A Review of Techniques for Sentinel Lymph Node Mapping with Special Reference to the Head and Neck Region

Abstract
Sentinel lymph node biopsy is an extensively explored diagnostic mean for reducing surgical risks while improving staging in different human malignancies. Several different techniques have been introduced to clinical use during last two decades. Methods as ink lymphography and lymphoscintigraphy have already established their position in sentinel nodes mapping. Novel approaches as CT, MRI-lymphography, contrast enhanced ultrasound and near-infrared fluorescence imaging possesses prominent potential advantages and demand further experience gaining. A review of the clinically utilized techniques is presented a special attention is drawn to their usefulness in the head and neck carcinomas work-up.

Keywords: Sentinel lymph node; Lymphography; Lymphoscintigraphy; CT; MRI; Ultrasound; Near-infrared fluorescence

Abbreviations: SCC: Squamous Cell Carcinoma; SLN: Sentinel Lymph Node; Tc-99m: Technetium; CT: Computed Tomography; SPECT: Single-Photon Emission Computed Tomography; MRI: Magnetic Resonance Imaging; US: Ultrasound; UPIO: Ultrasmall Paramagnetic Iron Oxide; CEUS: Contrast Enhanced Ultrasound; NIF: Near-Infrared Fluorescence

Introduction
To date the mainstay of management of the majority of malignant head and neck tumors is surgical treatment alone or in combination with radio-and/or chemotherapy. The basis of surgery for oral carcinoma consists of two usually significantly invasive procedures which consist of the ablation of the disease at the primary site and regional lymphadenectomy. Depending on the status of the regional lymph nodes in a given patient the extent and potential morbidity of the neck dissection varies [1]. The possible complications of neck dissection are: wound healing impairment [2]; major vessels damage [3], internal jugular vein thrombosis [4]; postoperative rupture of the carotid artery or internal jugular vein [5]; damage to the accessory, hypoglossal superior laryngeal and phrenic nerves as well the sympathetic trunk and brachial plexus, and possibility of provoking a vasovagal response [6-8]; chylus fistula and chylothorax [9]; increase of intracranial pressure [10]; visual loss [11]; lymphedema [12] and fracture of the clavicle [13].

Squamous cell carcinoma (SCC) accounts for more than 90% of the head and neck malignancies and it have a high potency for regional lymphogenic spread. The presence of neck lymph node metastasis is the one most important factor to determine prognosis and treatment strategy. Modern conventional investigation methods commonly used for tumor staging possess low sensitivity for neck metastasis detection, as up to 40% of cN0 necks are left undetected of occult disease, which presents sizes of less than 3mm. Correspondingly the current treatment of regional lymphatic basin in this group of patients depends of a choice between possible under treatment of 30-40% of patients with undetected metastases and overtreatment of the 60-70% of them [14].

The sentinel lymph node (SLN) apprehension was first addressed by Gould’s report on the James Ewing Society in 1960 and later expanded by Cabanas in 1977. Initially investigated in melanoma [15] and breast cancer [16] patients in short time SLN biopsy gained a considerable interest in head and neck research. The concept of SLN may be regarded as a modern approach enrooted and established by continuous anatomic and clinical
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Methods of Sentinel Lymph Node Identification

Lymphography is defined as visualization of the lymphatic channels and lymph nodes after injection of a contrast (usually radiopaque) material in a lymphatic vessel [22]. It is well recognized that regardless of chemical or physical features of the material injected for the means of contrast, the dominating factor that controls transport through lymphatics is the size or the hydrodynamic diameter of its particles. These properties range between contrasts used in different lymphographic modes. A general rule is that for agent’s which particles’ size is less than 10 nm (macromolecules) there will be high diffusion of the contrast from the lymphatic vessels and fast migration through the system. The mid-range agents with particles’ size from 50 to approximately 200 nm (nanoparticles) exhibit slower migration speed and stronger retention in the vessels. Particles greater than 500 nm (microparticles) will be slowly retained in the vessels the migration speed will be very low with contrast reaching only the first echelon node/s [23]. According to the time of performance the SLN imaging may be preoperative or intraoperative, depending on the means of delivery of the contrast medium into the lymphatic vessel lymphographic techniques are generally divided into direct and indirect [22, 24].

Direct lymphography (lymphangiography) was employed by Cabanas (1977) in his SLN pioneer study in penile carcinoma. It requires an iodinated contrast agent injection straight into the lymphatics [25]. This is complimented with X-rays, CT or MRI scans [26,27]. The iodinated contrast agent stays in the vessels for months which allows for long-term follow up but makes repetitive injections impossible. Such features as invasive and technically difficult cannulation of the lymphatic vessel and rare cases of infection, the dominating factor influencing results. Combining lymphoscintigraphy with single-photon emission computed tomography (SPECT) allowed for 3D images reconstruction and to improve the accuracy up to 95%. However, poor spatial resolution of the acquired images remained not satisfactory (1-2cm) [42-44]. Another moderate limitation of the technique is the so called “shine through” effect, which is encountered in identifying the SLN in level I in cases with primary tumor situated in the mouth floor. The radiation counts of the primary site overshadowing the sentinel node site background counts which bring difficulties for its detection by the gamma-probe. To overcome these conditions several solutions have been proposed: the use of lead shielding; removing the primary tumor prior to SLN; and mandatory level I dissection [45]. A large trial reported that the ability to identify the sentinel node was lower in patients with mouth of the floor SCC compared to other oral cavity sites (88% vs. 96%, p=0.138). The authors recommended the use of SLN biopsy as a single staging procedure in all oral cavity subsites except of mouth floor [46]. Possibly this matter may be at least in part accounted for often overlooked lingual lymph nodes which in a recent anatomical study were reported to be present in 23.8% of individuals and can serve as SLNs for some tongue and sublingual subsites except of mouth floor [47].

Indirect lymphography involves several modalities related to SLN mapping. The contrast agent is injected intravenously, intradermally, intramucosally, or subcutaneously and is drained to the lymphatic vasculature with sequential progression to the closest regional (sentinel) lymph node/s. Indirect lymphographic procedures are less invasive, easy to reproduce and cause no direct damage to the lymphatics. Indirect ink lymphography exploits blue dyes (Evans blue, isosulfan blue, methylene blue, patent blue) for visualization of lymphatic drainage after interstitial injection. Intraoperative injections of blue dye which later were adopted in various locations [15,30]. While indirect lymphography achieves accuracy up to 96% in melanoma of the head and neck its use in solid tumors of oral cavity requiring injections in the mucosa is restricted [31-34]. Generally dyes bear characteristics which dispose their very rapid migration through the lymphatic vessels, low invasive, easy reproducible and cheap but if used alone their productivity in SLN identifying is somehow limited to superficial tissue plains, in particular above the deep fascia on the trunk and the extremities and some allergic reaction on methylene blue, isosulfan blue and patent blue dyes reported in number of papers limited this technique to implement to all patients [35-37]. At present dye injections are used in combination with other more up to date methods for better intraoperative orientation.

Radioisotope technique (lymphoscintigraphy) is the most commonly used indirect lymphography modality which involves preoperative radioactive tracer injections (such as Technetium [Tc99m] sulfur colloid) with intraoperative localization of the nodes with external radiation detectors (gamma-probe). The fragments of this tracer or radiocolloids are nanoparticles with respective hydrodynamic behavior [38,39]. Gamma radiation is highly penetrating; tissue depth and density are no obstacles for Tc99m-based lymphoscintigraphy. The patterns of injected tracer distributions are imaged in 2D definition which precludes accurate location of the lymph node. A meta-analysis of SLN biopsy with lymphoscintigraphy for oral and oropharyngeal cancers proved a pooled sensitivity of 93% and negative predictive value rates from 88 to 100% [40]. Recent multicenter trial from Europe revealed sensitivity of 80% and negative predictive value 88% [41]. In general overall accuracy, sensitivity, and negative predictive values for lymphoscintigraphy sentinel lymph node biopsy are above 90% [14]. Surgeon experience in this technique is one important factor influencing results. Combining lymphoscintigraphy with single-photon emission computed tomography (SPECT) allowed for 3D images reconstruction and to improve the accuracy up to 95%. However, poor spatial resolution of the acquired images remained not satisfactory (1-2cm) [42-44]. Another moderate limitation of the technique is the so called “shine through” effect, which is encountered in identifying the SLN in level I in cases with primary tumor situated in the mouth floor. The radiation counts of the primary site overshadowing the sentinel node site background counts which bring difficulties for its detection by the gamma-probe. To overcome these conditions several solutions have been proposed: the use of lead shielding; removing the primary tumor prior to SLN; and mandatory level I dissection [45]. A large trial reported that the ability to identify the sentinel node was lower in patients with mouth of the floor SCC compared to other oral cavity sites (88% vs. 96%, p=0.138). The authors recommended the use of SLN biopsy as a single staging procedure in all oral cavity subsites except of mouth floor [46]. Possibly this matter may be at least in part accounted for often overlooked lingual lymph nodes which in a recent anatomical study were reported to be present in 23.8% of individuals and can serve as SLNs for some tongue and mouth floor cancers [47]. Apart from this radioisotope technique craves radiation exposure to both patients and the medical staff and requires special protection and waste disposal policy [48].

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While conventional CT and MRI have limited ability for neck metastasis detection and are used mainly for the primary site lesion extension assessment and staging [49] some researchers investigated their usefulness in SLN detection.

CT-lymphography was recently utilized for oral cancer SLN explorations [50], Saito, et al. [51] observed an occult metastasis in a node located in the floor of the mouth, i.e. lingual lymph node with no other regional disease, Honda, et al. [52] conducted a study on 31 cNO patients with oral tongue SCC to assess the properties of CT-lymphography for SLN spotting. The authors reported high negative predictive value of 95, 8%, and sensitivity value of 90.3%. Also a simple way for localizing the node has been introduced – a lattice marker was attached to the neck skin during the scan. The SLN location was indicated precisely by the crossing points of the lattice marker and the CT plane light [52]. Overall CT lymphography has the advantage of clear detection of lymph nodes lying in the proximity of the primary tumor, with continuous anatomic visualization [53,54]. Iodine-based contrasts used for CT-lymphography are small sized agents creating very short imaging windows with 1 minute clearance with high reported safety [55,56].

MRI lymphography has been used for SLN detection mainly in breast [57], and pelvic tumors [58]. One feature of these techniques is the possibility of differentiating between benign and malignant nodes depending on the node’s imaging pattern. Two commonly used agents for enhanced MRI are ultrasmall paramagnetic iron oxide (USPIO) and gadolinium chelates. USPIO is specific for the reticuloendothelial system. Malignant lymph nodes mainly consist of tumor cells these do not take up this contrast because of lacking reticuloendothelial system activity that can be detected by shorter T2 relaxation time [59]. As well as USPIO the gadolinium chelates have a small particle size which leads to poor contrast distribution in a short time after injection. Accuracy of contrast distribution in a short time after injection. Accuracy of CT-lymphography for SLN spotting. The authors reported high negative predictive value of 95, 8%, and sensitivity value of 90.3%. Also a simple way for localizing the node has been introduced – a lattice marker was attached to the neck skin during the scan. The SLN location was indicated precisely by the crossing points of the lattice marker and the CT plane light [52]. Overall CT lymphography has the advantage of clear detection of lymph nodes lying in the proximity of the primary tumor, with continuous anatomic visualization [53,54]. Iodine-based contrasts used for CT-lymphography are small sized agents creating very short imaging windows with 1 minute clearance with high reported safety [55,56].

Near-infrared fluorescence [NIF] a novel approach that allows optical imaging during surgery was firstly introduced by Kitai, et al. [69] in 2005 for breast cancer. Near-infrared light (700-800nm) is characterized by high tissue penetration rate up to 30mm with decreasing quality of visualization in deeper layers [70]. This technique combines advantage of dye in vivo visualization with excellent Intraoperatively the location of peritumoral injected contrast’s NIF fluorescence signal is captured and envisioned in real-time with an external fluorescence detector and clear definition of anatomical background [71]. In superficial lesions NIF technique allows transcutanous illumination of the lymphatic channels and nodes which grants optimal incision design and placement thereby reduces operating time [72,73]. Indocyanine green is used as a contrast a low molecular weight organic molecule, which fluorescence in the near-infrared, it’s a negatively charged, amphiphilic, water-soluble tricarbocyanine with very low toxicity, high safety and low number of allergic reactions [73,74-76]. Safety, reliability and high identification rates have been observed in preliminary studies of NIF used for SLN identification in head and neck cancer patients [77-79]. Lately Christensen et al. [80] have described a combined method of NIF and lymphoscintigraphy with preoperative SLN identification in all of 30 patients and intraoperative detection rate of the preoperatively defined nodes was 97% [66/68 nodes] [80]. In general this technique has prominent advantages and further clinical research is desired for its practical implementation.

When reviewing the properties of these different procedures one should point out that while ink lymphography, lymphoscintigraphy NIF require in fact only a surgeon trained in sentinel lymph node biopsy the currently evolving CT-, MRI- and US-lymphography requires a close cooperation between the surgeon and the radiologist. Hence the radiologist must also be familiar with lymphography principles and be ready to spot the contrast distribution in a short time after injection. Accuracy of SLN detection relies heavily on the experience of the centre and the clinician with a particular technique. Further clinical research is needed and sufficient learning curve must to be achieved in the newly introduced techniques which in their turn are aimed for achieving less invasiveness, easy reproducibility and better imaging quality.

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