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GUIDELINES

EANM-EORTC general recommendations for sentinel node diagnostics in melanoma

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Abstract The accurate diagnosis of a sentinel node in melanoma includes a sequence of procedures from different medical specialities (nuclear medicine, surgery, oncology, and pathology). The items covered are presented in 11 sections and a reference list: (1) definition of a sentinel node, (2) clinical indications, (3) radiopharmaceuticals and activity injected, (4) dosimetry, (5) injection technique, (6) image acquisition and interpretation, (7) report and display,

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(8) use of dye, (9) gamma probe detection, (10) surgical techniques in sentinel node biopsy, and (11) pathological evaluation of melanoma-draining sentinel lymph nodes. If specific recommendations given cannot be based on evidence from original, scientific studies, referral is given to "general consensus" and similar expressions. The recommendations are designed to assist in the practice of referral to, performance, interpretation and reporting of all steps of the sentinel node procedure in the hope of setting state-of-the-art standards for good-quality evaluation of possible spread to the lymphatic system in intermediate-to-high risk melanoma without clinical signs of dissemination.

Keywords Blue dye · Gamma probe · Lymphoscintigraphy · Melanoma · Pathology · Sentinel node

Abbreviations

AJCC	American Joint Committee on Cancer
CLND	Completion regional lymph node dissection
CT	Computed tomography
EANM	European Association of Nuclear Medicine
EORTC	European Organisation for Research and Treatment of Cancer
FNAB	Fine needle aspiration biopsy
H&E	Haematoxilin-eosin
HSA	Human serum albumin
ICRP	International Committee of Radiation Protection
MUMP	Melanocytic lesion of uncertain malignant potential
NSN	Non-sentinel lymph node

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PACS	Picture Archiving and Communication System
QA	Quality assurance
QC	Quality control
RES	Reticulo-endothelial system
RT-PCR	Reverse trascriptase polymerase chain reaction
SN	Sentinel lymph node
SNB	Sentinel lymph node biopsy
SPECT	Single photon emission computed tomography
US	Ultrasound

Preamble

In the frame of a collaborative approach, the European Organization of Research and Treatment of Cancer (EORTC) Melanoma Group and the European Association of Nuclear Medicine (EANM) Oncology Committee agreed to develop a document outlining recommendations for the practice of sentinel node diagnostics in melanoma. The document was prepared by a multidisciplinary, international working group comprising scientists and clinicians from many different European and other countries, all with sub-speciality expertise within the different fields involved in SN diagnostics. The product of the collaboration may be taken as a source of concepts on the different aspects of sentinel node staging. Clinicians, nuclear medicine and pathology specialists have to interact to optimize the final result, which is to offer to patients a reliable answer on the staging of their disease.

The aim of the authors has been to present state-of-the-art applications and protocols approved by experts in the field, adapted to European practice, based on evidence from original scientific studies. The recommendations are designed to assist physicians and other healthcare professionals in referral to, performance, interpretation and reporting of the different components for sentinel node diagnostics for optimal staging in melanoma patients without known clinical dissemination [1]. In the preparation of this document, several different approaches and even controversies appeared. Where more than one option seems to be practised, and none has been shown to be superior to the others, we hope that we succeeded in specifically expressing this state of knowledge. Every effort has been made to avoid conflicts of interest arising from non-academic and non-clinical relationships. The document has been approved by officers and other responsible individuals from either organization and reviewed by the editorial office of the Eur J Nucl Med Mol Imaging.

Definition of a sentinel node

Introduction

In 1992, Morton, Cochran and co-workers [2] defined a sentinel node (SN) as being "the initial lymph node upon which the primary tumour drains" (Fig. 1). It has been modified to and is now generally accepted as representing "a node upon which a lymph vessel originating in the tumour drains directly". The word "directly" emphasizes the fact that a tumour may drain directly to more than one lymph node (Fig. 1). One of them may receive the tracer before the other(s), but all are directly at risk of receiving malignant cells. The definition of a SN should encompass all nodes directly at risk of receiving tumour cells. The essential element is that there should be a lymphatic channel that connects the tumour with the node. After passing through the SN(s) lymph will pass onwards to so-called second-tier or second-echelon nodes higher up in the lymph node basin.

The definition in clinical practice

There is no consensus about a *practical definition*, and no definition has been shown to be superior in practice compared with other definitions. It can be noted that:

- Scintigraphic images indicate the area to explore
- Early, dynamic lymphoscintigraphy images usually visualise the lymphatic channels and indicate the

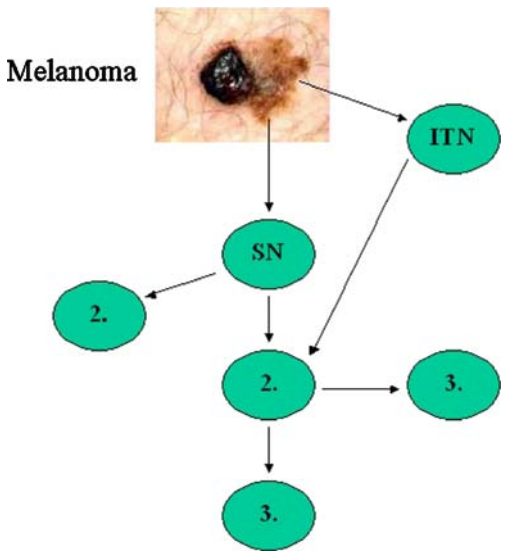

Fig. 1 Schematic presentation of lymph node drainage pattern from a melanoma, with the direct drainage to one sentinel node (SN) and to one interval node (ITN). From the SN drainage it goes on to two second-chelon or second-tier (2.) nodes, from one of those nodes drainage goes further to two third tier (3.) nodes

number of nodes on a direct drainage pathway and distinguish these from second-tier nodes
- The gamma ray detection probe can pinpoint the location of radioactive nodes
- A small incision and careful dissection allows the surgeon to detect blue lymph vessels just under the subcutaneous fascia and to follow them visually to the nodes that receive drainage directly from the primary lesion
- Careful palpation and/or preoperative ultrasound (US) of the drainage basin may identify nodes that are massively involved and fail to pick up the tracer fluids [3, 4]

Suggested other definitions of a SN in the literature include "the node closest to the primary lesion", "the first node visualised at lymphoscintigraphy", "the hottest node", "all radioactive nodes", "all blue nodes", or "all nodes with a count rate that is a certain factor higher than that of the background or compared to other nodes" [5, 6]. None of the definitions is perfect. They all have their flaws [5]. A combination of the definitions given above has lead to the often-quoted, thumb rules in relation to the modality, shown in Table 1.

Different detection techniques may be used and have an influence on the SN identification [2, 7, 8]. The definitions reflect to a high degree aspects of the *technology* that is applied to find the one on a direct drainage pathway. The tools have their limitations, and the tools should not be confused with the goal. The goal is to find the node on a direct drainage pathway, the tools being used to facilitate identification of the node on a direct drainage pathway. The recommendation for the clinician should be practical and keeping the concept in mind. Although excellent results have been described using blue dye or a probe alone, it is preferable to use a combination of the available detection techniques in the repertoire [8].

Pitfalls, errors

- The nearest node is not always on a direct drainage pathway from the tumour
- The tumour-positive node is not always the one with the highest count rate
- The first node that is visualised *is* a SN but it may not be the only one. Some of the radiocolloid may pass through the first node and lodge in a subsequent node
- A true SN may not be radioactive (obstructed lymph vessels)
- Not every radioactive node is a SN
- A true SN may not be blue
- A blue node may not be a true SN

Interval nodes

The so-called interval or in-transit nodes are lymph nodes lying along the course of a lymphatic collecting vessel, often in subcutaneous fat [9], between the primary tumour and the draining lymph node basin. Such nodes are on a direct drainage pathway from the tumour and should be considered to be SNs. They are clinically as important as SNs in recognized lymph node basins. They are reported to be present in between 3 and 10% of the patients [9–12]. Interval nodes should be removed along with the SNs in standard node basins, since they contain metastatic disease almost as often and may represent the only metastatic nodes [9, 12].

Summary

A sentinel node is defined as "a node upon which a lymph vessel originating in the tumour drains directly". The essential element is that there should be a lymphatic

Table 1 Definition characteristics of a sentinel node, according to modality

Modality	Practical definition
Lymphoscintigraphy; early dynamic images	The first node visualised
	The nodes draining directly the primary tumour
Lymphoscintigraphy; static images or gamma probe	The hottest nodes
	Hot nodes containing more than 10% of the activity in the hottest node in the lymphatic basin*
Blue dye	Any blue node draining directly the primary tumour
Palpation	Any palpable node should be considered as a SN, which may not be imaged due to lymphatic obstruction
Ultrasound	Preoperative evaluation of the SNs with ultrasound and FNAB may be used to select patients with a malignant SN to proceed directly to CLND (cf. Sect. 6: Image acquisition and interpretation)

*An empirical threshold used by several authors [171]. It may be used as suggesting a threshold for SNs, with less activity suggesting a second-tier node, unless dynamic imaging or blue dye suggest a direct drainage from the tumour

channel that directly connects the tumour with the node, including possible interval nodes. There are many suggestions in literature but no consensus about a *practical definition*. The practical approach should include a combination of available detection techniques; lymphoscintigraphy, blue dye, gamma probe, and palpation. Ultrasound may be added to detect a possible malignant SN.

Clinical indications

Introduction

All patients with invasive melanoma are at risk for developing metastases to the lymph nodes draining the melanoma site, which is the commonest site of first recurrence [13]. The most important prognostic factor (besides characteristics of the primary melanoma) is the metastatic status of the regional lymph nodes [14], and the risk of lymph node metastases increases with increasing Breslow thickness of the melanoma [15, 16]. Sentinel lymph node biopsy (SNB) is a minimally invasive procedure used to accurately stage nodal basins at risk for harboring occult metastases in patients without clinical spread of the disease [2, 17]. However, it must be noted that no overall survival benefit has yet been shown for SNB [18] so the main goals of SNB are staging and better local tumour control as well as better selection for completion regional lymph node dissection (CLND) to reduce morbidity.

Indications for SNB

SNB should be offered to patients with clinically localized disease and invasive melanoma, depending on different histopathologic characteristics of the primary melanoma, including:

- *Intermediate thickness 1–4 mm:* The risk of lymph node metastases increases from 8 to 30% (5), and there is consensus that these patients should be given the option for SNB for staging purposes
- *Thickness >4 mm:* Thick melanomas have a high risk of distant metastases, and the risk of lymph node metastases is around 40%, still the metastatic lymph nodes are usually not clinically palpable. Therefore the status of SN still offers very good prognostic information [16] and may be offered
- *Thickness ≤1 mm:* SNB may be offered to patients with melanomas 0.76–1 mm in thickness, since the risk for regional lymph node metastases is approximately 5% [19–21]. SNB is generally not recommended in patients with a melanoma of ≤0.75 mm since the risk of metastases to the lymph nodes draining the melanoma is around 1%. However, the indication for SNB in both subgroups may be modified by:
- *Clark level, ulceration and mitotic rate:* In the 6th version of the American Joint Committee on Cancer (AJCC) staging system, the Clark level is an independent prognostic factor for thin melanomas (but not for thicker melanomas), and patients having melanomas with a Clark level IV-V are often selected for SNB, as are patients with histopathological ulceration [14]. In the forthcoming 7th version of the AJCC staging system, expected to be published in 2009, the Clark level will be removed and mitotic rate (≥ 1 per mm^2) will be introduced as a prognostic factor

In light of the above-mentioned evidence, it is recommended that patients diagnosed with melanoma in clinical *stage T1b–T4b, N0 and M0* should be given an option of SNB.

SNB may be offered to patients for evaluation of melanocytic lesions of uncertain metastatic potential (MUMP) (cf. Sect. 11: Pathological evaluation of melanoma draining sentinel lymph nodes).

Contraindications for SNB

- *Poor general health status, grave concurrent disease and poor patient compliance*: These factors are usually contraindications for SNB
- *Known dissemination*: Systemic spread of the disease or clinically or sonographically apparent lymph node metastases (with or without fine needle aspiration biopsy (FNAB)) are contraindications. Preoperative ultrasound examinations of the relevant lymph node basins can in addition to staging usually avoid the pitfall that a lymph node completely filled with metastatic masses may be missed by SNB due to an altered lymphatic drainage pattern
- *Prior wide local excision:* Since the lymphatic flow from the primary tumour site may be considerably changed, SNB following wide excision may be contraindicated because the SNB may not show a reliable result [22, 23]

Special precautions

Pregnancy The incidence of melanoma during pregnancy is estimated at approximately slightly below 1 per 1,000 pregnancies [24]. Two issues have to be considered in pregnant women with melanoma before SNB is performed: The risk to the fetus related to radiation exposure and the necessity for the mother for staging of the melanoma. The risk of fetal damage is discussed in Sect. 4: Dosimetry, the conclusion being that the risk associated with SNB is

negligible. No relationship between pregnancy and worsened patient survival from melanoma has been shown [25]. Thus the indication for SNB in pregnant women is not influenced by pregnancy, but the procedure should be modified to minimize radiation exposure to the fetus and avoid the risk of anaphylaxis with blue dye (cf. Sect. 4: Dosimetry and Sect. 8: Use of dye).

Lactation SNB can be performed, but it is recommended that breastfeeding is discontinued prior to and 24 hours after the procedure (cf. Sect. 4: Dosimetry and Sect. 8: Use of dye).

Infants Melanoma in infants is extremely rare; therefore general guidelines for the SN procedure cannot be given. Each case should be diagnosed and treated individually. Concerning the risk related to radiation exposure, see above about the considerations for fetal exposure.

Children and adolescents Melanoma in children and adolescents is rare. It has been suggested that lymph node metastases are more prevalent in children with melanoma compared to adults. However, survival is still related to tumour thickness [26, 27]. Therefore children with melanoma should be offered SNB according to the recommendations for adults (cf. Sect. 4: Dosimetry).

Summary

SNB should be offered to patients diagnosed with melanoma in clinical stage T1b–T4b, N0 and M0 and may be offered to patients with melanocytic lesions of uncertain metastatic potential. SNB should not be used in patients with known dissemination. SNB can be used in small children as well as pregnant and lactating women with special precautions.

Radiopharmaceuticals and activity injected

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 [28, 29]. The radiocolloids that best display lymphatic vessels are small, with particle sizes in the range of 5–50 nm. With small particles, imaging can be performed 1–2 h after injection at which time the surface location of the SN is marked on the skin. If medium-size particles (50–200 nm) are to be injected, migration from the injection site is slower and accumulation in the SN may be prolonged. If nodes are not clearly depicted at 1–2 h after injection, more delayed images should be acquired at 4–6 h or even 24 h after injection just before surgery. Large-particle radiocolloids (>200 nm) may have difficulty moving through the interstitial matrix and enter the lymphatic capillaries only in small numbers. Thus, lymphatic collecting vessels may not be seen on dynamic imaging and most of the injected dose tends to remain at the injection site, with only a small amount reaching the SN (cf. Sect. 6: Image acquisition and interpretation) [30–32].

Radiopharmaceuticals

Over the last 15 years, the choice of radiopharmaceutical has narrowed but still varies among geographical regions, with the main tracers being ^{99m}Tc-human serum albumin (HSA) colloid (albumin nanocolloid, Nanocoll, Sentiscint) in Europe, ^{99m}Tc-sulphide colloid (with or without filtration through a 0.1- or 0.2-μm membrane filter) in North America, and ^{99m}Tc-antimony trisulphide colloid in Australia [28, 29]. ^{99m}Tc-rhenium sulphide colloid (Nanocis) has also been used [33].

It has been suggested that the optimal particle size for SN detection is 100–200 nm [29]. The size range of Nanocoll has been reported to be 5–80 nm [29, 34], although other authors have reported mean particle diameters of 6–12 nm [35–37]. The sizes of other ^{99m}Tc colloids commonly used in Europe are a median diameter of 100 nm for Nanocis [38] and a range of 100–600 nm (median 200 nm) for Sentiscint [39, 40]. There is no documented difference in the clinical outcome with all three ^{99m}Tc-colloids despite their apparent deviation from the proposed optimal size range.

Following intradermal injection in patients with melanoma, ^{99m}Tc-Nanocoll has a median transit time to the SN of 10 min [41]; after 4 h the accumulation in the SN is 2.1 ± 0.8% injected dose per gram [42], and the half-time for washout of activity from the node is 7.5 h [41].

Activity amount and labelling

^{99m}Tc labelling of HSA colloid proceeds within 10 min at room temperature while sulphide, rhenium, and antimony colloids require heating. It is important to pay attention to specific activity (number of decays per second per amount of substance) and number of particles administered. Based on the assumption of a limited clearing capacity of the macrophages in the SN, it has been suggested that the maximum activity of ^{99m}Tc should be loaded onto the smallest number of particles [29]. Labelling at higher specific activity has been demonstrated to result in higher nodal count rate for the

same administered activity [35]. Although the kit reconstitution instructions allow addition of 185 to 5,550 MBq in a volume of 1 to 5 ml [34, 39], it is recommended that ^{99m}Tc-HSA colloid be prepared at a minimum activity concentration of 100 MBq/ml (i.e. to deliver 20 MBq in 0.2 ml) at the time of injection and, wherever possible, the maximum reconstitution volume be used (e.g. ≥500 MBq in 5 ml).

There is not a consensus on the activity of radiocolloid to inject. The total activity to be injected should be adjusted to the time course of the SNB and varies from approx. 5 to 120 MBq. One- and 2-day procedures are shown to be equally efficient for SN detection. However, some show that if a next-day surgery procedure (2-day protocol) is used, injected activity should be calculated (adjusted for physical decay) to exceed 10 MBq in the patient on day 2 [43]. The specific activity should be calculated according to the volume of the (2–4) aliquots to be injected (cf. Sect. 5: Injection technique).

Quality control and stability

The EANM Guidelines on current good radiopharmacy practice (cGRPP) in the preparation of radiopharmaceuticals [44] recommend that labelling efficiency be checked on each preparation. The labelling efficiency should be >95%, determined by planar (thin-layer) chromatography, and the stated expiry time is 6 h for Nanocoll and Sentiscint and 4 h for Nanocis, although extended stability of Nanocoll has been demonstrated [45]. The presence of larger particles can be excluded by passage through a membrane filter of an appropriate pore diameter [36].

Drug interactions, adverse effects

No interactions of drugs with radiocolloids are expected due to local intradermal or subcutaneous application. Adverse effects are rare and mild following interstitial application of radiocolloids, although allergic reactions have been reported with ^{99m}Tc HSA colloid [46, 47]. The incidence of allergic reactions is too low to quantify, but appropriate medicine should be kept available during the procedure.

Summary

Different ^{99m}Tc-labelled colloids with particle sizes from 5 to 600 nm may be used for SN procedures, the most commonly used agent in Europe being Nanocoll followed by Sentiscint and Nanocis. The latter requires heating while the others label at room temperature. Labelling efficiency should be checked on each preparation. The size of the particles may have an influence on optimal timing at image acquisition. Activity injected should depend on time to imaging and surgery. Adverse effects including allergic reactions have been reported.

Dosimetry

Introduction

The use of radioactive colloids for SN biopsy needs the optimization of radiation safety issues for patients, for the staff in nuclear medicine departments, in the operating theatre, pathology laboratories and also the disposal of radioactive waste.

Patients

Adults For a regular nuclear medicine department SN lymphoscintigraphy is a procedure using low activity. The estimated local radiation dose varies depending on the volume of tracer, the application of multiple injections, administered activity and retention time. [48]. However, melanoma originates from skin tissue that is relatively less radiosensitive than many other tissues; the tissue weighting factor defined by the International Committee of Radiation Protection (ICRP) for the determination of effective dose is 0.01 for skin compared to 0.12 for breast [49], hence, in patients with melanoma, the local radiation dose contributes little to the effective dose. It has been calculated that a 500-mSv absorbed radiation dose to the skin at a depth of 70 μm averaged over 10 cm^2 skin area is comparable with an effective dose of 17 μSv [50].

The different radiopharmaceuticals used for SN imaging are associated with minor differences for the dosimetry. Absorbed dose at the injection site with respect to the most common radiocolloids is shown in Table 2 [48, 51, 52]. These doses are below the threshold for deterministic radiation effects with proposed activities injected [53]. In determining the equivalent dose, the radiolabelled colloid migrates minimally throughout the bloodstream or reticuloendothelial system (RES), or beyond the sentinel and second-echelon lymph nodes. The effective dose for a breast procedure has been reported to be about 0.32 mSv

Table 2 Local absorbed dose to injection site with respect to the mostly used radiopharmaceuticals

Radiocolloid	Injection volume	Local tissue dose
^{99m}Tc nanocolloid	0.1 ml	20–44 mGy/MBq
^{99m}Tc antimony sulphur colloid	0.1 ml	27 mGy/MBq

[48, 54]. The effective dose has been calculated as 0.0019 mSv/MBq in a 'worst-case' calculation for melanoma, assuming that 20% of the administered activity has been absorbed in the RES systemically [30] (Table 3). It should be noted that adoption of SPECT/CT imaging protocols for SN in melanoma will increase both local radiation dose and effective dose due to inclusion of the CT procedure; the dosimetry being dependent upon both the site of the melanoma and the CT acquisition parameters selected.

Children Melanoma is a rare occurrence in children. Using the same assumptions outlined above no special dosimetric precautions is needed for children (Table 3).

Pregnant women Pregnant patients could be offered the SN biopsy after careful counseling regarding the safety and efficacy of the procedure. According to ICRP publications, the risk to the fetus is considered negligible for investigations exposing a fetus to <1 mSv [55]. In this respect, the dose to the patient from a lymphoscintigraphy procedure is rather small (0.4 mSv) [56]. Maximum calculated doses to the fetus for a breast cancer SN procedure using 92.5 MBq tracer were all within 4.3 mGy, far below the 50 mGy threshold equivalent dose for deterministic damage to the fetus [57]. The dose to the fetus from a SN examination in melanoma will generally be below the 1 mSv limit for increased stochastic risk generally applied to fetal radiation hygiene. Only in a melanoma located rather close the fetus (over the lower abdomen or back) the theoretical risk of exceeding 1 mSv is a relevant question. In such a case, the two important modifications that may reduce fetal radiation exposure will be (1) to reduce activity injected, preferably less than 30–40 MBq, and to collect the image data twice the normal duration [58] and (2) short time interval—always following a 1-day protocol—from injection to operation.[48, 56, 59].

Lactating women The presence of ^{99m}Tc in breast milk has not been reported, but it has been recommended that lactation be suspended for nursing mothers for 24 h after radiopharmaceutical administration, since radiocolloid will be excreted from the breast milk during this period [56].

Table 3 Radiation dosimetry for adults and children >5 years of age [30]

	Administered activity	Organ receiving the largest radiation dose mGy/MBq	Effective dose mSv/MBq
Adults	15–35 MBq Intradermal	Spleen 0.015	0.0019
Children >5 years	15–35 MBq Intradermal	Spleen 0.050	0.0036

Staff dosimetry

Within the EU, national implementations of the following EU Directives apply with respect to radiation protection aspects of the clinical practice of nuclear medicine. Applying the 1990 Recommendations of the ICRP [60], the Basic Safety Standards Directive [61] enforces a general radiation protection framework to ensure the safety of employees and the public. The Medical Exposures Directive [62] reinforces the need for justification, optimization and limitation of all exposures and places additional specific requirements on stated duty holders, especially in respect to the practical aspects of a medical exposure—its referral, individual justification and execution—including the training and competence of all staff whose actions contribute to the procedure(s) performed.

Staff in nuclear medicine department To comply with regulatory requirements, including those mandated by the Medical Exposures Directive within the EU and those in force elsewhere [63], radiocolloid administration and preoperative diagnosis will be performed by trained nuclear medicine personnel working in controlled environments. The administered activities in lymphoscintigraphy are low compared with those used in most other nuclear medicine procedures. Any increase in the occupational exposure of nuclear medicine staff due to a SN procedure will be minimal as they are already categorised as radiation workers. The highest doses received by the hands of the staff have been recorded for the physician who administers the tracer [64], however, it is far below the ICRP annual dose limits for the extremities of a radiation worker [60]. One potential cause of significant exposure exists however—if transmission imaging using a radioactive ^{57}Co flood source is performed, the source must not be held directly during image acquisition.

Staff in operating room Radiation exposure to operating room personnel arising from the handling of radioactive specimens from SN procedures is minimal. Studies demonstrate that the occupational doses are insignificant, the mean whole body dose received by surgical staff has been measured to be <1 μSv per operation [48, 65–67], with the maximum effective dose to the surgeons involved reported to be <2 μSv [48, 54, 65]. The radiation dose to the hands of the surgeon has been estimated to be 5–94 μSv per patient [30]. When the surgical procedure is performed 24 h after injection the absorbed doses to the hands of the medical staff may potentially be minimized [64, 68]. The

monitoring of operating room personnel for occupational exposure to radiation is unnecessary during SNB procedures. Additional shielding and monitoring devices are not required in the operating room.

Pregnant staff in operating room One circumstance requiring specific consideration is that of the pregnant female surgeon or scrub nurse regularly performing or assisting the procedure. A pregnant surgeon who participates in <100 SN operations will stay below the limit of radiation exposure as recommended for pregnant women [67].

Staff in pathology department The pathology staff usually spends a shorter time manipulating the radioactive tissue specimens than the does the surgeon, and at a longer time interval after injection; their exposure will therefore be lower. Even personnel performing an unusually high number of procedures receive radiation doses well below established limits for members of the general public [69]. Under any circumstances, radiation exposure to the pathology staff is low and should not normally require badge monitoring.

Radiation safety precautions

Labelling the pathology specimens When transporting the specimens to the laboratory, many institutions seal them in suitable containers with outer labels indicating radioactive content [68], however labelling is not required if the surface dose rate is <5 μGy/h [70]. Even if an institution does not label specimens, all personnel handling them must be properly trained and authorized and the specimens should be transferred promptly.

Radioactive clinical waste While surgical instruments and pathology slides appear to stay at background radiation levels, measurable contamination of absorptive surgical sponges and other materials used in the handling of radioactive tissues is observed, especially when they are used in the vicinity of the injection site [48, 71]. Although a negligible contamination hazard, this would constitute radioactive clinical waste. It is advisable to monitor these materials for contamination and if contaminated the trash should be held for decay-in-storage before disposal.

Summary

Radiolocalization of SN in melanoma patients is associated with low levels of radiation exposure. While lymphatic mapping is not contraindicated in pregnant patients, it is common to halve the dose activity and same day surgery is preferred. Radiation exposure monitoring or limitation of the number of performed SN procedures as well as additional shielding is not required for staff in the operating room and pathology department.

Injection technique

Introduction

There is general consensus about intradermal injection of the radiocolloid producing 2–4 wheals, dependent on the location of the tumour/scar [72]. In contrast, there is no general consensus about the activity to be injected (cf. Sect. 3: Radiopharmaceuticals and activity injected), and the number of aliquots to be injected also varies in different centres, and evidence clearly documenting what should be preferred is not available. The drainage directions in the different parts of the body should be kept in mind [31]. Thus the injection technique is particularly important for melanomas located in regions characterized by ambiguous lymphatic drainage not only in relation to right or left side with respect to the midline, but also in terms of frontal versus dorsal oriented lymph flow for the trunk and head-and-neck region [73].

Patient preparation

For adult patients, no special preparation is needed with the common locations of the tumour/scar. Local anaesthesia with anesthetic cream or spray may be advisable in special cases including mucosal tumours, and in general for melanomas in small children.

Syringe, needle, volume and activity

Syringe Tuberculin *syringes* that have virtually no dead space are recommended; otherwise a 0.1 ml of air can be drawn into the syringe behind the radiocolloid to ensure no tracer is left in the dead space.

Needle The intradermal injection should be performed using a 25- or 27-gauge (G) needle. The needle is inserted in a direction as tangent as possible to the skin surface for a few millimeters inside the skin; this technique entails small volumes of injectate, just enough to produce a visible wheal in the skin.

Volume Small volumes (0.1–0.2 ml for each aliquot, depending on the thickness of the skin) are recommended in order not to collapse lymphatics and to avoid rupture of the wheal on the skin surface, which will cause contamination and loss of activity for the drainage visualisation [74].

Activity The total activity to be injected (cf. Sect. 3: Radiopharmaceuticals and activity injected) must be divided into the number of aliquots to be injected around the tumour/scar according to the body location, which means that the specific activity may vary among patients [72].

Contamination

Care must be taken to avoid contamination of the patient's skin during the procedure by placement of a sheet over the injected region and of a swab over the needle puncture before the needle is withdrawn from the skin. After injection the skin should once more be inspected for possible contamination.

Injection site and depth, and number of injections

There is agreement to inject the tracer at a distance of 0.1–1 cm from the scar or the tumour margin. The number of radiocolloid aliquots to be injected vary (2–4) according to the anatomic region being explored. The tracer should be administered on each side of the tumour/scar keeping as a reference the orientation of the surgical scar [75, 76].

Head-neck and trunk In patients with melanomas located in the head, neck and trunk, four separate tracer injections must be given, roughly equatorially, around the lesion (at 3, 6, 9, 12 h) because the drainage may be both cranial and caudal and across the midline of the body [77].

Extremities Two injections will usually be enough on the extremities, given medial and lateral to the tumour/scar.

Summary

Four wheals (on the extremities only two) should be produced by intradermal injections of 0.1–0.2 ml radiocolloid around the tumour/scar at a distance of 0.1–1 cm. A tuberculin syringe and a 25- or 27-G needle with minimal dead space should be used, and care must also be taken to avoid skin contamination.

Image acquisition and interpretation

Introduction

The important goal of imaging during lymphoscintigraphy for SNB is to accurately identify all SNs, but only true SNs, and to mark the surface projection of them on the patient's skin. Lymphatic drainage is unpredictable in individual patients, and accurate mapping of the precise pattern of lymphatic drainage from the melanoma site is therefore the very important contribution of lymphoscintigraphy [10, 78–84].

Camera equipment

Gamma camera Scintillation cameras are required to allow intensity settings to be varied to highlight SNs versus any second-tier nodes that may be present [31, 85–87]. A large field-of-view camera head is preferable, and dual-headed cameras will save time. Single photon emission computed tomography (SPECT) or combined SPECT-CT imaging may improve anatomical information and be helpful in certain regions such as the head-neck region, in the suprainguinal region, and in all mucosal melanomas [88–90].

Collimator About 90–95% of the injected activity remains at the injection site with the rest passing via the lymphatic collectors to accumulate in SNs. A small and variable amount of tracer passes through the SNs to second-tier nodes [30, 31, 85]. This means that the images intrinsically have very little anatomical information and are of unusually high contrast. High or ultrahigh resolution collimators are thus important to prevent or reduce septum penetration giving star artifacts, and in case of early dynamic imaging, to clearly define the lymphatic collectors as they pass toward and reach the SNs. Lead shielding covering the injection site may occasionally be helpful during static image acquisition in order to detect nodes close to the injection site, especially in case of high activity retention in the injected depots. However, the lead shield itself carries the risk of masking draining lymph nodes [91].

Body contouring Use of flood sources or alternative techniques (cf. below) is helpful for better anatomical information for the surgeon, thereby speeding the surgical location of the SN [92].

Dynamic (early) imaging

Some recommend dynamic imaging for all regions of the body [78] while others limit early imaging to certain indications, in particular if dye injection is not to be done (e.g. in head-neck melanoma, risk of dye allergy etc.). Dynamic imaging is obviously more time consuming for the nuclear medicine department, since the patient has to be under the gamma camera already during the injection. This should be compared with more precise anatomic information from imaging, with subsequent better identification of nodes close to the injection site, and better identification of a second-tier node, which on delayed images may appear quite hot and be falsely interpreted as SNs. Thus both

sensitivity and specificity are improved. The clinical impact on postoperative morbidity, recurrence rate, and possible reduction of operation time remains to be determined.

A 10-min dynamic image at 1 frame/min in a 128×128 matrix in word mode is suggested to determine where the lymphatic collectors are headed. Further 5-min static images in word mode 256×256 should be acquired over the node field to identify the collectors as they reach the actual SNs. This is important since sometimes, especially in the groin for leg melanomas, some tracer will be seen passing through the SN on to a second-tier node.

A dual-headed camera can be advantageous in allowing different views simultaneously during the early imaging phase. Anterior or posterior view together with a lateral view is often helpful in the groin to identify collectors passing to deep iliac or obturator nodes. In the head-neck region it allows other views such as oblique or vertex views to be combined with or added to the lateral view (Table 4).

Static (delayed) imaging

Time interval from injection to imaging In general, we recommend that the patient be studied in the morning if surgery is to be performed on the afternoon the same day, in the afternoon if surgery is planned for the next morning. If no extra activity of tracer is to be administered, in order to have an activity amount of more than 10 MBq when the patient is operated [93] the activity amount should be modified according to the time of surgery. The larger the particles, the longer time interval (cf. Sect. 3: Radiopharmaceuticals and activity injected).

Static images should be done to ensure that all SNs are identified and marked (Table 4). Some use static images for

Table 4 Summary of early dynamic imaging and delayed static imaging or scanning in different regions of the body

Tumour location	Obligatory imaging	Optional imaging	
		Dynamic[1] - static, planar imaging[2]	SPECT or SPECT-CT[3-4]
Trunk	*Either:* Static: Ant. axilla Ant. groin Post. trunk	Dynamic imaging: Over injection site	Dependent on findings at planar scint
		Static: Lateral axilla (triangular intermuscular space)	
		Static: Lateral mid trunk (paravertebral, retroperitoneal)	
		Static: Lateral neck	
	Or: Body scanning: Ant-post. from neck to groin		
Hand and forearm	Static: Ant. axilla and neck Medial elbow	Dynamic imaging: Over injection site	Axilla and neck
Arm	Static: Ant. axilla and neck Lateral: axilla (triangular, intermuscular space)	Dynamic imaging: Over injection site	
Foot and leg	Static imaging Anterior groin Posterior knee (poplit.)	Dynamic imaging: Over injection site	Groin
Head and neck	Dynamic imaging: Over injection site Static: Ant-post Lateral	Static: Vertex Oblique Sub-mental	Head-neck

[1] Dynamic imaging: 10 min, 1 frame/min in 128×128 matrix, followed by a 5- min image (256×256 matrix) over injection site; [2] Static images may be 5–10 min/region in 256×256 matrix; or scanning e.g. 32 min/m, 256×256 (up to x1024) matrix; [3] SPECT, e.g. 128×128, 180° in anterior L–mode rotation, 3° angle step with a 20–25 s/frame; [4] CT depends on equipment

all relevant body regions, whereas others in truncal melanoma prefer scanning from the neck to the groin.

Body contouring To facilitate topographic localization, a body outlining is performed using various techniques [94].

- A handheld point source can achieve good, identifiable outlines.
- A ^{57}Co flood source can be used for simultaneous transmission imaging providing the body silhouette. In each single view or in combination with scanning of the trunk the ^{57}Co-source is placed under the imaging bed. Because of the risk of occasional loss of faint nodes when using ^{57}Co flood, some authors suggest repeating the delayed scans without the transmission source [30, 78].
- A ^{153}Gd source is available in some dual-head cameras for attenuation correction of SPECT images. The source produces body outline transmission images. Lymph nodes and transmission images are acquired simultaneously, and images can be acquired in any angle desired. A ^{153}Gd-line source gives a clear body outline.
- SPECT-CT: see just below.

SPECT and SPECT-CT SPECT imaging improves SN identification and overcomes some of the limitations of planar SN scintigraphic mapping. However, reconstructed images obtained by acquisition of SPECT alone after radiocolloid injection, are not informative, unless combined with anatomical data such as corresponding CT images. Hybrid imaging is acquired with SPECT-CT enabling transmission (CT, low- or full-dose) and emission (SPECT), performed at the same setting without changing patient position. It allows for correct image fusion, provided no motion artifacts.

The benefit of SPECT-CT for SN identification is derived from the improved lesion detectability of SPECT itself, from the improved quality of the SPECT image achieved by using the CT for attenuation correction, and from the improved anatomic localization of nodes, by the CT data as well as by the three-dimensional data of the three SPECT reconstructed planes [88–90, 95]. The clinical implications are not yet documented, but appear obvious in more complicated anatomic situations.

SPECT acquisition parameters for SN detection may be performed using a matrix size of 128×128, 180° in the anterior L-mode rotation, and a 3° angle step with a 20–25-s time frame. CT acquisition is performed either as a low-dose or full-dose CT. With the low-dose CT, acquisition may be performed over 220°, 16 s for each transaxial slice [88, 89, 96]. With the full-dose CT, the acquisition is dependent on the number of slices of the CT scanner.

Skin marking The surface location of the SNs should be marked on the skin, and the depth of the node described or indicated using an orthogonal view. Marking all hot nodes seen on delayed imaging is a wrong approach and will result in unnecessary removal of second-tier nodes, which will then be associated with increased morbidity [31, 78, 80, 85–87] (cf. Sect. 1: Definition of a sentinel node). If more than one node is found in the same region, some prefer to mark just the hottest node(s) and then describe and display the other nodes on accompanying image print-outs. A small amount of tracer in a needle hub or tracer source is placed on the skin mark and the depth of the node can be measured on this orthogonal image using electronic callipers. It is important to mark the patient in the same position as the surgeon will use at operation, which is a matter of communication with the local surgical team.

Image interpretation

Dynamic, early imaging SNs are identified by a lymphatic collector passing directly from the primary melanoma site to them. Primary melanoma sites may drain to more than one SN in a node basin and to more than one nodal basin [97–99]. Thus there may be multiple SNs in an individual patient.

Delayed, static imaging A node that appears in a separate node field only on delayed image is also a SN unless it is on the same path as the SN seen on dynamic scans. If dynamic imaging has not been performed, SNs should be described and marked according to the recommendations, as given in Sect. 1: Definition of a sentinel node.

Non-visualisation of a sentinel node

If during dynamic image acquisition no transport of tracer is observed from the injection site, massage with a gloved hand for 5 min may help [100]. If a collector is seen to taper off or stop along a path, then massage could be performed along the line of the collector. The lymphatic flow is very susceptible to external pressure. If nodes are not clearly depicted earlier, more delayed images may be considered up to 24 h after injection. Re-injection of tracer may also be considered.

Interval nodes and nodes in unexpected locations

Any node seen along the path of the lymph collector between the primary melanoma site and a known node field (interval or interval SN, cf. Sect. 1: Definition of a sentinel node) receiving direct lymphatic drainage from the melanoma site should be marked as SNs, regardless of their location. SNs in unexpected locations may be found in the

triangular intermuscular space behind the axilla, and may also occur as para-aortic, paravertebral and retro-peritoneal nodes that can drain the skin of the posterior loin, as intercostal nodes, and rarely as internal mammary nodes [11, 31, 78, 101–105].

Ultrasound (US) and fine needle aspiration biopsy (FNAB) Preoperative evaluation of the SNs with ultrasound and FNAB may be used to select patients with a malignant SN to proceed directly to CLND, thereby avoiding SNB and a second operation [4, 106, 107]. The predictive value of a positive FNAB is nearly 100%. However, the predictive value of a negative test is much lower, since many small, metastatic deposits in the SNs cannot be visualised at ultrasound. The sensitivities and specificities reported are around 47–72% and 82–100%, respectively.

Potential pitfalls in the interpretation of lymphoscintigraphy

False-negative interpretation SNs can be missed at scintigraphy and/or surgery, typically in cases where

- the node is obscured by another node or the injection site
- the SN contains too little radioactivity
- two adjacent lymph nodes are thought to be a single node [78, 108]

False-positive interpretation may be caused by

- second-tier nodes, in particular if dynamic imaging is not performed
- lymphangioma or lymphatic lakes (focal dilatations of lymphatic collecting vessels) mistaken for a SN. However, in contrast to SN, the activity rapidly passes through the lymph vessel so that these lakes are usually not visible on delayed images. A true SN remains hot on delayed scans
- skin folds and other tissues containing radioactivity, but no node [78, 108]. The use of SPECT-CT has been reported to improve interpretation overcoming some limitations of planar data [88]
- skin contamination from the injection, which is typically seen as a very hot, focal accumulation close to the injection site and easy to remove by washing the skin
- urinary contamination on delayed imaging (some radioactivity is excreted via the kidneys), another cause of skin contamination typically in the groin region

Summary

The aim of imaging in SNB in melanoma is to ensure that all true SNs are described. The node(s) directly draining the primary melanoma site in each basin should be marked accurately on the skin. Dynamic imaging is strongly recommended in the head-neck region and is optional, but generally advantageous, in other regions of the body if logistics permit. Delayed scanning, performed with a large field-of-view, preferably dual-headed camera, must be tailored for each patient so that all possible drainage regions are covered. Hybrid imaging with SPECT/CT is increasingly used, improving anatomical information. Without dynamic imaging second-tier nodes may probably quite often be misinterpreted as true SNs. Other false-positive and false-negative cases are encountered, although not very often. In the few cases, in which no SN is visualised, repeated imaging and/or injection must be considered.

Report and display

Introduction

Preoperative lymphoscintigraphy with SN marking enables surgeons to assess the histological status of both predictable and unpredictable lymph node sites. The report and imaging display are essential parts of the SN procedure [31]. We recommend that the report must include description of injection and images (planar/tomographic/±CT), a good display (paper/film/electronic) of the images (early dynamic and delayed static), and marking on the skin (cf. Sect. 6: Image acquisition and interpretation) showing the location of SNs.

Report

Preoperative lymphoscintigraphy is the first step in the lymphatic mapping procedure, and the images are considered a "road map" guiding the surgeon. The lymphoscintigraphy report should include the following information (at the same time in agreement with local traditions and regulations):

- *Radiopharmaceutical used and injection technique:* The report must include information about the radiopharmaceutical, the activity amount and volume administered, and the location, depth, and number of injections. The date and time of the procedure (injection and imaging) should be reported, as well as the name of the person who reviewed the study. Use of local anaesthetics should be mentioned
- *Images:* The visualised lymphatic channels (especially during the dynamic phase of lymphoscintigraphy) should be described. The report of visualised ducts will give the surgeon an accurate idea about the first-echelon

node(s) and distinguish these from secondary nodes. Dynamic images aid in identifying a SN as a node on a direct drainage pathway. When drainage to more than one anatomic region is seen (e.g. axilla and groin), each of these regions must have at least one SN [73, 78, 88, 92, 97, 109, 110]
- *Number of SNs* in each basin
- *Location of the SNs.* Description is important to avoid the risk of misinterpretation of images by the surgeon
- *Nodes not believed to represent SNs*: second-tier nodes
- *Interval nodes:* Identification of SNs in unusual locations, including interval nodes (cf. Sect. 1: Definition of a sentinel node)
- *Non-nodal, focal accumulations:* Focal activity accumulations are sometimes observed in skin folds, or they may represent radiopharmaceutical contamination or lymphangioma. Such focal accumulations may cause misinterpretation, in particular on conventional planar images [88]
- *SPECT and SPECT-CT:* Information from possible SPECT or SPECT/CT imaging should be added. Images may be more difficult to interpret for the surgeon; therefore the description of these images is important [111]
- *Uncertainties:* In some cases the interpretation is uncertain, which should be conveyed to the surgeon
- *Conclusion:* The report should end with a conclusion

If the operation is performed before a final, typed report is available, the results of the study must be communicated to the surgeon in a preliminary report including all necessary information. A brief written report with annotated images should be sent to the operating room with the patient, or annotated images may be sent after verbal communication with the surgeon.

Display of images

Hardcopy and/or picture archiving and communication system (PACS) softcopy output of the emission and/or combined (emission + transmission with flood source) image data as well as images of relevant, fused SPECT/CT slices in the three planes (if performed) should be available to the surgeon before surgery.

Dynamic images Early images depicting lymphatic vessels may be important for the identification of SN vs. second-tier nodes and should therefore be displayed. However, it may be more comprehensive, space demanding and sometimes difficult to display in an easily interpretable form accompanying the report. It is important that possible sources of error (non-nodal, focal accumulations, etc.) of the interpretation by the surgeon are commented in the report [112].

Static planar images Late anterior and lateral images with body contouring, (cf. Sect. 6: Image acquisition and interpretation) are usually sufficient. Rarely other views may be helpful, but if used for special circumstances, it is crucial to indicate on the image the projection applied. It may be useful, in case of the presence of several SNs, to indicate which nodes have been marked on the skin, if marking is not done for all nodes.

SPECT/CT If acquired, the image format will depend on local display handling.

Summary

The report should contain written information on the acquisition of the study, description and images displaying lymphatic vessels and number and location of SNs, comments on SNs in unusual locations or secondary nodes, and marking of nodes on the skin. A report, preliminary or final, including images, must be available for the surgeon at the time of operation.

Use of dye

Introduction

The blue dye may assist in visual confirmation of the afferent lymphatics from the primary tumour site to the SN. The blue dye travels through the lymph nodes without being trapped. The combination of scintigraphy, gamma probe and blue dye yields the highest accuracy in SN identification [17, 113] and is therefore recommended. Blue dye is especially useful in cases of a primary melanoma in close proximity to its regional nodal basin, where the injection depots of the radiocolloid can cause a high background radioactivity interfering with the gamma probe location of SNs.

Blue dyes

Isosulfan Blue (Lymphazurin) and Patent Blue V, two chemically related triphenylmethane dyes, have traditionally been the dyes of choice for SNB. Methylene Blue has also been tested because it is less expensive, has a lower risk of anaphylaxis (see below) and seems to be as accurate for SNB as the two others [114]. However, Methylene Blue can cause cutaneous necrosis in the site of injection [115].

After intradermal administration, the dye enters and travels through the lymphatic vessels. The particle size is large enough to pass slowly through the SN [116, 117].

Some of the injected triphenylmethane dye binds weakly to interstitial proteins, mostly albumin, and this protein binding probably causes the blue coloring of SNs [118]. Half an hour, 1 and 24 h after injection, approximately one-third, two-thirds, and all the dye will have passed through the SNs, respectively [117]. Methylene Blue does not bind to proteins [118].

After lymphatic uptake, blue dyes circulate through the venous system to the general circulation, causing transitory blue discoloration in some patients, rarely lasting weeks or even months. The patients should be informed about that risk and the risk of blue coloured urine for up to 24 h after administration because of urinary excretion of the dye.

Injection volume, technique and timing

Blue dye is injected into the dermis 10–20 min prior to the operation in a volume of 0.5–1 ml around the primary tumour or scar. Inadvertent subcutaneous injection may lead to identification of an incorrect SN because of inadequate lymphatic migration of the dye [119].

The time interval from blue dye injection to surgery depends largely on the distance between the primary melanoma and the lymphatic basin(s) to be explored. Lymphatic flow is faster in the lower than in the upper extremities, slowest in the head and neck region and intermediate on trunk [80].

Some surgeons prefer one injection in the middle of the primary scar, while others, concerned about possible interference by scar tissue with the lymphatic drainage, recommend two to four injections around the biopsy scar at a distance of up to 5 mm from the scar. There is no evidence of the superiority of either technique. The injection should be performed after the patient is anaesthetized (locally or universally) to avoid a painful injection. The injection, if properly done, should produce a small blue wheal in the epidermis and quickly show the most proximate lymphatic roots. A 1 ml insulin type syringe is preferable because it allows better pressure control, and its small needle (27 G) is most apt to penetrate into the dermis. Five minutes of massage of the injection site enhances movement of the dye through the lymphatics to the SN [100]. Within 5–15 min, the SN is colored. Washout is evident after approximately 45 min [118].

Contraindications for blue dye

- Pregnancy because of the risk for an anaphylactic reaction
- Earlier allergic reaction to blue dye. Since many items contain triphenylmethane related compounds, sensitization to Isosulfan Blue and Patent Blue can occur without known prior use, allergy history or positive skin test
- Severe renal impairment is a contraindication for Methylene Blue use

Adverse effects and special precautions

The toxicity of blue dyes is low. Serious adverse effects are rare, especially for Methylene Blue, and are generally not serious:

- Allergic reactions such as urticaria and rash, blue hives and rarely also life-threatening anaphylaxis with pulmonary edema, hypotension, vascular collapse. Allergic reactions are seen with isosulfan and patent blue dyes in up to 2% [120, 121]. The risk of allergic reactions increases with increasing volume of injected blue dye [122]. Therefore the staff must be aware of (and trained in) recognizing and treating allergic reactions
- Blue coloured urine during the first 24–48 h [120]
- Protracted blue discoloration of the skin. Therefore many centres avoid using blue dye in the head and neck region [123]
- Interference with transcutaneous oximetry during anesthesia. The blue discoloration may mimic a true intraoperative hypoxic event [113]
- Cutaneous necrosis: Methylene Blue can cause cutaneous necrosis at the site of injection [115]
- Lactation: Safety in the newborn has not been established; hence it is recommended that breastfeeding is discontinued

Summary

The use of blue dye should routinely be used as a visual confirmation of the afferent lymphatics and the SN, complementary to the lymphoscintigraphy and gamma probe techniques. Because of the risk of prolonged coloring, many hesitate to use blue dye in the head and neck region. Some use one central intradermal injection; others prefer 2–4 intradermal injections around the scar. Anaphylaxis is seen in approximately 1%, less for Methylene Blue dye.

Gamma probe detection

Introduction

This section includes technical information on the important features of the gamma probe technology, quality control (QC), use of the device including problems in SN localization, and common sources of error in the use of

gamma probes. Specific training and information will be needed for each different type of gamma probe due to the wide range of features on each probe, and also for different operative procedures. In many countries, effective training to achieve competency 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 to enable the probe to be used to search for locations of radioactive accumulation (Fig. 2).

The hand-held probe is connected to a power supply and a unit that receives the electrical signals that come from the radiation detector. This unit is the analyser, and the analyser and the hand-held probe forms the probe system. Power to the whole system may be by mains connection or battery. The batteries may be rechargeable, and practical concerns determine which is best for a particular practice, such as the need to ensure that the batteries are charged prior to use.

The analyser provides a responding count rate from measured gamma rays emitted from the activity, usually by audible pitch or volume variation and by a visual display as a dial or digital count rate. The probe technology is described in a number of reference books [124–126].

Probe size and shape

Probes typically have outer dimensions of 12–15 mm. Smaller diameter means a combination of a smaller diameter detector giving less sensitivity, and possibly thinner shielding giving greater penetration of gamma rays through the walls. Probes needed for access between ribs will therefore generally have poorer performance than larger probes, and in general the slight improvement in spatial resolution will be lost by the significant loss in sensitivity.

Some probe tips are angled relative to the handle, and this can be of benefit when looking around laterally in an incision site. Some people have difficulty visualising the direction when using an angled probe, so each surgeon must identify his or her own preference.

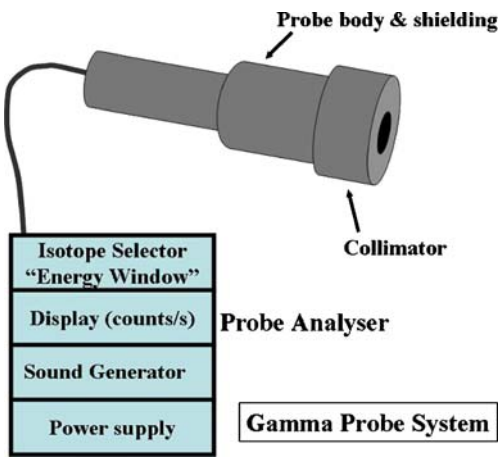

Fig. 2 Schematic view of a gamma probe system

Probe performance

The probe performance is described in terms of its spatial resolution and its count sensitivity [127–129]. The spatial resolution indicates how spread out the signal is from a point source; the sensitivity is the number of counts per second for a given activity. 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. Resolution worsens with increasing distance; so at 60 mm depth the apparent source width will be about double that at 30-mm depth. Many nodes contain well below 1% of the injected activity, and with a 6.01 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 99mTc for a 3-cm-deep node. For a 3-cm-deep node with 1% uptake from a 40-MBq injection of radiocolloid, with surgery at 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 longer delay from surgery there may be a much lower count rate and difficult localisation. With experience, localisations at low count rates are possible, but with greater variability, longer search time and less confidence than at higher count rates.

The probe also picks up photons 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. The adequate shielding of the probe from radiation penetrating through the side is therefore important. The rejection of scatter is achieved by having a probe with a good "energy resolution", and with a narrow energy window. Good energy resolution is a very important characteristic of the probe, and is often overlooked.

Probe controls

The probe analyser has a number of settings that affect the practical performance of the probe, and therefore the ease with which the surgeon can localise the nodes.

Energy window setting The energy window determines whether a gamma ray is counted or not, depending on the gamma ray energy, and has a fixed energy level of 140 keV for 99mTc, but with a variable "width". The wider the energy window the higher the sensitivity, but the more scatter that is detected. Thus it can be hard to identify a low-uptake node if it is close to the injection site, and in this case the amount of detected scattered radiation may be of a similar level to that from the node. The manufacturer will recommend a standard window setting, and possibly a "high sensitivity" setting in which a wider window is used. The high sensitivity setting should be used with caution, and is of most value for low-uptake nodes remote from the injection site.

Collimation Collimators are sometimes removable, and this gives a great gain in sensitivity at a loss of spatial resolution. Most probes benefit from the use of collimation, though if low activity nodes cannot be found then removal of the collimation can help if the node is remote from the injection site.

Additional shielding The direct penetration of gamma rays through the side of the probe may be reduced by the use of a small metal plate (tungsten or lead) held over the injection site, though this will probably only be of any significant effect for nodes very close to the injection site, or if the probe is poorly constructed with too little shielding.

Integration time Some systems allow the averaging of the count rate over a certain period of time in order to give a signal with less variability. Integration times of more than 1 s 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 will give an audible response from low to high pitch over a given minimum to maximum count rate range, say 100 counts/s to 1,000 counts/s. Counts below 100 cps will all have the same low pitch, and any count rate above 1,000 cps will have the highest pitch. The range of the current audible signal will require changing, sometimes also during the operation, to be appropriate to the count rate being detected at the time. The correct setting of the range is extremely important, since an incorrect setting may lead to nodes not being found since the pitch variation may not be sufficient. Some systems allow "auto-ranging", automatically changing the pitch range to the detected counts, but this can be confusing when trying to get a sense of the absolute count at any point.

Care of the probe and quality assurance

Quality Assurance (QA) All radiation detectors must be checked and managed within a QA program. Surgeons are advised to work closely with their nuclear medicine colleagues and medical physicist in setting up QC procedures.

Recommendations are:

- On purchase, tests of performance are advised to give a reference value for sensitivity and spatial resolution, and to form a base line 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
- Visual inspection for damage, particularly to cables and connectors, is the main practical check, and all users must be aware that the probe detector is fragile and is likely to be damaged if dropped onto a hard surface
- In the operating theatre the general functioning of the probe can be roughly checked by pointing the probe at the injection site before searching for nodes. This is a very crude practical check and not a substitute for QC checks; even a 50% loss in sensitivity would not have any effect on the general response to the injection site

Sterility

Sterility of the probe is generally assured by putting the probe into a sterile sheath, though this makes the probe tip larger. The skin surface may be scanned before sheathing, and in that case the probe must be decontaminated between patients by wiping with e.g. 70% alcohol or an agent that is recommended by the supplier. Care must be taken when removing a sheath not to accidentally take off any removable collimator, since these are costly to replace.

Gamma probe and lymphoscintigraphy information

The first point of reference is the nuclear medicine report, and any skin marking, if lymphoscintigraphy has been performed. Different positioning of the patient between imaging and surgery should be avoided since it may mean that skin marks are several centimeters from the "true" skin position in the theatre, so a search with the probe before incision is always required [7, 130, 131].

The time delay from imaging to surgery also means that the actual distribution of activity may be significantly different from imaging to surgery, perhaps with fall or loss of activity in the marked SN and increased accumulation in other sentinel or secondary nodes.

Searching with the probe

Before the search for the SN, the surgeon can point the probe at the injection site, to confirm that the probe gives a response. Searching within the lymphatic basin should be

slow and methodical, with the initial count range set to the lowest and the volume turned up. The theoretically optimal search pattern is to start at the position in the basin closest to the injection site, to place the probe tip perpendicular and in contact with the skin, and to move it in a raster pattern consisting of roughly parallel lines, at right angles to the direction towards the injection site, and with about 2 cm between each search line (Fig. 3). When the surgeon identifies a possible rise in activity in one scan line direction, the optimal search path is to then find the peak count in that direction and then to scan perpendicular to that scan line.

The speed of scanning should be no more than a few cm/s, but too-slow movement will lead to a loss of information from the change in pitch, which comes with movement as the counts rise and fall as the probe moves over a hot node. The drop in counts as the probe is angulated whilst over a hot spot can confirm location.

The relation to the injection site is not very important for injection sites more than about 30 cm from the drainage basin, but for closer distances this pattern ensures a roughly constant background due to scatter. Particular care must be taken in situations in which the injection site may be in the field of view of the probe. Once the skin surface location of a node is found, the incision site can be planned. Different users will come to their own favourite ways of using the probe, but the search strategy recommended above is the theoretical optimal, and is a good technique to follow until significant experience is gained.

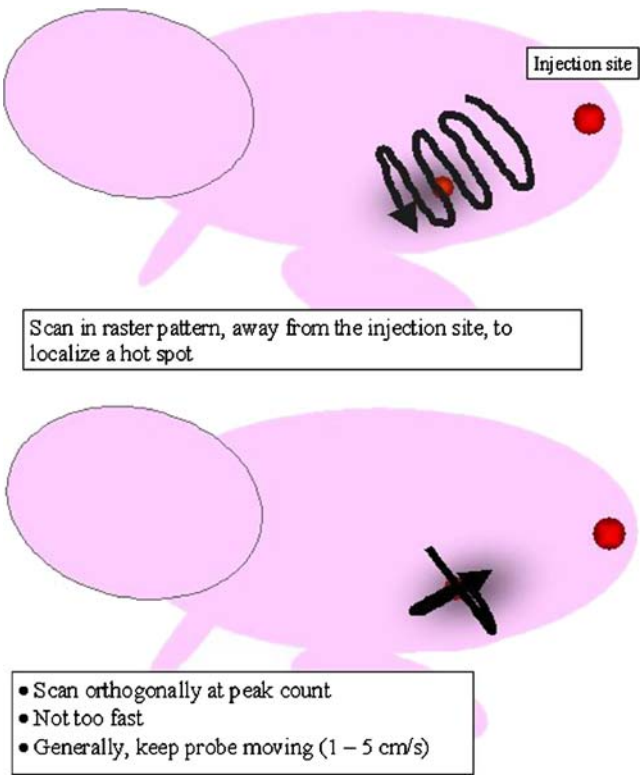

Fig. 3 Schematic view of systematic searching with the gamma probe

Use of the probe in the exploration and dissection

The tissues will move during dissection, and the probe can be used to guide the dissection path. The surgeon may sometimes find a sudden loss of counts since they may have gone past the node, and retraction of the probe will restore the count rate.

When a piece of tissue appears to contain radioactivity, it should be carefully investigated before being dissected free. Placing the probe tip against the suspected hot node, and then angling the probe quite steeply in different directions can avoid the mistake of detecting counts from tissues distal to the suspected tissue or from the injection site.

When a node is dissected free it can be checked for activity by placing it on a surface away from the patient, to avoid interference from activity in the patient, or by placing the tissue on the upturned probe tip (facing the ceiling). If an accurate count rate is required for documentation purposes, or research, a probe count should be taken away from other radioactive sources and with the node at least 2 cm from the probe tip, since positional variation is very large for sources closer to the probe tip. The probe system can be set to collect counts over a fixed time period, say 10 s, with the greater count having reduced statistical error compared to the continuously displayed count rate.

Inspecting the basin for other nodes

When a hot node has been removed, the wound site should be checked for remaining activity. Even if one hot node was clearly noted on lymphoscintigraphy, in practice this could actually have been two close nodes seen as one due to the limited spatial resolution of the gamma camera. Nodes closer than about 15–20 mm may well appear as one node, so after removal of one node in a limited number of cases another hot node may still be present in a close location.

When other sources of activity are found in the lymphatic basin, the decision of whether to remove them will depend upon the report from lymphoscintigraphy, and the working definition of “nodes to remove”.

Common sources of error or problems

Damage to probe: dropping the probe will usually cause it to stop functioning. Ensure that staff is aware of fragility, and perform QA before each operating list. A spare probe can easily be interchanged.

Cable damage: poor handling of the cable and connectors is to be avoided by careful handling, aided by staff training.

Incorrect collimation: for systems with removable collimation, the failure to put on the collimator will reduce the spatial resolution considerably, and may cause localisation difficulties.

Energy window incorrectly set: this may be caused by simple user error, selecting the wrong isotope, but is more likely to happen if the QA procedures are done with a ^{57}Co isotope and the procedure specifies that the energy window is set to ^{57}Co. To avoid this, perform all QA procedures on the ^{99m}Tc window, unless the window is unusually narrow, in which case more attention must be paid to resetting the window after QA.

Failure to identify a node on the skin scan: methodical and slow searching is essential for some low-activity nodes, or if the scatter background is high. Deep nodes (> 30 mm) may be hard to localise, but if there is image information from lymphoscintigraphy to direct the incision then as the probe approaches the node during dissection the counts will rise rapidly.

Removal of tissue with no activity: the surgeon must carefully assess if the tissue being identified for dissection is hot. Scatter, or shine through from a distant injection site, must be excluded before the "node" is removed.

Summary

The gamma probe is a sensitive and effective tool for identifying radioactive lymph nodes, particularly when combined with image information from lymphoscintigraphy. The device is simple, yet understanding the principles and obtaining good basic training is essential for its effective use, and is often required by law. Good support from radiation experts in the nuclear medicine or medical physics department can be valuable, particularly for routine QA, optimisation and purchase advice on performance. A methodical approach to localisation is required, with awareness of the major pitfalls, from technical and patient-related variations. The surgeon must be clear on the working definition of a SN, and how the probe information is used to identify nodes to produce a low false-negative rate.

Surgical technique in sentinel node biopsy

Introduction

The principle of SNB is to remove all true SNs with minimal dissection, causing as little disturbance as possible to the surrounding tissue but without compromising the accuracy of the procedure. There is consensus that the best approach is a combination of blue dye and the radiocolloid techniques including lymphoscintigraphy and gamma probe [132]. There are, however, a number of other questions concerning the surgical technique of SNB. No randomized trials focusing on the specific surgical technique have been performed, and the literature is rather sparse on the technical aspects of SNB.

Learning curve

Lymphatic mapping and SNB can be successfully learned and applied in a standardized fashion with high accuracy and SN identification rates of 97–100%. Isotope and dye in combination gives the highest detection rate and is recommended instead of using any one approach alone [132]. There is a definite learning curve (cf. Sect. 9: Gamma probe detection) with a drop in the false-negative rate from 10% for the first 25 cases to 5% for the following cases [17].

General or local anaesthesia?

Most surgeons prefer general anaesthesia for SNB; however some use local anaesthesia, often with the addition of sedation [27, 133]. Some argue that the local anaesthesia can interfere with lymph flow and make it more difficult to identify blue-stained lymphatic vessels.

Surgical technique

With the marked growth in the number of SNB procedures, the challenges of the logistics between the nuclear medicine department and the surgical facilities have increased considerably. It seems that 1- and 2-day procedures are equally efficient for SN detection. However, injected activity should be calculated to exceed 10 MBq in the patient at surgery (cf. Sect. 3: Radiopharmaceuticals and activity injected).

The surgeon uses the lymphoscintigram, the skin marks over the SN(s) and the gamma probe to plan the incision which should allow for possible later CLND if the SN proves to be positive. A small incision parallel to the assumed direction of the lymph flow will allow the surgeon to detect the greenish small lymph vessels just under the subcutaneous fascia and to follow them visually and with the gamma probe to the draining SN(s). It is important to use a meticulous dissection technique to avoid contamination of isotope and dye in the wound by damage of the afferent lymph vessels and avoiding bleeding for better vision. The main principle is to cause as little disturbance as possible to the surrounding tissue, thereby diminishing the risk of short- and long-term complications. The complication

rate after SNB is usually less than 10% and less serious compared to up to 40% after CLND. The complications after SNB are usually early and minor; bleeding, infection and small seromas (especially in the groin), very rarely deep venous thrombosis or lymphoedema [17].

Some surgeons use a knife, some diathermy for the dissection, but hardly any comments exist on these issues in the literature. It is advisable to ligate the afferent and efferent lymph node vessels before cutting the SN free, either ligating lymphatics with a suture or preferably with a clip as shown to be effective for other surgical procedures [134]. There is no consensus on whether to use drains or prophylactic antibiotics peroperatively [135].

When the hot and/or blue SNs have been removed, it is recommended to check for residual radioactivity of the lymphatic basin, and with the finger to identify possible large hard non-blue and non-radioactive nodes (especially if no pre-operative US is performed). These may be SNs full of metastatic cells and thereby no longer capable of receiving any lymph drainage from the primary tumour site [3] and should therefore be removed as well.

When the primary tumour site is located close to the lymph node basin it is reasonable to perform the wide local excision of the primary melanoma site before the SNB in order to remove the radioactive disturbance from the injection site of the probe detection of the SNs.

Should sentinel node biopsy in all lymph node basins be performed?

SN detection rates from both the groin and the axilla are favourable in most reports with only a 3% false-negative rate overall; 1% for the groin and 5% for the axilla [11, 17]. After an initial hesitation to operate in the head-neck region by some because of false-negative rates up to 15% [11], several reports have now shown acceptable results with only slightly lower SN detection rates compared to the groin and axilla [136]. Parotid SNs are often superficially located and should be removed. SNB in the head-neck region should only be performed by experienced surgeons because of the proximity to important structures such as the accessory and facial nerves. SPECT-CT is recommended in this region (cf. Sect. 6: Image acquisition and interpretation).

Interval nodes and SNs in unusual sites such as the triangular intermuscular space, flank, etc. should be removed if possible since they may contain metastases and may be the only metastatic nodes [9, 11]. Popliteal and epitrochleal SNs visualised by lymphoscintigraphy should be removed. There is no consensus as to whether external iliac or obturator SNs should be removed when visualised by scintigraphy [137, 138]. However, they can be removed simply via a small muscle-splitting incision above the inguinal ligament with upward retraction of the peritoneum and contents. Deeply located SNs in the abdomen are usually not removed but may be followed with regular imaging (CT, PET).

Summary

A small incision and meticulous dissection assisted by blue dye and the gamma probe should be used to remove SNs according to the definition. Interval nodes and SNs in unusual sites should be removed as well. If no preoperative ultrasound examination is performed, enlarged hard nodes found by palpation in the wound should also be removed. Wide local excision should be performed before the SNB when the injection site is close to the lymph node basin to avoid disturbance from the radioactivity at the injection site. Afferent and efferent lymphatics should be ligated or clipped prior to division

Pathological evaluation of melanoma-draining sentinel lymph nodes[1]

Introduction

Accurate histopathologic assessment of the SN that drains primary cutaneous melanoma is essential for individualized prediction of likely clinical outcome, and for determination of the need for immediate CLND. Despite the use of SNB for more than 16 years, no consensus has developed on the "correct" way to sample the SN. The views expressed below are based on the literature and a meeting[1] organized by the EANM and the International Sentinel Node Society, in an attempt to approach a consensus. Figure 4 summarizes practical data that may be included in a report of pathological evaluation of SN(s).

Gross assessment of sentinel nodes

SNs are transported to the laboratory unfixed or in formalin. Ideally, the surgeon should mark each SN with clips or stitches to highlight the point of entry of afferent lymphatics, blue-stained areas and areas suspicious for tumour and, as evidence to support the sentinel status of the submitted node, record detected radioactivity in the requisition.

[1] Considerations from a meeting of experts convened by the European Association for Nuclear Medicine and the International Sentinel Node Society, at the Sixth Meeting of the International Sentinel Node Society Meeting in Sydney, Australia in February 2008

Worksheet for Evaluation of Sentinel Node Pathology

Patient Name: _____ Date of Birth: _____

Hospital Number: _____ Pathology Number: _____

Anatomic site of Sentinel Node: _____

Specimen Type:

Surgeon Dissected Node(s): Number of Nodes: Color: Radioactivity*

Fatty Nodule: Number of Nodes:* Color: Radioactivity**

	Node 1	Node 2	Node 3	Node 4	Node 5	Node 6
Node Length (mm)						
Node Width (mm)						
Bisected (yes/no)						
1 mm slices (number)						
Number of tumour foci						
Subcapsular tumour (yes/no)						
Parenchymal tumour (yes/no)						
Extracapsular spread (yes/no)						
Diameter largest deposit (mm)						
Depth of tumour (from capsule) (mm)						
Tumour as % nodal area						
Tumour immunophenotype						
S-100 +/-						
Mart-1 +/-						
HMB-45 +/-						
Other +/-						
Other +/-						
Nevus: Capsule +/- trabeculum						

Fig. 4 Standardized form for reporting characteristics of the SN. This template should be modified to fit local requirements. *After dissection in Pathology **Radioactive count from requisition sheet

Most patients have one or two SNs from one basin, but more basins and more nodes are encountered [2, 8]. If more than three lymph nodes from one basin are present, the specimen is probably the result of partial CLND rather than SNB. Surgeons should be aware of the labour and expense issues that relate to the special processing of multiple lymph nodes. The advantage of SNB as originally described is that it provides a limited specimen that can be examined in greater detail than was customary previously in evaluations of multiple lymph nodes from lymphadenectomy specimens.

SN specimens should have neither crush nor cautery artifacts. SNs may be partially dissected free of associated fat, but leaving some so that the lymphatics can be assessed for the presence of tumour. During dissection, the SN is examined for blue coloration and measured (maximum length x width in mm). The node is bisected through its longest meridian because melanoma cells first invade the subcapsular sinus at the point of entry of afferent lymphatics [139]. Cut surfaces are carefully examined for metastases or collections of melanin or carbon pigment when used by the surgeon. Imprints may be made for cytology (see below). SN halves are fixed in formalin for 12–24 h (less if received in formalin) prior to processing, section cutting and staining.

Laboratory confirmation that a submitted node is a sentinel node

Because technical problems can affect the accuracy of nuclear medicine and surgical procedures, nodes claimed as sentinel may not be true SNs. For example, a tumour-replaced lymph node can alter lymph flow and thereby lead to inaccurate identification of the SN. Preoperative ultrasonography can identify nodal metastases larger than 5 mm (and there are claims that some smaller deposits may be detected in this way); if these putative tumour deposits are confirmed as melanoma by fine needle biopsy, the patient may be considered for CLND instead of SNB. The accuracy of currently used mapping agents is time-dependent. The blue dye often dissipates before the SN arrives in the laboratory, and it may also move to second-tier (non-sentinel) nodes. The radioactive isotope decays rapidly from the peak emission values measured in the operating room, and few laboratories are, in any case, equipped to measure radioactivity. A stable, inert marker that selectively accumulates in SN and is readily visible on standard microscopy would permit confirmation of SN status.

Carbon particles injected intradermally with blue dye accumulate preferentially and usually exclusively in SN [140]. Particles accumulate in subcapsular sinuses and lymphoid tissues around the entry point of afferent lymphatics. The location of these particles also indicates the intranodal areas most likely to harbor metastatic tumour cells [141]. This approach cannot be used in patients with carbon-based permanent black tattoos, because tattoo pigment often tracks to regional lymph nodes. Drug regulations vary and this use of carbon particles is currently not permitted in some countries.

In Australia, antimony sulphur colloid is routinely used for lymphoscintigraphy prior to SN biopsy. Scolyer et al. [142] report the use of inductively coupled plasma mass spectrometry of tissue sections to confirm SN status by detection of increased amounts of antimony but this is not suggested as a routine procedure.

Intraoperative evaluation of sentinel nodes

SNB was developed using intraoperative frozen sections because CLND could be performed immediately if the SN contained a tumour. However, further experience has shown that frozen sections are unreliable. Preparation of a full-face frozen section often requires discarding substantial nodal tissue during cutting of the frozen block. Since SN metastases are frequently small and close to the nodal meridian, diagnostic tissue might be discarded during preparation of frozen sections. In addition, identification of melanoma cells is always more difficult in frozen tissue sections than in slides from well-fixed tissues. Melanoma-draining SNs should be evaluated as sections from well fixed paraffin-embedded tissues. If intraoperative assessment is requested, visual assessment of the cut face of the node is recommended. Cell smears may be obtained by scraping the nodal cut surfaces, or tumour imprints prepared by pressing the cut surfaces of SN onto glass slides for cytological evaluation.

The need to evaluate multiple levels of the sentinel node

Multiple sections of each half of the SN should be stained with haematoxilin-eosin (H&E). The number of stained sections and the thickness between sections remain debatable.

In early studies of nodal micrometastases, Cochran et al. [139] showed that early melanoma metastases are predominantly identifiable in tissues adjacent to the longest nodal meridian; these investigators have consistently recommended examination of ten full-face serial sections cut from both faces of the node (Fig. 5, left side) and stained by H&E (Sects. 1, 3, 5 and 10), S-100 (Sect. 2), HMB45 (Sect. 4) and MART-1 (Sect. 6) [2, 143–145]. If no tumour cells are found in nodal sections from a patient with a high-risk primary melanoma (as assessed by micrometer-measured thickness), further sectioning of the remaining nodal tissue may be undertaken, although the yield from this additional evaluation is low. Antibodies to tyrosinase and cocktails of antibodies to multiple melanoma epitopes may also be used. This approach detects melanoma in 16–20% of SNB specimens, a figure closely similar to the rate of ipsilateral regional nodal failure in patients treated by wide excision alone [146] .

More recently, Starz et al. [147], Cook et al. [148], Spanknebel et al. [149], Abrahamsen et al. [150] and Riber-Hansen et al. [151] have shown that evaluation of sections deeper in the SN can identify additional metastases in some patients. Determination of the clinical significance of detection of these additional melanoma cells, which are usually small metastases, will require prolonged follow-up.

According to Cook et al. [148], examination of six pairs of sections cut at 50-μm intervals and stained respectively with H&E and S100 can detect melanoma in up to 33.8% of SNs. Spare sections are made available at each level to help resolve difficult cases by additional immunohistochemistry. These studies were prompted by the practical difficulty of accurately identifying the true median plane and to try and reconcile differences in detection rates between histopathology and molecular techniques reverse trascriptase polymerase chain reaction (RT-PCR). A modification of this protocol (Fig. 5, right side) has been adopted by EORTC and is a requirement for patients entering EORTC clinical trials.

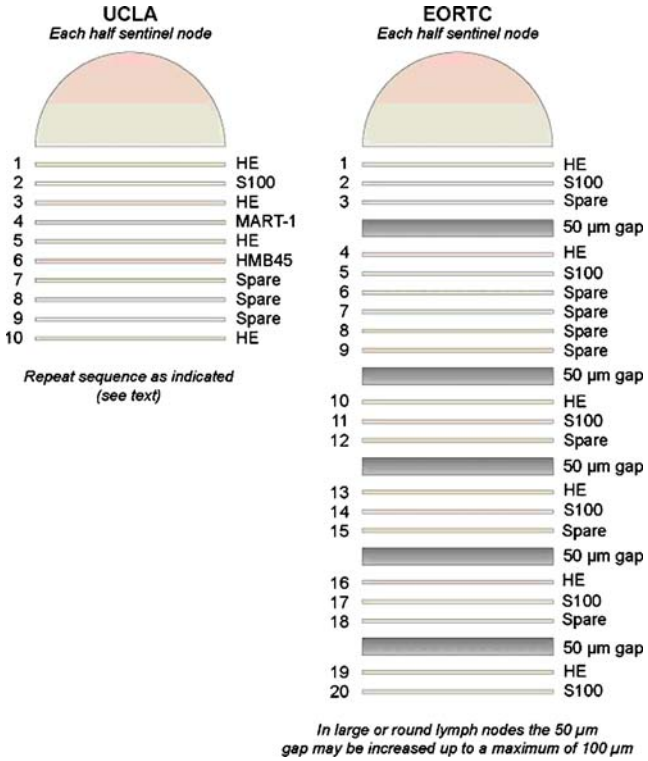

Fig. 5 Two sampling protocols: The *left column* depicts the long-standing recommendations from UCLA that the two halves of the SN are intensively sampled using H&E and Immunostaining on sequential sections. This approach intensively samples the perimeridional tissues, uses three separate immunomarkers of varying sensitivity and specificity, but does not evaluate the more peripheral parts of the node. This technique detects melanoma in 16–20% of SNs, a frequency identical to the ipsilateral failure rate of patients treated by wide excision alone. The *right column* depicts the node-sampling technique adopted by the EORTC Melanoma Group. This uses H&E staining and S-100 protein (spare sections may be used for additional immunomarkers). It samples more peripheral portions of the two halves of the SN and is reported to detect melanoma in up to 33.8% of SNs. The clinical significance of the additional positive nodes detected in this way will become clear with extended follow-up studies that are in progress. This approach calls for some additional technical effort and a small increment in pathologist work

What to look for microscopically

Immunohistochemically stained sections are usually screened first. The entire slide is scanned at low power and the different nodal compartments are assessed, with initial scrutiny of the subcapsular sinus. Tumour cells may occupy substantial areas of the node or be few and dispersed singly or as microcolonies in subcapsular sinuses, lymphoid parenchyma, deeper sinuses and afferent lymphatics (the last with the same clinical implication as intranodal tumour) (Fig. 6a–d). High-power (x400) fields are examined to confirm the nature of single or clustered tumour cells. Extracapsular invasion is infrequent but should be recorded [152].

The role of immunohistochemistry in evaluation of sentinel nodes

Single tumour cells or small micrometastases can be difficult to identify in H&E-stained sections; even experienced pathologists may miss up to 12% of tumour-positive SNB specimens when evaluation does not include immunohistochemistry [152]. The choice of immunohistochemical reagents is important [153]. Antibodies to S-100 protein are almost 100% sensitive for melanoma but not very specific because they also stain paracortical dendritic leukocytes, some sinus histiocytes, fat cells, Schwann cells of node-associated nerves and capsular/trabecular nevocytes [154]. However, the staining properties and the morphology of reactive cells usually permit recognition of cell type.

MART-1 and HMB-45 (Fig. 5) are less sensitive but more specific than S-100. These epitopes are more specific for cells of melanocytic lineage but are not expressed by up to 25% of melanomas [153]. Combinations of antibodies (antibody cocktails) are no more sensitive than S100 and do not allow immunophenotypic differentiation of melanoma cells and nevus cells.

False-positive assessment of SNs arises from misidentification of non-melanoma cells. It is reported that confusion between melanin-containing macrophages and immunopositive melanoma cells may be reduced by using a colored chromagen such as aminoethylcarbazole or alkaline phosphatase followed by “fast red”, instead of brown diaminobenzidine. However, participants find red chromogens more difficult to interpret. Other potential sources of false-positives are S-100-reactive dendritic cells, capsular and trabecular nevi, histiocytes, intranodal and perinodal nerves, ganglion cells and mast cells but distinction is usually straightforward on morphological grounds. SNB specimens from older patients may show extracellular HMB45 reactivity in focally calcified trabeculae of inguinal or pelvic nodes.

Nodal nevi versus nodal metastatic melanoma

Discrimination of benign nevocytes from metastatic melanoma cells requires evaluation of nodal architecture and the cytology and immunophenotype of the cells under consideration [144]. Melanoma cells are usually larger than nevocytes and are commonly located in the subcapsular sinus and lymphoid tissues of the node. Unlike nevocytes, they are seldom seen in the nodal capsule other than within afferent lymphatics. Cytological features that distinguish melanoma cells from nevocytes include large size, high nuclear-to-cytoplasmic ratio, prominent nucleoli, mitotic figures (especially atypical forms), and the fine melanin granules (single melanized melanosomes) that indicate

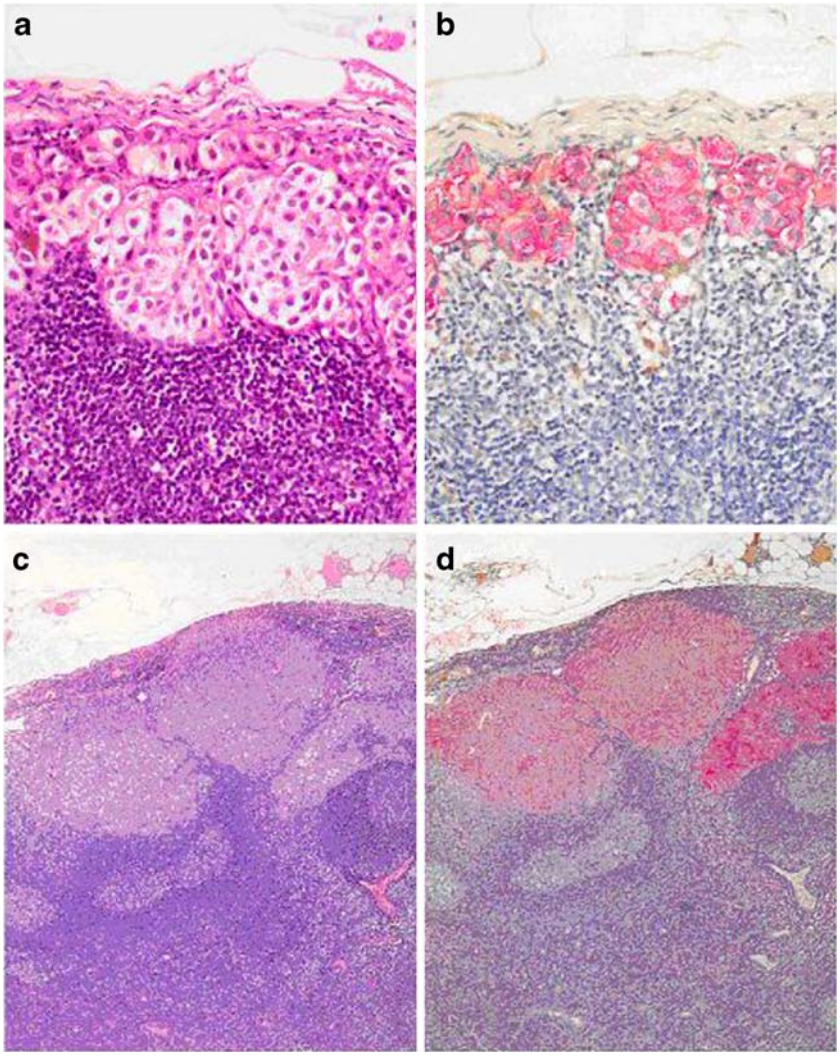

Fig. 6 Patterns of melanoma metastases in the SN: **a** Melanoma cells occupy and expand the subcapsular sinus (H&E). **b** Melanoma cells occupy and expand the subcapsular sinus (HMB-45). **c** Nodules of metastatic melanoma occupy the subcapsular sinus and extend into the underlying nodal parenchyma (H&E). **d** Nodules of metastatic melanoma occupy the subcapsular sinus and extend into the underlying nodal parenchyma (S-100 protein)

melanin synthesis within a cell. Coarse melanin granules (melanosomal aggregates) are characteristic of macrophages (melanophages). Up to 25% of melanoma patients have capsular or trabecular nevocytes in one or more regional node(s). The location of nevocytes in connective tissue is usually obvious. Extension of nevocytes into perivascular stroma or ultrafine reticulations of the trabeculae can make interpretation difficult because an apparent location in the parenchyma is more characteristic of melanoma [144, 155].

Connective tissue stains such as Masson trichrome and reticulin may help by disclosing the arborizing pattern of nodal stroma. Nevocytes stain positive for S-100 (nucleus and cytoplasm), MART-1 (cytoplasm) and p16 [156], usually have weak or absent reactivity for HMB45 and are negative for Ki67. Nevertheless, discrimination of nevus cells from melanoma cells on the basis of cytology is often relatively straightforward on H&E preparations.

Sentinel node biopsy for evaluation of melanocytic lesions of uncertain metastatic potential

Sometimes the morphology and immunophenotype of the primary cutaneous lesion are not adequate to distinguish a melanoma from a nevus [157–161]. In this case, the margins of excision should be increased for the primary and the patient can be offered SNB. Additional nodal dissection is not required if the SN is lesion-free or demonstrates exclusively capsular or trabecular nevus cells. If the node contains tumour that is truly located in the parenchyma, CLND and adjuvant therapy may be offered because a parenchymal location favors metastatic disease.

The use of SNB in the evaluation of melanocytic lesions of uncertain malignant potential (MUMP) is complicated by the fact that some patients with spitzoid lesions and atypical cellular blue nevi have ostensibly parenchymal tumour deposits that resemble lymph node metastases and yet are clinically benign. Some argue that SNB is not appropriate

for lesions that have limited malignant potential and are not customarily treated by regional nodal surgery. This subject clearly needs to be more formally evaluated and an excellent first step would be the formation of an international registry of such cases, as was proposed at the International Sentinel Node Society Meeting in Sydney (February 2008).

Molecular biology as a supplement to histology

Conventional microscopy may fail to detect melanoma in SNs of patients with limited and occult tumour burden. This argues for more extensive nodal sampling [148] and/or approaches such as reverse transcriptase polymerase chain reaction (RT-PCR) [141]. The possibility that RT-PCR might identify melanoma cells in SNs with no histological or immunohistological evidence of tumour has attracted considerable interest. However, there is currently no compelling reason to abandon microscopy and analyze SN exclusively by RT-PCR. The techniques commonly used to extract mRNA for evaluation by RT-PCR destroy the tissue and prohibit identification of the specific cell from which the enhanced signal was derived. A molecular signal for a melanoma-associated marker might also derive from capsular and trabecular nevocytes, Schwann cells of intranodal nerves or macrophages that have ingested melanosomes or other organelles from melanoma cells. Concerns have been expressed that overinterpretation of RT-PCR results carries the risk of overtreatment [162]. The efficacy and clinical relevance of molecular analysis of SN are being studied in the second Multicenter Selective Lymphadenectomy Trial (MSLT-II) sponsored by the National Cancer Institute in the United States (Principal Investigator: Donald L. Morton, John Wayne Cancer Institute, Santa Monica, CA, USA; Clinical Trials Identifier NCT00389571).

Measurement and distribution of nodal tumour

Pathologists may refine assessment of prognosis by measuring the size and/or distribution of SN metastases. Starz et al. [147] reported that the micrometer depth of tumour penetration from the interior of the SN capsule correlated directly with the likelihood of non-SN (NSN) metastases in the same regional node basin. Wagner et al. [163] correlated SN tumour volume with outcome, and Cochran et al. [152] calculated the percentage of the SN replaced by melanoma [164] to predict likelihood of NSN metastasis, recurrence and death from melanoma. Dewar et al. [165] noted that the distribution of metastases in the SN was also strongly predictive of NSN metastases, those confined to the subcapsular zone were not associated with further metastases, whereas parenchymal metastases often were. Van Akooi et al. [166] have shown that the maximum dimension of the largest nodal tumour deposit is a relevant prognostic parameter. Metastases <0.1 mm in diameter were infrequently associated with metastases in NSNs and unfavorable clinical outcomes during the early years of follow-up. Govindarajan et al. [167] also reported that SN metastases less than 0.2 mm in diameter were not associated with further disease, but Scheri et al. [168] have shown that even very small SN metastases can be associated with reduced survival.

It is highly probable that in future treatment, decisions (e.g. the need for CLND) will depend on tumour burden and distribution in the SN [166, 169]. Currently, however, none of the available measures of tumour burden or disposition in the SN, individually or in combination, can be used as the sole basis for treatment decisions, although they can place patients in high- and low-risk categories for recurrence and death from melanoma. For this reason, pathologists may wish to provide information on (for example) the Starz micrometer depth [147, 170], diameter of the largest metastasis and whether the tumour is confined to the subcapsular sinus or extends into the nodal parenchyma.

Non-sentinel, metastatic nodes

Approximately 17–25% of patients with SN metastasis also have non-sentinel node (NSN) metastasis in the same drainage basin. NSN metastasis is usually limited to a single node and usually involves less of that node than does SN metastasis. Whereas SNs are studied in detail, most NSNs undergo a relatively superficial examination: bisection with both halves stained by H&E. There is some interest in the routine use of immunohistology to evaluate NSNs, but data from Scolyer et al. [155] suggest that the return from such studies would be low. Immunohistochemical study of NSNs might be more appropriate when SN tumour is substantial, but this possibility requires further study.

Summary

No consensus has yet been obtained about the correct analysis of SN pathology. The recommendations presented in this paper are based on available evidence and a recent expert conference organized for the purpose of development of consensus. The number of levels examined, application of different histochemical methods, and use of molecular biology are presented in relation to the impact on sensitivity and specificity of the diagnosis of a metastatic SN. The lack of knowledge about the impact on clinical outcome is emphasized. The problems related to discrimination of the presence of benign tissue from metastatic melanoma are discussed.

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