Image-Guided and Radioguided Surgery

16

Francesco Giammarile, Sergi Vidal-Sicart, Federica Orsini, Renato A. Valdés Olmos, and Giuliano Mariani

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F. Giammarile
Nuclear Medicine and Diagnostic Imaging Section, Division of Human Health, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna, Austria

S. Vidal-Sicart
Nuclear Medicine Department, University Hospital Clinic, Barcelona, Spain

F. Orsini
Nuclear Medicine Unit, "Maggiore della Carità" University Hospital, Novara, Italy

R. A. Valdés Olmos
Nuclear Medicine Section and Interventional Molecular Imaging Laboratory, Department of Radiology, Leiden University Medical Centre, Leiden, The Netherlands

Nuclear Medicine Department, Onze Lieve Vrouwe Gasthuis Hospital, Amsterdam, The Netherlands

G. Mariani (✉)
Regional Center of Nuclear Medicine, Department of Translational Research and Advanced Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy
e-mail: giuliano.mariani@med.unipi.it

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Learning Objectives

- To become familiar with the overall practice and relevance of image-guided and radioguided surgery in cancer patients.
- To acquire basic knowledge on the relevance of tumor status of regional lymph nodes in patients with solid epithelial tumors.
- To understand the pathophysiologic bases of the sentinel lymph node concept in oncologic surgery.
- To acquire basic knowledge on the clinical applications of sentinel lymph node biopsy in patients with breast cancer.
- To understand the pathophysiologic issues involved in the rationale and practice of radioguided sentinel lymph node biopsy in patients with breast cancer.
- To become familiar with the procedures involved in lymphatic mapping and radioguided sentinel lymph node biopsy in patients with breast cancer, including injection of the radioactive agent for lymphoscintigraphy, preoperative gamma camera imaging, and intraoperative radioguided sentinel lymph node localization aided by the use of the handheld gamma-detecting probe.
- To understand the rationale and acquire knowledge on the practice of radioguided occult lesion localization in patients with breast cancer with non-palpable breast lesions.
- To become familiar with the advantages of intraoperative imaging with portable gamma cameras in patients with breast cancer.
- To acquire basic knowledge on the clinical applications of sentinel lymph node biopsy in patients with cutaneous melanoma.
- To understand the pathophysiologic issues involved in the rationale and practice of radioguided sentinel lymph node biopsy in patients with cutaneous melanoma.
- To become familiar with the procedures involved in lymphatic mapping and radioguided sentinel lymph node biopsy in patients with cutaneous melanoma, including injection of the radioactive agent for lymphoscintigraphy, preoperative gamma camera imaging, and intraoperative radioguided sentinel lymph node localization aided by the use of the handheld gamma-detecting probe.
- To become familiar with the advantages of intraoperative imaging with portable gamma cameras in patients with cutaneous melanoma.
- To acquire basic knowledge on the clinical applications of sentinel lymph node biopsy in patients with head and neck cancers.
- To understand the pathophysiologic issues involved in the rationale and practice of radioguided sentinel lymph node biopsy in patients with head and neck cancers.
- To become familiar with the procedures involved in lymphatic mapping and radioguided sentinel lymph node biopsy in patients with head and neck cancers, including injection of the radioactive agent for lymphoscintigraphy, preoperative gamma camera imaging, and intraoperative radioguided sentinel lymph node localization aided by the use of the handheld gamma-detecting probe.
- To become familiar with the advantages of intraoperative imaging with portable gamma cameras in patients with head and neck cancers.
- To acquire basic knowledge on the clinical applications of sentinel lymph node biopsy in patients with gynecological cancers.
- To understand the pathophysiologic issues involved in the rationale and practice of radioguided sentinel lymph node biopsy in patients with gynecological cancers.
- To become familiar with the procedures involved in lymphatic mapping and radioguided sentinel lymph node biopsy in patients with gynecological cancers, including injection of the radioactive agent for lymphoscintigraphy, preoperative gamma camera imaging, and intraoperative radioguided sentinel lymph node localization aided by the use of the handheld gamma-detecting probe.
- To become familiar with the advantages of intraoperative imaging with portable gamma cameras in patients with gynecological cancers.

16.1 Introductory Background

Together with PET, radioguided surgery is the nuclear medicine application that has experienced the most relevant growth in the last 15 years. The simple term "radioguided surgery" actually constitutes a wide range of combined procedures based on synergistic collaboration between at least two different specialties, nuclear medicine and surgery, but often involves other specialties as well; correct application of such procedures ensues obvious immediate and long-term benefits to the patient.

Radioguided surgery includes a set of pre-, intra-, and postoperative techniques and procedures that are designed to optimize traditional oncologic surgery. All these technologies may be encompassed in the "guided intraoperative scintigraphic tumor targeting" (GOSTT) concept [1, 2]. As a common intrinsic feature, the GOSTT concept implies the preoperative administration of a radiopharmaceutical that, though possibly with different modalities, concentrates preferentially in a certain target tissue to be removed. Preoperative imaging (most commonly with a conventional gamma camera—sometimes with PET) provides useful information on the optimal surgical approach to be adopted for each individual patient, while the intraoperative use of a handheld radioactivity-detecting probe (the gamma probe) further guides the surgeon for the identification and removal of the target tissue that has preoperatively been "labeled" with the radiopharmaceutical.

Preferential accumulation of a radiopharmaceutical in the target lesion can be achieved by three main mechanisms: (1) interstitial administration of an adequate radiopharmaceutical (typically a radiocolloid) that depicts the pattern of lymphatic drainage from the site of a solid epithelial tumor, to identify the sentinel lymph node(s) draining from the primary tumor; (2) systemic administration of a tumor-seeking radiopharmaceutical that preferentially accumulates in the target lesion; and (3) direct intralesional administration of a radiopharmaceutical that, by virtue of a relatively large size, is retained virtually indefinitely at the injection site for the so-called radioguided occult lesion localization.

The sentinel lymph node (SLN) procedure is a diagnostic staging procedure that is applied in a variety of tumor types. The procedure aims to determine the tumor status of the SLN(s), defined as any lymph node on a direct drainage pathway from the primary tumor [3]. The concept is based on the premise that lymph flow from the primary tumor travels first to the SLN and then sequentially to the other regional lymph nodes—a process that underlies the concept of "orderly progression" of metastatic tumor cells along a certain path of lymphatic drainage (Fig. 16.1). Therefore, the histopathologic status of this node should reflect the histopathologic status of the entire nodal basin. This concept translates into the clinical practice as follows:

- If metastasis is found in the SLN(s), there is a high probability that other lymph nodes in the same lymphatic basin harbor metastasis as well.
- Current recommendations in case of metastatic SLN(s) call for proceeding to completion lymphadenectomy of that particular basin (except in some specific instances

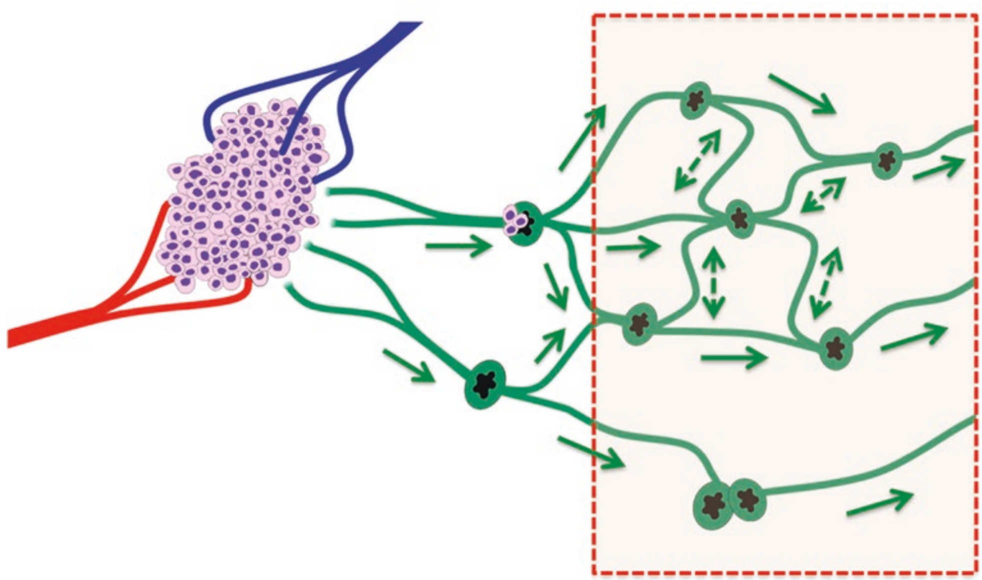


Fig. 16.1 The sentinel lymph node concept assumes that lymph drains from a solid tumor in an orderly fashion from lower-echelon to higher-echelon lymph nodes. Therefore, tumor cells migrating through lymphatic channels are most likely to be entrapped and possibly originate metastasis in the first node(s) they encounter, the sentinel node(s), before spreading to higher-echelon lymph nodes along the same lymphatic drainage pathway. Nevertheless, even in any given lymphatic basin, there can be more than one sentinel lymph node, as lymph can drain from the site of the primary tumor via different lymphatic channels toward the same basin. Furthermore, complex interconnections can exist at higher levels, with variable directions of lymph flow at intermediate levels. The pattern of lymph flow is even more complex when considering that lymph from the tumor can drain to more than one lymphatic basin, each one repeating the basic pattern represented here for a single basin (*reproduced with permission from: Giammarile F, Orsini F, Valdés Olmos RA, Vidal-Sicart S, Giuliano AE, Mariani G. Radioguided surgery for breast cancer. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1363–1400*)

still under investigation—see further below in the section on breast cancer).

- Lymphadenectomy of a certain lymphatic basin is burdened with a relatively high probability of immediate and delayed local complications.
- Completion lymphadenectomy in a basin with metastatic SLN(s) is performed either for purely staging purposes (as in patients with breast cancer) or for treatment purposes (as in patients with cutaneous melanoma).
- On the other hand, if no metastatic tumor cells are found in the SLN(s), it is very unlikely that other lymph nodes of that particular basin harbor metastasis.
- Therefore, completion lymphadenectomy can safely be omitted, thus sparing to these patients unnecessary additional surgery—with the associated complications.

The sentinel lymph node concept takes on a special pathophysiologic rather than a purely anatomic value. In particular, the sentinel lymph node is not always the node anatomically closest to the tumor but rather the first node that tumor cells migrating through the lymphatic channels draining the tumor site encounter along a certain drainage pathway (Fig. 16.2).

In addition to being routinely applied in patients with breast cancer and cutaneous melanoma, radioguided SLN biopsy is being validated in patients with a wide variety of other solid epithelial cancers. In particular, malignancies where the feasibility and/or clinical impact of radioguided sentinel lymph node biopsy has been more thoroughly investigated include head and neck cancers and gynecological cancers (vulvar, cervical, and endometrial). Other less validated clinical applications include prostate cancer, cancers of the gastrointestinal tract (esophagus, stomach, colorectal, anus), non-small cell lung cancer, differentiated thyroid carcinoma, and others.

Sentinel lymph node mapping in patients with penile cancer, an application with intermediate levels of evidence as regards clinical benefit to patients, is discussed in Chap. 35 of this book.

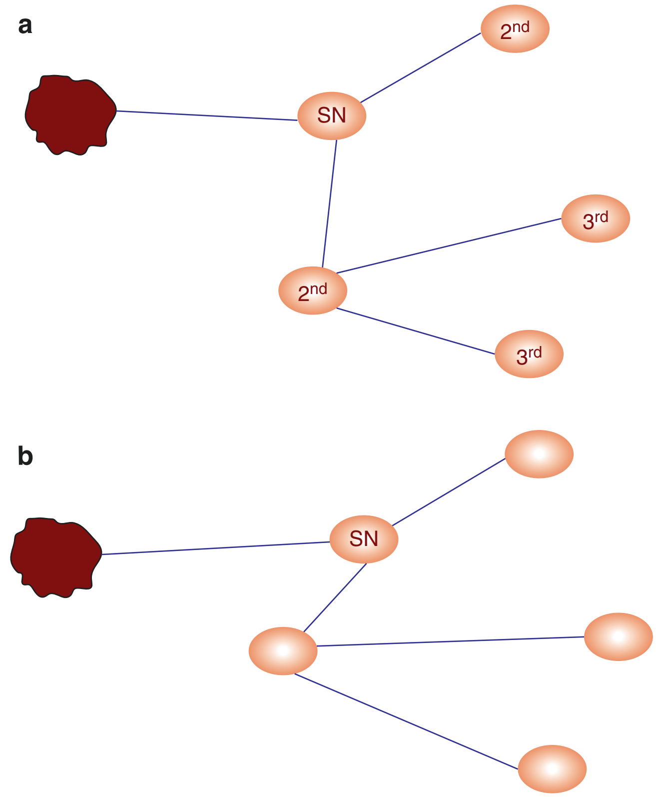


Fig. 16.2 (**a**) Lymphatic mapping for sentinel node biopsy assumes that lymph from a primary cancer (depicted with a dark red colour) drains to a particular regional lymph node before reaching higher-tier lymph nodes along the same lymphatic pathway. (**b**) The lymph node anatomically closest to the cancer is not necessarily directly at risk of receiving tumor cells. SN = sentinel lymph node, 2nd = second-tier lymph node, 3rd = third-tier lymph node (*modified from: Nieweg OE. The sentinel lymph node concept in oncologic surgery. In: Mariani G, Manca G, Orsini F, Vidal-Sicart S, Valdés Olmos RA, eds. Atlas of Lymphoscintigraphy and sentinel node mapping—A pictorial case-based approach. Milan: Springer-Verlag Italy; 2013:87–94*)

Key Learning Points

- Radioguided surgery includes a set of pre-, intra-, and postoperative techniques and procedures that are designed to optimize oncologic surgery.
- The fundamental common requisite for successful radioguided surgery is the use of a radiopharmaceutical that, following either systemic or interstitial administration, preferentially accumulates at the lesion/tissue to be excised.
- The rationale of sentinel lymph node biopsy in patients with epithelial solid cancers is based on the concept of "orderly progression" of metastatic tumor cells along a certain lymphatic pathway.
- A negative sentinel lymph node biopsy makes it highly unlikely that other lymph nodes in the same lymphatic basin harbor metastasis.
- Sentinel lymph node biopsy is currently *the* standard of care in patients with early breast cancer or cutaneous melanoma and extensively validated also in patients with head and neck cancers or with gynecological malignancies.
- Application of sentinel lymph node biopsy in other malignancies (prostate cancer, cancers of the gastrointestinal tract, differentiated thyroid cancer, non-small cell lung cancer, and others) is still under clinical validation for feasibility and accuracy.

16.2 Radioguided Surgery in Breast Cancer

16.2.1 The Clinical Problem

Breast cancer is the most frequent cancer diagnosed in women worldwide. In patients with breast cancer, accurate lymph node staging is essential for both prognosis (of early-stage disease) and treatment (for regional control of disease) [4].

Clinical examination (i.e., palpation) is not accurate enough for assessing the axillary lymph node status, and preoperative imaging modalities including PET/CT with [^{18}F]FDG and ultrasound have low sensitivities, especially in case of micrometastatic disease [5]. Thus, the traditional staging approach for patients with breast cancer requires axillary lymph node dissection (ALND) for extensive histologic evaluation of up to 15–20 lymph nodes. However, ALND results in a high incidence of postoperative complications that can reduce the quality of life. Furthermore, in early breast cancer, nearly 80% of axillary dissections reveal no metastasis and, therefore, could have been avoided [6].

Radioguided SLN biopsy has become a routine technique in breast cancer management, contributing to development of less invasive surgical procedures [7]. A systematic review showed an axillary 0.6% relapse rate in SLN-negative patients and no benefits from ALND in terms of survival after a negative SLN biopsy [8].

The main objective of SLN mapping (SLNM) and SLN biopsy (SLNB) in breast cancer patients is axillary staging. These procedures are an appropriate alternative to routine staging ALND for patients with early-stage biopsy-proven breast carcinoma without cytologically or histologically proven axillary lymph node metastases. Appropriately identified patients with negative SLNB do not need to undergo ALND. On the other hand, recent evidence suggests that ALND can safely be omitted also in patients with early breast cancer even in the case of positive SLNB, provided that some tumor-related features indicate a nonaggressive pattern of growth in that particular patient [9–12].

Despite the widespread application of SLNB for early-stage breast cancer, there is significant variation in performance characteristics reported for such procedures. The ranges of rates for false-negative findings and for SLN identifications emphasize the variability of this procedure. Learning curves for this technical procedure also vary. Nevertheless, once a multidisciplinary team is experienced with the procedure, reasonable levels of accuracy are achieved, with identification rates well above 95% reported routinely [13].

Key Learning Points

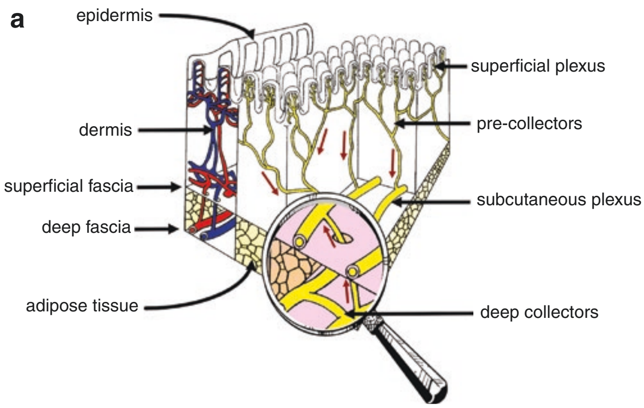
- In patients with breast cancer, accurate lymph node staging is important for both prognosis and treatment purposes.
- Clinical examination and diagnostic imaging are not accurate enough for reliable preoperative assessment of axillary lymph node staging in patients with early breast cancer.
- De novo axillary lymph node dissection (a futile surgery in nearly 80% of patients with early breast cancer) is burdened with a high incidence of early and late postoperative complications.
- Radioguided sentinel lymph node biopsy, a much less invasive procedure, identifies with high accuracy those 20% of early breast cancer patients with lymph node metastasis who actually need lymph node dissection.

16.2.2 Pathophysiologic Issues on Sentinel Lymph Node Mapping

The breast embryologically originates from ectodermal tissue, as a skin appendage, and therefore shares a pattern of lymphatic drainage with the overlying skin. The mammary gland is interposed between the superficial (subdermal) and the deep (subcutaneous) lymphatic plexuses; the two systems are interconnected by a dense network of lymphatic vessels (Fig. 16.3). Lymphatic vessels surrounding the mammary lobules predominantly drain to the subareolar Sappey's plexus, which is part of the superficial plexus of the skin. Most of the lymph produced in the breast is therefore drained to the subareolar region, progressing then toward the ipsilateral axillary nodes. A small fraction of the lymph produced in the breast (about 3%, mostly from the deeper portions of the breast parenchyma) drains instead to lymph nodes of the internal mammary chain, while an even smaller amount drains to other lymph nodes such as the intercostal and pectoral muscles, contralateral breast, or even abdominal lymph nodes.

Key Learning Points

- Most lymphatic drainage from the mammary gland flows toward axillary lymph nodes, after merging in the subareolar Sappey's plexus.
- About 3% only of the lymph produced in the breast (mostly originating from the deeper parenchyma) drains to lymph nodes of the internal mammary chain.



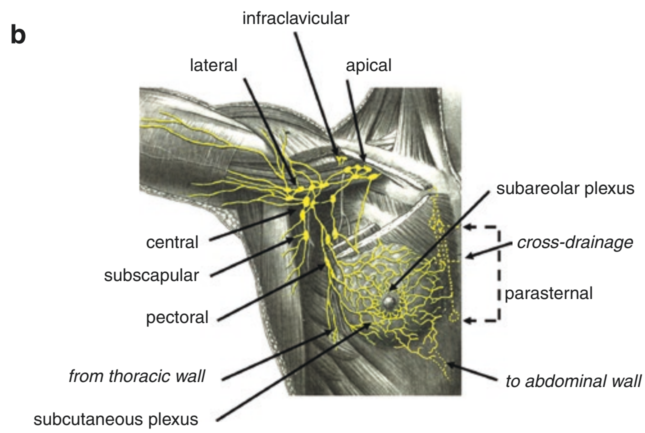


Fig. 16.3 (**a**) Schematic representation of blood circulation and lymphatic circulation in the skin. For easier comprehension, the lymph and blood vessel networks (which are actually embedded in each other) are represented separately, respectively, on the right (yellow) and on the left (red and blue). Its embryologic origin in the ectoderm places the mammary gland in an ideal space between the subcutaneous plexus and the deep lymphatic collectors (magnified insert). Branches of the periductal plexus drain lymph mostly toward the skin surface (via the subareolar plexus), while a minor component drains toward the deep collectors (which in turn drain toward the internal mammary chain). Dark red arrows indicate the direction of lymph flow at different levels, merging toward the subcutaneous plexus. Lymphatic mapping agents injected intradermally over the mammary gland drain to the subcutaneous plexus that also receives most of the lymph draining from the mammary gland. (**b**) Anatomic drawing of the lymphatic pathways and lymph node stations draining the mammary gland. Most of the lymph produced in the breast drains to the subareolar (or Sappey's) plexus before merging with the subcutaneous plexus of the overlying skin and then flowing mostly to the axilla. Lymph from deeper portion of the mammary gland drains either through the same pathway or through deep lymphatics that reach the parasternal lymph nodes (internal mammary chain) and even the contralateral side (*reproduced with permission from: Mariani G, Moresco L, Viale G, Villa G, Bagnasco M, Canavese G, et al. Radioguided sentinel lymph node biopsy in breast cancer surgery. J Nucl Med. 2001;42:1198–215*)

16.2.3 Procedures for Sentinel Lymph Node Mapping/Biopsy

Generally, SLNM and SLNB involve a continuum of procedures consisting in (1) interstitial tracer injection, (2) preoperative scintigraphic imaging, and (3) intraoperative gamma probe localization for surgical removal of the detected lymph nodes (LNs). Although there is consensus on some broad aspects of SLN protocols for breast cancer, consensus does not exist on all details. Controversies exist with regard to the particle size of the radiocolloid, the optimal route for injection, the timing and type of scintigraphy and intraoperative detection, and whether or not extra-axillary SLNs should be considered for harvesting and analysis. In addition, the specific radiotracer and technique used are guided by local availability, regulations, and practices [14].

16.2.3.1 Procedures in Nuclear Medicine

Three main parameters define an optimal tracer administration technique for radioguided SLNB: injection site, injected volume, and injected activity. A fourth parameter to be taken into account is the time elapsed between injection and surgery, as it directly influences the amount of radioactivity to be injected.

Radiopharmaceuticals

The ideal radiotracer should show rapid transit to SLNs with prolonged retention in the nodes. In general, the drainage, distribution, and clearance of radioactive colloids by the lymphatic system may vary depending on the size of the particles. Small particles are drained and cleared first; large particles are drained and cleared last and may be retained longer at the injection site. Nevertheless, it has been shown the success rate in the identification of axillary SLNs is not significantly affected by the particle size of the radiotracer. Thus, the selection of radiotracer is based more on local availability than on differences in SLN detection. However, there is general agreement that a 100–200-nm-sized radiocolloid should be considered the best compromise between fast lymphatic drainage and optimal retention in SLNs [15].

The radiopharmaceuticals most widely used for radioguided SLNB in breast cancer patients are ^{99m}Tc-sulfur colloid (particle size: 15–5000 nm, usually filtered to select a more restricted range of particle size), ^{99m}Tc-nanocolloidal albumin (5–100 nm), and ^{99m}Tc-antimony trisulfide (3–30 nm). New radiotracers have recently been developed for SLNM, most notably ^{99m}Tc-tilmanocept (or Lymphoseek®), which is composed of a dextran backbone with multiple mannose residues and linked to DTPA for ^{99m}Tc-labeling. Accumulation of this tracer in lymph nodes is not due to nonspecific phagocytosis by macrophages (as is the case for traditional ^{99m}Tc-radiocolloids) but to a ligand-receptor type of binding to the CD206 antigen expressed on the surface of macrophages and of dendritic cells present in lymph nodes. Such binding is non-saturable (at least with the amounts of radiotracer employed for SLNM) and results in a much more stable and prolonged retention in LNs than the phagocytosis mechanism that radiocolloids undergo. The potential advantages of its small molecular size (equivalent to 7.1 nm) and the receptor-targeted nature of the mannose moieties in ^{99m}Tc-tilmanocept include rapid transit from the primary site to the SLN as well as selective accumulation in that node, with limited pass-through to second-echelon nodes [16].

Activities and Volumes

Published evidence supports the use of small volumes with high specific activity to improve SLN detection. Consensus on the activity to be administered in a SLN procedure has not been reached. In current practice, a total injected activity of 5–30 MBq (depending on the elapsed time between scintigraphy and surgery) is generally considered sufficient for surgery planned for the same day. Prior-day injection has been shown to be technically feasible by adequately increasing the amount of radioactivity injected (up to 150 MBq), to compensate for physical decay in the intervening period between tracer injection and intraoperative detection.

Injection Procedure

The injection site/modality is another controversial issue. The most commonly used injection sites can be classified into two categories: deep (intratumoral and peritumoral) and superficial (intradermal, subdermal, subareolar, and periareolar) injection (Fig. 16.4).

Lymphatic circulation within solid tumors (including breast cancer) is generally grossly abnormal, disrupted, and inefficient. Thus, intratumoral administration requires the injection of high activities (up to 370 MBq) and large volumes (up to 4 mL). Injection of such large volumes can substantially increase interstitial pressure at the injection site, thus possibly altering the pattern of lymphatic drainage and forcing drainage routes different from those prevailing in baseline conditions. Furthermore, most of the injected radioactivity is retained at the injection site, often causing interference in SLN detection caused by the important "shine-through" effect during imaging as during intraoperative detection. Finally, slow drainage from the tumor can cause poor scintigraphic visualization of the lymphatic channels and possibly lead to failure of lymphoscintigraphic imaging and of intraoperative SLN identification. All the above considerations explain why, at present, most nuclear medicine centers prefer peritumoral or superficial injections rather than the originally proposed intratumoral route of administration.

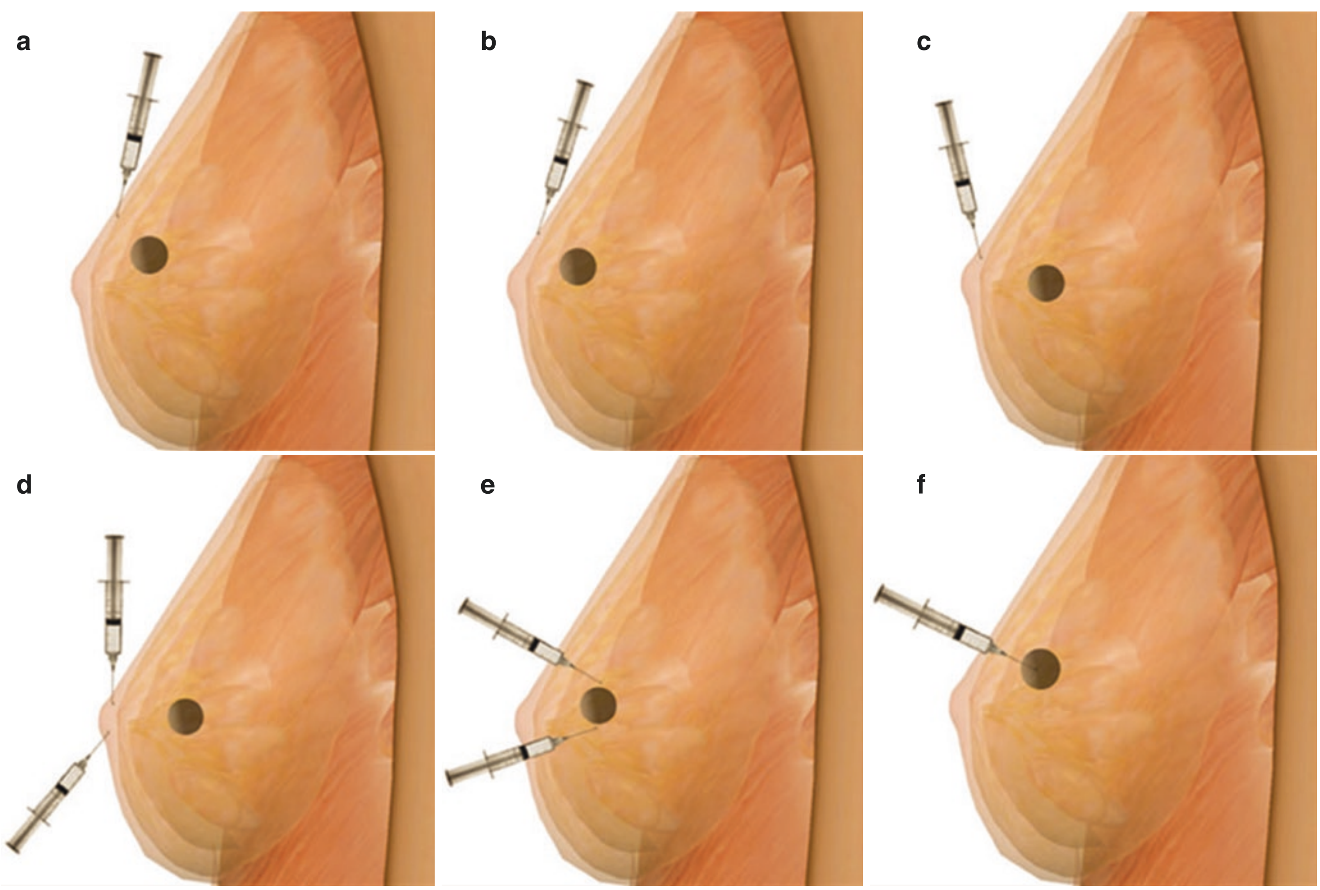


Fig. 16.4 Modalities of interstitial injection of the lymphatic mapping agent for SLN mapping in patients with breast cancer. Superficial injections (**a**–**d**) and deep injections (**e**, **f**): intradermal (**a**), subcutaneous (**b**), subareolar (**c**), periareolar (**d**), peritumoral (**e**), and intratumoral (**f**) (*reproduced with permission from: Giammarile F, Orsini F, Valdés Olmos RA, Vidal-Sicart S, Giuliano AE, Mariani G. Radioguided surgery for breast cancer. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1363–1400*)

Peritumoral injection, performed by depositing two aliquots on each side of the tumor, is considered the gold standard for accurate SLN detection, because the tracer is injected near the same lymph vessels draining the tumor and is able to reveal both extra-axillary and axillary drainage. However, this approach has been criticized especially in case of non-palpable and multicentric tumors. The use of peritumoral injections requires careful investigation of a patient's prior imaging and medical records, particularly if the tumor is non-palpable.

Intra-/subdermal administration is performed on the skin overlying the tumor, in such a way so as to produce a small wheal. More than one injection could be performed in adjacent sites. Periareolar administration is generally performed with two to four injections, each one at the edge of the areola at Sappey's plexus. The rationale of such administration stems from the fact that lymph (therefore also the radiocolloid) is drained from the intra-/subdermal space to the subcutaneous plexus, which is the merging point for the lymph originating from the underlying breast parenchyma. Superficial injection sites have numerous advantages, including simplicity, shorter time between injection and SLN identification, and increased radiotracer uptake in LNs, resulting in improved nodal identification rates. Nonetheless, it should be considered that superficial injection allows almost exclusive identification of axillary nodes.

Despite the discrepancies mentioned above, it is common experience that all injection modalities enable axillary SLN to be identified accurately, and satisfactory SLN detection rates have been reported for all injection approaches. Thus, if the goal of SLNM/SLNB is axillary staging only, superficial tracer injection (periareolar, subareolar, subdermal, intradermal) may be preferable to a deep injection (peritumoral, intratumoral), due to better and quicker visualization of axillary SLN.

Nevertheless, it should not be neglected that deep tracer injection results in improved detection of extra-axillary SLNs: after peritumoral administration, lymphoscintigraphy shows drainage to the internal mammary chain in 20–30% of the cases, while this fraction is much lower (<3%) after intra-/subdermal or periareolar administration. The combination of both injection techniques (deep and superficial) in the same patient may lead to an overall improvement in SLN detection.

Imaging Procedures

Lymphatic mapping enables to determine the number of lymph nodes that are on a direct drainage pathway from the tumor and thus to locate the SLNs. Preoperative imaging is strongly recommended due to variability in breast lymphatic drainage toward the axillary and extra-axillary LNs. Thus, preoperative lymphatic mapping has the potential to both improve accuracy (especially in extra-axillary LNs) and reduce morbidity relative to the use of handheld gamma probes alone [14]. Preoperative imaging also serves as quality control on the use of the appropriate tracer, failure of the injection, failure of the radiopharmaceutical, and management of the appropriate breast and axilla (injection of the proper side, left or right breast). Reasons not to use preoperative lymphoscintigraphy are logistical or because there is no definite evidence of a higher intraoperative success rate in the harvesting of axillary SLNs.

Although dynamic imaging acquired at a 1-min frame rate can provide information useful to SLN localization, this modality is not often used in SLN procedures for breast cancer, whereas it has a greater role in other malignancies such as cutaneous melanoma (see further below in this chapter). Thus, the standard technique for lymphatic mapping in patients with breast cancer relies on sequential planar images acquired with an adequate delay after interstitial injection of the radiopharmaceutical, complemented with SPECT/CT imaging—when indicated. Delay of acquisitions after radiotracer injection varies according to the radiopharmaceutical used (smaller particles migrate from the injection site faster than larger particles), the injection modality (speed of migration progressively increases passing from intratumoral to peritumoral, subcutaneous, and intra-/subdermal injection, respectively), and the patient's characteristics (lymphatic drainage can be slower in elderly or overweight patients). Lymphatic drainage and lymph node visualization is usually observed already 20–30 min after superficial injection, compared to 2–3 h following intratumoral injection. The amount of radiocolloid migrated to LNs represents about 1% of the injected activity after superficial administration, whereas it is about 0.1% after peritumoral administration.

A single-head or dual-head large field-of-view (FOV) gamma camera is generally used for imaging, using low-energy, high-resolution collimators and centering the energy window on the 140 keV photopeak of ^{99m}Tc (±5% or 10%).

Planar images are usually acquired at 15–30 min, then 2–4 h postinjection, and as needed thereafter up to 18–30 h (in case of 2-day protocol). At least two views are acquired, i.e., anterior and 45° anterior oblique (right or left depending on side of the tumor); a right or left lateral view is also recommended. The typical acquisition time is 3–5 min, and a 2 mm pixel size is recommended (or anyway the smallest pixel size available on the gamma camera), usually with 256 × 256 matrix size and zoom factor 1 (or, rarely, 128 × 128 matrix size with zoom factor 2).

Figure 16.5 shows different patterns of lymphatic drainage and SLN visualization obtained by planar lymphoscintigraphy. It should be noted that planar imaging alone does not provide the exact anatomical location of the detected SLNs. The crudest way to yield some anatomical correlates is to define the body contour by moving a ^{57}Co (or ^{99m}Tc) point source along the patient's body during image acquisition (Fig. 16.6). Alternatively, by placing a ^{57}Co flood source beneath the patient's body, a simultaneous emission and transmission image can be acquired where delineation of the

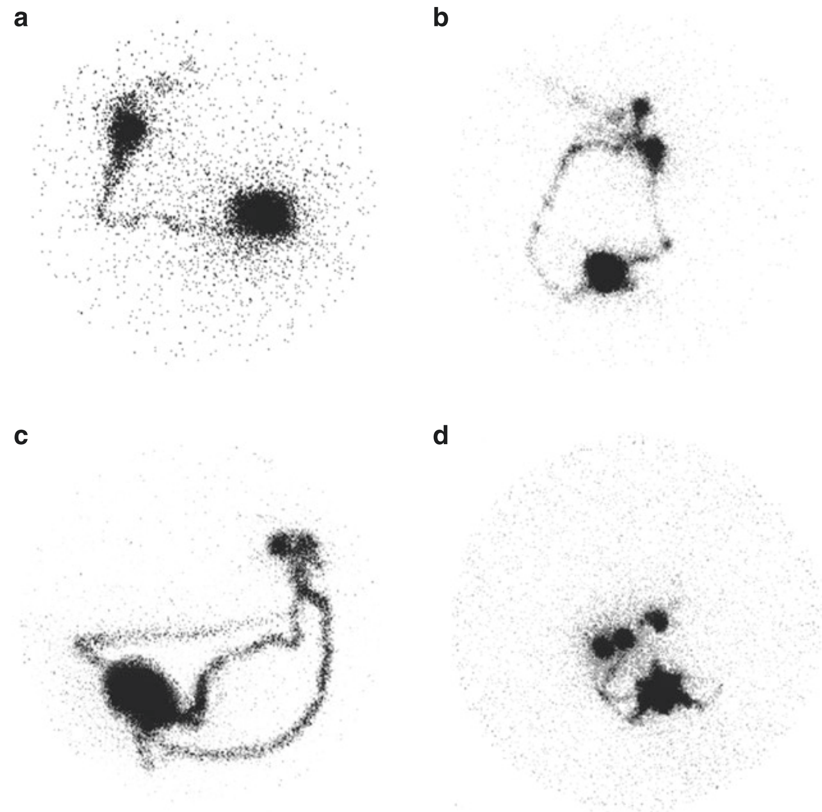


Fig. 16.5 Different patterns of lymphatic drainage visualized during lymphoscintigraphy acquired 30–60 min after intradermal injection of ^{99m}Tc-nanocolloidal albumin in patients with breast cancer. (**a**) Single lymphatic vessel leading to a single SLN, with faint visualization of higher-tier lymph nodes (right anterior oblique view). (**b**) Two separate lymphatic vessels widely diverging in their initial pathway and leading to two separate but adjacent SLNs, with faint visualization of higher-tier nodes (left anterior oblique view). (**c**) Three separate lymphatic vessels widely diverging in their initial pathway, leading to two separate but very close SLNs (left anterior oblique view). (**d**) Multiple lymphatic vessels leading to at least three separate SLNs (*reproduced with permission from: Mariani G, Moresco L, Viale G, Villa G, Bagnasco M, Canavese G, et al. Radioguided sentinel lymph node biopsy in breast cancer surgery. J Nucl Med. 2001;42:1198–215*)

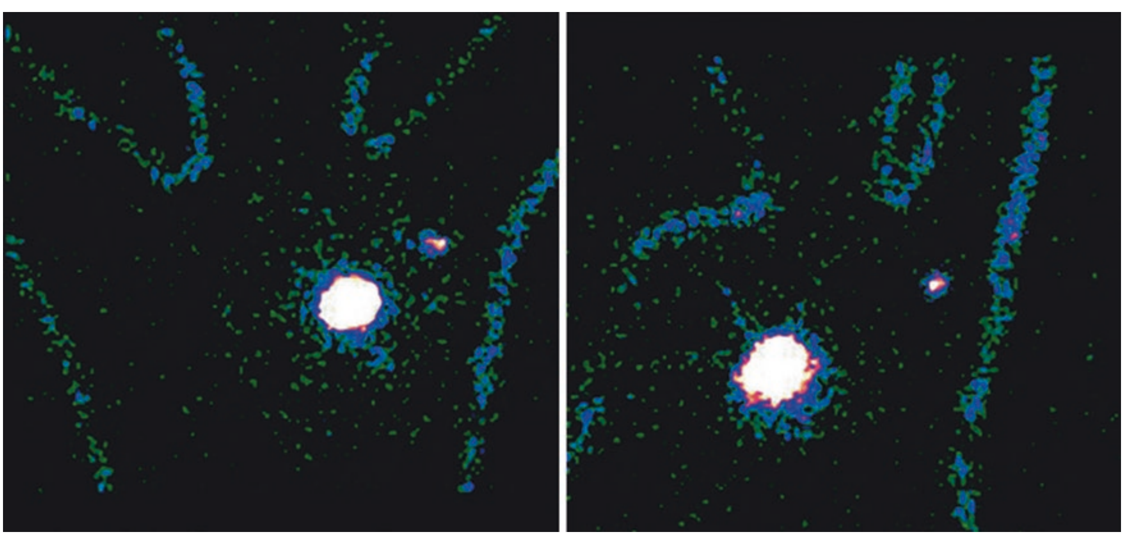


Fig. 16.6 Delineation of body contour obtained by moving a ^{57}Co point source along the body of the patient during acquisition of the planar images in a patient with cancer of the left breast; although appearing as a single large radioactive area in the breast, ^{99m}Tc-nanocolloidal albumin was injected at four spots periareorally. Images acquired in the anterior view (left panel) and in the left anterior oblique view (right panel) visualize migration of the radiocolloid to a single SLN in the left axilla (*reproduced with permission from: Giammarile F, Orsini F, Valdés Olmos RA, Vidal-Sicart S, Giuliano AE, Mariani G. Radioguided surgery for breast cancer. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1363–1400*)

body contour is based on attenuation of the ^{57}Co γ-rays passing through the patient's body (Fig. 16.7). By combining tomographic functional images registered with anatomic data from CT, SPECT/CT imaging provides better contrast and resolution than planar imaging and has the possibility to correct for attenuation and scatter (Fig. 16.8). In particular, fused SPECT/CT imaging considerably improves the topographic SLN localization within an anatomical landscape, thus providing a valuable road map to the surgeon. It has also been shown that SPECT/CT can detect in a substantial number of patients additional SLNs not visualized on planar images. In the majority of cases, the anatomic information provided by the fused SPECT/CT images provides useful preoperative complementary information resulting in better anatomical SLN localization (Fig. 16.9), reduced surgical time, and greater confidence of the surgeons with the technique. However, the added value of SPECT/CT imaging seems to be limited to a small fraction of the breast cancer patients undergoing SLNB and is associated with additional costs and requires extra time for imaging. For these reasons, specific indications for the use of SPECT/CT imaging should be defined, so that the majority of patients who will not benefit from this imaging technique are spared unnecessary costs and inconvenience.

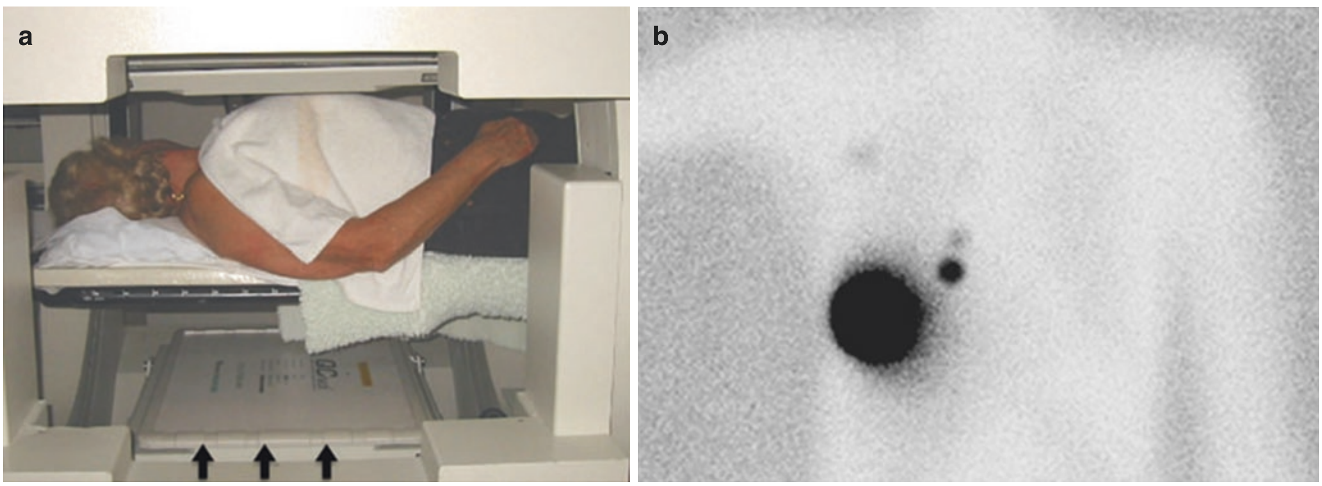


Fig. 16.7 Delineation of the body contour during acquisition of planar lymphoscintigraphy in a patient with cancer of the right breast. (**a**) The ^{57}Co flood source is placed opposite to the gamma camera head (black arrows in the lower part of the panel) during acquisition of the anterior planar image. (**b**) Lymphoscintigraphy obtained after intratumoral injection of ^{99m}Tc-nanocolloidal albumin depicts lymphatic drainage to SLNs in the right axilla and right internal mammary chain (*reproduced with permission from: Giammarile F, Orsini F, Valdés Olmos RA, Vidal-Sicart S, Giuliano AE, Mariani G. Radioguided surgery for breast cancer. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1363–1400*)

Fig. 16.8 Left panel: geometry of the gamma camera detectors for SPECT/CT acquisition in the same patient as in Fig. 16.7. Right panel: 3D surface volume rendering of the SPECT/CT acquisition clearly visualizing radiocolloid drainage to one right parasternal SLN (with a higher-tier lymph node) and to one right axillary SLN (*reproduced with permission from: Giammarile F, Orsini F, Valdés Olmos RA, Vidal-Sicart S, Giuliano AE, Mariani G. Radioguided surgery for breast cancer. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1363–1400*)

Key Learning Points

- Radiopharmaceuticals employed for lymphatic mapping in patients with breast cancer include ^{99m}Tc-radiocolloids with variable particle size (that are retained in lymph nodes because of nonspecific phagocytosis by macrophages) and ^{99m}Tc-tilmanocept (which is retained in lymph nodes because of binding to the CD206 antigen expressed by macrophages and dendritic cells).
- Particle size of radiocolloids is inversely related to the rate of drainage from the site of interstitial administration.
- Although the optimal modality of tracer injection is still debated, superficial injection (intra-/subdermal over the skin projection of the tumor, subareolar, or periareolar) is currently the preferred modality in most centers.
- Preoperative lymphoscintigraphy is an integral component of the whole lymphatic mapping procedure.
- Planar scintigraphy using variable angle views is generally sufficient for adequate visualization of axillary lymph nodes draining the breast.
- In patients with breast cancer, SPECT/CT imaging has a certain added value especially when planar scintigraphy fails to adequately visualize the lymphatic drainage pathways.
- When acquiring planar scintigraphy only, definition of the body contour yields useful anatomical correlates for the lymph nodes visualized during lymphoscintigraphy.

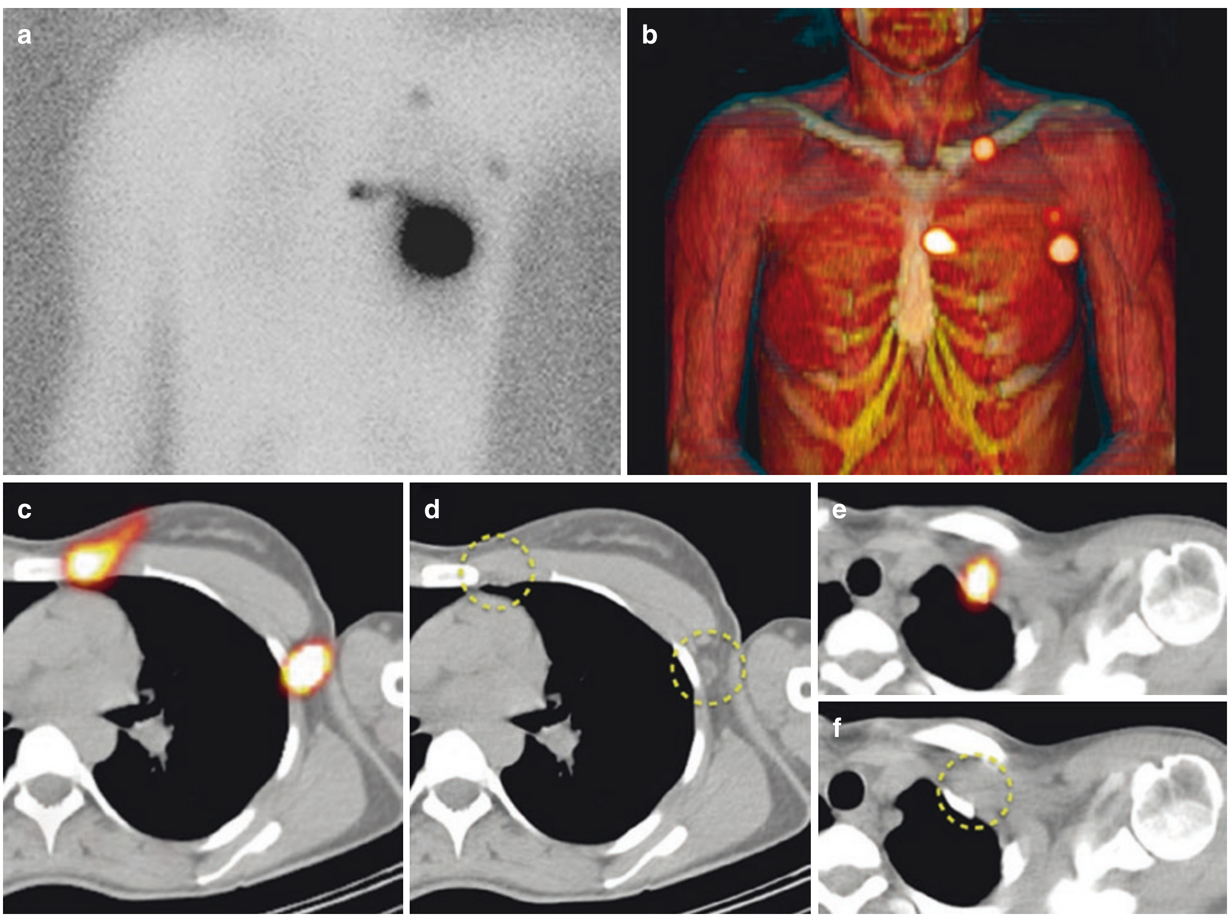


Fig. 16.9 Anatomical SLN localization obtained with SPECT/CT in a patient with cancer of the left breast. Following intratumoral injection of ^{99m}Tc-nanocolloidal albumin, planar imaging (**a**) visualizes lymphatic drainage to SLNs in the left axilla, periclavicular area, and internal mammary chain. Fused SPECT/CT imaging displayed with 3D surface volume rendering (**b**) better localizes the anatomical correlates of SLNs visualized on planar imaging. Transaxial fused SPECT/CT sections localize such SLNs in the second intercostal space and level I of the left axilla (**c**), as well as behind the left clavicle (**e**). The CT component of the SPECT/CT acquisition (**d** and **f**) provides purely anatomical evaluation of the radioactive lymph nodes, as indicated by dashed yellow circles (*reproduced with permission from: Giammarile F, Orsini F, Valdés Olmos RA, Vidal-Sicart S, Giuliano AE, Mariani G. Radioguided surgery for breast cancer. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1363–1400*)

16.2.3.2 Procedures in the Surgical Suite

Blue-Dye SLN Localization

There is general agreement that combined administration of radiocolloid and blue dye using either superficial injection or deep injection enhances SLN detection. The blue dye can be injected around the primary tumor 10–20 min prior to surgery in a volume of 2–5 mL. Within 5–15 min the SLNs are colored. Washout is evident after approximately 45 min.

Disadvantages of using blue dyes are as follows: (1) impossibility to evaluate extra-axillary nodes, (2) temporary blue tattooing of the skin or areola (for patients undergoing breast-conserving surgery), and (3) induction of anaphylactic reactions (that require resuscitation in 0.5–1.0% of patients and that contraindicate its use in pregnant women) [17].

Radioguided Surgery

Intraoperative SLN detection is usually guided by the use of a handheld gamma probe that provides both a readout count rate and an audible signal which is proportional to the count rate. Such probes are designed specifically for intraoperative use, and are therefore be able to detect the SLN from the skin surface as well as within the exposed surgical cavity.

In principle, SLNB requires the removal of all SLNs receiving direct lymphatic drainage from the site of the primary tumor. In practice, this is not always achieved. In cases with multiple radiolabeled LNs, it is often difficult to distinguish between true SLNs and second-tier LNs. The issue of how many LNs should be harvested when multiple radioactive LNs are found is still debated. In this regard, while removing too few LNs may lead to miss potential metastases in regional LNs, indiscriminate removal of all radioactive axillary LNs may cause morbidity similar to that experienced after conventional ALND (in addition to the unnecessarily increased burden for histopathologic analysis). Several operational definitions of the SLN have evolved over time in order to decide exactly which LNs should be removed to maximize the likelihood of locating the "true" biologic SLN and to minimize unnecessary removal of non-SLNs. Some authors base SLN identification on the absolute number of counts/sec recorded for the presumed nodes, while others consider the ratio of the "in vivo" or "ex vivo" radioactive counts in the SLNs relative to background or to neighboring non-SLNs. Empiric thresholds are widely employed, corresponding to (1) 10% or 20% of the count rate in the first LN removed (which is usually the most radioactive) or (2) at least tenfold the background count, taken at a location remote from the injection site.

When a hot SLN has been removed, the surgical bed should be checked to confirm its removal and to evaluate remaining activity. Finally, to minimize false-negative results, the open axilla should be palpated and suspicious lymph nodes harvested, even if these are neither hot nor blue. The use of dedicated small field-of-view gamma cameras facilitates assessment of complete removal of SLN(s).

SLN Non-Visualization or Failed Intraoperative Detection

In approximately 1–2% of the patients, SLNs will not be detected preoperatively or intraoperatively, and the status of axillary LNs cannot be determined with this approach. Old age, obesity, tumor location other than in the upper outer quadrant, and non-visualization of SLNs on preoperative lymphoscintigraphy may be associated with failed SLN localization. Deeply located SLNs are difficult to detect intraoperatively because of tissue attenuation; furthermore, the large amount of radioactivity retained at the injection site may cause adjacent SLNs to be hidden during imaging and during the intraoperative search because of the shine-through effect.

Patients who have been submitted to prior breast surgery or radiation may demonstrate lymphatic drainage to LNs in locations not typically seen in patients without a history of prior surgery. The lymphatic duct to the original SLN may be obstructed by tumor growth, or the original SLN may be entirely replaced by metastatic disease. As a consequence, lymphatic drainage may be either diverted to a non-SLN, or no lymph nodes may be visualized.

It is important to note that when a SLN is not detected intraoperatively, this corresponds to a failure of the method and not to a false-negative case (i.e., when axillary metastatic relapse is observed despite a negative SLNB).

The majority of patients with preoperative lymphoscintigraphic SLN non-visualization will have at least one SLN detected intraoperatively, either by a gamma probe alone or by a gamma probe combined with blue dye. While logistically difficult in most centers, a second radiotracer injection, perhaps with a different injection site/modality, may be useful to visualize previously non-visualized SLNs. In many patients in whom planar lymphoscintigraphy does not result in SLN visualization, SPECT/CT imaging does result in SLN detection.

Although there is no definitive consensus on what to do if a SLN cannot be visualized, the current standards of care recommend ALND when intraoperative SLN identification is not achieved.

Current recommendations regarding the use of SLNB in patients with breast cancer as jointly defined by the EANM and the SNMMI in their practice guidelines are summarized in Table 16.1 [14].

Table 16.1 Recommendations regarding use of sentinel lymph node biopsy in patients with breast cancer according to the joint EANM and SNMMI practice guidelines

Clinical condition	Use of sentinel lymph node biopsy
T1 or T2 tumor	Established
Older age	Established
Obesity	Established
Male breast cancer	Established
DCIS[a] with mastectomy	Established
Before neoadjuvant systemic therapy	Established
Multicentric/multifocal tumor	Controversial
T3 or T4 tumor	Controversial
DCIS[a] without mastectomy	Controversial (except for DCIS with suspected or proven microinvasion)
Suspicious, palpable axillary lymph nodes	Controversial
After neoadjuvant systemic therapy	Controversial
Pregnancy	Controversial
Evaluation of internal mammary lymph nodes	Controversial
Prior diagnostic/excisional breast biopsy	Controversial
Prior axillary surgery	Controversial
Prior nononcologic breast surgery	Controversial
Inflammatory breast cancer	Not recommended

Controversial indications are those for which SLN biopsy is not universally accepted or for which the evidence behind the practice is limited or entirely missing

Modified from Ref. [14]

[a]*DCIS* ductal carcinoma in situ

Key Learning Points

- Radioguidance provided by preoperative lymphoscintigraphy and by intraoperative gamma probe counting can be complemented by visual guidance based on administration of blue dye.
- Biopsy of the sentinel lymph node(s) is guided intraoperatively by a handheld gamma probe providing an instantaneous count rate.
- Radiocolloids with small particle size might not be retained efficiently in the first (sentinel) lymph node encountered along a certain pathway of lymphatic drainage, thus possibly visualizing multiple lymph nodes along that pathway.
- Although the issue is still debated, different parameters can be considered to decide which lymph nodes should be removed to maximize the likelihood of harvesting the "true" sentinel lymph node for analysis.
- In about 1–2% of patients with breast cancer, the sentinel lymph node cannot be detected on preoperative lymphoscintigraphy or intraoperatively with gamma probe guidance.
- Factors associated with failure of radioguided sentinel lymph node biopsy include old age, obesity, tumor location, and non-visualization on preoperative lymphoscintigraphy.

16.2.4 Radioguided Occult Lesion Localization (ROLL) with ^{99m}Tc-MAA

Hook-wire localization of non-palpable lesions has been for many years the most widely used preoperative technique. Although this is a reasonably effective technique, it involves some disadvantages. First, the entry site of the wire is often not at the ideal location for surgical incision at the time of surgery. This may lead to additional unnecessary dissection and suboptimal cosmetic results. The most important disadvantage, however, is the inaccuracy of localizing the target lesion percutaneously and during dissection. This results in non-negligible rates of reoperation after histologic demonstration of tumor involvement of the margins of the surgical specimen.

Intraoperative ultrasound imaging without preoperative wire localization has also been used to map excision of non-palpable breast lesions; however, this technique has limitations, as it is feasible only in patients whose breast lesion is detectable at ultrasound imaging.

The "radioguided occult lesion localization" (ROLL) approach has gained popularity for non-palpable tumor lesions, including breast cancer [18]. ROLL involves the intralesional injection of a small amount of radioactive tracer that does not migrate from the site of interstitial injection, typically ^{99m}Tc-MAA. Injection is performed on the same day or on the day before surgery, under mammographic or ultrasound guidance, with injected activities ranging from 2 to 15 MBq. Surgeons identify the lesion intraoperatively as a hot spot by using a handheld gamma probe, which allows accurate localization and removal of the lesion with minimal excision of healthy tissue.

ROLL is a well-tolerated and feasible technique for localizing early-stage breast cancer during breast-conserving surgery and is a suitable replacement for wire-guided localization. Reported advantages of the ROLL technique include (1) easy and precise intraoperative localization of the breast lesion; (2) complete lesion resection, with free margins and reduced needs for second operations in most of the cases; (3) improved centering of the lesion within the surgical specimen; and (4) possibility to choose the most suitable surgical approach (skin incision) minimizing surgical aggressiveness and optimizing the cosmetic results of surgery.

Key Learning Points

- Conventional techniques for localization of non-palpable breast lesions are affected by definite drawbacks that limit their accuracy and/or result in suboptimal cosmetic outcome.
- Radioguided occult lesion localization based on intralesional injection of radiolabeled particles that do not appreciably move from the injection site (such as ^{99m}Tc-MAA) has gained popularity because of its high feasibility, overall accuracy, and optimal cosmetic results.

16.2.4.1 ROLL with Radioactive Seeds

Alternatives to hook-wire localization of occult breast lesions include carbon trace as well as the use of sealed radioactive seeds. The seeds used to this purpose are similar to those used for brachytherapy in patients with prostate cancer, each seed consisting of a 4.5 × 0.8 mm titanium capsule containing a tiny ceramic cylinder enriched with ^{125}I. Iodine-125 has a long decay time (half-life of 59.4 days) and emits low-energy γ-photons (27 keV). The radioactive seed is placed in the center of the breast lesion using an 18 G needle fixed in a needle holder under mammographic or ultrasound guidance. During surgery, excision of the lesion is guided by using a handheld gamma probe.

It has been shown that radioguided seed localization in non-palpable breast lesions is at least equivalent to the hook-wire technique in terms of ease of procedure, reliable resection of the target lesion, volume of breast tissue excised, and histologically negative tissue margins; furthermore, the procedure can be combined with the standard technique of radioguided SLNM and SLNB [19].

16.2.4.2 Sentinel Node and Occult Lesion Localization (SNOLL)

Different techniques have been described to identify the SLNs in combination with ROLL, the so-called “sentinel node and occult lesion localization” (SNOLL) procedure. In particular, intratumoral injection of ^{99m}Tc-MAA for ROLL may be associated with subdermal injection of ^{99m}Tc-nanocolloid for radioguided SLNM and SLNB. Another possibility is to use a single intratumoral injection for both ROLL and SNOLL in the same session. In fact, following interstitial injection of even small-particle radiocolloids, only a minor fraction of the injected activity migrates through the lymphatic channels, thus making it possible to image the pathways of lymph drainage and to guide intraoperatively for the radioguided search of SLN(s). Most of the injected activity actually remains retained at the site of interstitial injection, thus making it possible to perform a ROLL procedure using the same handheld gamma probe used for radioguided SLNB.

Therefore, as a single combined procedure for intraoperative localization of the breast lesion and for SLNB, the SNOLL technique may improve the entire surgical procedure. The majority of the studies published so far show a high percentage of successful tumor resection and intraoperative SLN localization, with minimal failure rates [20].

When using the radioactive seeds for ROLL, the energy window of the handheld gamma probe can be switched between the 27 keV energy peak of the ^{125}I source and the 140 keV energy peak ^{99m}Tc, thus permitting to discriminate γ-ray emissions of the two radionuclides.

Key Learning Points

- Intralesional placement of radioactive seeds in the form of tiny sealed sources containing ^{125}I is an alternative to intralesional injection of ^{99m}Tc-MAA for radioguided occult lesion localization.
- The procedure of radioguided sentinel lymph node biopsy can be combined in the same patient with the procedure of radioguided occult lesion localization.
- Different combinations of radioactive agents have been proposed for simultaneous radioguided sentinel lymph node biopsy and radioguided occult lesion localization.

16.2.5 Added Value of Intraoperative Portable Gamma Cameras

Recently, several types of portable or handheld mini gamma cameras have become available for clinical practice; while some of these portable gamma cameras are not specifically designed for radioguided surgery, other models are focused on different applications of SLNB [21].

Appropriate perioperative use of a portable gamma camera enhances the reliability of the entire procedure of radioguided surgery, by providing high-resolution imaging of the surgical field. The use of a perioperative imaging device implies the possibility to better plan the surgical approach, to localize surgical targets in complex anatomical areas just before making the surgical incision, to monitor the lymphatic basin before and after removal of the hot lymph nodes, and, above all, to verify completeness of SLN excision.

16.2.6 Future Perspectives

Some of the competitive/complementary modalities that are emerging for different applications of image-guided surgery are particularly interesting, such as those based on indocyanine green fluorescence, contrast-enhanced ultrasound with microbubbles, and superparamagnetic iron oxide nanoparticles.

Although such novel approaches have clinical potential, they are generally burdened by relatively high failure rates and/or false-negative results and at present cannot challenge the existing standard procedures [22].

Particular attention is also being paid to the development of hybrid imaging agents that in principle combine the advantages of a multimodality approach, such as radioactive *and* fluorescent agents for SLNM (Fig. 16.10). Important advances in this technology lead to the development of hybrid intraoperative devices capable to detect simultaneously both an optical signal (i.e., the fluorescent guide) and a radioactive signal (i.e., intraoperative radioguidance).

Key Learning Points

- Portable small field-of-view gamma cameras can be employed intraoperatively for high-resolution imaging of the surgical field.
- Intraoperative imaging per se does not guide search for the sentinel lymph node(s), but rather it is employed for assessing completeness of radioguided sentinel lymph node excision.
- Newer and/or alternative approaches to sentinel lymph node biopsy are based on indocyanine green fluorescence, contrast-enhanced ultrasound with microbubbles, and superparamagnetic iron oxide nanoparticles.
- Hybrid imaging agents (e.g., radioactive *and* fluorescent) that combine the advantages of multimodality intraoperative detection are currently under clinical validation.

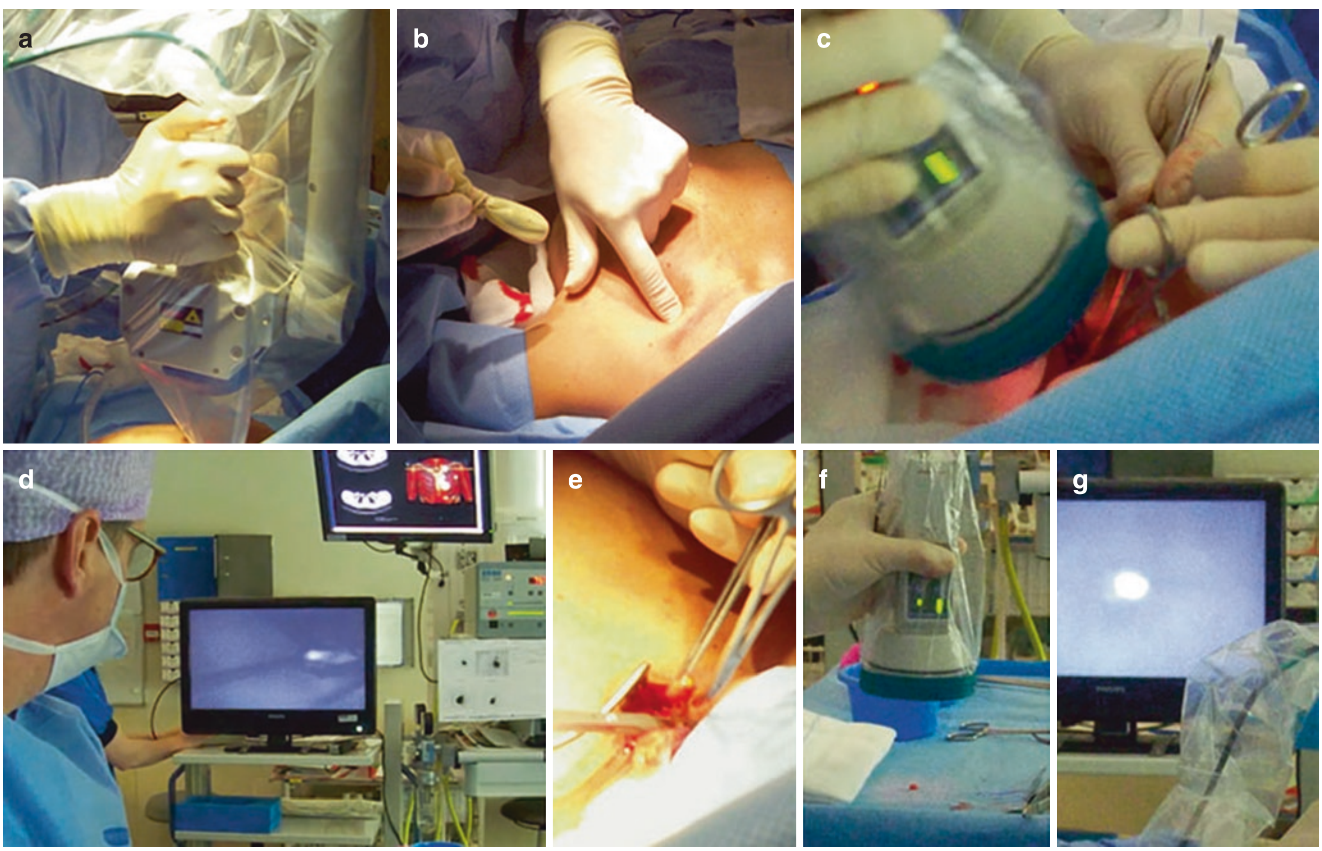


Fig. 16.10 Resection of an infraclavicular SLN in a patient with high-risk breast cancer using the hybrid tracer ICG-^{99m}Tc-nanocolloidal albumin. The SLN is first located on the skin projection using a portable gamma camera (**a**) and a handheld gamma probe (**b**). A portable near-infrared camera (**c**) is then used to depict the fluorescence signal on the screen (**d**). This enables to remove the SLN under combined radioguidance and optical guidance (**e**). Ex vivo control of the fluorescence signal is finally performed (**f** and **g**) (*reproduced with permission from: Giammarile F, Orsini F, Valdés Olmos RA, Vidal-Sicart S, Giuliano AE, Mariani G. Radioguided surgery for breast cancer. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1363–1400*)

16.3 Radioguided Sentinel Lymph Node Biopsy in Cutaneous Melanoma

16.3.1 The Clinical Problem

The incidence of melanoma is growing worldwide. Currently, the estimated incidence in Europe is 9 out of 100,000 inhabitants, with a mortality of 2.3/100,000. Every year 4% more people are diagnosed with the disease, thus making melanoma the cancer with the greatest increase in incidence.

Regional lymph nodes are frequently the first site of metastasis before systemic spreading of the disease. The presence or absence of lymph node metastases, even in the form of micrometastasis, is an important prognostic factor in early-stage melanoma patients [23]. Occult metastases in the regional nodes (clinically non-palpable and difficult to identify also with ultrasonography) are found in nearly 20% of patients who present with melanoma with Breslow thickness >1 mm. Therefore, accurate histopathological evaluation is required for early detection of metastasis. In the past, regional lymphadenectomy was routinely performed to stage clinically node-negative melanoma patients. However, this procedure very often constituted a clear, unnecessary overtreatment since the majority of patient (usually >75%) with <4 mm Breslow thickness melanomas were found not to have lymph node metastases [24, 25]. Therefore, de novo lymphadenectomy exposed many patients to unnecessary immediate and long-term surgical complications, such as possible wound infection and delayed wound healing, lymphedema, hematoma, seroma, pain, etc. Due to the above considerations and to recent developments of cancer surgeries toward less aggressive procedures, over the last 20 years, lymphadenectomy has gradually been replaced with SLNB, a minimally invasive method based on the concept of "orderly" progression of metastatic cells through the lymphatic system that allows accurate assessment of the lymph node status [26] without the burden of complications associated with lymphadenectomy [27].

16.3.2 Lymphatic System of the Skin

The skin has a very dense network of lymphatic capillaries and vessels. The paths followed by the collecting vessels on their way to the lymphatic basins vary from patient to patient and from a skin location to another. These pathways can sometimes be very complex and unexpected. In general, lymphatic vessels converge to form larger vessels or multiple vessels (as, e.g., in the upper thigh). These collecting vessels pass through the subcutaneous fat layer and penetrate into the deep fascia after reaching the lymph node basin.

The pattern of lymphatic drainage from the skin has been investigated with various modalities over the past centuries, mostly with postmortem studies. In the nineteenth century, Sappey defined lines that passed down the midline front and back, as also along a horizontal line around the waist from the umbilicus to the level of the second lumbar vertebra, stating that lymph channels did not cross these lines. This concept (the so-called Sappey's rule) was universally accepted over the next almost 100 years. After the introduction of clinical lymphoscintigraphy in the mid-twentieth century, it was frequently observed that Sappey's rules did not always prove to be correct (Fig. 16.11). In particular, there were "zones of ambiguity" on each side of Sappey's lines where the direction of lymphatic drainage was impossible to predict. Therefore, clinicians began to use lymphoscintigraphy in patients with melanomas located in those ambiguous areas to identify lymphatic basins potentially at risk to receive metastatic tumor cells.

Since lymphatic cutaneous drainage is highly variable, lymphoscintigraphy is currently a mandatory component of the radioguided SLNB procedure. The reproducibility of this technique is very high, ranging from 84% to 96% in several studies.

The predictability of lymphatic drainage in cutaneous melanoma depends on the location of the primary lesions, being approximately 98% in the lower limbs, 88% in the upper extremities, 56% in the anterior thorax, and 39% in the posterior trunk. Lymphatic drainage is almost completely unpredictable in the head and neck region. Nevertheless, learning the physiologic and "lymphoscintigraphic" drainage patterns constitutes at least a first estimation of the most likely draining basins, depending on the location of the primary tumor.

Key Learning Points

- In patients with cutaneous melanoma, sentinel lymph node biopsy has replaced de novo lymphadenectomy, which exposes patients to unnecessary immediate and long-term surgical complications and turns out to be futile surgery in >75% of patients with <4 mm Breslow thickness melanomas and a clinically negative lymph node basin.
- Widespread use of lymphoscintigraphy has demonstrated failure of the so-called Sappey's rule to accurately predict the pathway of lymphatic drainage for melanomas located in ambiguous areas around Sappey's lines (midline in the front and back and horizontal line around the waist).
- High intersubject variability in the patterns of lymphatic cutaneous drainage makes the use of preoperative lymphoscintigraphy a mandatory component of radioguided sentinel lymph node biopsy in patients with cutaneous melanoma.

16.3.3 Tracer Injection

Usually the radiotracer is administered intradermally with four or more aliquots (depending on the anatomic site) injected around the primary melanoma or the excisional biopsy site. According to the EANM guidelines [28], after

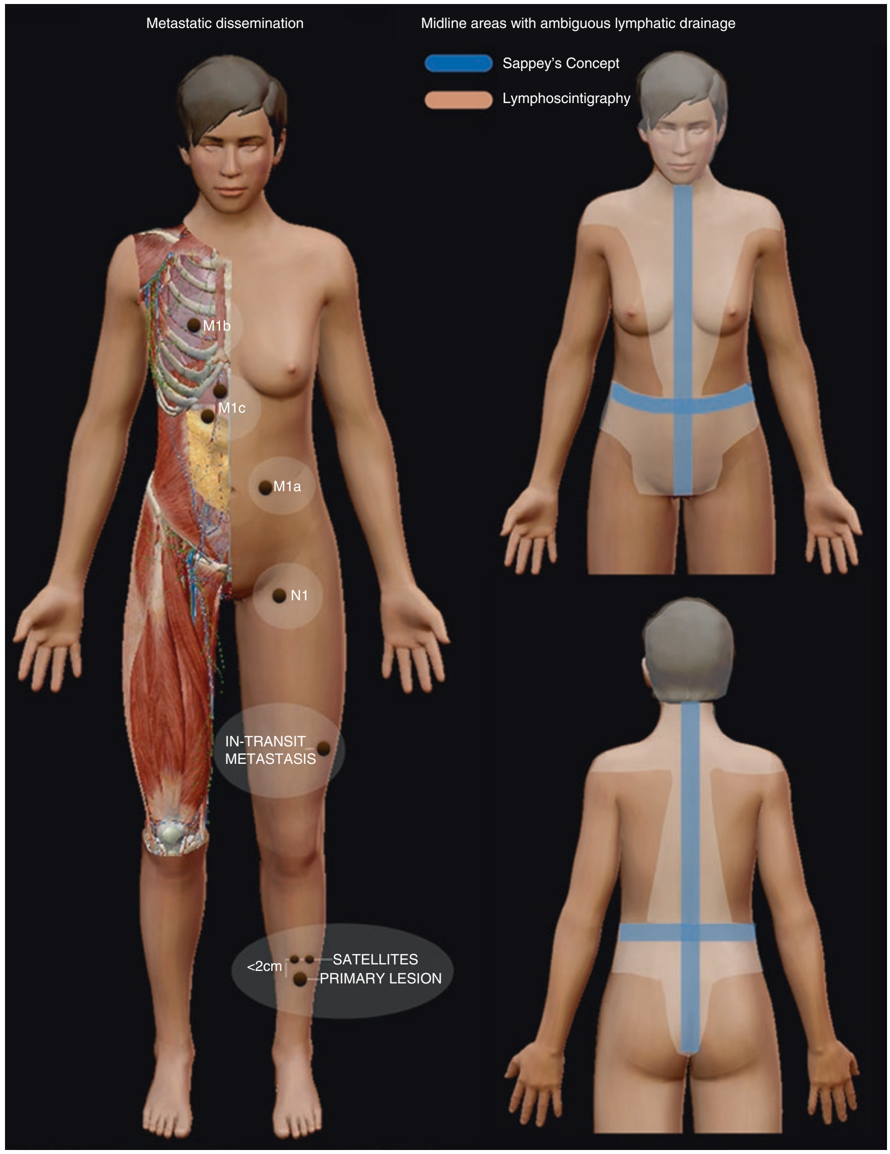


Fig. 16.11 On the left, metastatic dissemination in melanoma: N1, metastasis in draining lymph node basin; M1a, distant skin, subcutaneous, or nodal metastases; M1b, lung metastases; and M1c, all other visceral metastases. On the right, midline areas with ambiguous lymphatic drainage according to the classical skin watershed Sappey's concept determined postmortem (blue) and its actual extension according to recent lymphoscintigraphy findings (light brown) (*reproduced with permission from: Vidal-Sicart S, Orsini F, Giammarile F, Mariani G, Valdés Olmos RA. Radioguided surgery for malignant melanoma. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1401–32*)

injection into the dermis (within 1 cm from the melanoma or the excisional biopsy scar), the volume of radiotracer should raise a wheal. To avoid contamination of the patient's skin during the injections, the needle must be inserted in a tangent direction relative to the skin surface.

The radiotracer may be injected the day before surgery or on the same day of surgery (2-day or 1-day protocol, respectively). The injected activity varies from 5 MBq up to 120 MBq depending on the time elapsed between lymphoscintigraphy and surgery. The injected volume must be small (0.1–0.2 mL per aliquot) to avoid lymphatic collapse caused by sudden rise of interstitial pressure.

16.3.4 Lymphoscintigraphy

After injection, lymphatic imaging is performed to ascertain adequate drainage and uptake in the SLNs. Lymphoscintigraphy reflects the actual lymphatic drainage in that particular patient and enables to visualize the lymphatic basin at risk of metastases draining from the specific region of the skin where the melanoma is located [29].

All possible drainage regions must be covered during image acquisition. In the case of cutaneous melanoma, dynamic lymphoscintigraphy is crucial to identify regional lymphatic basins and to distinguish the true SLN from higher-echelon, non-SLNs that can be visualized along the same lymphatic duct. After the dynamic series, static early and delayed images are acquired, 30 min and 2 h postinjection, respectively (Fig. 16.12). When using a lymphatic mapping agent with fast migration, such as ^{99m}Tc-tilmanocept, SLNs can be clearly visualized as early as 15–20 min postinjection (Fig. 16.13). Finally, the site of each SLN visualized during lymphoscintigraphy must be marked on the skin with the aid of a ^{57}Co or ^{99m}Tc point source. The study is completed by performing external counting with a handheld gamma probe

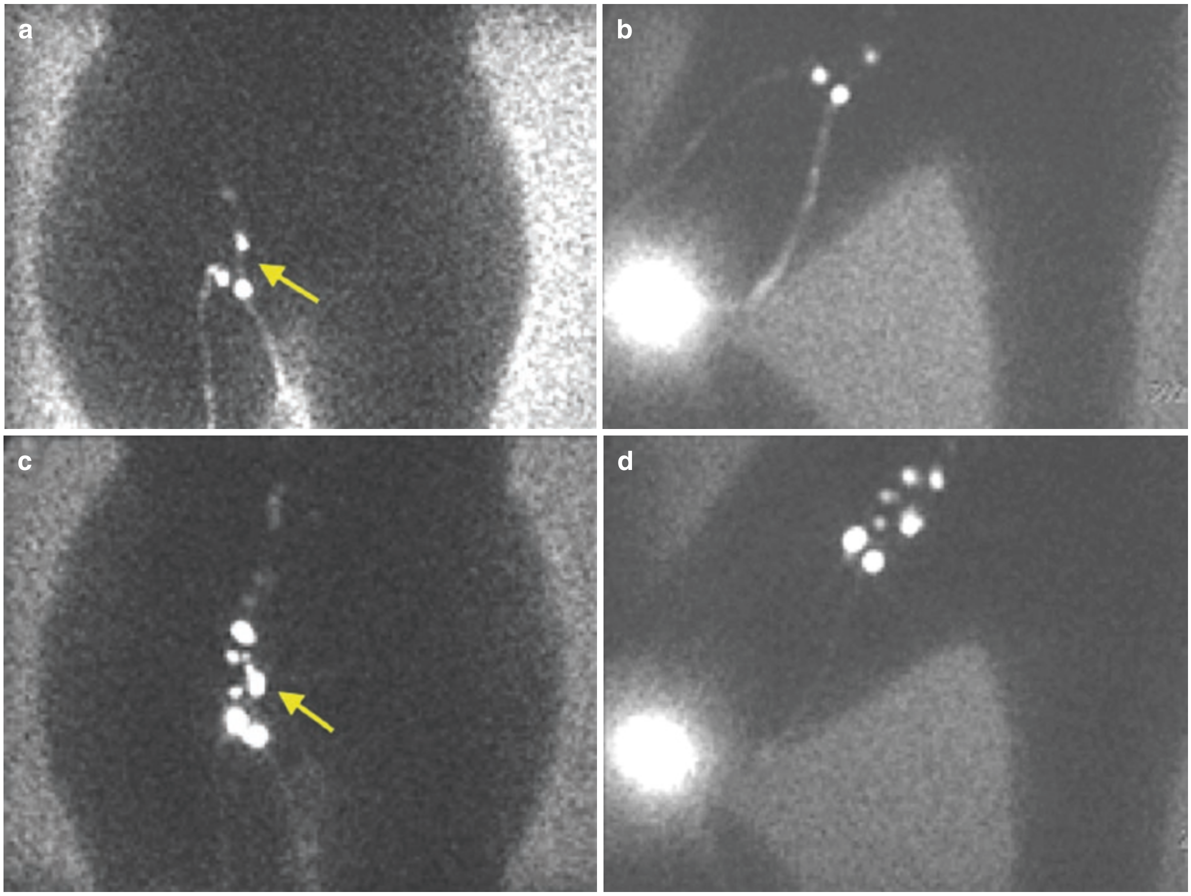


Fig. 16.12 Lymphoscintigraphy obtained in a patient with melanoma of the right lower thigh at sequential times (30 min and about 2 h, respectively) after perilesional injection of ^{99m}Tc-nanocolloidal albumin. Early (**a** and **b**) and delayed (**c** and **d**) planar images in anterior (left panel) and oblique views (right panels). In the early images, two well-depicted lymphatic channels directly connect the injection site with SLNs in right groin. The yellow arrow in (**a**) points to a faint lymphatic channel cranial to one of the two lower SLNs, leading to an additional lymph node, which could be a second-echelon node, but also a real SLN if this channel passes below the first depicted SLN directly from the injection site. The yellow arrow in (**c**) indicates high uptake in the aforementioned lymph node, as well as in other nodes upstream. Second-echelon nodes are more frequently observed in the groin than in other parts of the body, due to the particularly high velocity of lymph flow that occurs in the lower limb. In these cases, acquiring only delayed images would not be accurate, because it is difficult to distinguish between the real SLNs and second-tier lymph nodes (*reproduced with permission from: Vidal-Sicart S, Orsini F, Giammarile F, Mariani G, Valdés Olmos RA. Radioguided surgery for malignant melanoma. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1401–32*)

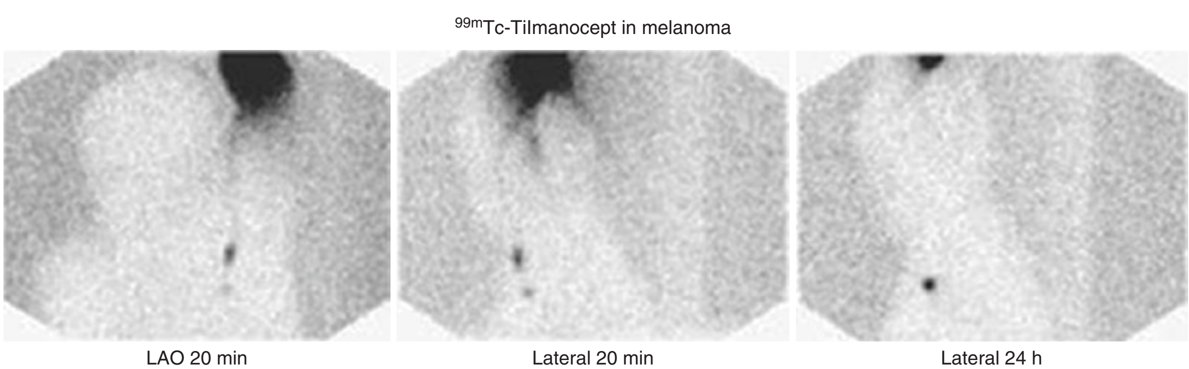


Fig. 16.13 Lymphoscintigraphy with ^{99m}Tc-tilmanocept obtained in a patient with melanoma of the left forearm. Planar images acquired with the left arm raised, site of injection remaining visible on the top of each image. A single axillary lymph node was visualized 20 min postinjection, along with some activity in the draining lymphatic pathway. Radioactivity in the SLN visualized at 20 min was well retained at 24 h, and the SLN to background count ratio was >30:1 intraoperatively (*modified from: Sondak VK, King DW, Zager JS, Schneebaum S, Kim J, Leong SP, et al. Combined analysis of phase III trials evaluating [^{99m}Tc] tilmanocept and vital blue dye for identification of sentinel lymph nodes in clinically node-negative cutaneous melanoma. Ann Surg Oncol. 2013;20:680–8*)

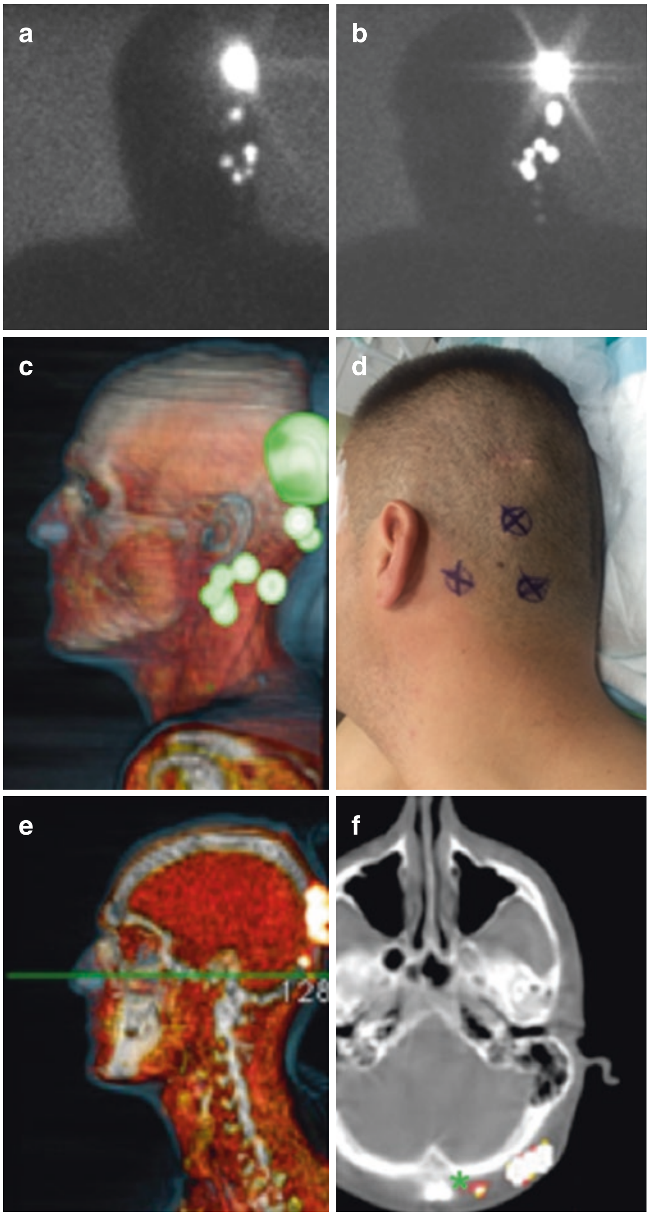


Fig. 16.14 Lymphoscintigraphy obtained after pericicatricial injection of ^{99m}Tc-nanocolloidal albumin in a patient operated because of melanoma in the left posterior parietal scalp. (**a**) Anterior and (**b**) left oblique planar views show several hot spots in the occipital and left cervical area. (**c**) 3D surface volume rendering after SPECT/CT acquisition. (**d**) Skin marks in the areas where SLNs were considered to be. (**e**) Sagittal fused SPECT/CT slice corresponding to the focus of uptake in the occipital area. (**f**) Transaxial fused SPECT/CT slice showing two separate foci of radiocolloid uptake in this area. The focus with faint uptake indicated by the green asterisk was a very tiny SLN (3 mm) that was positive for metastasis. In this case SPECT/CT imaging constitutes an added value over planar imaging alone that depicted only one hot spot associated with a large-sized SLN in the occipital area, masking activity in the adjacent tiny SLN (*reproduced with permission from: Vidal-Sicart S, Orsini F, Giammarile F, Mariani G, Valdés Olmos RA. Radioguided surgery for malignant melanoma. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1401–32*)

on the lymphatic basin visualized during lymphoscintigraphy, in order to confirm the exact projection on the skin surface of the SLN location [30, 31].

By depicting the lymph node stations at risk for metastasis, lymphoscintigraphy constitutes a lymphatic road map for surgeons. SPECT/CT added to planar lymphatic mapping allows a more accurate anatomical localization of SLNs and frequently detects SLNs not seen on planar imaging (Fig. 16.14); this imaging technique also reduces false-positive findings such as skin contamination.

By providing anatomical landmarks to be recognized by the surgeon in the operation room, it has been recognized that SPECT/CT improves and optimizes the overall surgical procedure (Fig. 16.15). In principle, SPECT/CT is recommended in all patients with melanoma as a complementary modality to planar imaging and is becoming mandatory in melanomas of the head and neck, of the trunk, or in any areas with high probability of unexpected lymphatic drainage [32–34].

Key Learning Points

- The radiopharmaceutical for lymphatic mapping (either ^{99m}Tc-radiocolloid or ^{99m}Tc-tilmanocept) is injected intradermally in multiple aliquots around the primary melanoma or (more frequently) around the excisional biopsy scar.
- In patients with cutaneous melanoma, lymphoscintigraphy usually includes an early dynamic acquisition that helps to identify regional lymphatic basins and to distinguish the true sentinel lymph node(s) from higher-echelon lymph nodes that can be visualized along the same pathway of lymphatic drainage.
- Delayed static images acquired at 30 min and 2 h postinjection are generally sufficient for adequate preoperative imaging.
- SPECT/CT imaging provides an added value over planar imaging, for more accurate anatomical localization of radioactive sentinel lymph node(s).
- SPECT/CT imaging is highly recommended in patients with melanomas of the head and neck region, of the trunk, or in areas with high probability of unexpected lymphatic drainage.

16.3.5 Intraoperative Counting/Detection

Intraoperative SLN detection is based on an acoustical and count-reading signal provided by the handheld gamma probe. Injection of a blue dye (patent blue, isosulfan, methylene blue) may be employed to complement with intraopera-

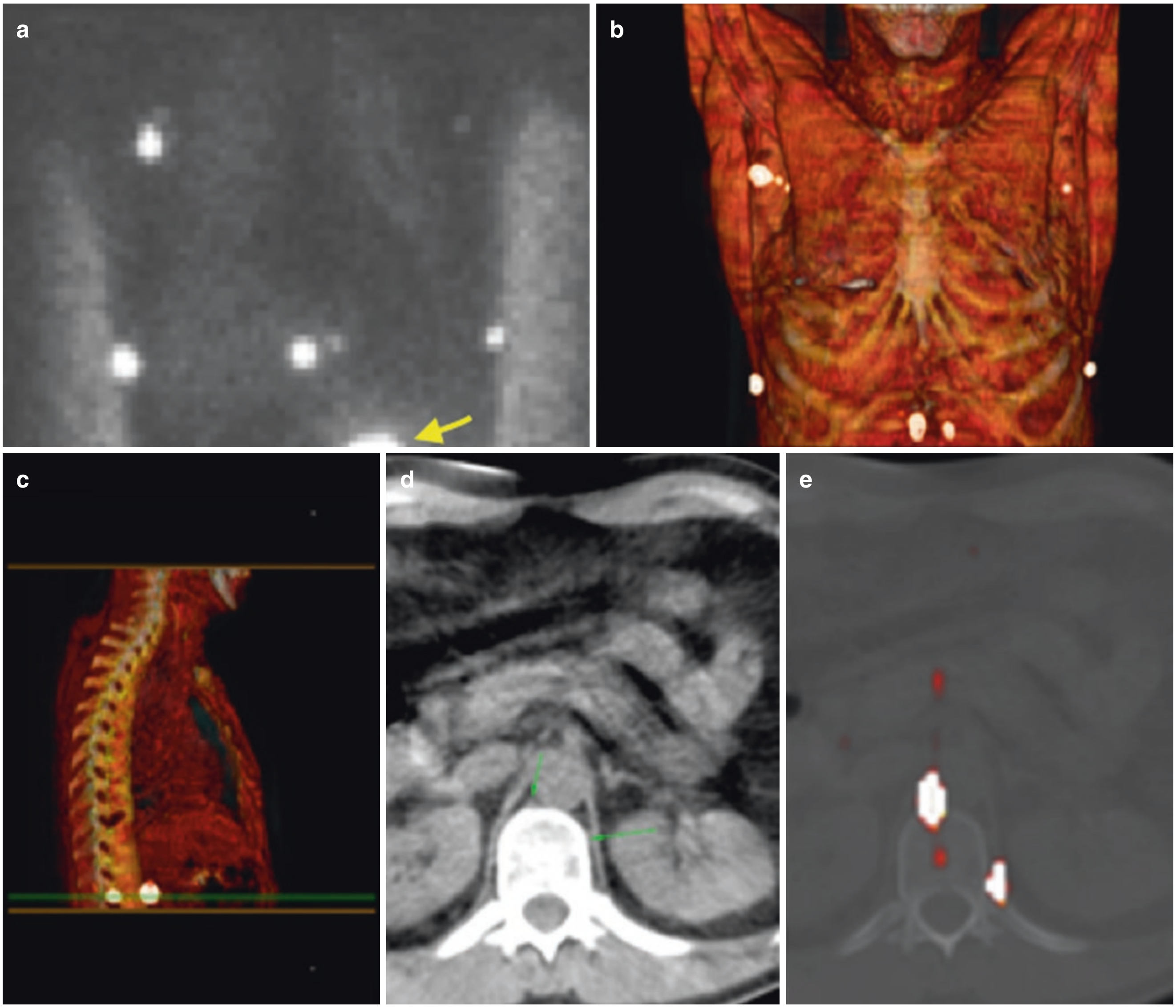


Fig. 16.15 Lymphoscintigraphy obtained after perilesional injection of ^{99m}Tc-nanocolloidal albumin in a patient with melanoma located in the left-central periumbilical area (yellow arrow in upper left panel). (**a**) The planar anterior view shows several hot spots in both axillae and para-costal areas and two hot spots central in the abdomen. All of them were considered as SLNs. (**b**) 3D surface volume rendering after SPECT/CT acquisition, showing the anatomical location of these nodes. (**c**) Sagittal fused SPECT/CT slice centered on the foci of "abdominal" uptake. (**d**) Transaxial slice of the CT component of the SPECT/CT acquisition, showing two tiny lymph nodes in para-aortic and paravertebral areas (green lines). (**e**) Transaxial fused SPECT/CT slice confirming obvious radiocolloid uptake in these locations—corresponding to SLNs; due their deep location, these SLNs were not harvested for analysis (*reproduced with permission from: Vidal-Sicart S, Orsini F, Giammarile F, Mariani G, Valdés Olmos RA. Radioguided surgery for malignant melanoma. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1401–32*)

tive visual guidance the radioguidance provided by the radionuclide procedure. According to current recommendations, all stained and/or radioactive lymph nodes retrieved during surgery should be harvested for analysis.

The standard device for intraoperative SLN detection is a non-imaging, handheld gamma probe which enables to identify the area with the highest activity (counts per second) in each lymphatic region and its correspondence with the skin mark(s) previously made in the nuclear medicine department as the last phase of lymphoscintigraphy. After incision in the marked area, in vivo radioactivity is measured in comparison with background activity (average count rates of the surrounding lymph node basin). After SLN identification, the gamma probe is used to assess the residual activity in the surgical field, so as to remove potential additional SLNs.

Consensus has not yet been achieved on the best procedure to define a count-rate threshold that would enable to

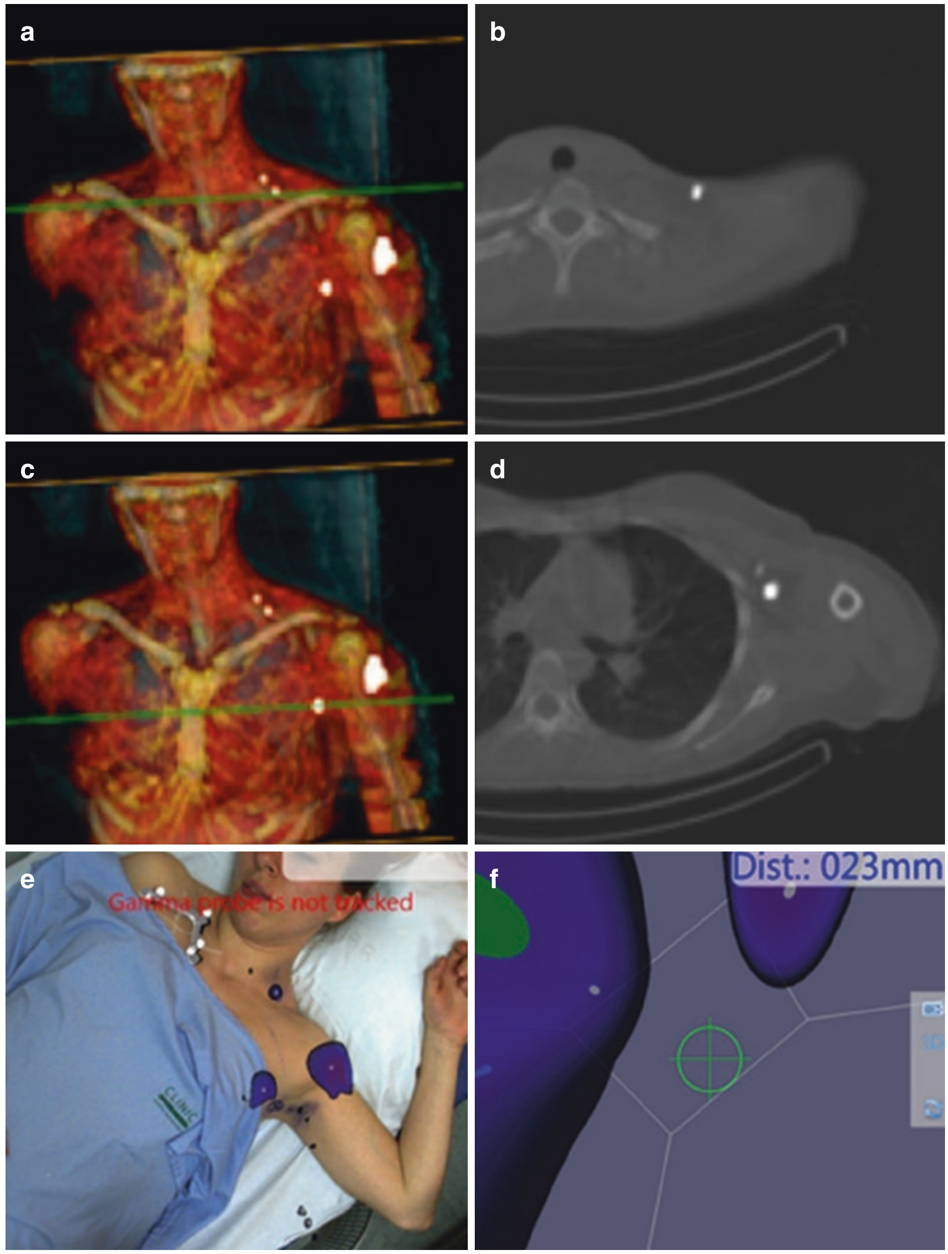


Fig. 16.16 Lymphoscintigraphy obtained after pericicatricial injection of ^{99m}Tc-nanocolloidal albumin in a patient operated because of melanoma of the left arm—just below the shoulder. 3D surface volume rendering images shown in (**a**) and (**c**) depict the site of radiocolloid injection and SLNs in the left axilla and left supraclavicular area (**a**, **c**). Transaxial fused SPECT/CT slices shown in (**b**) and (**d**) depict exact anatomical locations of such SLNs, demonstrating that in the left axilla there are actually two adjacent but separate radioactive lymph nodes. (**e**) Image produced by the freehand SPECT system, where the radioactive foci are displayed directly onto the patient's body (the infrared-based tracking device positioned on the patient's body is clearly seen in the right upper chest; a similar device is fixed on the handheld gamma probe). (**f**) The freehand SPECT device allows virtual reconstruction providing real-time information about depth within the of the radioactive foci (*reproduced with permission from: Vidal-Sicart S, Orsini F, Giammarile F, Mariani G, Valdés Olmos RA. Radioguided surgery for malignant melanoma. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1401–32*)

identify the true SLNs and therefore to avoid unnecessary harvesting of non-SLNs, based on the absolute number of counts in the radioactive lymph nodes. The ratio of in vivo or ex vivo radioactive counts in the lymph node relative to background has also been proposed to the same purpose. Finally, the 10% rule recommends that all lymph nodes counting 10% or higher than the ex vivo hottest SLN should be harvested, whereas, according to other authors, a 20% threshold would be optimal for this purpose. Whatever is the protocol adopted, the surgeon must harvest for analysis also any additional lymph node(s) in the surgical field that appear enlarged or suspicious on palpation, even if they do not prove to be radioactive or blue-stained [28].

Recently, portable small FOV gamma cameras have been introduced to improve intraoperative detection of SLNs in addition to the handheld gamma probe. Although these devices are too bulky for direct handling in the exposed surgical field, they allow real-time SLN visualization and therefore the possibility to assess completeness of lymph node resection. These dedicated small FOV portable gamma cameras can be used in combination with fluorescence cameras for simultaneous dual-signature lymph node detection after injection of a hybrid (such as ICG-^{99m}Tc-nanocolloid) tracer for optical *and* radioactive guidance. Development of hybrid instrumentation for simultaneous detection of the radioactive and the optical signal with a single small FOV imaging device is also underway.

The freehand SPECT technique has recently been introduced to allow for intraoperative 3D navigation by using an infrared system to track the handheld gamma probe relative to the patient's body. While the surgeon is scanning the area of interest with the probe, relative positions of the patient and of the counting device are continuously being recorded by the tracking system, thus permitting direct visualization of the distribution of radioactivity in the surgical field and providing assessment of exact depth of the lesion (Fig. 16.16).

16.3.5.1 Harvesting and Analysis of Sentinel Lymph Nodes

Most protocols discourage the use of intraoperative frozen section pathology to evaluate SLNs immediately after harvesting, because this procedure may miss the lymph node's subcapsular region (the area most commonly involved in metastases). Furthermore, at variance with breast cancer, immunohistochemistry is necessary for accurate pathologic analysis of melanomas (see further below). Finally, due to the process, some tissue will be discarded, and occult metastatic cells may be missed. A possible alternative is touch-preparation cytology.

There is not yet a universally accepted protocol for processing SLNs in melanoma patients. In addition to conventional staining with hematoxylin and eosin (H&E), diagnostic sensitivity *and* specificity of pathologic analysis are greatly enhanced by performing immunohistochemistry with antibodies against the S100 antigen (one of the most sensitive markers for melanoma) and against the melanoma antigen recognized by T cells (MART-1, one of the most specific markers for melanoma) and/or against tyrosinase [35]. Finally, the extremely high sensitivity of molecular analysis using PCR-based techniques greatly facilitates the detection of melanoma cells (or cell debris containing nuclear material) within an excised SLN [36, 37].

Key Learning Points

- Injection of a blue dye may be employed to complement with intraoperative visual guidance the radioguidance provided by the radionuclide procedure.
- According to current recommendations, all stained and/or radioactive lymph nodes retrieved during surgery should be harvested for analysis.
- Although the best count-rate threshold identifying the true sentinel lymph node(s) has not yet been univocally defined, different parameters have been proposed, mostly based on the ratio of count rate of additional radioactive lymph nodes relative to the hottest lymph node retrieved (10% rule or 20% rule).
- Intraoperative high-resolution imaging with portable, small field-of-view gamma cameras does not guide per se search for the sentinel lymph node(s), but rather it is employed for assessing completeness of radioguided sentinel lymph node excision.
- Hybrid imaging agents (e.g., radioactive *and* fluorescent) that combine the advantages of multimodality intraoperative detection are currently under clinical validation.
- Sentinel lymph node(s) harvested during surgery must be analyzed using both conventional hematoxylin and eosin staining and immunohistochemistry with antibodies against the S100 antigen and the MART-1 antigen and/or against tyrosinase.
- Molecular analysis based on PCR techniques is extremely sensitive in the detection of melanoma cells (or cell debris containing nuclear material) within an excised sentinel lymph node.

16.3.6 Accuracy of Sentinel Lymph Node Biopsy and Long-Term Prognosis

SLNB identifies about 20% of patients with clinically occult nodal metastasis at diagnosis, thus preventing the development of regional lymph node involvement by performing selective lymph node dissection at the involved lymphatic basin; on the other hand, it avoids futile surgery in patients with pathologically negative SLNs, i.e., those patients who would not benefit from lymphadenectomy.

Currently, regional lymphadenectomy is performed only in patients with metastatic SLN, although recent studies based on long-term follow-up raise some concern that the false-negative rate is actually higher than it was reported by initial validation studies based on regional lymphadenectomy—which was performed irrespective of the SLNB findings. On the other hand, there is wide variability in the values reported for the false-negative rate, ranging from 5.6% to a surprisingly high value of 21%; these data suggest that in some patients micrometastases were not correctly identified during the SLN procedure and then progressed over time to palpable disease [28, 38].

The Multicenter Selective Lymphadenectomy Trial (MSLT-I) demonstrated that, in selected patients with intermediate-thickness cutaneous melanoma, clinical management based on SLNB findings prolongs disease-free survival and melanoma-specific overall survival, whereas SLNB has not been demonstrated to achieve similar clinical benefits in patients with higher Breslow thickness [39].

Particular attention must be paid to melanomas with lymphatic drainage to multiple basins, as frequently observed in the torso and head and neck region. In fact, patients with truncal melanomas and multiple lymphatic basin drainage have less favorable survival than patients with a single draining lymphatic basin. Truncal melanomas are also associated with an increased risk of nodal metastasis. Furthermore, SLNs in uncommon sites must be harvested for analysis, since they may contain metastasis at nearly the same frequency (about 20%) as SLNs in common lymphatic drainage basins.

When SLNs in both common and uncommon drainage basins cannot be harvested because of surgical difficulties, close clinical and ultrasound-based monitoring of the lymphatic stations at risk is recommended during follow-up [40–42].

16.3.7 Indications and Contraindications

The SLNB procedure must be performed after histological confirmation that the primary lesion is indeed a malignant melanoma. In the common clinical practice, it is generally combined with wide local excision of the scar from prior biopsy of the suspected cutaneous lesion, with 1–2 cm margins depending on the melanoma Breslow thickness.

According to current guidelines, SLNB is indicated for staging melanoma patients with clinically lymph node-negative lymphatic stations and intermediate Breslow thickness (1.01–4 mm).

When thickness of the primary melanoma is <1 mm or > 4 mm, some controversy remains, and each individual case should be considered taking into account different factors. In particular, SLNB should be offered to selected patients with thin melanoma and high-risk features (ulceration, mitotic rate ≥1 mm^2, or >50% regression), especially in the subgroup of patients with Breslow thickness between 0.75 and 0.99 mm. It may also be considered in patients where exact thickness cannot be reliably assessed because of inadvertent shaving or cauterization before definitive biopsy of the suspected lesion. SLNB can be considered also for melanomas with >4 mm Breslow thickness, in order to obtain more accurate information and for local control.

Contraindications for SLNB include poor general health status, local or systemic spread of disease ascertained by other diagnostic procedures, and prior extensive surgery in the region of the primary tumor or of the lymph node basin. In case of concurrent primary melanoma and satellitosis or “in-transit” metastases, SLNB should not be considered due to the fact that these patients are staged as having stage III disease [28, 43].

Key Learning Points

- Sentinel lymph node biopsy identifies about 20% of patients with clinically occult nodal metastasis at diagnosis and avoids futile surgery in patients with negative sentinel lymph node(s).
- There might be a non-negligible rate of false-negative sentinel lymph node biopsies in patients with cutaneous melanoma.
- Clinical management based on sentinel lymph node findings prolongs disease-free survival and melanoma-specific overall survival in patients with intermediate-thickness cutaneous melanoma, whereas evidence is not stringent in patients with higher Breslow thickness.
- Sentinel lymph node biopsy is indicated in melanoma patients with clinically lymph node-negative lymphatic stations and intermediate Breslow thickness (1.01–4 mm).
- Controversy remains when the primary melanoma is <1 mm or >4 mm; different factors should be considered in these patients, based on histology of the primary tumor.

16.4 Radioguided Sentinel Lymph Node Biopsy in Head and Neck Cancers

16.4.1 The Clinical Problem

New cases of oral cavity, pharynx, and larynx cancers in the United States in 2017 were estimated at about 63,000 (73% men, 27% women), 27% of them being located in the pharynx, 26% in the tongue, 21% in the mouth, 21% in the larynx, and 5% at other sites. These tumors account for about 3.5% of all new cancers cases, and about 13,350 deaths were expected for these cancers in 2017 [44]. Squamous cell carcinoma (or its variants) is the most frequent histologic type of these tumors (over 90% of the cases).

Metastasis to neck lymph nodes is a major determinant for the prognosis of oral, oropharyngeal, and other head and neck cancers, as the disease-free survival rate decreases to approximately 50% when even a single lymph node harbors metastasis. The extent of lymph node involvement can be considered as an indirect index of the systemic tumor burden and is a crucial factor of tumor staging, being closely correlated to overall survival and constituting a main determinant of treatment planning [45].

Preoperative evaluation of patients with newly diagnosed head and neck cancers includes physical examination, ultrasound, CT, MRI, and/or PET/CT; all these modalities have suboptimal sensitivity for the detection of microscopic lymph node involvement, as occult metastasis is found in 15–30% of patients with clinically node-negative head and neck squamous cell carcinoma [46].

Although debate is still ongoing about performing prophylactic lymph node neck adenectomy versus a wait-and-see approach [47], selective neck dissection is generally recommended in all patients with clinically negative lymph node status. However, lymph node metastasis is found in only about 30% of the patients with early oral squamous cell carcinoma so treated, thus resulting in overtreatment in over 70% of patients. Prophylactic neck lymphadenectomy has been developed based on the common pathways for spread of all head and neck cancers to regional nodes, and it consists of surgical removal of those nodes most commonly involved with metastasis specifically from head and neck cancers. The extent of lymphadenectomy varies according to location of the primary tumor. For example, in case of oral cancers, it includes all lymph nodes of levels I, II, and III and sometimes also the superior part of level V. In case of pharyngeal and laryngeal cancers, selective neck dissection includes levels II, III, IV, and VI when appropriate [48].

Due to the anatomical complexity and unpredictable individual variability of lymphatic drainage in the individual patients (that can be found in up to 20–30% of patients), the therapeutic value of selective neck dissection is limited by the occurrence of 10–20% of "skip metastases" that have bypassed the expected first nodal basin.

In this scenario, radioguided SLNB constitutes an alternative to selective neck dissection, as it guides surgery to a personalized identification of lymphatic neck drainage and it allows detection of occult cervical metastasis in patients with early head and neck cancers. In particular, a negative SLNB prevents the unnecessary removal of functional lymph nodes and limits the extent of neck dissection surgery. Compared to selective neck dissection, patients also usually experience fewer complications such as sensory disturbances (skin numbness); overall duration of surgery is also considerably shortened [49].

Application of SLNB technique has been investigated mostly for those head and neck cancers whose anatomical location allows direct and easy access to the tumor for injection of the lymphatic mapping agents. For this reason the procedure has already been sufficiently standardized in patients with oral and oropharyngeal squamous cell carcinoma with accessible subsites, while it is still experimental in head and neck cancers arising at other sites.

In patients with early oral and oropharyngeal cancer, SLNB has shown results comparable to those of selective neck dissection in terms of regional control, as demonstrated by multiple validation trials [50, 51].

Key Learning Points

- Metastasis to neck lymph nodes is a major determinant for the prognosis and for treatment planning in patients with oral, oropharyngeal, and other head and neck cancers.
- Preoperative evaluation with even the most advanced imaging techniques has suboptimal sensitivity for the detection of microscopic lymph node involvement.
- Although selective neck dissection is recommended in patients with clinically negative lymph node status, lymph node metastasis is found in only about 30% of the patients with early oral squamous cell carcinoma, thus resulting in overtreatment in over 70% of such patients.
- Radioguided sentinel lymph node biopsy constitutes a valid alternative to selective neck dissection, as it personalizes identification of lymphatic neck drainage and it allows detection of occult cervical metastasis in patients with early head and neck cancers.
- The procedure has already been sufficiently standardized in patients with oral and oropharyngeal squamous cell carcinoma with accessible subsites, whereas it is still under experimental in head and neck cancers arising at other sites.

16.4.2 Lymphatic System of the Head and Neck

The head and neck region contains over 300 lymph nodes, constituting approximately 30% of lymph nodes in the whole human body. Their anatomy is quite complex due to the close proximity with different tissues and vital organs—often of small size.

Concerning in particular lymphatic anatomy of the cervical region, a useful schematic classification into specific anatomic subsites groups them into seven levels on each side of the neck, Robbins' classification [52] (Fig. 16.17). In the anterosuperior region of the neck, submental and submandibular lymph nodes are included in level I, respectively, as sublevel IA and sublevel IB, divided by the anterior belly of the digastric muscle. In the lateral region of the neck, upper jugular nodes extend from the base of the skull up to the inferior border of the hyoid bone and are classified as level II; the vertical plane defined by the posterior surface of the submandibular gland divides lymph nodes located anteriorly (sublevel IIA) from nodes located posteriorly (sublevel IIB). The middle jugular lymph nodes are included in level III, the space from the hyoid bone up to the inferior border of the cricoid cartilage. Level IV includes lower jugular nodes, from the inferior border of the cricoid cartilage to the clavicle. The posterior border of the sternocleidomastoid muscle constitutes the posterior border of levels II, III, and IV. Those lymph nodes located posteriorly to the posterior border of the sternocleidomastoid muscle are classified as level V; in turn, sublevel

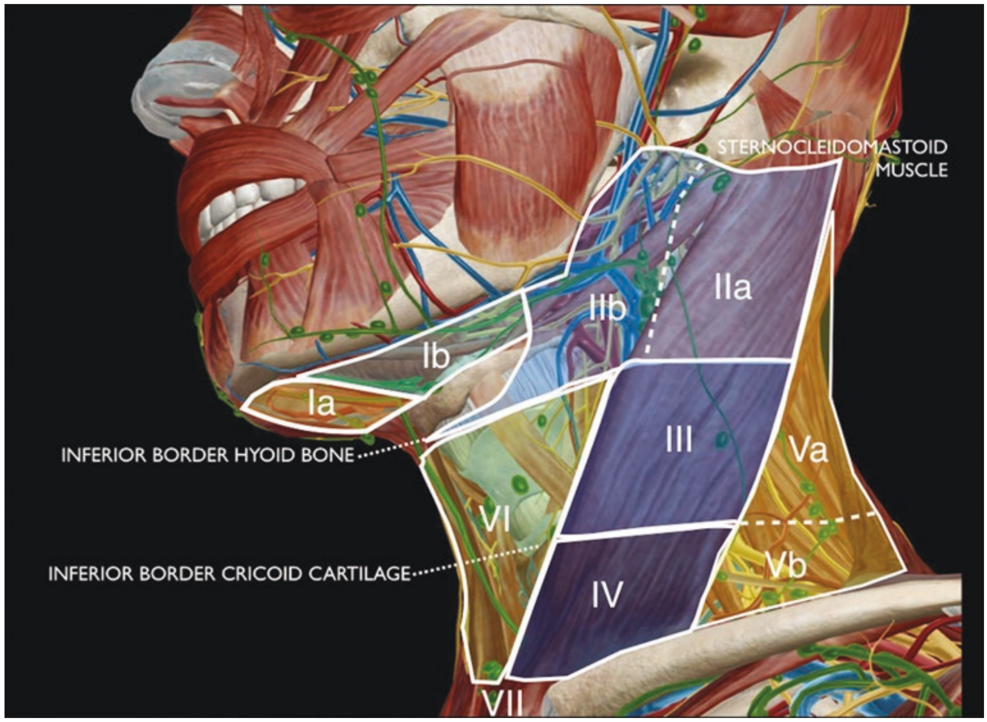


Fig. 16.17 Lymph node levels of the neck according to Robbins' classification

VA includes the spinal accessory nodes, whereas sublevel VB includes the nodes following the transverse cervical vessels and the supraclavicular nodes. Pretracheal, paratracheal, precricoid, and perithyroidal nodes (including the lymph nodes along the recurrent laryngeal nerves) constitute level VI. Finally, level VII includes the superior mediastinal lymph nodes [53].

The patterns of lymph drainage from the oral cavity, oropharynx, larynx, and hypopharynx exhibit numerous inter- and intraindividual variations, even from the same primary tumor site. For example, drainage from the anterior floor of the mouth and lingual apex is expected to submandibular nodes; however, bilateral drainage to higher-level lymph nodes and to the middle jugular chains is not infrequently observed. Moreover, lymphatic drainage from a primary tumor located in the midline may be directed to either the left or the right side. On the other hand, lateralized malignancies of the tongue or floor of the mouth often show pure contralateral drainage [54–56].

Key Learning Points

- Anatomy of the lymphatic system in the head and neck region (which constitutes about 30% of all lymph nodes in the whole human body) is quite complex.
- Robbins' classification levels for each side of the neck constitute a well-validated basis for staging patients with cancers of the head and neck.
- The patterns of lymph drainage from the oral cavity, oropharynx, larynx, and hypopharynx exhibit numerous inter- and intraindividual variations, even from the same primary tumor site.
- Lymph originating in midline structures (tongue and floor of the mouth) frequently drains to contralateral lymph nodes.

16.4.3 Modalities of Tracer Injection

A variety of radiopharmaceuticals have been used for imaging the pattern of lymph flow in the head and neck region. In addition to the established radiocolloid lymphatic mapping agents (primarily ^{99m}Tc-albumin nanocolloid and ^{99m}Tc-sulfur colloid), a new tracer targeting the mannose receptors located on macrophages and other cells in lymph nodes, ^{99m}Tc-tilmanocept, has more recently been approved; the particularly small particles of this agent result in rapid clearance from the site of interstitial injection, while avid binding to the CD206 receptors expressed on macrophages' membranes results in reduced or absent drainage to second-echelon lymph nodes and efficient SLN retention, thus facilitating SLN detection both preoperatively and intraoperatively [57, 58].

As an alternative to the radioactive label, near-infrared fluorescent tracers (such as indocyanine green—ICG) have been used that are potentially helpful for SLN biopsy [59]; moreover, the combination of fluorescent tracers with conventional ^{99m}Tc-radiocolloids is an attractive option for improving the SLN detection rate in patients with head and neck malignancies, by combining radioguidance with optical guidance [60].

The technique of tracer injection may have a relevant impact on the acquisition time and image quality during preoperative lymphatic mapping. In case of oropharyngeal cancers, the tracer should be injected intramucosally in the healthy mucosa surrounding the malignant lesion or scar margin, considering that in the subepithelial stroma there is a high concentration of lymphatic capillaries, which provides a larger area for faster lymph drainage. Intratumoral or deep injections should be avoided, because bleeding at the injection site results in lower image quality and difficult SLN identification.

Tracer injection is usually performed with small syringes with minimal dead space; alternatively, 0.1 mL of air may be drawn into the syringe behind the radiocolloid suspension to ensure complete administration. A 25 G or 27 G needle should be used, total injected activities varying from 15 to 120 MBq, depending on size and location of the primary tumor. Furthermore, the injected activity should be adjusted according to the timing of lymphoscintigraphy with respect to surgery; in particular, greater activities are required if surgery is scheduled the day after lymphoscintigraphy (2-day protocol), in order to ensure that the remaining activity exceeds 10 MBq at the time of surgery.

Small volumes (0.1–0.2 mL per aliquot) should be injected at a short distance from the primary lesion, in order to avoid masking of SLN(s) possibly located in the vicinity of the injection site; small volume is recommended also to minimize spilling of the radiotracer (due to resistance to injection of, e.g., the tongue tissue) causing contamination of the field. The number of aliquots to be injected varies from two to four, depending on size and location of the lesion. The use of a local anesthetic for topical application (10% xylocaine spray) a few minutes before tracer injection is recommended for oral cavity tumors.

The most frequent pitfall possibly occurring during lymphoscintigraphy is skin or mucosal radioactive contamination due to spillage of the lymphatic mapping agent either during injection or immediately thereafter. Oral contamination can be reduced by inviting the patient to use a mouthwash or to rinse the mouth before swallowing.

Key Learning Points

- The lymphatic mapping radiopharmaceutical (either ^{99m}Tc-colloid or ^{99m}Tc-tilmanocept) is generally injected intramucosally in multiple aliquots around the tumor site.
- The use of hybrid radioactive *and* fluorescent lymphatic mapping agents (potentially combining the advantages of radioguidance with those of visual guidance) is currently under clinical investigation.
- For cancers of the mouth, cautions must be adopted to minimize spilling of the radiotracer causing contamination of the field.

16.4.4 Lymphoscintigraphy

Preoperative lymphoscintigraphy provides images of lymphatic drainage in the tumor region. SLNs are visualized as "hot spots" along a certain pathway of lymphatic drainage. Accurate gamma camera imaging plays an important role in lymphatic mapping, since the resulting images are used to direct the surgeon to the site(s) of SLN(s).

With the patient positioned as comfortably as possible on the imaging table (usually in the supine position—to reproduce positioning during surgery), acquisitions are generally performed using a large FOV gamma camera to visualize all the possible routes of lymphatic drainage. Dynamic acquisition for 20–30 min starting immediately after radiotracer injection shows the drainage pattern and helps to distinguish SLNs from second-echelon nodes. SLNs are generally identified 15–60 min after radiotracer injection as one or more hot spots to which lymphatic drainage passes and may be multiple, ipsilateral, and/or contralateral to the primary tumor, in one or more areas of the neck. Static images in the anterior and lateral views are subsequently acquired; the skin projection of SLNs can be marked with the aid of an external radioactive marker, such as a ^{57}Co-source pen. If SLNs are not clearly visualized, static imaging can be repeated later 2–4 h postinjection or even just before surgery.

Repeat radiotracer injection and imaging may be considered in case of totally absent visualization of lymph nodes; however, proceeding to neck dissection is preferred in these cases in order to avoid erroneous staging information caused by a false-negative SLN biopsy. It must be considered that accurate preoperative localization and marking of the skin projection of SLN location correlate well with the precision of the surgical procedure.

Although SLNs can often be adequately localized with planar imaging alone, the use of SPECT/CT imaging facilitates in the majority of cases the procedure of SLNB, particularly when considering the complex anatomical region of the head and neck and the proximity of some primary lesions to SLNs and to other important anatomic structures. SPECT/CT is useful to identify additional SLNs (otherwise missed on planar imaging, especially when radioactive lymph nodes are adjacent to the injection site) (Fig. 16.18), as well as to exclude ambiguous imaging in case of radiotracer leakage or contamination. Furthermore, SPECT/CT imaging allows planning of the best surgical approach, thanks to the anatomical details provided by CT in hybrid images and thanks to volume rendering with 3D images; for instance, it can clarify if SLNs are located superficially underneath the skin or hidden below in deep tissues or in proximity or not to vital vascular and neural structures [61, 62]. Nevertheless, it must be emphasized that SPECT/CT imaging does not replace planar lymphoscintigraphy—but rather it complements planar imaging.

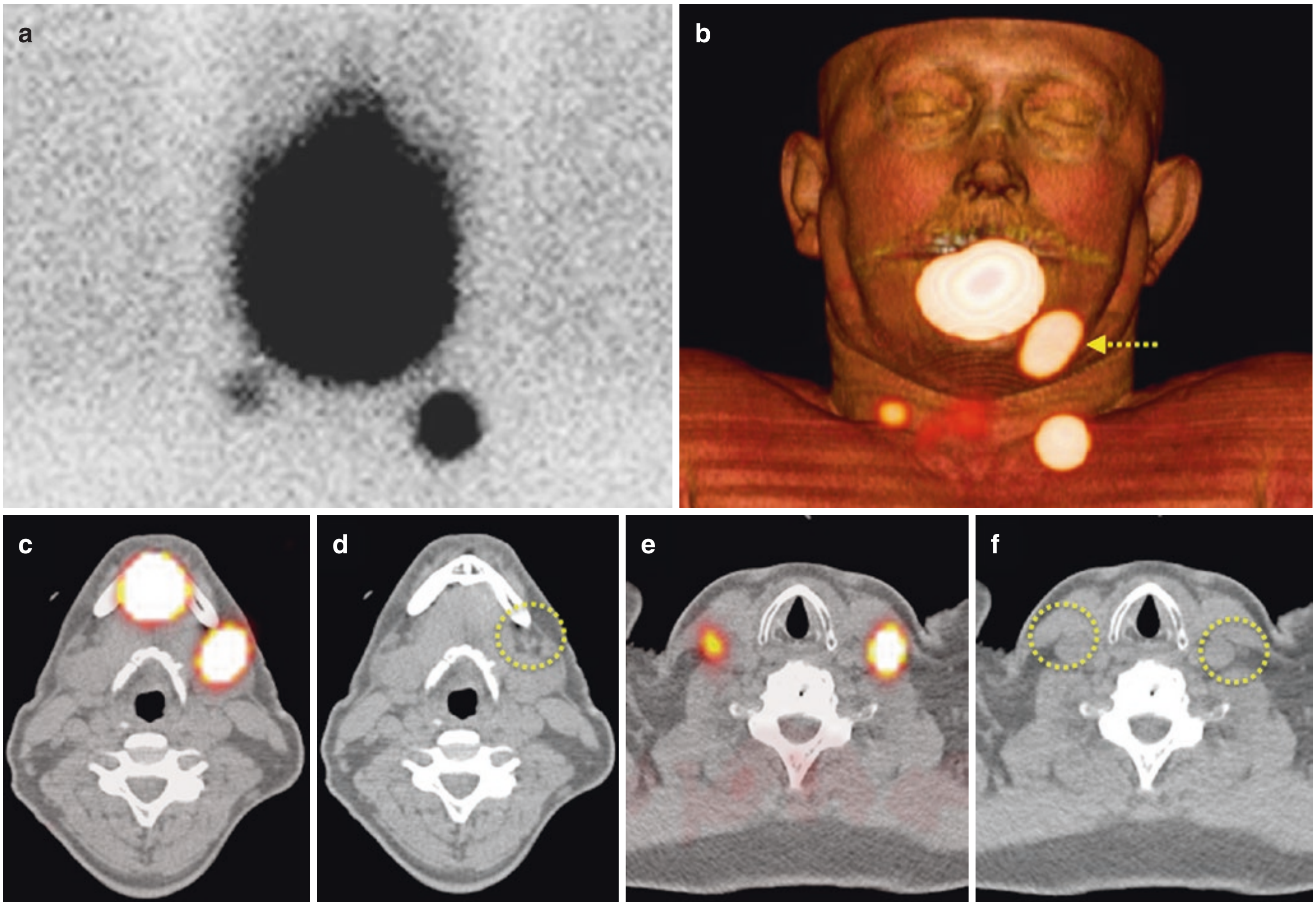


Fig. 16.18 Lymphoscintigraphy obtained after perilesional, submucosal injection of ^{99m}Tc-nanocolloidal albumin in a patient with carcinoma of the tongue. (**a**) Planar imaging shows bilateral drainage of the lymphatic mapping agent; however, due to proximity with the site of radiocolloid injection (large area of intense activity), it is difficult to ascertain the exact number and anatomical location of the radioactive lymph nodes. (**b**) 3D surface volume rendering depicts an additional SLN, left from the injection site (yellow arrow). Transaxial fused SPECT/CT slices (**c** and **e**) and corresponding CT slices (**d** and **f**) better localize SLNs in the submandibular area (yellow dashed circle in **d**) and in the lower neck (yellow dashed circles in **f**) (*reproduced with permission from: Orsini F, Puta E, Valdés Olmos RA, Vidal-Sicart S, Giammarile F, Mariani G. Radioguided surgery for head and neck. In: Strauss HW, Mariani G, Volterrani D, Larson SM, eds. Nuclear oncology—From pathophysiology to clinical applications. New York, NY: Springer; 2017:1433–49*)

Key Learning Points

- In patients with head and neck cancers, lymphoscintigraphy should include an early dynamic acquisition, which helps to distinguish true sentinel lymph nodes from second-echelon nodes visualized along the same pathway of lymphatic drainage.
- Static images in the anterior and lateral views are subsequently acquired, images being recorded even up to 2–4 h after injection if necessary when sentinel lymph nodes are not clearly visualized.
- SPECT/CT imaging facilitates in the majority of cases preoperative lymphatic mapping, because of the complex anatomical region of the head and neck and the proximity of some primary lesions to SLNs and to other important anatomic structures.

16.4.5 Intraoperative Gamma Probe Counting/Detection

A handheld γ-detecting probe is routinely used for intraoperative detection of the SLN(s) in the surgical field. The injection of blue dye at the time of surgery in addition to radioguidance with the intraoperative gamma probe is optional and may be a useful adjunct to aid SLN localization and harvesting. After SLN removal, the resection site and other cervical regions are explored with the gamma probe for any significant residual radioactivity; in this case further radioactive lymph nodes may be retrieved and harvested. An ex vivo counting ratio of 10:1/20:1 with respect to background identifies a hot spot as a radioactive SLN to be analyzed for the presence of metastatic tumor cells.

Sometimes the SLNs can be difficult to identify due to close proximity with the peritumoral injection site (the so-called shine-through effect), which can limit detection of SLNs, especially in patients with tumors of the mouth floor. Also after removing radioactive SLNs, activity remaining at the injection site can impair measurement of residual activity in the excision surgical field. For these reasons, the primary cancer should be resected before searching for the SLN(s).

Deeply located SLNs can be difficult to detect because of tissue attenuation; in these cases SPECT/CT imaging considerably helps in evaluating depth of radioactive lymph nodes. A number of portable and handheld mini gamma cameras have been developed to provide direct intraoperative visualization of radioactive foci, with the purpose of improving detection of SLNs. Using these devices, the entire lymph node excision procedure in the head and neck area can be directly monitored in the surgical room. Moreover, by acquiring images of absent or residual radioactivity after excision of the SLNs, the dedicated mini gamma camera can help to assess completeness of the procedure [63, 64].

Recently the use of intraoperative gamma cameras has been combined with fluorescence cameras for synchronous SLN signal detection using the abovementioned hybrid lymphatic mapping radiotracers combined with indocyanine green [65]. A further technology called freehand SPECT (fhSPECT) has been introduced for navigational surgery, combining the acoustic information of a conventional gamma probe and intraoperative 3D images with real-time visualization of radiotracer distribution within the surgical field.

Key Learning Points

- The injection of blue dye at the time of surgery in addition to radioguidance with the intraoperative gamma probe may be a useful adjunct to aid sentinel lymph node localization and harvesting.
- Any lymph node with an ex vivo counting ratio of 10:1/20:1 versus background should be harvested for analysis.
- Intraoperative high-resolution imaging with portable, small field-of-view gamma cameras does not guide per se search for the sentinel lymph node(s), but rather it is employed for assessing completeness of radioguided sentinel lymph node excision.
- The so-called "freehand SPECT" technology allows navigational surgery, combining the acoustic information of a conventional gamma probe and intraoperative 3D images with real-time visualization of radiotracer distribution within the surgical field.

16.4.6 Retrieval and Analysis of Sentinel Lymph Nodes

Histopathologic analysis of SLNs is extremely effective and can detect the presence of micrometastasis (<2 mm) and of clusters of several cells or even isolated tumor cells. By focusing on only a few lymph nodes, the pathologist can completely dissect and examine at 50–100 μm intervals each SLN. Sections may be stained with hematoxylin and eosin (H&E) for conventional light microscopy; if such examination is negative, adjacent sections may be used for immunohistochemistry.

Complete neck dissection is performed if the SLNB procedure detects invasion of at least one sample (10–15% of cases). If bilateral drainage is seen on lymphoscintigraphy, both hemi-necks are usually operated on. Most lymph node procedures are performed in a second step following exhaustive SLNs pathologic analysis indicating micro- or macrometastasis. Other techniques, such as frozen sections, imprint cytology, and molecular analysis with RT-PCR for cytokeratin, have been described. The clinical role of these methods remains uncertain due to the fact that, at present, the prognostic significance of micrometastasis and isolated tumor cells is unknown [66].

16.4.7 Accuracy of Sentinel Lymph Node Biopsy and Long-Term Prognosis

After the SLN concept in oral cancer was validated in several studies in which all patients underwent elective neck dissection following SLNB, several long-term prognostic studies have been performed. In 91–99% of the cases, the SLN was successfully harvested; a sensitivity of 86–95% and a negative predictive value of 88–100% have been reported; 9–37% of patients were upstaged as a result of the SLNB findings [45, 67–73].

These data confirm that the technique is a reliable means of personalizing treatment by detecting lymph node metastases, provided that the team is experienced and adheres to good practice rules. It must be considered that, compared to the conventional surgical approach, the SLNB technique is less invasive, more cost-effective, and beneficial to the patient's quality of life, by reducing morbidity and improving cosmetic outcome.

Failure to visualize and harvest SLNs may be related to their close proximity to the injection site (e.g., floor of mouth tumors). In fact, lower sensitivity rates are reported for floor of mouth cancers as compared to other oral subsites: 80–86% versus 94–97%, respectively. In these patients, the close spatial relation between the injection site and the SLN seems to result in a lower identification rate due to the "shine-through effect."

Another cause of false-negative SLNB is massive metastasis in lymph nodes that may block lymphatic drainage along that particular pathway and reroute it to other lymphatic pathways; this event causes non-visualization of the true SLNs (containing metastasis) and possible visualization of alternative routes of lymphatic drainage to metastasis-free lymph nodes.

As to histopathologic analysis of SLNs, the significance of isolated tumor cells is still controversial. When it was considered as an indication for neck dissection, a significant difference was found at 3 years between cases with isolated tumor cells alone versus micro- or macrometastasis, suggesting better prognosis in the former case [68]. Other studies suggested that in the classification of SLNs as harboring isolated tumor cells, micro- and macrometastasis could be important to evaluate if treatment of the neck is always necessary after a positive SLNB. Additional non-SLN metastases were found in 31% of neck dissections following a positive SLNB, respectively, in 13%, 20%, and 40% of patients with isolated tumor cells, micrometastasis, and macrometastasis in the SLN [74].

16.4.8 Indications and Contraindications

Current indications for SLNB include early oral and selected oropharyngeal squamous cell carcinomas, with negative lymph node status by palpation and imaging evaluation (CT, MR imaging, or PET/CT), staged as clinical T1 or T2 and N0 cancer [75].

T1 and T2 patients have resectable tumors with size <4 cm in maximum diameter. In fact, in case of larger tumors, it is difficult to perform tracer injection all around the tumor; furthermore, large tumors tend to drain to multiple lymphatic basins and in the majority of patients require a neck dissection for access to the primary tumor or for defect reconstruction. The indication, generally achieved in a multidisciplinary oncology team meeting, may be extended to patients whose necks underwent prior surgery or radiation therapy. However, these applications of the SLNB technique, while clinically attractive, remain largely unexplored; at the moment, levels of evidence in this regard are low, since the prior interventions can distort the normal lymphatic pathways and give rise to unexpected patterns of metastasis [76, 77].

In pregnant women and children, the urgency and the necessity of staging the neck should be discussed. In particular, SLNB protocols should be modified to minimize risks of radiation exposure and of blue-dye administration. SLNB can safely be performed in lactating women, but it is advised that breastfeeding be discontinued for about 2 days following the procedure [75].

Key Learning Points

- Complete neck dissection is performed in case of a tumor-positive sentinel lymph node biopsy, as observed in about 10–15% of cases.
- If bilateral drainage is seen on lymphoscintigraphy, both hemi-necks are usually operated on, even if the sentinel lymph node on one side does not harbor tumor cells.
- Sentinel lymph node biopsy is a reliable means of personalizing treatment in patients with head and neck cancers—particularly squamous cell carcinoma of the oral cavity.
- Possible causes of failure or of false negativity of sentinel lymph node biopsy include close proximity to the injection site and massive metastasis in lymph nodes that may block lymphatic drainage along a pathway of lymphatic drainage and reroute it to other lymphatic pathways.
- Sentinel lymph node biopsy is currently indicated in patients with early oral and selected oropharyngeal squamous cell carcinomas and clinically negative lymph node status, staged as clinical T1 or T2.

16.5 Radioguided Sentinel Lymph Node Biopsy in Gynecological Cancers

16.5.1 The Clinical Problem

Vulvar cancer has a low incidence in the most developed countries; for instance, about 6000 new cases overall were estimated in the United States for the year 2016. The presence of lymph node metastasis is the most important prognostic factor, because the 5-year survival rate drops from 95% to 60% when lymph nodes harbor metastases. On the other hand, the surgical complications of bilateral inguinal lymphadenectomy lead to high morbidity in these patients, who are generally >70 years old and frequently present with other clinical comorbidities.

There were nearly 13,000 new cases of cervical cancer in the United States in 2016, with more than 4000 deaths. Prognosis of early-stage cancer depends on the lymph node status, tumor size, depth of stromal invasion, and presence of lymphovascular involvement. Pelvic lymphatic metastases are found in 11–21% of patients with FIGO stage IB and in 40% of patients with FIGO stage IIB, with possible spread to the para-aortic region. Considering these figures, SLNB has been proposed as an alternative to de novo pelvic lymphadenectomy in an attempt to reduce surgical morbidity.

Endometrial cancer is the most frequent gynecological cancer, with 60,000 new cases estimated in the United States in 2016 and nearly 10,500 deaths. Lymph node metastasis is present in 10% of stage I patients, and the 5-year survival rate drops to 50% when pelvic or para-aortic node lymph nodes contain metastases. The treatment of choice for endometrial cancer is modified radical abdominal hysterectomy and bilateral salpingo-oophorectomy. However, the role of lymphadenectomy remains controversial. Radical pelvic and para-aortic lymphadenectomies represent the standard treatment in the high-risk group (grade 3, >50% myometrial invasion or papillary serous, carcinosarcoma, and clear-cell cancer histology). Currently, there is no international consensus on the optimal extent of surgery for endometrial cancer staging. Thus, the SLN procedure is being explored as possible alternative option to de novo pelvic and para-aortic lymphadenectomy [78, 79].

In general, radioguided SLNB in patients with gynecological cancers involves a more complex approach than in patients with breast cancer or melanoma, due to a variety of anatomical and pathophysiological factors.

16.5.2 Lymphatic System of Intrapelvic Female Organs

Lymphatic drainage from the vulva normally flows to the inguino-femoral lymph nodes, either on one side only or bilaterally. In particular, lymph originating from the labia flows to the superficial inguinal nodes, proceeding then to the deep inguinal nodes and to the pelvic lymph nodes, whereas some midline structures, such as the clitoris, can drain directly to deep lymph nodes. Tumors located in the midline usually drain to both inguinal sides.

The cervix is a deep midline structure whose lymph drains laterally to the parametria, to the external iliac lymph nodes, and to the obturator lymph nodes. The expected drainage is bilateral migration to the external iliac lymph nodes. Obturator fossa lymph nodes are the most common group, followed by progression of lymph to the common iliac and para-aortic lymph nodes as the second-echelon group. Direct para-aortic drainage has been observed in 1% of cases. Parametrial lymph nodes can be very difficult to evaluate during lymphoscintigraphy and radioguided SLNB, due to their proximity to the site of injection of the lymphatic mapping agent.

Lymphatic drainage from the corpus uteri is in some respect different from cervical drainage. In particular, lymph from the two lower thirds of the corpus drains in a similar manner as lymph from the cervix. Whereas, lymph from the upper third of the corpus uteri drains directly to the para-aortic lymph nodes [79].

Key Learning Points

- Gynecological malignancies where sentinel lymph node biopsy can be employed include vulvar cancer, cervical cancer, and endometrial cancer.
- Lymph originating in the vulva normally drains to the inguino-femoral lymph nodes, proceeding from superficial to deep inguinal nodes and to pelvic lymph nodes.
- The expected lymphatic drainage from the cervix is bilaterally to the parametria, to the external iliac nodes, and to the obturator lymph node, progressing thereon to the common iliac and para-aortic lymph nodes.
- Lymph from the two lower thirds of the corpus uteri drains in a similar manner as lymph from the cervix, whereas lymph from the upper third drains directly to the para-aortic lymph nodes.

16.5.3 Modalities of Tracer Injection

Administration of the lymphatic mapping agent (e.g., a ^{99m}Tc-colloid) in patients with vulvar malignancies is performed through three to four intradermal/intramucosal injections (0.1–0.2 mL each aliquot) around the primary lesion or the excision scar (Fig. 16.19).

In patients with cervical cancer, lymphatic mapping is performed by peritumoral or periorificial injection of four radiopharmaceutical aliquots. In the case of previous conization, pericicatricial injection at the four quadrants is recommended. Usually, a total volume of 2 mL is applied.

One of the most controversial issues for accurate lymphatic mapping in patients with endometrial cancer concerns the best injection site/modality. Different approaches have been proposed to this purpose, such as cervical injection, endometrial peritumoral injection assisted by hysteroscopy, and myometrial/subserosal injection. The cervical approach (similar to the technique adopted for cervical cancer, although with somewhat deeper punctions) is the easiest way to inject the lymphatic mapping agent. It is performed periorificially into the four quadrants. Endometrial injection during hysteroscopy allows direct injection around the tumor. If it is performed the day before surgery, this procedure requires particular coordination among the gynecology, anesthesia, and nuclear medicine departments. If it is performed shortly prior to the start of surgery, it is problematic to acquire lymphoscintigraphy and SPECT/CT. The third option, radiotracer injection into the corpus uteri in a myometrial or subserosal location, is usually adopted during surgery. However, it is also possible

Fig. 16.19 Modality of injection of the lymphatic mapping agent in vulvar cancer. Submucosal injection in a patient with a midline lesion in the left labia minora. Since bilateral lymphatic drainage is expected in the majority of these patients, it is important to inject the radiocolloid around the tumor, although this can be difficult in the medial part (right panel)

to inject the tracer guided by transvaginal ultrasonography the day prior to surgery [78].

Key Learning Points
- In patients with vulvar cancer, the lymphatic mapping agent is administered through 3–4 intradermal/intramucosal injections around the primary lesion or the excision scar.
- In patients with cervical cancer, the lymphatic mapping agent is injected peritumorally or periorificially, at the four quadrants.
- The optimal modality of injection in patients with endometrial cancer is still debated, and different approaches are being explored (e.g., cervical injection, endometrial injection during hysteroscopy).

16.5.4 Lymphoscintigraphy

In the case of vulvar cancer, a dynamic acquisition is recorded immediately after tracer injection, usually for about 10 min. Early (15 min) and delayed (2 h) static planar images are then obtained (Fig. 16.20). The lymph node that first receives a direct lymphatic channel from the tumor or shows an increase in uptake in the delayed images is usually considered as a SLN.

In patients with cervical cancer (Fig. 16.21) or with endometrial cancer (Fig. 16.22), planar imaging is based on 3–5 min anterior and lateral views acquired at 30 min (early) and 60–120 min (delayed) after radiotracer injection. Early images can depict the lymphatic duct(s) and the first-draining lymph node(s). Delayed images may discriminate the SLN from second-echelon nodes. Planar images cannot, however, provide precise anatomical landmarks [78]. This limitation is overcome by SPECT/CT imaging obtained just after delayed imaging. In vulvar cancer, lymphatic drainage is mainly directed to Daseler's medial inguinal region (83%), while drainage to the lateral inferior groin is minimal (0.5%) [79].

SPECT/CT is crucial to adopt the optimal approach to inguinal lymphadenectomy in patients with positive SLNB. SPECT/CT imaging is mandatory in case of cervical and endometrial malignancies, since it allows correction for tissue attenuation and may thus lead to detection of additional SLN(s). Moreover, by providing accurate anatomical localization, it plays an important role in planning surgery and shortening the operative time [80].

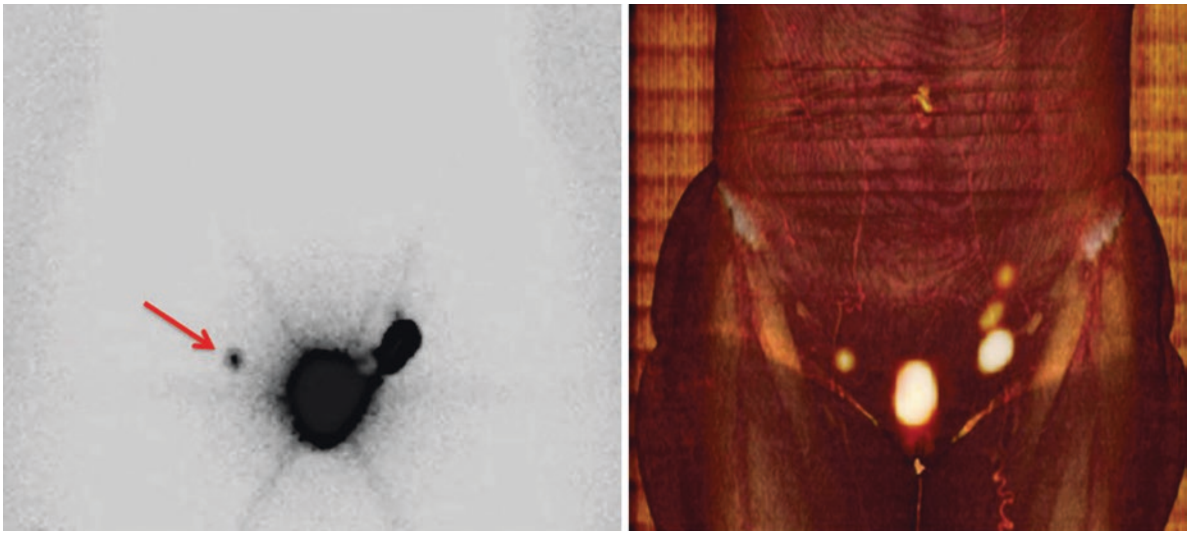


Fig. 16.20 Lymphoscintigraphy obtained after perilesional, submucosal injection of ^{99m}Tc-nanocolloidal albumin in the same patient with vulvar cancer as depicted in Fig. 16.19. The delayed planar image (left panel) shows a predominantly left inguinal lymphatic drainage. However, a faint uptake on the right inguinal side is observed as well (red arrow). The 3D surface volume rendering image obtained after SPECT/CT acquisition more accurately visualizes the radioactive lymph nodes (right panel)

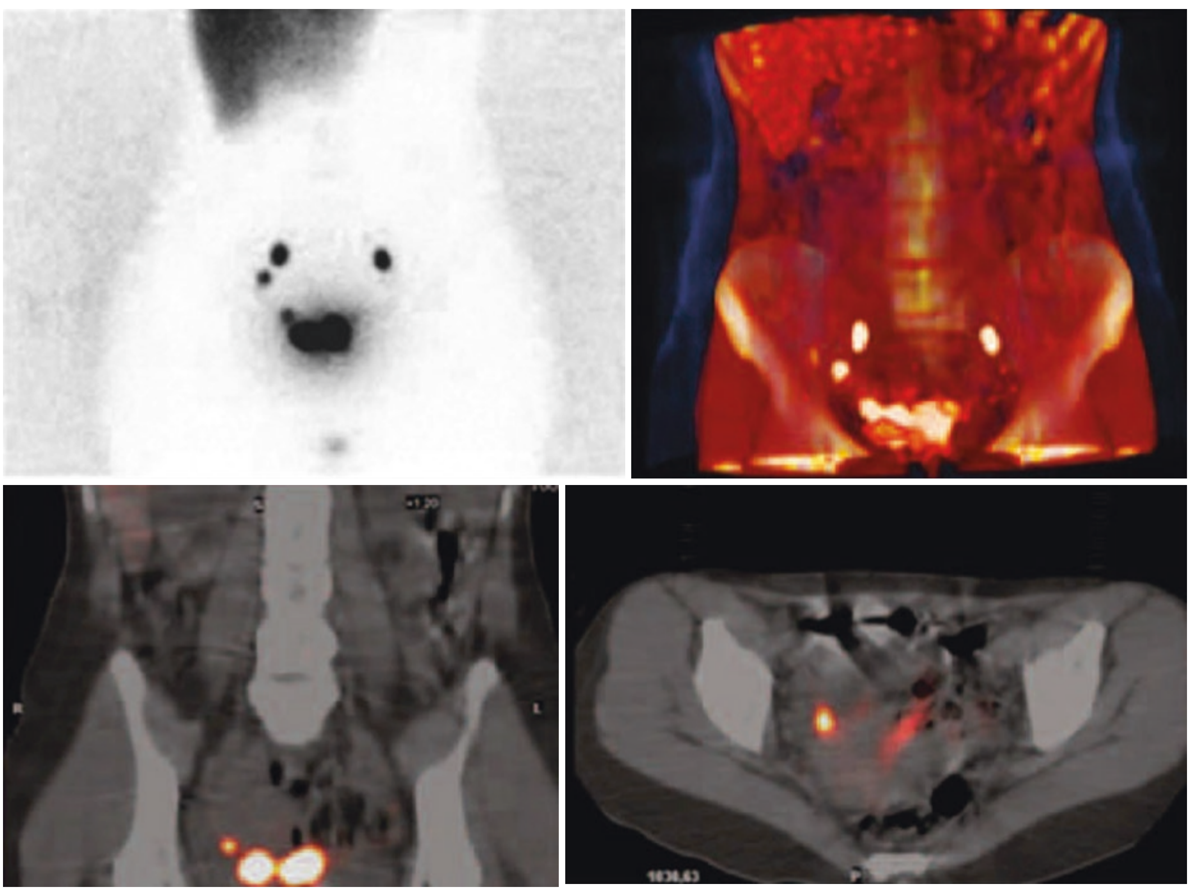


Fig. 16.21 Lymphoscintigraphy obtained after perilesional injection of ^{99m}Tc-nanocolloidal albumin in the patient with cervical cancer. Upper left panel: planar imaging shows bilateral lymphatic drainage with two radioactive lymph nodes in the right pelvis and one in the left pelvis. Note a hot spot near the injection site, depicted on the right-hand side. Upper right panel: 3D surface volume rendering obtained after SPECT/CT acquisition shows the anatomical distribution of the radioactive lymph nodes. Coronal fused SPECT/CT slice (lower left panel) and transaxial fused SPECT/CT slice (lower right panel) depict more precisely the location of the radioactive lymph nodes, one of which (considered a true SLN) is located in the right parametrium (*reproduced with permission from: Paredes P, Vidal-Sicart S. Preoperative and intraoperative lymphatic mapping for radioguided sentinel node biopsy in cancers of the female reproductive system. In: Mariani G, Manca G, Orsini F, Vidal-Sicart S, Valdés Olmos RA, eds. Atlas of lymphoscintigraphy and Sentinel Node Mapping—A pictorial case-based approach. Milan: Springer 2013:249–68*)

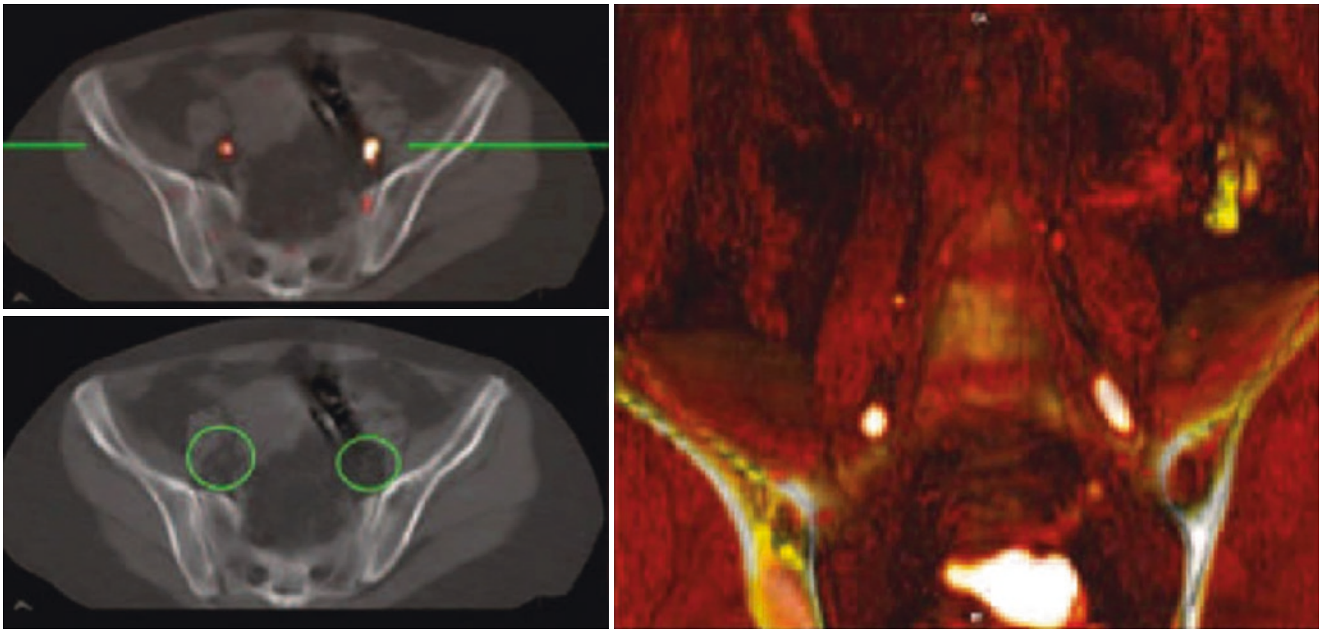


Fig. 16.22 Lymphoscintigraphy obtained after cervical injection of ^{99m}Tc-nanocolloidal albumin in the patient with endometrial cancer. The transaxial fused SPECT/CT slice (upper left panel) shows radiocolloid uptake in SLNs located in both external iliac areas; the lymph nodes are depicted in the CT component of the SPECT/CT acquisition (green circles in the lower left pane). Three-dimensional surface volume rendering obtained after SPECT/CT acquisition provides a good overview of the anatomical location of the radioactive lymph nodes near major blood vessels (*reproduced with permission from: Paredes P, Vidal-Sicart S. Preoperative and intraoperative lymphatic mapping for radioguided sentinel node biopsy in cancers of the female reproductive system. In: Mariani G, Manca G, Orsini F, Vidal-Sicart S, Valdés Olmos RA, eds. Atlas of lymphoscintigraphy and Sentinel Node Mapping—A pictorial case-based approach. Milan: Springer 2013:249–68*)

Key Learning Points

- In patients with vulvar cancer, an early dynamic acquisition helps to identify the lymphatic basins at risk and also to recognize higher-echelon lymph nodes visualized after visualization of the "true" sentinel lymph node along a certain pathway of lymphatic drainage; static planar images acquired up to about 2 h postinjection are usually sufficient for adequate lymphoscintigraphic mapping in these patients.
- Lymphoscintigraphy can be more complex in patients with cervical cancer or endometrial cancer, in whom SPECT/CT imaging acquired in addition to early and delayed planar imaging provides the anatomical correlates that are especially useful to guide the surgeon to retrieve the sentinel lymph node(s).

16.5.5 Intraoperative Gamma Probe/Gamma Camera Detection

SLN detection using blue dyes alone in patients with vulvar or cervical cancer ranges from 75% to 95%. Although in most cases the same SLNs are identified by the blue dye and by the radioactive lymphatic mapping agent, radioguided SLNB has in general better performance than optical guidance alone with blue dyes. Nevertheless, current standard practice in patients with vulvar or cervical cancer includes the simultaneous injection of both types of lymphatic mapping agent, radiopharmaceutical and blue dye, for combined radioguidance and optical guidance. The use of fluorophores (mainly indocyanine green, ICG) has recently been advocated in this scenario, with better results than with the blue dye [78, 79].

In patients with vulvar tumors, skin marks on the cutaneous projection of the SLN(s) can indicate the best location for surgical incision and subsequent use of the handheld gamma probe for exploring the surgical field. Conversely, in cervical and endometrial tumors, laparoscopy is the most frequent surgical approach; therefore, cutaneous marks are not useful as a guide. Moreover, specifically designed gamma probes must be used during laparoscopy. In particular, these probes must be inserted through a 10–12-mm-diameter trocar, must be long enough to reach the area of interest (up to a distance of 25 cm from the abdominal surface), and must have suitable maneuverability so to cover an area with a radius of 15–20 cm. Laparoscopic gamma probes have been developed taking these issues into consideration, in particular the limitation on maneuverability that may impede SLN identification. In these probes the angle of the detector relative to the tip varies from 0° to 45–90°, according to specific uses in different phases of the same surgical session.

In clinical practice, possible interferences during laparoscopic gamma probe scanning must be kept in mind. In particular, the uterus is usually enlarged in patients with endometrial cancer, thus increasing the interference on SLN detection. Furthermore, laparoscopic gamma probe scanning along the intra-abdominal lymphatic pathways can be affected by activity accumulated in the ureter or by liver activity due to radiocolloid uptake in the reticuloendothelial system [78–82].

Due to anatomical complexity of the area, activity remaining at the injection site can mask activity coming from the SLN, as in the case of, e.g., an inguino-femoral SLN adjacent to the radiotracer injection site in a patient with vulvar cancer or parametrial SLNs in cervical/endometrial cancer. In such instance, intraoperative visual guidance (e.g., with blue dye—or with fluorescent agents) is particularly useful. Furthermore, intraoperative use of gamma probes can be supplemented with the use of portable gamma cameras or other techniques such as intraoperative freehand SPECT. The main advantages of using a portable gamma camera for SLN detection in gynecological cancers are (1) greater sensitivity for localizing parametrial lymph nodes, (2) better discrimination of interference from liver activity when resecting para-aortic lymph nodes, and (3) better ability to ascertain the completeness of SLN excision.

Intraoperative freehand SPECT can provide some advantages also in this setting, as it yields virtually real-time information on precise SLN's depth and on completeness of SLN resection. Moreover, SPECT/CT data obtained during lymphoscintigraphy with a large field-of-view gamma camera can be uploaded and included in the image display to provide anatomical landmarks and the possibility of intraoperative navigation [79, 80].

Key Learning Points

- In patients with vulvar or cervical cancer, sentinel lymph node biopsy is often performed combining preoperative lymphatic mapping and intraoperative gamma probe guidance with visual guidance using a blue dye (or more recently a fluorescent agent).
- The most frequent approach to sentinel lymph node biopsy in patients with cervical or endometrial cancer is laparoscopic surgery, using specifically designed gamma probes that can be inserted in the abdominal cavity through a laparoscopic trocar and reach deep intra-abdominal sites.
- Intraoperative gamma probe guidance can be somewhat hampered by interferences in counting caused by proximity with radioactivity either at the injection site or at physiologic sites of accumulation other than lymph nodes.
- Visual guidance, especially with the use of a fluorescent agent, turns out to be particularly useful to overcome these interferences.
- Other imaging modalities possibly employed during surgery (such as dedicated small field-of-view gamma cameras or freehand SPECT) can be useful to assess completeness of sentinel lymph node excision.

16.5.6 Retrieval and Analysis of Sentinel Lymph Nodes

The optimal method for histopathological examination of SLNs in patients with gynecological cancers has not been established. Considering that routine staining with hematoxylin and eosin (H&E) may not allow the identification of micrometastasis (<2 mm in diameter), ultrastaging histologic techniques can be used when H&E staining is negative for metastasis. In particular, immunohistochemical staining can be employed for enhanced sensitivity in detecting tumor cells.

There is current consensus on the rationale of performing lymphadenectomy of the basin where the SLN contains micrometastasis, although it can be argued that the benefit of increased detection of metastatic involvement in the SLN outweighs potential harms, including overtreatment of patients with micrometastasis [83].

In patients with cervical cancer, hysterectomy is performed only when no metastases are found in the pelvic lymph nodes; ultrastaging with immunohistochemistry has resulted in increased detection of micrometastases in 25–30% of patients in whom conventional H&E staining had revealed no SLN metastasis. In this regard, the recurrence-free survival rate is significantly reduced in patients with micrometastases, thus suggesting that even this low metastatic load is an independent prognostic factor in cervical cancer.

The added value of immunohistochemistry is relevant also in patients with endometrial cancer, since nearly 20% of patients are upstaged because of the detection of micrometastases. In low-risk endometrial cancer (grades 1–2, with less than 50% myometrial invasion), ultrastaging increases by about 50% the number of positive SLNs versus standard histology. Nevertheless, there is no consensus yet on how to treat patients with endometrial cancer and micrometastasis only in SLN(s) [78].

16.5.7 Accuracy of Sentinel Lymph Node Biopsy and Long-Term Prognosis

Lymphatic mapping with SLNB may modify the management of patients with gynecologic tumors by allowing accurate stratification according to lymph node metastatic status and possibly sparing in many patients the potential morbidity of unnecessary lymphadenectomy.

The rate of radioguided SLN identification is high in patients with vulvar and cervical cancer, in whom lymph node status plays a key role for the selection of further surgery or changes in patient's management. In the current clinical practice, patients with early vulvar cancer are treated with de novo bilateral inguino-femoral lymphadenectomy, which is burdened by high comorbidity; however, only 10–26% of the patients actually have inguinal metastases. In a recent meta-analysis, the overall SLN detection rate per groin was 86.9% with combined blue dye and radiocolloid. The false-negative rate resulting from 25 studies using the combined dual tracer procedure was 6.6%, with an average 3.4% recurrence rate in the groin after a negative SLNB; nevertheless, the GROINSS-V study reported that women with multifocal disease had a higher rate of false-negative SLNB, 11.8% [84]. In these patients SLNB resulted in a lower incidence of morbidity than de novo inguino-femoral lymphadenectomy. The wound breakdown rate was 11.7% versus 34%, cellulitis 4.5% versus 21.3%, and lymphedema 1.9% versus 25.2%.

Cervical cancer is usually treated with radical hysterectomy and pelvic lymphadenectomy. However, the great majority of stage Ib patients does not benefit from lymphadenectomy, as only 15% of them have lymphatic metastasis at diagnosis. During surgery, a positive SLNB avoids hysterectomy or trachelectomy, while para-aortic lymphadenectomy is performed because of the higher risk of further lymph node involvement; afterwards, chemoradiation therapy becomes the treatment of choice. On the contrary, when SLNB is negative for metastasis, hysterectomy or trachelectomy can be performed as planned.

A meta-analysis showed that the highest SLN detection rates were obtained with the combined radiotracer and blue-dye procedure. The pooled SLN detection rate was 92.2% with a 95% negative predictive value for cervical tumors <2 cm. Bilateral SLN visualization is associated with fewer false-negative results.

A novel lymphatic mapping modality that employs ICG dye with near-infrared fluorescence imaging is gaining widespread use because of the possibility of real-time imaging during surgery and higher overall and bilateral SLN detection rates than with the blue dye [78].

Early-stage, low-risk endometrial cancer requires pelvic lymphadenectomy for staging, although this procedure is a matter of controversy. Patients in the high-risk group (grade 3 endometrioid carcinoma with myometrial infiltration over 50% or endometrial serous and clear-cell carcinoma) require staging based on pelvic and para-aortic lymph node dissection. Randomized clinical trials demonstrated that there is no advantage in performing systemic lymphadenectomy in patients with early-stage endometrial cancer, and the debate whether lymphadenectomy should be the standard approach continues. Other studies showed that SLNB predicts lymph node status in patients with early-stage endometrial cancer and that lymph node involvement was more accurately detected by ultrastaging.

The SENTI-ENDO multicenter study assessed more than 120 patients with endometrial cancer using a double-tracer cervical injection. SLN was detected in 88.8% of patients and 17% presented pelvic lymph node metastases. The negative predictive value was 97%, with three cases of false-negative results. It was concluded that SLNB could be an alternative to lymphadenectomy in patients with low- or intermediate-risk endometrial cancer. However, the real incidence of metastasis in para-aortic lymph nodes could be underestimated [78, 79, 81, 82].

Key Learning Points

- Analysis of the harvested sentinel lymph nodes in patients with gynecological malignancies is based on conventional hematoxylin and eosin staining, although immunohistochemistry is more sensitive for the detection of micrometastasis <2 mm or isolated tumor cells.
- There is no univocal consensus on the optimal therapeutic strategy to be adopted on the basis of the results of sentinel lymph node biopsy in patients with gynecological malignancies, especially when micrometastasis only is found in sentinel lymph node(s).
- Nonetheless, sentinel lymph node biopsy has a definite impact in patients with gynecological malignancies.
- The false-negative rate of sentinel lymph node biopsy in patients with vulvar cancer is low when using the combined radioguidance and visual blue-dye guidance procedure.
- Sentinel lymph node biopsy yields important information in patients with cervical cancer, with high detection rate and low false-negative rates when using the combined radioguidance and visual blue-dye guidance procedure.
- Visual guidance with a fluorescent agent is a useful complement to radioguidance for sentinel lymph node biopsy in patients with cervical cancer.
- Sentinel lymph node is feasible and can represent an alternative to lymphadenectomy in patients with low- or intermediate-risk endometrial cancer.

16.5.8 Indications and Contraindications

According to the EANM guidelines published in 2014, the indications for SLNB in patients with gynecological cancers can be summarized as follows:

- Early cervical cancer (Ia2/Ib1, IIa1 stages).
- Stage I and II high-risk endometrial cancer, i.e., endometrioid cancer with the following features: >50% myometrial invasion or poorly differentiated (grade 3) or serous papillary, clear-cell, or carcinosarcoma histological subtype.
- Squamous cell vulvar carcinoma Ib/II <4 cm in size, without presurgical lymph node metastases.
- Although based on limited experience, SLNB in vulvar melanoma is also accepted with the same indications as in cutaneous melanoma.

Contraindications for SLNB in patients with gynecological cancers include the following conditions:

- Suspected extrauterine involvement.
- Presence of pathological pelvic or para-aortic lymph nodes on radiological or other imaging investigation.
- Prior history of surgery or radiotherapy of the lymph node areas under evaluation.
- General contraindication to surgical treatment (related to age or medical conditions).

Key Learning Points

- Sentinel lymph node biopsy in patients with gynecological malignancies is indicated for early cervical cancer, stage I and II high-risk endometrial cancer, and clinically lymph node-negative squamous cell vulvar cancer <4 cm in size.
- Besides established general contraindications to surgery, sentinel lymph node biopsy is contraindicated when extrauterine involvement is suspected and there are preoperative evidence of pathological pelvic or para-aortic lymph nodes and prior history of surgery or radiotherapy of the lymph node areas under evaluation.

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