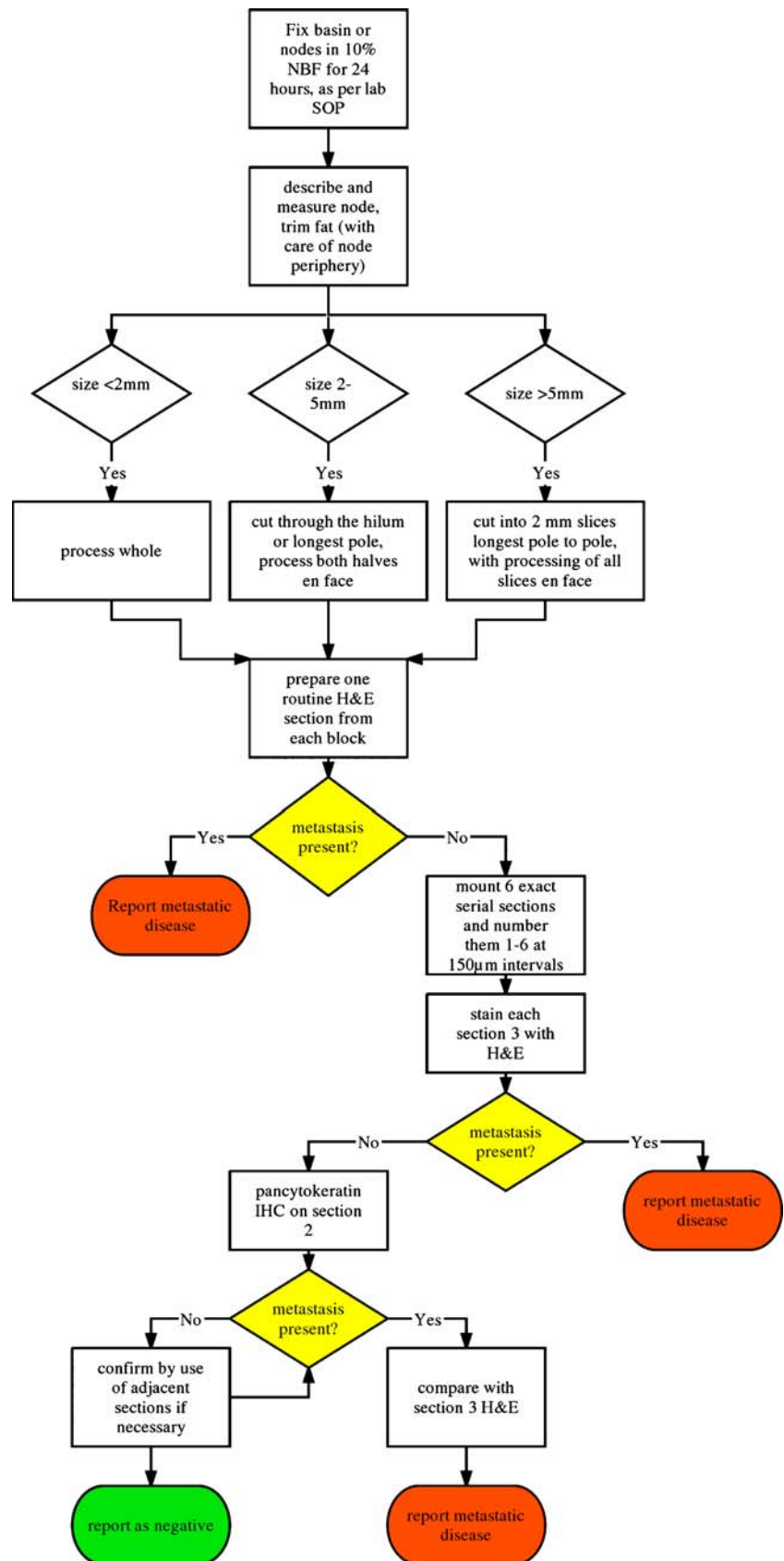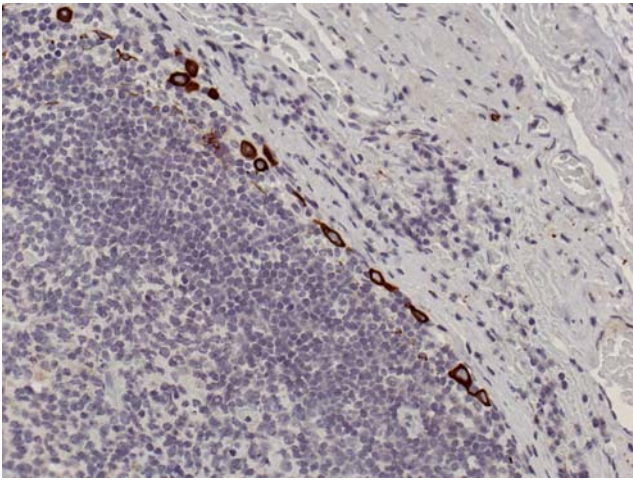
Fig. 4 Isolated tumour cells stained by AE1/AE3 in a SLN

number 3 sections are stained by the H&E method, and metastatic disease reported if present. If negative or equivocal, immunocytochemistry (IHC) is performed on all number 2 sections using a pancytokeratin antibody (see below), and the slides examined for positivity. IHC-positive slides are compared with the adjacent section 3 H&E slide. The remaining sections may be used if required.

Much of the published literature has utilized AE1/AE3 pancytokeratin antibody. However, concern has been expressed regarding the specificity of this anticytokeratin cocktail. Cross-reactivity is a problem seen with a number of pan-cytokeratin antibodies, and may mandate the use of more than one antibody in SNB protocols to clearly delineate micrometastases and isolated tumour cells from other elements in the node, such as dendritic cells and macrophages. However, presently it is reasonable to assume that any reputable commercially available pancytokeratin
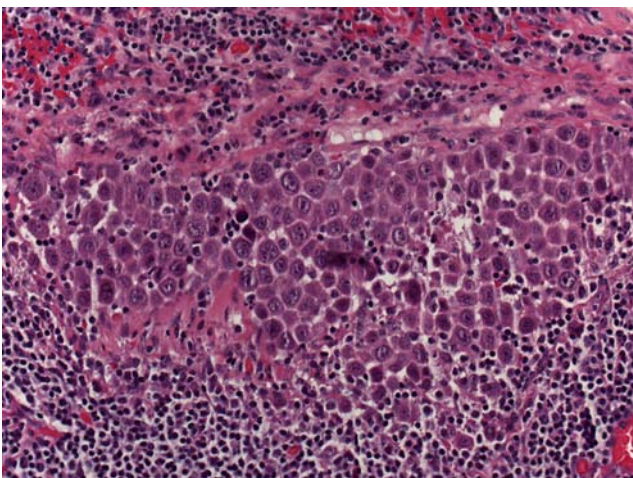
antibody (such as AE1/AE3 or MNF116) may be used. It is important to recognize artefacts and any cytokeratin-positive components should always be compared with adjacent sections stained by H&E (Figs. 4, 5, 6, 7, 8 and 9).

Microscopy

Sections should be examined using a good-quality bright-field microscope and equivocal findings discussed with an experienced colleague. Where pancytokeratin-positive cells are present, it is essential that adjacent sections are stained to allow morphological comparison.
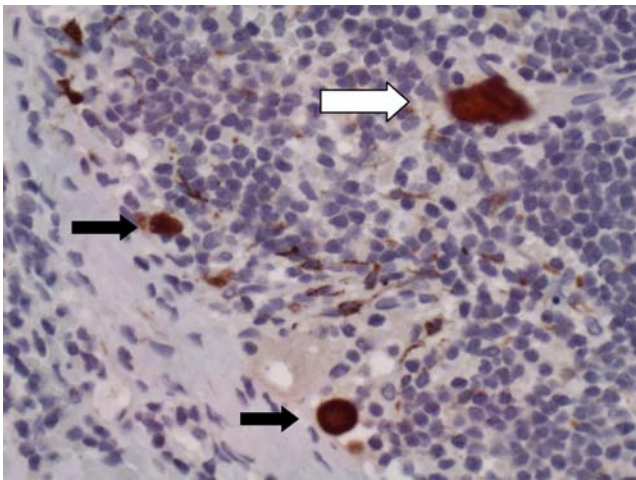
Report

The side, number and level of each node basin in the neck must be recorded. A diagram provided by the surgical team should be incorporated into the pathology record where possible.

The report must include details of the numbers of nodes found in each individual basin and which nodes were hot, blue, both, or unlabelled. The dimensions of each node must be included and the macroscopic appearance of the gross and cut surfaces stated.

- Macrometastasis
  - Note the largest dimension of the metastatic deposit in each node, and whether extracapsular spread is present or not.

- Micrometastasis
  - Should be recorded, even in the presence of macro-metastasis.
  - The largest dimension should be recorded.
  - Anatomical location within the node: capsular, subcapsule, parenchymal.
  - Unifocal or multifocal. It is often not possible to be confident of the exact numbers.
  - Presence of extracapsular spread. This is permitted if the deposit is peripherally located and is associated with a reactive stromal response.

- Isolated tumour cells
  - If evident, should be recorded, even in the presence of macro or micrometastasis.
  - If cohesive, the size of the largest deposit should be stated.
  - If dispersed, note the anatomical distribution.

- Benign inclusions including naevus cells, salivary inclusions and false-positive cytokeratin artefacts (e.g. dendritic cells or scattered nonviable anucleate cells) should be recorded.
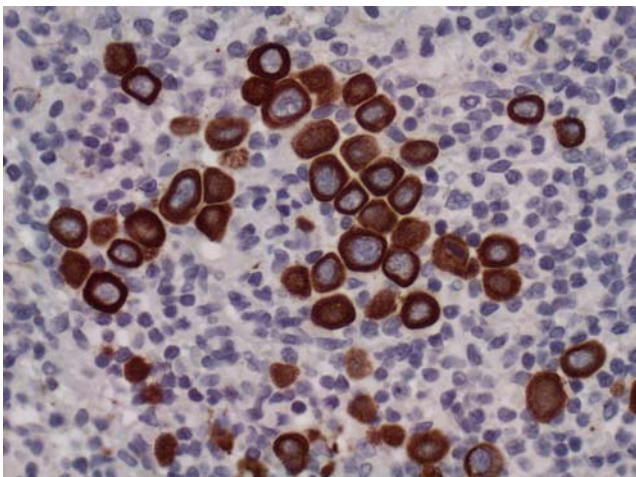

Fig. 5 A micrometastasis found in the seventh level of a SLN that was clear on initial sectioning. Viable nucleated squamous cells are present in a cohesive group. Section quality is suboptimal and recutting is not possible

Fig. 6 Cytokeratin-positive cells in a SLN stained by CKC pancyto-keratin. The *white arrow* shows a contaminant squame (this can be ascertained by the geometric outline, lack of nucleus and by focusing at high power). The *black arrows* show nonnucleated individual tumour cells, and dendritic cells can be seen in the background

Fig. 8 Multinucleated and mononuclear macrophages revealed by the detailed protocol in a SLN that was clear in the first sections. These were suspicious for isolated tumour cells within the island

The histopathological features of isolated tumour cells need careful description, as the UICC size cut-off by necessity encompasses small metastases which vary greatly in size and presumably in biological potential. Positive findings using nonmorphological methods in the absence of histologically proven metastasis are generally considered as isolated tumour cells.
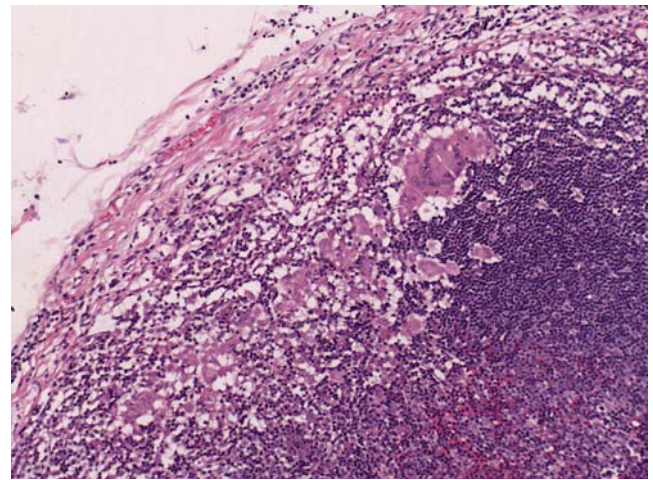
Other methodologies

Frozen sections

The use of frozen section evaluation for SLNs has been described in a number of recent reports, and the results

appear promising with negative predictive values ranging from 83% to 99% [64, 125, 126]. These results are in contrast to an earlier report by Civantos et al. who described poor sensitivity of frozen section compared with step-serial sectioning in a series of 43 patients with oral cancer [127]. The main advantage to the technique is that it may allow a majority of patients to undergo a single-stage procedure. While the use of frozen section evaluation is advocated by some authors, it has not yet gained universal acceptance.

Imprint cytology

The use of imprint cytology in conjunction with frozen sections in the assessment of SLNs has been described in other tumours such as breast adenocarcinoma. One study in OSCC based on 30 cases demonstrated high sensitivity and

Fig. 7 A group of nucleated isolated tumour cells stained by AE1/AE3 in a SLN
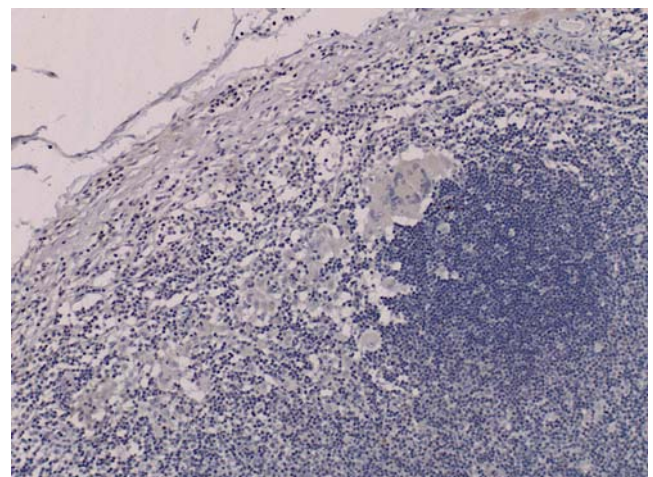
Fig. 9 Adjacent field to Fig. 5 stained by AE1/AE3, showing absence of tumour cells

specificity [128] though a recent study showed frozen section evaluation to be a more accurate method of intra-operative diagnosis [125]. Imprint cytology has an advantage over frozen section evaluation in that no tissue is lost in generation of the sample; however, much larger studies are required before considering adoption into the protocol.

Reverse transcriptase polymerase chain reaction

Methods with increased sensitivity, such as cytokeratin RT-PCR, have been suggested. The small number of studies published demonstrate expression of cytokeratins in nodes which were metastasis-negative on initial assessment. However, only a proportion of the nodes demonstrated metastases on serial sectioning [129, 130]. The clinical role of these methods in the future remains uncertain given the ongoing concerns regarding their specificity together with the associated medicolegal problems, given that significant tissue must be used which has not been assessed histopathologically.

Burden of work

The authors recognize that on average 2.5 sentinel node basins are yielded per neck side. The protocol above may produce up to 12 levels per node. If three nodes are present in each basin there could be 180 slides to examine. On the other hand, the majority of SLNs are small and the nodes from a single basin can often be grouped into one cassette (using ink to identify 'hot' and 'blue' nodes) saving laboratory time and effort. In addition, the described protocol has the ability to detect all micrometastases.

According to Cochran's principle, metastatic deposits tend to cluster in the plane of the hilum, and some authors argue the case for examination of bisected SLNs at three or six levels only [131]. Although such a protocol can theoretically miss a micrometastasis [132], it may turn out that no useful information is added by levelling through the block beyond six levels. However, several studies have shown that additional SLNs are upstaged by step serial sectioning with cytokeratin immunohistochemistry, compared with H&E only [81].

Outcome analysis

The success of this multidisciplinary staging technique depends on good communication between all of the individual components: imaging, surgery, and histopathology. The multidisciplinary team setting should be utilized for the discussion of every patient, and regular audit of patient outcomes against published data should be carried out. SLNs should be successfully located and harvested in >90% of patients [28]. The accuracy of the technique can be assessed by the proportion of patients whose SLN contains metastases, which should match that of END (20–30%, depending on patient population and tumour size [133]). Lastly, the rate of false-negatives (SLN-negative patients who develop early recurrent disease) should be <5% [134].

Further studies are required to determine the full clinical significance of micrometastasis and individual tumour cells in OSCC. The biological potential of the tumour cells may vary with different types of tumours, and clinical decisions currently have to be made on the basis of grade, stage and margin status of the primary lesion as well as on microscopic findings in the SLN [135, 136]. The optimum protocol will hopefully emerge from the large-scale trials and studies currently in progress [81, 109, 111, 122, 123]. One of the major aims of SNB in OSCC is to achieve better staging, and thorough pathological examination of SLNs remains the standard.

Summary

Successful application of the SNB technique is dependent on good communication between all members of the multidisciplinary team, and this joint guideline represents an extension of that approach. SNB provides an additional tool for staging patients with early OSCC. However, it is not without limitations, and all practitioners of SNB must be aware of these. It is hoped that this document will serve as a reference outlining the optimal practice for the provision of SNB in patients with OSCC, based on the best currently available evidence. As such, the use of the above protocol is recommended until further data, in the form of large prospective studies currently underway, becomes available.

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