Novel techniques for sentinel node biopsy in head and neck cancer

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NOVEL TECHNIQUES FOR SENTINEL NODE BIOPSY IN HEAD AND NECK CANCER

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Abstract

The most important prognostic indicator in early cancer is whether the disease has metastasised to regional lymph nodes. Pre-operative imaging is not sensitive enough to detect micrometastatic deposits therefore most patients judged to have more than 20% risk of disease spread will have elective surgical removal of the draining lymph node basins in order to reduce the risk of leaving tumour behind. Such operations can be lengthy and associated morbidity can reduce the patient's quality of life. In the majority of these elective nodal clearances histopathological analysis is clear of disease in the majority suggesting that the surgery could be omitted without affecting the patient's disease-free survival.

Sentinel node biopsy (SNB) is a surgical stating test in which the tumour draining lymph nodes can be thoroughly investigated for metastasis. If free of disease there is no indication to subject the patient to further surgery. SNB has been validated in oral cancer, but there is a false negative rate of up to 14%. Recent developments may improve the accuracy of the SNB test.

This work evaluated new sentinel node technologies (navigation surgery, fluorescence imaging, and improved tracer formulations) in oral cancer, and opened up new applications for the test in other head and neck cancers (salivary, thyroid and larynx). These refinements in sentinel node process may allow many patients suffering with early cancer to benefit from personalised staging and treatment.

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Abbreviations

Abbreviation	Meaning
AJCC	American joint committee on cancer
AMIGO	Advanced multimodal image guided operating
ANOVA	Analysis of variance analysis
CK	Cytokeratin
CND	Completion neck dissection
CS	Clare Schilling
CSI	Clinical spectral imager
CT	Computed tomography
DCT	Differentiated thyroid cancer
END	Elective neck dissection
ENT	Ear nose and throat
fhSPECT	Freehand SPECT (Single-photon emission tomography)
FL	Fluorescence
FNAC	Fine needle aspiration cytology
FNR	False negative rate
FS	Frozen section
GSTT	Guys and St Thomas' NHS Trust
HNSCC	Head and neck squamous cell carcinoma
ICD	International statistical classification of disease
ICG	Indocyanine green
LSG	Lymphoscintigraphy
MDM	Multidisciplinary meeting
MHRA	Medicines health regulatory authority
MM	Professor Mark McGurk
MRI	Magnetic resonance imaging
MRND	Modified radical neck dissection
NIR	Near-infrared
NICE	National institute of health and care excellence
OMFS	Oral and maxillofacial surgery
OSCC	Oral squamous cell carcinoma
OSNA	One step nucleic acid amplification
PET	Positron emission tomography
PIS	Patient information sheet
PLND	Pelvic lymph node dissection
PVB	Patent blue dye
RCT	Randomised controlled trial
RT	Radiotherapy
SBR	Signal to background ratio
SCM	Sternocleidomastoid muscle
SNB/SLNB	Sentinel node biopsy/Sentinel lymph node biopsy
SPECT/CT	Single-photon emission tomography/Computed tomography
Tc	Technetium
TMN	Tumour, node and metastasis
TORS	Transoral robotic surgery
TRAQ	Trust risk and quality
US	Ultrasound
WHO	World health organisation
Zr	Zirconium

1.1 Head and Neck cancer

The medical field of “Head and Neck” describes a surgical subspecialty comprising oral and maxillofacial surgery (OMFS), ear nose and throat (ENT) and plastic surgeons who perform ablative and reconstructive surgery in a range of neoplasms located above thoracic inlet excluding primary intracranial tumours. Head and neck surgeons predominantly deal with malignant tumours as categorised according to the International Statistical Classification of Disease and Related Health Problems version 10 (ICD-10[1]). Globally head and neck cancer is the sixth most common form of cancer, with annual worldwide incidence of 650,000 and causing 350,000 deaths per year[2]. Malignancies can develop in a variety of structures such as the upper aerodigestive tract, craniofacial skeleton, paranasal sinuses, salivary and thyroid glands as well as skin, soft tissue, haematological and metastatic tumours. This heterogeneous group treated under the umbrella of head and neck surgery can develop from a variety of cell types, each with a specific set of biological risk factors and natural history (Table 1. Head and Neck Cancer). The most prevalent malignancies (>90%) found the head and neck arise in epithelium exposed to carcinogens resulting in squamous cell carcinoma[3].

Almost two-thirds of patients with head and neck squamous cell carcinoma (HNSCC) present with locally advanced disease and a further 10% with distant metastasis[4]. Such patients staged III and IV respectively by the TNM (American Joint Committee on Cancer[5]) classification system who are suitable

for active treatment will usually be offered a multimodal approach. Despite this the outlook is poor with a 35-52%[6-9] five year overall survival rate. Conversely the treatment for early HNSCC is usually single modality and 5 year survival is achieved in 90% of patients with stage I and 70% with stage II disease[3].

Table 1-1 Overview of primary head and neck cancer

Anatomical location (ICD-10 code)	Predominant histopathological subtypes	Incidence 1.Worldwide annual incidence[10] 2.UK age standardised observed rate/100,000 males in 2014[11]	Risk factors[12]	Five year survival rate (UK[13])
Oral Cancer (ICD-10 C00, C02-C06, C14)	Squamous cell carcinoma (SCC) 93% Adenocarcinoma 2.8% Mucoepidermoid 1.4% Adenoid cystic 1% [14]	Worldwide 275,000[10] UK 21.38/100,000[11]	Alcohol Tobacco Betel nut chewing Premalignant conditions Dietary deficiencies	Stage I 82.8% Stage IV 27.8%
Nasopharynx (NPC), oropharynx hypopharynx (ICD:10 C09-10, 11-13)	Squamous cell carcinoma (95%) Nonkeratinising squamous cell carcinoma (75-99% NPC)[12])	Worldwide 130,300[10] UK (2006[15]) Nasopharynx 0.4/100,000 Oropharynx 2.25/100,000 Hypopharynx 0.66/100,000	Alcohol Tobacco Viral (HPV, EBV) Genetic (NPC)	Stage 1 56% Stage IV 42%[16]
Laryngeal Cancer (ICD-10 C32)	Squamous Cell (80-95%[12])	Worldwide 157,000[17] UK 8.36/100,000[11]	Alcohol Inhaled tobacco Industrial exposure Ionising radiation Gastric reflux Viral (HPV)	Stage I 91.1% Stage IV 41.9%
Salivary Gland Cancer (ICD-10: C07-8)	Mucoepidermoid 26% Adenoid cystic 22% Adenocarcinoma 17%[18]	Worldwide 4,000[19] UK 1.38/100,000[18]	Ionizing radiation Viral (EBV, CMV) Industrial exposure Hormonal	Stage I 95.6% Stage IV 28.6%[20]
Thyroid Cancer (ICD-10 C73)	Papillary 83.2% Follicular 6.5% Hurthle 3.2% Medullary1.7%[14]	Worldwide 298,000[17] UK 3.19/100,000[11]	Ionising radiation Family history (MEN)	Stage I 99.9% Stage IV 55%[21]

In addition to undergoing treatment for a life-threatening disease, head and neck cancer survivors uniquely have to cope with facial disfigurement and functional impediments such as changes in speech and swallow. Furthermore surgery to the neck performed to remove metastatic or 'at-risk' lymph nodes is associated with long-term neck and shoulder pain, reduced shoulder mobility, weakness of facial muscles, and scarring. This treatment related morbidity significantly reduces patient's quality of life[22, 23]. Treatment related morbidity can be reduced by tailoring the extent of surgery or radiotherapy to the clinically apparent disease however this requires an accurate patient-specific approach to ensure that those with occult metastatic disease are not disadvantaged. It is this health need that has led to the consideration of adopting novel staging techniques such as sentinel node biopsy (SNB) in the management of head and neck cancer.

Each tumour has different controversies and neck management uncertainties that are further discussed below.

1.2 Oral cancer

Malignant tumours arising in the oral cavity are overwhelmingly epithelial in origin and are commonly referred to as oral squamous cell carcinoma (OSCC). It should be noted that although tumours of the lip are grouped with the oral cavity by ICD-10, the triggers and biological behaviour are more akin to skin SCC and thus they are managed differently to other oral tumours.

In western countries intraoral sites of predilection are the lateral/ventral tongue (40%) and the floor of mouth (30%) [24, 25]. In Asian populations there is a higher rate of buccal tumours, the location of 40% of OSCC in Sri-Lanka[10].

Although the development of OSCC is a multifactorial process, irreversible cell changes are triggered by a sustained and dose-dependent physical contact with carcinogens[26, 27]. The geographical anatomical variation relates to cultural preference for such substances. In western countries exposure with either alcohol and/or tobacco is found in over 90% of cases, and in countries where betel chewing is endemic (mainly the Indian subcontinent) it is implicated in up to 50% of cases [27].

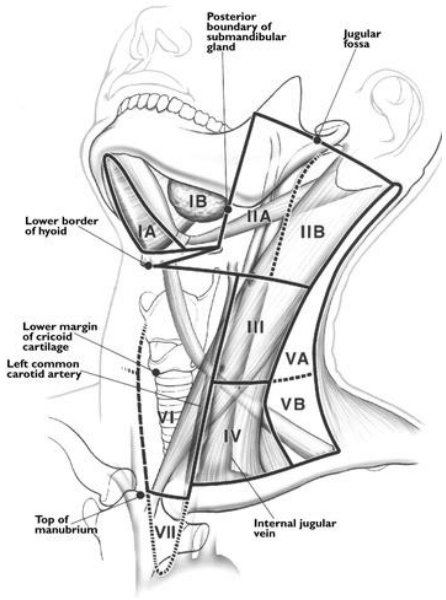
Table 1-2 - American Joint Committee on Cancer (AJCC) cancer staging manual 7th edition (2010)[28]. TNM classification for oral, salivary, and differentiated thyroid cancer

T- Tumour			
	Oral	**Salivary**	**Thyroid (differentiated)**
Tx	No available information on the primary tumour		
T0	No evidence of primary tumour		
Tis	Carcinoma in situ	N/A	
T1	Tumour less than 2cm in greatest dimension	< 2cm and no extraparenchymal spread	T1a ≥1cm T1b ≥1 ≤2
T2	Tumour 2-4 cm in greatest dimension	2-4cm and no extraparenchymal spread	2-4cm limited to thyroid
T3	Tumour greater than 4cm in greatest dimension	>4cm and/or extraparenchymal spread	>4cm and/or minimal extrathyroid spread
T4a (resectable)	Tumour invades adjacent structures (cortical bone, extrinsic muscles of the tongue, maxillary sinus, skin)	Invades skin, mandible, ear canal, facial nerve	Invades subcutaneous soft tissue, larynx, trachea, oesophagus, or recurrent laryngeal nerve
T4b (irresectable)	Tumour invading masticator space, pterygoid plate, skull base and/or encases internal carotid artery	Invades base of skull, pterygoid plates, or encases carotid Artery	Invades prevertebral fascia or encases carotid Artery/vertebral vessels.
N-Node			
Nx	Regional nodes not assessed		
N0	No region lymph node metastasis		
N1	Metastasis in a single ipsilateral lymph node of 3cm or less in maximum dimension		N1a Metastasis to level VI N1b Metastasis unilateral/bilateral cervical nodes, or superior mediastinal nodes
N2a	Metastasis in a single ipsilateral lymph node more than 3cm but less than 6cm in maximum dimension		N/A
N2b	Metastasis in multiple ipsilateral lymph node more than 3cm but less than 6cm in maximum dimension		N/A
N2c	Metastasis in bilateral or contralateral lymph node more than 3cm but less than 6cm in maximum dimension		
N3	Metastasis in a lymph node more than 6cm in greatest dimension		
M – Metastasis			
Mx	Distant metastasis cannot be assessed		
M0	No distant metastasis		
M1	Distant metastasis		

Patients diagnosed with OSCC are clinically staged (by examination and imaging) pre-operatively according to the TMN classification (Table 1.2).

In the UK surgery is the mainstay of treatment in all but palliative cases of OSCC. Adjuvant radiotherapy or chemoradiation are only indicated if there are adverse features that increase the chance of recurrence[29, 30]. The primary tumour is excised with a wide pathological margin (>5mm) necessitating reconstruction with pedicled or free flap in cases where primary closure is not possible. Surgical resection of regional lymph nodes (neck dissection) is performed at the same time as resection of the primary tumour with the additional benefit of access to neck vessels in patients requiring microvascular reconstruction. Patients with clinically diagnosed cervical metastasis undergo therapeutic neck dissection to remove all metastatic nodes plus other involved structures with a clear margin (Table 1.3. Classification of neck dissection), management of the clinically N0 neck is more controversial.

Table 1-3 Classification of neck dissection based on recommendations of American Head and Neck Society (AHNS)[31]

Neck Dissection	**Structures dissected**	**Neck Levels** Image source; American Journal of Roentgenology 2000[32]
Selective Neck Dissection (SND)	One or more neck levels dissected. E.g. Supraomohyoid (levels I-III)	
Modified radical neck dissection (MRND) Type I	Dissection of levels I-V with preservation of the spinal accessory nerve (XI)	
Modified radical neck dissection (MRND) Type II	Dissection of levels I-V with preservation of XI and the internal jugular vein (IJV) or sternocleidomastoid muscle (SCM)	
Modified radical neck dissection (MRND) Type III	Dissection of levels I-V with preservation of IX, IJV and SCM.	
Radical Neck Dissection	En bloc clearance of levels I-V, spinal accessory nerve, IJV, SCM	
Central Compartment Neck Dissection	Selective dissection of level VI and VII	
Extended Neck Dissection	Inclusion of non-lymphatic structures e.g. carotid artery or additional lymphatic structures outside defined neck levels.	

Analysis has shown that despite clinical examination and multimodality imaging there is a limit of sensitivity for pre-operative diagnosis of small metastasis [33-35]. Metanalysis looked at the sensitivity of occult metastasis detection by different imaging modalities in necks that had been classified N0 by clinical examination, finding pooled estimates of 52%, 65%, 66%, and 66%, for CT, MRI, PET and US, respectively[36].

When occult neck metastasis are left untreated until they become clinically apparent (i.e. the patient undergoes a therapeutic rather than elective neck dissection) there is a significant detriment to outcome as demonstrated by a prospective randomised controlled trial comparing the two strategies in early (T1-T2) oral cancer[37]. In this trial the overall survival after elective neck dissection (END) was 80% compared to 67.5% in the therapeutic neck

dissection arm (hazard ratio 0.64, p=0.01). However, neck metastasis developed in (108/253) 43% of patients randomized to the therapeutic surgery arm. This would appear to represent the 'true metastasis' rate, and the cohort in whom resection of the cervical lymph nodes is indicated.

Neck dissection is associated with complications such as chyle leak, wound infection, sensory disturbance, shoulder and neck dysfunction and scar[38, 39], and so ideally it should only be undertaken in patients for whom there will be a oncological benefit. Furthermore, elective neck dissection may give false reassurance that all occult metastasis are removed reliably, leaving a disease free neck. The combined results of two major cancer centres (Memorial Sloan-Kettering, New York USA and Princess Margaret, Toronto Canada) reported that in a cohort of 164 patients with tongue tumours staged pT1-T2 N0 by elective neck dissection, 18% developed isolated recurrence in the neck[40]. Over one-third of the recurrences (39%) were in the contralateral neck and when a subset of the neck dissections (52/164) were investigated by immunohistochemistry and serial sectioning previously undetected metastasis were identified in 15% of cases. These data suggest that blanket ipsilateral END will still miss metastasis in a small but significant group of patients.

In contrast, sentinel node biopsy (SNB) is a diagnostic test designed to detect occult metastasis. It provides accurate staging and identifies those patients with early metastatic disease that will benefit from lymphadenectomy and/or adjuvant treatment.

The Sentinel European Node Trial (SENT[41]) showed that sentinel node biopsy (SNB) is a reliable staging tool in the cN0 neck in early oral cancer. The

sensitivity for detection of metastasis was 88%, but there was a false negative rate (FNR) of 14%. SNB is an operator sensitive procedure but the merit of the test has been appreciated by National Institute for Health and Care Excellence (NICE). After a cost-effectiveness analysis NICE recommended that from January 2016, SNB is offered to UK patients with T1-T2 OSCC with clinically N0 necks who do not require microvascular reconstruction[42].

1.3 Salivary gland cancer

Malignant neoplasms of the salivary glands are a heterogeneous group. In 2005 the World Health Organisation (WHO) classified salivary gland malignancy into 23 different entities[12], the most common of which are listed in Table 1.1. These tumours can be classified according to which gland they arise in (parotid, submandibular, sublingual, minor), and also by cell origin.

There are geographical variations in the prevalence of histological subtypes[43, 44], but the anatomical distribution always favours the parotid gland, which is the location of 60-80% of a salivary tumours[12, 19, 43]. A further 7-11% occur in the submandibular gland, 9-23% in the minor salivary gland and 1% in the sublingual gland.

Histopathologically primary salivary malignancy can derive from epithelial cells (Mucoepidermoid carcinoma, acinic cell, adenoid cystic, adenocarcinoma), mesenchymal cells (sarcomas, histiocytomas), and haematolymphoid cells (lymphomas) [45].

As well as being a histologically diverse group, salivary gland malignancies are rare. The combined global incidence of benign and malignant salivary tumours

is 0.4-2.6 cases per 100,000, just 6% of all head and neck tumours [43, 46, 47]. The majority of salivary tumours are benign although this varies by gland, as demonstrated by the '25:50:75 rule' which although historical gives a rough guide to the propensity of malignancy in tumours of the parotid, submandibular and sublingual glands respectively [18]. In total salivary gland malignancy represents 0.3% of all cancer types. Because of the diversity and scarcity of salivary tumours there is a deficit of high level evidence regarding treatment, a fact recognised by recently published UK management guidelines[48].

Surgery is the primary treatment modality, with wide excision of the tumour and therapeutic neck dissection in the case of lymph node metastasis. The extent of margin is debatable, particularly in relation to adenoid cystic carcinoma, which has an indistinct tumour edge with beads of tumour cells permeating the surrounding tissue. Post-operative radiotherapy is indicated in patients with radiosensitive tumours over 4cm in size or with high-grade features, extraparenchymal or nodal extracapsular spread, or multiple nodal metastases.
The case for elective neck dissection is not clear. Most clinicians would perform END in the case of T3-4 and high grade tumours. Nevertheless, Nobis et al. showed that up to 30% of patients with low-grade malignancies harboured cervical metastasis. Furthermore several case series looking at END in relation to parotid malignancies found that many of the metastatic nodes were outside the traditional neck dissection fields. Stenner et al[49] found that up to three quarters of the positive lymph nodes were intra- or periparotid. This has important implications in parotid surgery. In the case of a small <4cm (T1-2) tumour, a subtotal superficial parotidectomy may be sufficient to clear the primary disease so minimising the risk of morbidity to the facial nerve. But if

one is concerned about positive intraparotid lymph nodes then an argument could be made for a total parotidectomy. The risk versus benefit of removing the deep lobe is uncertain as cadaveric studies have shown that the deep lobe does not contain any lymph nodes in up to 93% of patients[50, 51].

Consequently the same question arises as in managing early SCC: "what is the optimum method of identifying and removing occult lymph node metastasis?"

1.4 Thyroid cancer

Thyroid tumours are classified by the World Health Organisation as endocrine tumours rather than head and neck tumours [52]. In many countries surgery for thyroid malignancy is centralised but can fall under the care of head and neck surgeons, ENT surgeons, endocrine and general surgeons. In the UK surgeons operating on thyroid malignancies are required to provide consultant level outcome data and interrogation of the UK Registry of Endocrine and Thyroid Surgery (UKRETS) found that there are 147 surgeons fully registered in the UK, sixteen of whom identify as head and neck surgeons[53].

Thyroid tumours are epithelial in origin; commonly follicular cells which give rise to differentiated follicular and papillary carcinoma (Table 1.1). Poorly differentiated and anaplastic tumours also arise from follicular cells and may represent progression of differentiated thyroid cancer (DTC), whereas medullary carcinomas arise from parafollicular 'C' cells.

Thyroid cancer is the most common type of endocrine malignancy, and constitutes 1.7% of all malignancies worldwide. There is considerable geographic variation with higher incidence in the developed world, although

there is a consistently higher incidence in females. The incidence of thyroid cancer has increased over the last 3 decades but cure rate for DTC remains high with an 80-90% ten year survival rate, even in patients with regional metastatic disease.

Fine needle aspiration cytology (FNAC) guided by ultrasound scan is the investigation of choice but it is not always possible to differentiate between benign and malignant disease (particularly follicular adenoma or carcinoma). It is not uncommon for patients to undergo a diagnostic thyroid lobectomy, which may be converted to a staged completion thryroidectomy once the pathology has been reviewed. Staging is according to TMN (Table 1.2).

Occult lymph node metastasis are thought to occur in 20-50% of DTC[54], however the significance of this spread is debatable. Most patients will receive post-operative radioiodine, which ablates both residual thyroid tissue and metastatic disease. This offers excellent tumour control, but there is a small subset of patients who relapse. Criteria for these 'high-risk' patients include male gender, age over 45 years, tumour over 4cm and extra capsular or extra-thyroid disease. Elective central neck dissection (clearance of level VI and VII) is recommended in high-risk patients, although the procedure is associated with an increased risk of recurrent laryngeal nerve damage and hypoparathyroidism[55, 56].

On the opposite end of the spectrum there are clinical trials in progress looking at whether patients with low risk disease can be spared iodine treatment[57].

There is lack of consensus about the extent of lymph node sampling required to stage both high and low risk patients, to which SNB may offer a solution.

1.5 Laryngeal cancer

The larynx extends from the tip of the epiglottis to the cricoid cartilage and is divided into the supraglottis, glottis and subglottis each with their own TNM staging. Most laryngeal cancers arise in the supraglottic and glottic regions and 95% are squamous cell in origin (Table 1.4). Men are more commonly affected than women and the risk of developing the disease are increased by the synergistic effects of alcohol and smoking (Table 1.1).

Table 1-4 - American Joint Committee on Cancer (AJCC) cancer staging manual 7th edition (2010)[28]. TNM staging of Laryngeal Cancer.

T- Tumour			
	Supraglottis	**Glottis**	**Subglottis**
T1	One subsite, normal cord mobility	Limited to vocal cord(s) with normal mobility	Limited to subglottis
T2	More than one subsite, no fixity of larynx	Extends to supra- or subglottis with normal (T2a) or impaired (T2b) vocal cord mobility	Extends to vocal cord(s) with normal or impaired mobility
T3	Vocal cord fixation and/or post cricoid, pre-epiglottic or paraglottic spread with minor cartilage invasion	Invades inner cortex of laryngeal cartilage	Limited to larynx with vocal cord fixation
T4a	Invades through cartilage, extends beyond larynx	Invades through cartilage, extends beyond larynx	Invades through cartilage, extends beyond larynx
T4b	Invades prevertebral space, mediastinum, carotid artery	Invades prevertebral space, mediastinum, carotid artery	Invades prevertebral space, mediastinum, carotid artery

The treatment of laryngeal cancer is very much dependent upon tumour size with the emphasis upon preserving laryngeal function. Small tumours up to T2 can be treated by either radiotherapy, transoral laser microsurgery (TLM) or transoral robotic surgery. Traditionally more advanced tumours (T2b-3) are offered chemoradiotherapy, however certain cases may be suitable for function

sparing surgery such as vertical partial laryngectomy (VPL). Laryngectomy is recommended when tumour has spread through cartilage and into the soft tissues of the neck[58]. In node positive disease, it is recommended[58] that levels II–V should be treated on the involved side. Elective treatment of the N0 neck is recommend in T3 and T4 disease. Radiotherapy (RT) or surgery ± post-operative RT is provided to at least lymph node levels II, III and IV bilaterally.

The incidence of occult cervical metastasis in T3/T4 disease is reported as 21-78%[59-61], although the reported range is wide there is no doubt that some patients are undergoing unnecessary treatment with the additional burden of a bilateral neck dissection. Additionally there is controversy over the management of paratracheal nodes which are found to harbour occult metastasis in 9-27% of cases but do not form part of the routine neck dissection[62]. SNB again offers the possibility of bespoke treatment for this group of patients.

1.6 Development of sentinel node biopsy

In the 18th century the discovery that malignant disease spread via lymphatics set the intellectual framework for the move to radical surgical treatments which started in the 1930's with Halstead and radical mastectomy and in the head and neck by Butlin and Crile. Both life and surgery were harsh in this period and the significant surgical morbidity this approach engendered was accepted. It was only late in the last century that sentinel node biopsy was adopted as staging tool for patients with early cancer.

In the 1960s Ernest Gould, head of surgery at Washington Hospital, described for the first time intraoperative node sampling for metastasis by frozen section. He would dissect the “angular node” during parotidectomy and based on the histology would decide whether to proceed with radical neck dissection or not[63]. Intellectually there was acceptance that lymph node metastasis which were not yet clinically apparent could be detected microscopically, but the challenge was in sampling the right nodes.

The sentinel node concept was further explored in penile cancer by Cabanas in the 1970s[64] when he performed lymphangiograms of the dorsal lymphatics of the penis. Cabanas questioned the routine use of bilateral groin lymphadenectomy for both clinically suspicious and clinically normal nodes. He felt that lymphatic metastasis was an embolic mechanical process, and would usually be a late phenomenon in penile tumours due to thick fascial planes acting as a natural barrier. At the time there was no consensus about the best management of the groin nodes in penile cancer and patients suffered considerably from lymphoedema of the lower limbs when groin dissection was undertaken. He developed a technique using a radiopaque dye and plain x-ray but this was unwieldy and the impact of his reports was limited to targeted nodal resection. In 1992 Morton et al [65]published their data on the application of sentinel node biopsy in the management of melanoma. They confirmed the basic concept that if the SLN contained no cancer cells, then one can reliably assume that all the other nodes in the drainage area are cancer-free. They reported a 99% sentinel node identification rate with a false negative rate of <1%, which was highly persuasive that SNB was safe to use as a staging tool in its own right. If their results are calculated by today’s standards in fact their

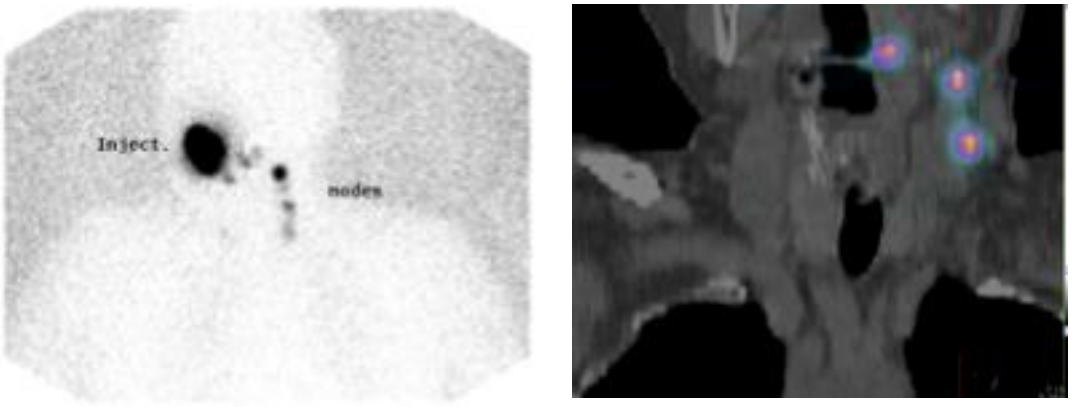
sentinel node identification rate was 86% (194/223 patients) and false negative rate of 4% (47 true positive and 2 false negative results) but nevertheless these impressive results paved the way for the adoption of SNB into routine care. Morton et al had introduced important development to the technique – the use of vital blue dye -, which allowed the sentinel node to be identified by colour. This optical tracer was better than Cabanas' x-ray method, but required the identification of the fine lymphatic channels draining the dye. This in turn drew criticism for the extensive dissection required to expose the channels [66] and detection was difficult when there was haemorrhage in the operative field. Furthermore the dye quickly flowed through the sentinel node to lower echelon nodes and Morton attributed this sampling error for the two false negative results, recommending top up injections during the procedure.

The next advance was made by Krag et al who investigated the use of technetium labelled sulphur colloid for SNB, a substance already licensed for the investigation of lymphatic abnormalities [67, 68]. The radiolabelled colloid had two advantages, the general position of the SN could be identified on the patient by lymphoscintigraphy (LSG) prior to surgery and secondly the radiation could be traced trough the tissues during surgery by means of a hand-held gamma probe. This is not the whole answer for without a visual marker it is still difficult to identify the sentinel node in situation of low signal to background ratio.

The relative advantages of the two tracers (blue dye & radiolabelled colloid) either together or alone have been debated [67, 69-72] but the SNB technique has now has been validated through large prospective international studies [73-

75]. Consequently SNB is now widely adopted in the management of breast cancer and melanoma with agreed guidelines that include both tracer modalities[76, 77] resulting in an average false negative rate in breast cancer of 7% [78]and 12.5% in melanoma[79].

Pre-operative sentinel node localisation had been aided by the recent advent of Single Positron Emission Computed Tomography/CT (SPECT CT), a fusion of gamma radiation images with conventional CT (Figure 1.1). This gives added anatomical detail on the position of the radioactive focus (sentinel node) in three dimensions and is a useful addition to the SNB protocol in a number of different tumour types[80] There is advantage particularly in node localisation in deep cavity tumours [81-83]and overweight patients[84].



A. Planar lymphoscintigraphy images of anterior floor of mouth tumour.

B. SPECT/CT images of patient in showing improved anatomical localisation of the sentinel nodes compared to image A.

Figure 1-1 - Lymphoscintigraphy (A) and SPECT/CT (B) images from patient with an anterior floor of mouth tumour.

1.7 Physiology of sentinel nodes

The process by which tumour disseminates around the body, and particularly the ability form metastatic lymph node deposits is due to a highly complex interplay between tumour and host cytokine signals that allows transformation of tumour cells between epithelial and mesenchymal phenotype. The mechanisms by which invasion of the lymphatic system and formation of a viable growth within the lymph node occurs is the subject of much debate, but there is an element of lymph-node 'preparedness' to support metastatic growth. Much work has looked at the lymph node signalling microenvironment but few have related this to changes in the physiological functioning of the metastatic and pre-metastatic lymph node. In 2003 a group in Washington university cancer research centre investigated Eμ-c-*myc* transgenic mice that develop a highly metastatic strain of lymphoma[85]. They found active lymphatic sinus growth within the lymph nodes of the mice before the tumour had metastasised[86]. These abnormally enlarged lymphatic channels resulted in a 23-fold increased flow of subcutaneously injected lymphatic tracer (TRITC-dextran) through the lymph nodes when compared to wild type littermate controls. The investigators proposed that metastasis to draining lymph nodes is facilitated by lymph node lymphangeogenesis actively increasing flow, thereby promoting dissemination and seeding of tumour cells. Lymph node lymphangeogenesis seems to be triggered via c-Myc oncogene overexpression stimulating the vascular endothelial growth factor pathway (VEGF-C and VEGF-D), a mechanism supported by the high levels of c-Myc-expressing B cells within the enlarged lymphatic vessels.

Subsequent work was undertaken using a B16-F10 metastatic melanoma model which reliably causes metastasis to the draining lymph nodes and lungs in mice by two months after injection[87]. Harrell et al[88] injected the hind footpads of mice with B16 melanoma cells on one side and sterile saline on the opposite side. They investigated the vascular changes around the tumour and draining lymph nodes 13-22 days after inoculation. Findings showed significantly increased and enlarged lymphatic sinuses in both the cortex and medulla of the popliteal nodes but not the inguinal nodes of the tumour containing leg; changes that occurred before metastasis were established. Furthermore functional imaging studies of lymphatic flow were undertaken using near-infrared fluorescent nanoparticle (quantum dot) preparation injected in both footpads. Tracer was detectable in the tumour draining lymph nodes within two minutes of injection whereas it took 30 minutes for signal to reach the lymph node of the non-tumour side, a more than twenty fold difference in flow rate. In this study significant changes in the immune cell population within the tumour draining lymph nodes were seen; namely an eightfold increase in the B cell population by flow cytometry and histologically these were found in both the enlarged lymphatic channels of the cortex and medulla of the lymph node.

The importance of these findings is critical to understanding the reliability of the sentinel node procedure. Prior to this information it was felt that lymphatic fluid followed a pressure gradient from the high interstitial intratumoural pressure driving the flow of cells into the lower pressure lymphatic system[89]. This theory did not explain why drainage would be to the same sentinel nodes if for example the position of the patient were changed diverting the flow through alternative interconnecting lymphatic vessels. However, understanding the

phenomenon of pre-metastatic sentinel node lymphangiogenesis has explained how sentinel node biopsy maps an active process directing lymphatic tracer to specific sentinel nodes.

1.8 Summary and formulation of research questions

SNB has a sound physiological basis that applies to solid tumours that spread via the lymphatic route. The technique is in its infancy in tumours of the head and neck but has potential to answer some of the management related controversies as outlined above. The promise of reducing treatment related morbidity through harnessing technology to provide individualized treatment embodies the ethos of 21st century medicine in the developed world. However, there are a number of potential areas where the SNB technique can be improved. Presently in oral cancer the false negative rate of SNB is 14%. In contrast, the equivalent failure rate of elective neck dissection is reported between 6% and18%[37, 40, 90-92]%, with most papers reporting neck recurrence after END in the pN0 neck to be ≤12%[93]. On balance a slightly higher failure rate may be accepted for SNB in return for the improved morbidity and treatment cost, but ideally it should be explored if it is possible to reduce the FNR to a level that is the same or better than the alternative treatment. The first question is therefore – "is there a way to improve the reliability of SNB detection in head and neck cancer?"

The second issue is that of accessibility –both accessibility to the tumour and accessibility to services to facilitate the SNB procedure. Literature suggests that SNB is a test that harnesses the innate physiological behaviour of lymphatic spread in cancer, and should be applicable to many tumours. Currently

tumours that cannot be accessed for injection until patients are under a general anaesthetic are exempted from this procedure as the interruption to surgery involved in transferring the patient to the Nuclear Medicine department for imaging renders the workflow unmanageable. However, if the imaging process which tracks the movement of tracer from tumour to SN could be transferred to the operating theatre, the possibility of using SNB in a range of tumours is expanded. Furthermore the wider applicability of SNB in the context of worldwide healthcare could be unlocked if the necessity for very expensive fixed gantry nuclear medicine imaging equipment is replaced by cheaper portable technology. The latter can be transferred between the outpatient and theatre setting providing optimum flexibility. The second question to explore is therefore; “is there a way to identify the SN in the operating theatre by using purely intra-operative techniques?”

Chapter 2 Sentinel node biopsy technique - review of the literature and protocol development

This chapter is a review of the current guidelines and best practice for SNB, which has been undertaken in order to formulate a gold-standard protocol by which to test potential improvements. Additionally, a search of literature relating to innovative and experimental techniques in SNB has been carried out and these data are presented in table form. Information is presented in three sections exploring sentinel node tracers, sentinel node imaging, and pathological analysis of sentinel nodes.

2.1 Tracers used in sentinel node biopsy

Radiotracers used with or without the addition of an optical tracer form the standard for SNB in the head and neck. Blue dye (Patent blue V sodium Guebert 2.5%, PVD) is the most commonly used optical tracer[41, 94, 95], although there is increasing use of fluorescent optical tracers[96]. Hybrid tracers that incorporate more than one modality are not widely used at present but may offer an improvement to current practice[97].

2.1.1 Radiotracers

A radioactive tracer is a compound incorporating a radioisotope (radionuclide) that allows localisation of the tracer by detection of emitted radioactive decay. The radionuclide is added to a ligand whose structure is dependent upon the

function the tracer is expected to perform; in the case of SNB this is a charged protein in solution.

In the UK Nanocoll®(GE Healthcare Ltd) is the only currently available tracer for use in SNB. Nanocoll is supplied as 500mcg cold kit for radiopharmaceutical preparation. Nanocoll, human serum albumin derived from blood donations, is a powder for reconstitution into colloidal suspension. It is used clinically when combined with Sodium Pertechnetate (99mTc) to form injectable technetium-99m albumin nanocolloid. Sodium Pertechnetate is produced separately in a technitium-99m-generator by decay of Molybdenum (^{99}Mo)[98].

The radiopharmaceutical product is a heterogeneous suspension of albumin particles of which 95% are <80nm in diameter. When introduced subcutaneously into connective tissue interstitial fluid 30-40% of the administered technetium-99m albumin colloidal particles (less than 100 nm) are filtered through the lymphatic capillaries and vessels to lymph nodes. Here the particles are trapped within the reticular cells and hence the lymphatic drainage can be mapped. At the injection site some of the remaining tracer is phagocytosed into local histiocytes and a smaller fraction passes into the circulating blood and is broken down via the reticuloendothelial system (RES) or excreted by the kidneys[98].

2.1.2 Tracer Injection technique and dose

The dose and method of injection of 99mTc-Nanocoll is dependent upon the tumour and for non-skin head and neck tumours the best described technique is related to oral squamous cell carcinoma[94]. These 2009 guidelines provide a step-by-step method for tracer injection, which has remained uncontested. In summary, for oral tumours optimal images can be obtained by peritumoural submucosal injection using 20-40MBq for the one-day and 40-80MBq for the two-day protocol. The total injected activity is adjusted according to the timing of imaging with respect to surgery. In general, higher doses are required for a 2-day protocol, in order to ensure the activity exceeds 10 MBq at the time of surgery. Small volumes of 0.1–0.2 ml per aliquot are recommended to minimize contamination (spilled tracer) due to the resistance of the tissue. The injected volume should not exceed 0.2 - 0.3 ml[94].

Tuberculin syringes with minimal dead space are used (Perfektum® tuberculin syringe 1.0mL capacity, Sigma-Adrich). The tracer is either prepared into four separate syringes where the small volume (usually 0.1ml Nanocoll) will require a small air bubble to aid expulsion of the tracer through the syringe dead space. If a single syringe is used for all injections (usually a two day protocol volume of 0.4-0.8ml) then the air bubble is not necessary. Radiotracer is injected at 0.1–0.5 cm from the tumour. Tracer is administered keeping as a reference the clock face orientation by four separate submucosal injections (at 3, 6, 9 and 12 o'clock). Following injection, bleeding is controlled with a gauze swab, and the patient asked to rinse the mouth to minimize pooling of the radiotracer in the oral cavity. Residual gamma activity

in the syringes is measured to accurately calculate the total injected tracer activity[94].

2.1.3 Optical tracers

2.1.3.1 Blue dye (PVD)

Patent blue V sodium Guebert 2.5% (PVD, Guebert, Roissy France) is a triarylmethane dye also known as the food additive E131. It is licensed for sentinel node biopsy in the UK. The dye is presented in a 2mL clear glass ampoule and is suitable for immediate injection (up to 10mls of intravenous or 1-2mls for subcutaneous route). The dye has no pharmacological effect and is passively excreted in a dose dependent manner in the urine and stools in the 24-48 hours following injection. Allergic reaction has been noted at a rate of 0.09% in two major studies of SNB in breast cancer[99]. Of these, 7% were serious anaphylactic reactions requiring ITU input although there were no fatalities.

PVD is rapidly transported via the lymphatic system and is detected by discoloration of the lymph node or the lymphatic channels, which can be dissected to the lymph node. The tracer can only be seen in contrast to the surrounding structures under white light, and will be affected by haemorrhage or overlying tissue.

The dye does exhibit some weak autofluorescence when excited at 635nm wavelength with emission at 660nm, but the signal is less bright than indocyanine green[100].

PVD will discolour the injection site, which is deterrent to some surgeons who believe this hampers their ability to judge the tumour margin. There is no evidence to show that that use of PVD increases the risk of a close tumour margin[41].

During surgery the intention is to inject PVD in the same location and method as the radiopharmaceutical. Due the small molecular size of PVD it is transported rapidly and not retained within the lymph node, thus the timing of injection in relation to surgery is critical in order to capture the dye as it passes through the node.

PVD has a proven safety and efficacy record in sentinel node biopsy surgery however there are a number of disadvantages to its use. These could be avoided by using alternative tracers that are retained within the node, and/or have a signal that penetrates through overlying tissue.

2.1.3.2 Indocyanine Green (ICG)

ICG (PULSION Medical Systems AG, Munich, Germany) is a near infrared (NIR) tricarbocyanine fluorescent dye, indocyanine green $C_{43}H_{47}N_2NaO_6S_2$ with structural formula shown in figure 2.1.

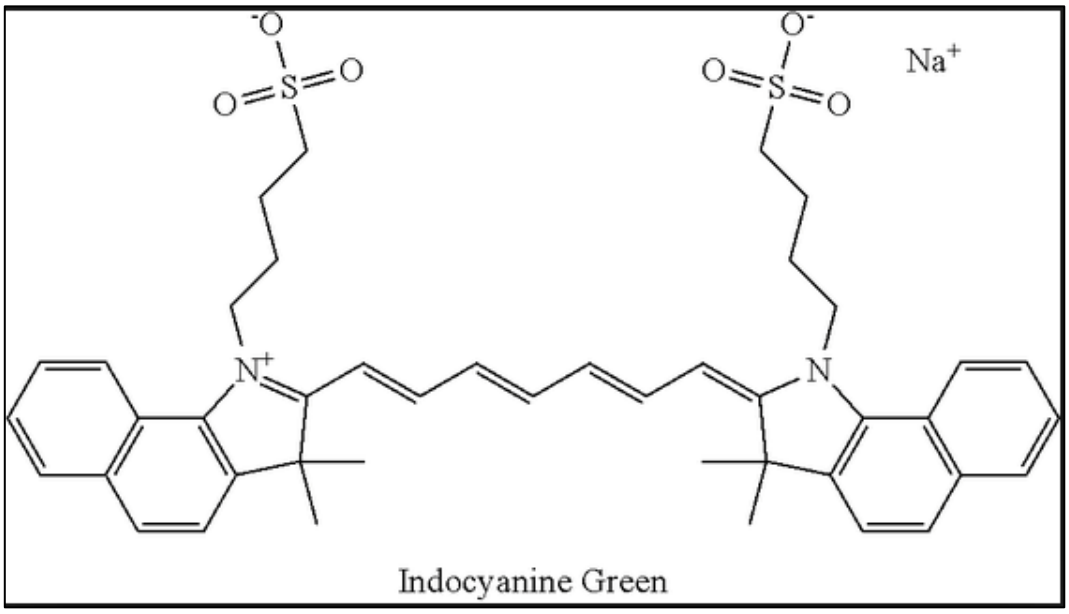


Figure 2-1 - Structural formula of Indocyanine Green (ICG, PULSION medical systems, Germany)[101]

ICG has been used in vascular perfusion studies of the micro and macro circulation since the 1970s[102]. ICG is a licensed product for vascular studies in the UK[103],and is also used in studies of the lymphatic system in patients with lymphoedema[104] but it has not been widely used for sentinel node biopsy. The product is dispensed as 25 or 50mg of dark green powder stored in an amber glass vial. The powder is reconstituted with 5/10mls of sterile water for injection (0.5% w/v, Ranbaxy UK) to give a solution of 5mg/ml. When injected in vivo (free ICG), the dye binds to plasma proteins and remains within the intravascular space without crossing the blood-brain barrier. ICG is not metabolized and is excreted into bile without enterohepatic circulation. Subcutaneous injection of ICG leads to its rapid drainage into the lymphatic system and the superficial network of lymphatic channels can be mapped under near infrared (NIR) light. Free ICG readily binds to albumin within the lymphatic fluid and is washed into the lymph

nodes. ICG retention within the lymph node is dependent upon the size of protein to which it is attached. Unlike PVD, the fluorescent optical signal has some penetration of the overlying tissues (up to 1cm)[105] reducing the amount of tissue dissection required to identify the sentinel node. Moreover the excitation and emission spectra of ICG do not overlap with tissue autofluorescence (figure 2.2) resulting in a high specificity for the labeled node.

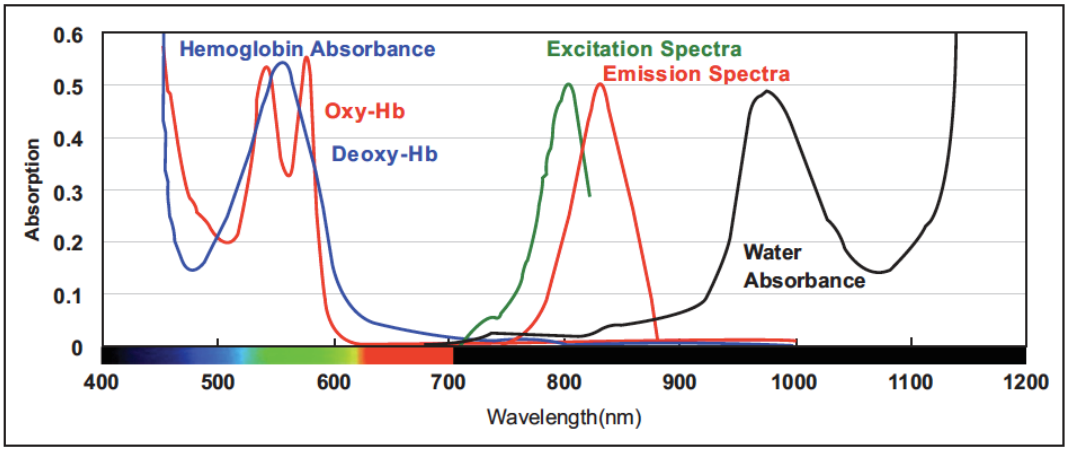


Figure 2-2 Excitation and emission spectra of ICG (Excitation and Emission wavelength are drawn by green and red line respectively) and Haemoglobin (blue/red lines), and water (black line) absorbance spectra. [106]

Free ICG has been used as sole tracer for sentinel node biopsy with mixed results. The optimum dose is uncertain, and there are many imaging devices available. Recent studies that have compared free ICG to other tracers or investigated the optimum dose of ICG are listed in table 2.1 (Table 2.1. Results of Sentinel Node Biopsy using free ICG). It is worth noting that each study has used a different near-infared imaging device. Furthermore some of the study protocols were such that there may have been some binding of the free ICG to the radiotracer, although this was not the aim in any of these studies.

Table 2-1 - Results of Sentinel Node Biopsy using free ICG

Reference	Patients Tumour type	Dose of tracer (free ICG mg/ml)	Imaging Device	Study results and conclusion
He K et al. Trans Res 2016[107]	99 Breast	1ml (5mg/ml)	FIRE (custom built)	All patients had blue dye and free ICG by separate injection. ICG had 99% SLN detection rate, compared to detection 91% by blue dye. All SN+(35 cases) were detected by ICG, 92% by blue dye. ICG is more accurate than blue dye. ICG^{99m}Tc-Nanocoll followed by LSG and SPECT/CT.
Stoffels et al. JAMA Surgery 2015[96]	80 melanoma	Not recorded	Fluobeam (Fluoptics)	Concordance of separately injected free ICG and ^{99m}Tc labelled Nanocolloid was less than 90% ($p<0.001$) and free ICG performed particularly poorly in pre-operative localisation (21% of basins). Accurate SNB requires radiotracer as well as fluorescent signal.
How J et al. Gynecol oncol 2015[108]	100 Cervical and Uterine	ICG (0.1 ml, 0.25 mg/ml	Firefly (Da Vinci)	Feasibility of cervical injection in patients undergoing hysterectomy for endometrial cancer. Three tracers injected simultaneously (one syringe) but not formally mixed. Blue dye performed less well than the other tracers (71% detection) whereas ICG and ^{99m}Tc Nanocoll were similar 87% and 88% detection rate, but results should be interpreted cautiously as there may be partial binding of the two compounds.
Samorani et al. EJSO 2015[109]	301 Breast	ICG (0.4-1.2 ml of 5mg/ml)	PDE Hamamatsu	98.7% concordance between ICG and ^{99m}Tc labelled Nanocolloid injected up to 18 hours apart. ICG detected more SN than ^{99m}Tc Nanocolloid 583 vs. 452, and more positive sentinel nodes 70 vs. 55. Authors suggest that ICG could be used to replace nanocolloid tracer injection in breast SNB.
Cloyd et al. J Surg Oncol 2014[110]	52 Melanoma	ICG 2mls of 0.25mg/ml	SPY Elite™, Novadaq	Patients underwent lymphoscintigraphy with standard radiotracer followed by separate injection of blue dye and ICG. Sentinel node identification rate was 96.2% by radiotracer, 59.6% for blue dye, and 88.5% for ICG ($P<0.05$ for ICG vs. blue dye). Radio signal is still required to localize the sentinel node but blue dye can be safely omitted form the protocol as it offers no advantage over ICG.
Nakamura Anitcancer Research 2015[111]	19 Oral/or pharyngeal	ICG 2ml of 2.5mg/ml	HyperEye medical system (HEMS, Japan)	Patient either had SNB by free ICG alone (n=2), radioisotope alone (n=13) or a both (n=4). Seven additional fluorescent sentinel nodes were identified in the 4 patients who had ICG and isotope, none were positive for metastasis. Poor concordance in combined cases, but procedure significantly quicker. Free ICG can identify additional nodes but clinical significance uncertain.
Yamashita Ann Surg Oncol (2012)[112]	31 Lung cancer	ICG 2ml of 5mg/ml	Charge-coupled device (CCD) Olympus	All patients had intraoperative thorascopic injection of free ICG. SN identification rate was 80% (25/31) but ICG correctly identified the position of the positive nodes in the one study patient with metastatic disease. Adhesions and tracer leakage were the cause of failure in 6/31 patients.
Gilmore et al. J	33 lung cancer	Dose escalatio	FLARE open	SN identification was ICG dose dependent with higher rate of identification over 1000 mg. All positive SNs

Thoracic and Cardio Surg 2013[113]		n 3.8mg to 2500mg	surgery or thoracoscopic NIR camera, Novadaq	were fluorescent with an average of 3 fluorescent nodes compared to 6.5 in completion nodal dissection.

These data suggest that free ICG seems to work particularly well in breast cancer but is less successful in tumours where the lymph nodes are either close to the tumour or buried deep in the tissue. In these cases reliance on optical tracer alone is not possible and radiotracer is required to guide the surgeon to the local vicinity of the node. Fine dissection can then be undertaken guided by the fluorescence signal. Blue dye universally performed worse than ICG and many studies concluded that it could be omitted from practice if ICG is used as an alternative optical tracer.

2.1.4 Hybrid Tracers

Hybrid tracers are compounds that harness more than one modality, when applied to sentinel node biopsy multimodality usually refers to a combination of radio- and optical tracer. Currently there are no commercially produced multimodal tracers, but they can be assembled from mixing available licensed products, namely ICG and ^{99m}Tc-Nanocoll.

The multimodal tracer derived from is a mixture of Technetium-99m labelled Nanocolloid and indocyanine green (ICG) is referred to as ICG-^{99m}Tc-Nanocoll (Figure 2.3). The major advantage of the hybrid ICG-^{99m}Tc-Nanocoll tracer over free optical tracer is that the stable compound is trapped within the sentinel nodes resulting in high level of concordance of fluorescent and

gamma signal (>95)[114], reducing the possibility of removing second echelon nodes that contain optical tracer only.

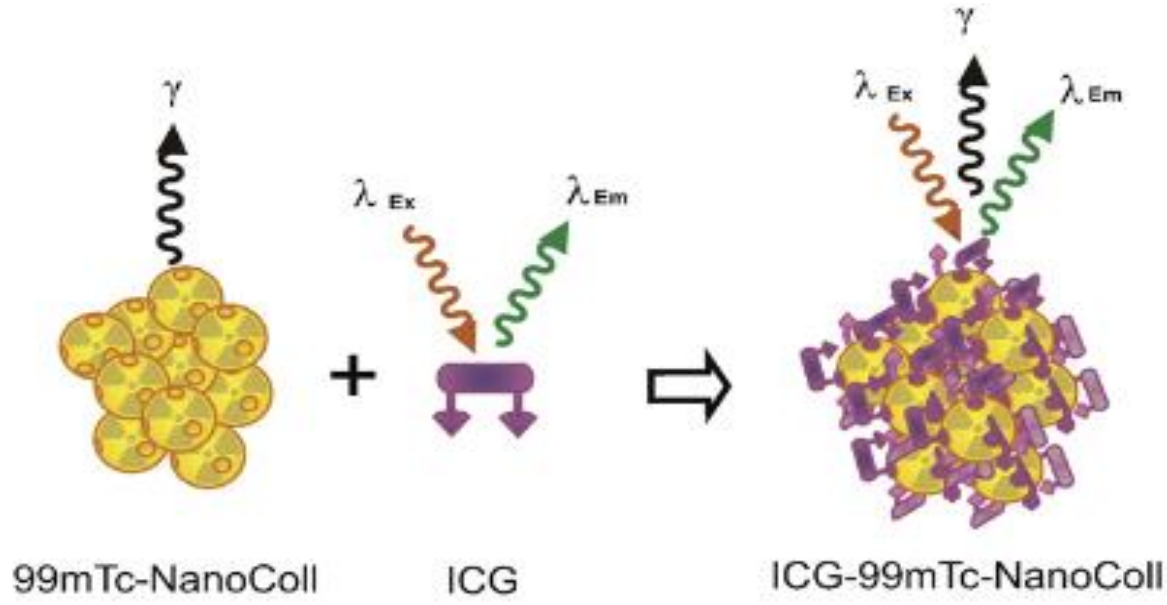


Figure 2-3 Schematic of multimodal tracer ICG-99mTc-Nanocoll[115]

Preclinical development of ICG-99mTc-Nanocoll was undertaken at the Netherland Cancer Institute[115], where it was shown that the hybrid imaging agent allowed combined pre- and intraoperative identification of SNs in mouse models for prostate and breast cancer [97, 115].

i) Mouse Breast Cancer Model

Both healthy mice and a mouse mammary tumour model (KEP1-Luc) underwent injection of either ICG or ^{Tc99}Tc-nanocolloid into tumour or normal mammary tissue (10 animals in total). Following mammary gland injection imaging was undertaken with either an IVIS In-vivo camera or SPECT/CT before the animals were sacrificed and lymph node dissection performed. The fluorescence intensity and radioactivity counts were measured.

In a second experiment peritumoural injection was undertaken using the

multimodal tracer ICG-^{99m}Tc-Nanocoll. All tumour-bearing mice (n=5) showed highly reproducible tracer uptake in the SLN starting around 6 min post-injection, with maximum values at 30 minutes. An important additional advantage of this multimodal imaging agent was the enhanced signal intensity over ‘free’ ICG. Using the same molar amount of dye (7.8 × 10−6 mmol), free ICG achieved a signal intensity of 30–200%, whereas the multimodal imaging agent performed significantly better (1252–8080%; p = 0.027). Excluding one animal with advanced metastatic spread, this results in an average 86- fold increase in signal-to-background ratio.

ii) Mouse Prostate Cancer Model

Male TRAMP mice expressing the SV40 T antigen (TRAMP+) spontaneously develop autochthonous prostate tumours with metastases occurring at 24 to 28 weeks of age. The lymphatic pathway from the prostate gland drains via the lumbar LNs to the renal LNs. In this study, TRAMP+ mice between 25 and 30 weeks of age and age-matched nontransgenic (TRAMP−) mice were injected intratumorally (TRAMP+) or into the prostate (TRAMP−) with cocktail solutions:), NC (^{99m}Tc-Nanocoll/ICG), and Human serum albumin, HSA (^{99m}Tc-Vasculosis/ICG) or dye injection alone (PVD, ICG). Distribution of the imaging agents to the lymph nodes was assessed at different time points after injection from 15 minutes to 4 hours. The cocktail tracer or optical dye injections were given to 30 animals and the combined fluorescence and radio signals were detectable in vivo and ex vivo by IVIS camera and SPECT/CT. The relative intensities of the fluorescent and radioactive signals were measured at different time points (Cocktail injections were prepared by diluting 10 µl of ICG solution in 30 µl of the

respective tracer solution ^{99m}Tc-Nanocoll (1.11×10− 7-mmol albumin; 8 MBq or 0.21 mCi). Results showed that there was good correlation between the fluorescent and radioactive signals in the cocktail group (correlation coefficient R ≥ 0.92). The fluorescent signal intensity was higher in the cocktail group compared to dye alone, particularly at the later time from injection (60 and 240 minutes).

Clinical studies using the multimodal tracer have also been taken forward by the Dutch group. The Netherland Cancer Institute have published data on its use in head and neck, breast, melanoma, gynaecological and urological cancers (Table 2.2). They estimate to have used the tracer in >700 patients, although not all cases have been reported in scientific literature. They have not noted any adverse effects related to the use of ICG ^{99m}Tc-Nanocoll.

In one study they twice irradiated a group of patients to understand if there was any difference in the sentinel node labelling abilities of ^{99m}Tc-Nanocoll if it was combined with ICG or not. They found that the addition of ICG did not alter the in vivo behaviour of ^{99m}Tc-Nanocoll and in fact the sentinel node detection on SPECT/CT was identical after both doses[116].

Moreover interactions between ICG and Nanocoll prevent self-quenching of ICG in these particles, resulting in a high fluorescent intensity, giving an 86-fold improvement in the signal to background ratios compared to ‘free’ ICG. In the ICG–

99mTc -nanocolloid complex, the ratio of ICG to human serum albumin is 18:1[117]. While the amount of 99mTc-nanocolloid is identical to that used for radiologically guided SNB, the dose of ICG used (<0.05 mg) is substantially lower than the amount of ICG allowed for intravenous use.

Table 2-2 - Patients studies using ICG-99mTC-Nanocolloid reported in the literature: Publications where it was thought the same patient group was republished have been left out.

Author	Patient Group	Study Protocol	Results summary
Christensen et al. Ann surg oncol (2016)	30 oral cancer	All patients received ICG^{99m}Tc-Nanocoll followed by LSG and SPECT/CT.	Intraoperative detection by gamma probe and NIR imaging devices located 97% of nodes identified pre-operatively. Fluorescence alone identified an additional 11/96 nodes. 10% of patients had transcutaneous fluorescence detectable.
Van den Berg et al. Radiology 2015[118]	104 Melanoma patients	All patients received ICG^{99m}Tc-Nanocoll followed by LSG and SPECT/CT. Blue dye injected in non-facial tumours (n=69)	Using pre-operative imaging as a guide, 96.7%, 93.8% and 61.7% of the target nodes were identified intraoperatively by fluorescence, gamma signal and blue dye respectively. In five cases fluorescent nodes could be seen transcutaneously, in a further five fluorescent signal allowed identification of nodes not located with gamma due to high background signal.
Stoffels et al. Eur J of Molecular imaging 2015 [116]	40 patients with cutaneous malignancies of the head and neck	RCT of hybrid tracer vs. radiotracer alone.	100% intraoperative identification of SN with hybrid tracer compared to 95% with standard tracer.
Verbeek et al. Int J Gynae cancer 2015[119]	12 patients with vulval cancer	All study patients received CG^{99m}Tc-Nanocoll and blue dye, results compared to historical data using ICG:HSA and free ICG.	100% concordance of ICG and ^{99m} Tc signals using the hybrid tracer compared to 62% detection rate for blue dye. Intraoperative NIR fluorescence based SLN detection rates were 75%, 83%, and 100% for ICG alone, ICG:HSA, and ICG^{99m} Tc-nanocolloid, respectively (P = 0.21)
Bourbon-Arce M et al, Rev Esp Med Nucl Imagen Mol. Sept 2014[120]	25 patients with head and neck tumours (16 melanoma and 9 oral cavity)	All patients injected with ICG^{99m}Tc-Nanocoll, comparison of conventional LSG, SPECT/CT, gamma imaging and fluorescence detection	26% additional SNs were found using the multimodal approach
Brouwer O.R et al, J Nuc Med (2012)[114]	N=25 10 with melanoma of head and neck 6 with melanoma of	All patients had standard injection with ^{99m}Tc-Nanocolloid (average dose 71MBq) followed by SPECT/CT. All patients were further re-injected with	Identical drainage pattern was found between the ^{99m}Tc-Nanocolloid and ICG^{99m}Tc-Nanocolloid. All excised radioactive lymph nodes were fluorescent.

	the trunk 9 with penile cancer	ICG-^{99m}Tc Nanocoll within 24 hours (average dose 74MBq)	
Van der Poel et al. Eur Urol(2011)[121]	N=11 patients undergoing robot assisted laparoscopic prostatectomy (RALP) for cN0 prostate cancer.	All had pre-operative injection with ICG-^{99m}Tc Nanocoll. Pre-operative SPECT/CT was performed. Intraoperative SN identification undertaken with gamma probe and NIR laparoscopy combined with LN dissection.	NIR fluorescence intraoperatively identified 23/27 SNs found on pre-operative imaging. Where fluorescence did not aid retrieval the SN was embedded in fat, which attenuated the signal.
Brouwer OR et al. Ann Surg Onc (2012[122])	N=11 patients with melanoma undergoing SNB.	All patients had ICG-Tc99m Nanocoll injected. 7/11 also had blue dye injection.	100% of sentinel nodes were radioactive and fluorescent. 43% of SN in 7 patients were blue.
van Leeuwen AC et al. J Biomed Optics (2011)[97]	N=6 patients with T2b or Gleason >6 prostate cancer.	Pilot study in detection of fluorescent lymph nodes. ICG-99mTc-nanocolloid injected into prostate gland pre-operatively and Sentinella used to detect fluorescence ex vivo in the pelvic node resection specimen.	Fluorescence was detected in the resected tissue. Fluorescence correlated with the radioactivity.
Frontado M et al.[123]	N=20 patients with oral cavity, melanoma and penile tumours	Patients injected with ICG-99mTc-nanocolloid and blue dye. Correlation of blue dye and fluorescence n the sentinel nodes.	97% of the SNs were fluorescent while only 39.2% were stained blue
Van den Berg, N et al. Eur J Nuc Med Mol Imaging (2012)[124]	N=14 patients with oral cavity tumours clinically stage N0.	All patients injected with ICG-99mTc.nanocolloid and SPECT/CT prior to SNB.	43 SLN identified, 4 of which were close to injection site and identified in vivo by fluorescence only.
Schaafsma et al. Br J Surg (2013)[125]	N=32 patients with breast cancer	Patients injected with blue dye and two different particle density of ICG labelled 99mTc-Nanocoll.	NIR fluorescence aided intraoperative identification of sentinel nodes. Up to 29 hours post injection. There was no difference in the sentinel node imaging using the two formulations.

2.1.5 Selective lymph node tracers

Tilmanocept (Lymphoseek – Norgine Healthcare) is a new sentinel node tracer designed for uptake into lymph node via binding to macrophage CD206 mannose receptor, resulting in preferential uptake into the sentinel node [22].

This tracer offers a potential advantage over traditional tracers by clearing the injection site rapidly whilst being selectively retained within the sentinel nodes[126]. This should reduce the 'shine-through' effect in which nodes near to the tumour can be missed due to high signal at the injection site. It has shown impressive results in reducing the FNR for SNB in oral tumours from 9%(NEO3-05 [15]) to 2.56% (NEO3-06 [23]). In these successive studies all patients underwent concurrent neck dissection and so no there was no control group to allow assessment the patient benefit. Lymphoseek has regulatory approval in the USA and Europe and but it will not be available for routine clinical use in the UK until late 2017, falling outside the recruitment period of this research.

2.1.6 Sentinel node tracers – summary

Traditional sentinel node imaging using radionuclide has a long history and a reliable outcome, allowing both pre- and intraoperative identification of the sentinel nodes. The major disadvantage is if there is shine through effect (nodes cannot be discerned from the general radiation blush at the injection site), in which case the addition of optical tracer is advantageous. For this reason blue dye is still widely used, but this review has shown the low specificity of the dye for the sentinel node. In contrast the literature has shown that fluorescence out performs PVD in sentinel node identification, but in its free state ICG is not retained within the sentinel node and may lead to unnecessary removal of additional non-sentinel nodes. In contrast the hybrid tracer ICG^{99m}Tc-Nanocoll, incorporating both radionuclide and fluorescent

modalities combines a gamma and fluorescence signal, which is retained within the sentinel node. Head and neck tumours are the ideal anatomical site to benefit from this due to the proximity of the injection site and the sentinel nodes. Literature review confirms that ICG^{99m}Tc-Nanocoll has shown promising results in the identification of additional sentinel nodes in oral cancer. The hybrid tracer has not been used in the UK, nor has it been applied to non-oral head and neck tumours. Therefore an aim of this work will be to introduce the tracer to the UK for the first time, to apply it to non-oral cancer head and neck tumours and to develop a protocol for intraoperative imaging, that would allow repeatable testing across different centres and different tumours.

Following injection of the tracer, the next step in the SNB process is the imaging protocol, which is discussed in detail in section 2.2.

2.2 Sentinel node imaging

Sentinel node imaging has evolved from the plain x-rays used in Cabanas' work[64] through to lymphoscintigraphy and subsequently to combination modality of single-photon emission computed tomography (SPECT) and x-ray computed tomography (CT) resulting in hybrid SPECT/CT[127]. Although x-rays are no longer used, Lymphoscintigraphy another type of planar (two dimensional) imaging is routinely performed and increasingly supplemented by SPECT/CT for the additional anatomical detail provided[128].

New developments in traditional hybrid imaging such as PET/CT[129], PET/MRI[130] as well as novel modality fusion such as

SPECT/Ultrasound[131] may have a potential role in sentinel node biopsy but access to these facilities are at present very limited. Freehand SPECT (fhSPECT) is a recently developed mobile imaging system that seems to offer a reliable intraoperative 3-D imaging[132].

2.2.1 Lymphoscintigraphy

Lymphoscintigraphy (LSG) comprises both static and dynamic imaging phases, allowing collection of complimentary information to distinguish between sentinel and second echelon nodes.

The dynamic imaging phase commences immediately following radiotracer injection, with frames (images) acquired every 10-20 seconds for 20-30 minutes. The patient is positioned under the imaging gantry as quickly as possible after the tracer has been given, so as not to miss the appearance of in-transit nodes and to capture the exact order of appearance of subsequent nodes[133, 134]. Once dynamic imaging is completed the static phase follows on and collects images at a much longer frame speed (300 seconds) recording a summation of the emission of tracer and providing a map of the intensity within the field of interest[135]. The images show just the hotspots without anatomical reference and therefore require more than one view to orientate the reporter. After LSG imaging is complete, SPECT/CT can be undertaken but it must be noted that the temporal element of the imaging process cannot be captured by SPECT/CT due to the delay between injection and imaging.

Although lymphoscintigraphy is a reliable test and much favoured by nuclear medicine physicians[136], data does not support a great advantage of this test over other modalities in the head and neck. A recent review of sentinel node outcomes across 14 European centres found that the false negative rate of LSG was 13.5% compared to 10% when SPECT/CT was used although the difference did not reach statistical significance (p=0.297)[136].

Flach et al[137] looked at the inter-observer and inter-institution variability in reporting sentinel nodes using a series of nine OSCC patient who had undergone LSG and SPECT/CT imaging. They identified a schism in reporting which reflected a split in the fundamental ideology of sentinel node mapping. On the one hand some teams would label just the first node to appear, others would highlight nodes they felt had a direct drainage pathway from the tumour (although definition of this introduced further variables) whilst others still would label any node that shows tracer activity as a sentinel node. Moreover some teams would limit the number of nodes they would consider SN irrespective of the images shown by the scans and there was also considerable disagreement over the status of hotspots seen in the contralateral neck. These disagreements (amongst very experienced practitioners) would seem to erode the value of the functional and temporal aspect of lymphoscintigraphy perhaps rendering it the preserve of purists.

Certainly there would be no advantage in using a modality that identifies more negative nodes than LSG without improving the accuracy of positive node identification, and those who favour LSG as a sole imaging tool reference

recent data showing that LSG identifies fewer nodes than SPECT/CT[138, 139]. Unfortunately neither of these studies reported sensitivity of each modality in detecting positive sentinel nodes.

The major disadvantage of LSG is the lack of anatomical detail and the resolution of the images that makes it difficult to distinguish between one hot focus and a cluster of closely related nodes. It is unlikely that this is relevant in clinical practice as the surgeon will be drawn to the correct region by either modality and the number of nodes excised will depend upon a combination of intraoperative gamma counts, anatomy, and operator interpretation. The situation of a 'missed node' is the most significant outcome by which to assess the reliability of the test but this cannot be measured in the majority of cases where all nodes are negative for metastasis and therefore could very well be picked by a random choice without detriment to the patient.

2.2.2 SPECT and SPECT/CT

Single-photon emission computed tomography (SPECT) detection is undertaken by rotating gamma cameras that allow computation of summated radionuclide signal providing excellent spatial resolution and sensitivity[140]. Wagner et al.[141] first described the advantages of using the combined functional and anatomical information provided by fusion of SPECT and conventional CT (SPECT/CT) over planar lymphoscintigraphy for oral cancer. They found that SPECT/CT identified 49 draining "sentinel" nodes in 30 patients. Lymphoscintigraphy identified just 38/49 nodes, although only one third of the group went on to have pathological confirmation by biopsy. They

found the advantage of SPECT/CT over planar imaging was in identifying nodes close to the injection site, however it should be noted that the protocol did not include immediate imaging by LSG, rather a delay of one hour post injection which may account for this discrepancy of higher echelon nodes.

Subsequent studies showed conflicting results of the utility of SPECT/CT over planar lymphoscintigraphy[128, 142], and although it is not considered essential for accurate SNB [94], it remains a popular investigation allowing surgical access planning by anatomical localisation of the SN in relation to critical structures[143]. In many cases SPECT/CT is performed in addition to LSG in solely to confirm the anatomical localisation of the nodes already identified by planar imaging[137].

Although SPECT/CT is useful in determining the surgical approach for retrieval of the targets, the patient is imaged in standard anatomical positions meaning targets can be displaced during the operation particularly when tissues are moved or removed. An ideal imaging tool would be able to update during the procedure to reflect the exact status after surgical steps are undertaken. Moreover in relation to SNB imaging data that is contemporaneously obtained allows the possibility of delivering and tracking the radioisotope in real-time during the operation.

The challenge in moving SPECT imaging out of the nuclear medicine department is changing from a gantry-based design (whereby the detectors are rotated around the patient) to a hand-held detector that can be controlled

by the operator. There are fully integrated operating theatres which would allow for gantry-based detectors AMIGO – Advanced Multimodal Image Guided Operating), but these are expensive, require large amounts of space, and also interfere with the flow of surgery[144]

2.2.3 Freehand single-photon emission computed tomography (fhSPECT)

The prospect of an intraoperative detector was first reported in 1989 whereby a catheter mounted detector was used to locate lung tumours via bronchoscopic approach[145]. The first patent filed for a hand-held detector with tomographic capability was in 2001[146] and in 2002 Benlloch et al.[147] described a gamma camera able to process stereoscopic images with a resolution of 2mm in phantom models.

In 2005 a group based at Technische Universität München in Munich, Germany (subsequently forming a commercial operation, SurgicEye GmbH, Germany), devised a technique of combining a handheld nuclear detector (gamma probe) and a patient position-tracking device that would allow generation of 3D nuclear images in a process they christened freehand SPECT (fhSPECT)[148]. Although many less measurements points are taken by the handheld device (thousands compared to nearly a million in the gantry-based system), the operator is able to move very close to the region of interest thus allowing a comparable level of accuracy. Table 2.3[149] shows the relative technical limits of freehand versus gantry-based imaging.

Table 2-3 - Comparison of freehand and gantry based nuclear medicine imaging, taken from Chapter 4.Intraopertive 3D nuclear imaging and its hybrid extensions in “Gamma cameras for interventional and intraoperative imaging, CRC Press 2016[149].

Property	Gantry-Based Imaging	Freehand Imaging
Calculation of relative position of projections	Per construction	Using tracking
Angular coverage of projections	Full angle	Limited angle
Symmetry of projections	Per construction	Impossible
Applicable reconstruction algorithm	Analytic or iterative	Iterative
Pre-computation/measurement of system matrix for iterative reconstruction	Possible	Impossible
Weight of detector	~1000 kg	0.2–2 kg
Field of view of detector	~30 × 30 cm^2	~5 × 5 cm^2
Statistics of acquired data	High	Low
Distance between detector and anatomy	Far	Close

2.2.3.1 Declipse®SPECT System

First introduced in 2007[150] fhSPECT has evolved in the past seven years from a prototype to a commercial product, declipse®SPECT cart system (SurgicEye GmbH, Germany). The set up for the fhSPECT system is shown in figures 2.4 – 2.6. The detector comprises a gamma probe (one-pixel gamma detector) that provides continuous data collection (updating 20 times per second with accuracy of 0.2mm[150]) whilst the position is tracked via infrared signal reflected through fiducial markers (navigation i-spheres). To ensure accuracy of the equipment calibration is undertaken prior to each procedure, confirming the localisation of a known source. Image reconstruction is undertaken via iterative algorithms and the image is overlaid on a video image of the patient.

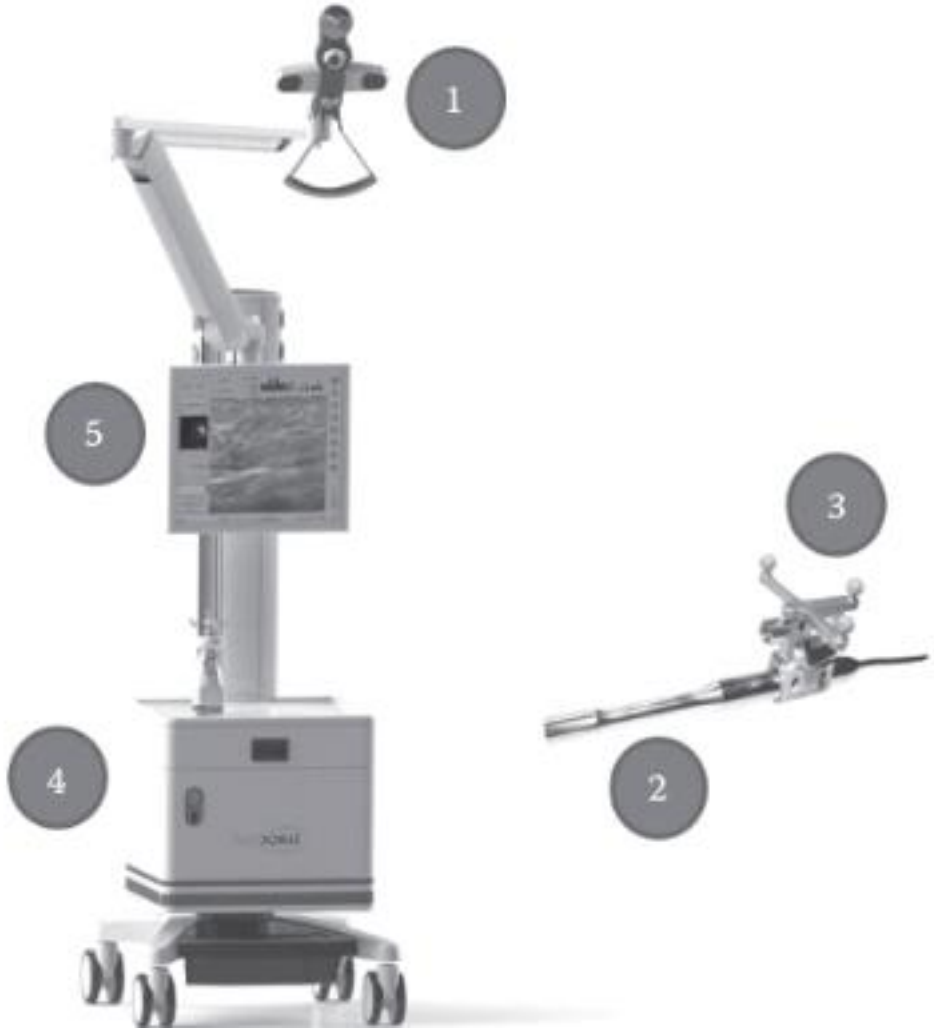


Figure 2-4 - Components of freehand single-photon emission computed tomography device (fhSPECT), declipseSPECT, image provided by SurgicEye GmbH, Germany.

1) Optical tracking system comprising video camera (centre) and two offset infrared cameras The NDI Vicra video camera has resolution of 1032(h) x 778(v) at 30 frames per second and 8 bit colour resolution.
2) Gamma probe (Gamma probe system SG-04, Crystal Photonics GmbH) attached via cord to control panel shown in figure 2.2.
3) Tracking target mounted onto gamma probe. Disposable fiducial markers (navigation i-spheres) on a rigid three-pronged frame
4) Central processing unit (medical PC 4) loaded onto medically certified cart suitable for transfer into the operating theatre
5) Touch screen display showing real-time video with overlay of SPECT images.

Figure 2-5 - Control unit CXS-SG04 (CE 0633) Crystal photonics GmbH Germany for gamma probe shown in figure 2.4.

The control panel selects for nuclide, sample time and pitch of the counts per second. The readings undergo linear algorithmic processing by the central processing unit in figure 2.1 to produce the 3D image.

Figure 2-6 - Patient-tracking device. Lightweight sterilisable frame with mounted disposable navigation i-spheres (fiducial markers).

The stent is taped to the patient near to the region of interest. The stent can also be placed on the patient during SPECT/CT where implanted stainless steel (316) rods allow localisation via downloaded images in declipse®SPECT central processing unit, allowing comparison of the fhSPECT and SPECT/CT images.

2.2.3.2 Augmented reality image fusion

Augmented reality (AR) is the fusion of digital computer-generated information with a real-world view to form a composite image. The Declipse®SPECT system produces an augmented reality image of the radiation hotspots

superimposed onto a real-time video image of the patient, which is displayed on a screen on the cart system (Figure 2.7 A and B).

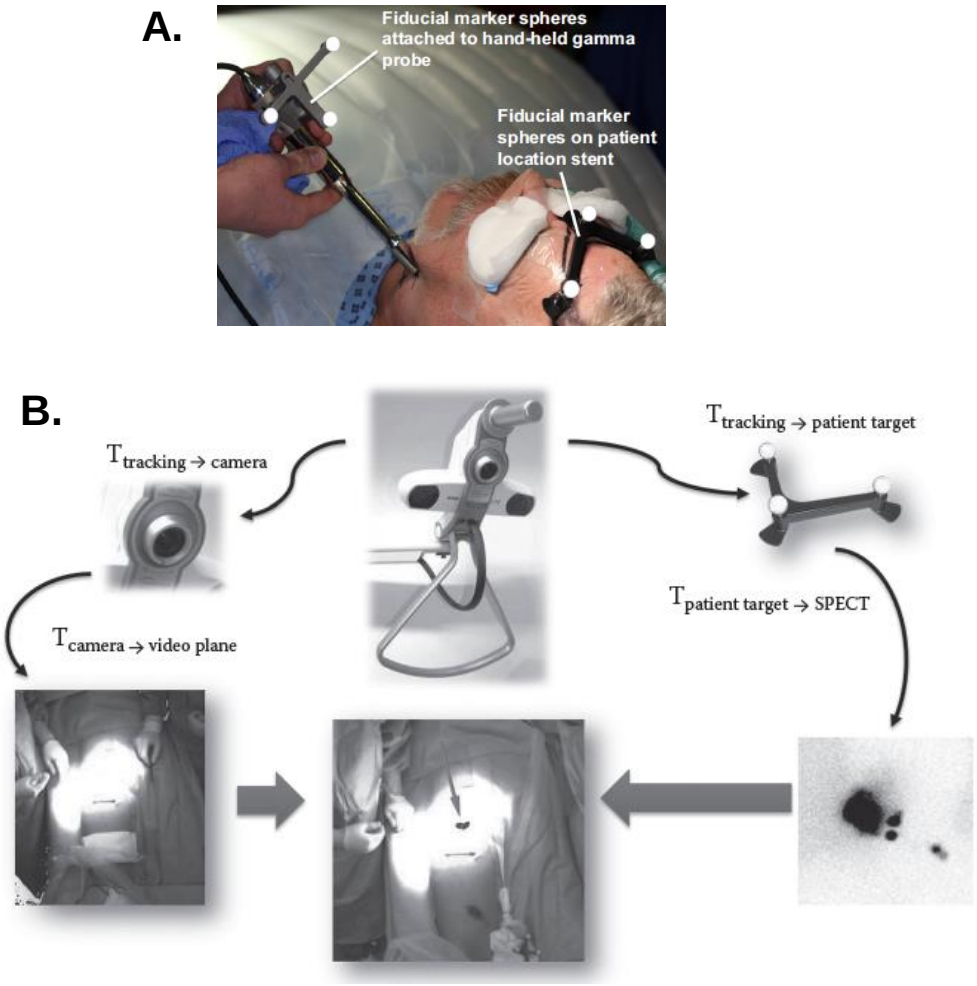


Figure 2-7 - Production of augmented reality images by DeclipseSPECT system

A) Probe and patient tracking devices
B) Freehand SPECT translated and rotated to the coordinate system of the camera and finally projected on the image plane (image from Chapter 4, Intraoperative 3D nuclear imaging and its hybrid extensions, Gamma Cameras for interventional and intraoperative imaging, CRC press 2016)[149]

The images are created by 'scanning' the patient methodically with a hand-held gamma probe that is passed over the area of interest from several different angles, taking care to avoid directing the probe tip towards the injection site. The algorithm commences after a 5 second countdown instigated by the operator holding the probe close to a gamma source. The

processed augmented reality image appears on the screen immediately. The image is continuously refined as more counts are accumulated, and the operator continues the scan until a clear image of the radiation hotspots are obtained or a minimum of 2000 counts are processed. Typical scanning time is 2-3 minutes. If the navigation spheres are obstructed during the scan an alarm sounds to warn the operator that tracking of the probe or patient position has been interrupted and counts are not taken until the connection is re-established. Once the scan has been completed the results are saved automatically and used to create a surgical report which includes the exact time and length of the scan, the number of hotspots found and a screen shot is automatically stored. Once the scan is saved the operator can flip between the augmented reality image (figure 2.8A) and a 3D view (2.8B) and the distance between the probe tip and the hot spot is shown in the top right hand corner of the screen allowing three-dimensional navigation to the radioactive hotspot.

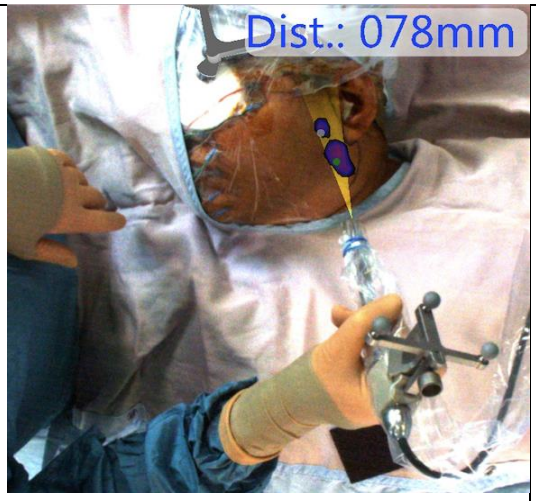


Figure 2.8.A. Augmented reality image of fhSPECT scan result

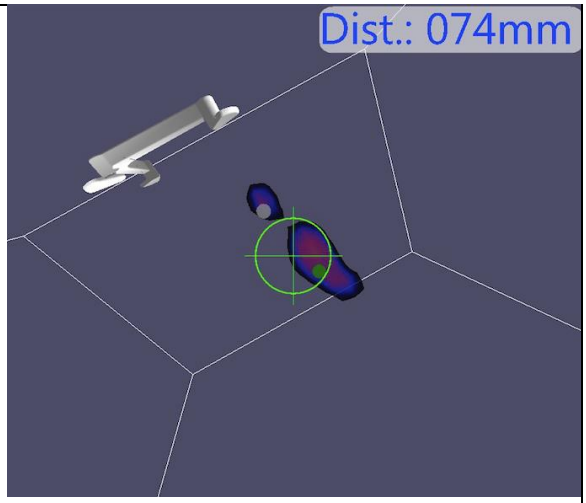


Figure 2.8B. Three dimensional image view of the same patient showing the patient reference target and hotspots only.

Figure 2-8 Augmented reality and '3D mode' images

Automatic screen shots are saved every three minutes during the procedure and additional images can be taken by the operator as required. Scans can be performed at any point during the procedure, for example once the sentinel lymph nodes have been removed to ensure that there are no remaining hotspots, although the previous scans cannot be re-upload when a new scan is taken. All scans appear on the operation report.

2.2.4 Utility of fhSPECT localisation of sentinel nodes.

Review of the literature reveal a small number of case reports and pilot studies using fhSPECT (with Declipse®SPECT) for SNB detection in breast cancer[148, 151-154], melanoma[155, 156] prostate cancer[157, 158] and oropharyngeal tumours [159].

There are four publications detailing the utility of fhSPECT in oral cancer. In 2014 Bluemel et al.[160] based in Marburg, Germany, reported a case report of fhSPECT in a patient with an anterior floor of mouth tumour in which the system detected sentinel node identified by pre-operative lymphoscintigraphy and SPECT/CT. This led to a case series of twenty-three oral cancer patients in whom pre-operative scanning by fhSPECT had a 98% detection rate (outperforming lymphoscintigraphy but not SPECT/CT) however, the investigators were not blinded to the results of the conventional nuclear medicine imaging prior to scanning with fhSPECT [161]. Additionally, all the patients in these two reports had concurrent neck dissection, potentially masking any failings in the technique.

Around the same time a Dutch group were exploring the use of the Declipse®SPECT system, reporting their first case in 2013[132] progressing to a case series of 66 patients with cT1-T2 N0 oral cancer who underwent SNB using fhSPECT. In this study fhSPECT identified 94% of the sentinel nodes that had been identified pre-operatively, however it should be noted that the position of the nodes had been marked on the neck by the nuclear medicine team prior to the surgeon scanning with the Declipse®SPECT system. Investigators asked the surgeons to rate on a three-point scale the usefulness of the system in identifying and retrieving the nodes during surgery and found that it provided additional useful information in 24% of cases.

2.2.5 PET Localisation of sentinel nodes

Images produced by fusion of positron emission tomography (PET) and sequential or simultaneous MRI or CT scans are useful not only for staging but are increasingly under investigation with respect to biomarkers such as hypoxia, angiogenesis and capillary permeability in assessing response to treatment and diagnosis of recurrence[130, 162, 163]. Heuveling et al.[164] Investigated the possibility of a PET/CT lymphoscintigraphy using a novel tracer 89Zr-nanocolloid albumin, for five patients with oral cancer who also underwent traditional gamma camera imaging by SPECT/CT using 99mTc_nanocolloid. They found that PET/CT lymphoscintigraphy not only identified the same SN hotspots as SPECT/CT but also located additional SN close to the injection site. The authors noted they were able to identify lymphatic channels draining into the lymph nodes that could not be seen on the SPECT/CT. This advantage however did not translate to the operating

room as there is currently no appropriate intraoperative detection device for the 89Zr labelled tracer, reducing the translatability of this technique to clinical practice.

2.2.6 Sentinel node imaging – summary

Lymphoscintigraphy and SPECT/CT are widely used together for standard SN imaging.

At the present time there is little evidence to support a move away from these modalities, but the development of freehand SPECT may allow an alternative method of sentinel node imaging that can be performed in the operating theatre. To date this modality has not been tested against the gold standard of lymphoscintigraphy and SPECT/CT in a blinded trial. An aim of this project will be to test the ability of fhSPECT to reliably detect sentinel nodes in patients with oral cancer by a surgeon who is blinded to the results of pre-operative nuclear medicine imaging. A further aim is to extend the role of fhSPECT in other tumour groups.

2.3 Pathological analysis of sentinel nodes

The key hallmark of the SNB pathology protocol is a highly detailed interrogation of the tissue. Excised sentinel nodes undergo an increasingly intricate and expensive number of tests to detect metastasis. If no metastases are found at the early stages (simple bisection and H&E staining, the same as in a neck dissection), this triggers more in-depth analysis with the intention that a micro metastasis (defined as viable metastatic cells measuring ≥0.2

mm) could not be missed. This means most protocols would serially section the nodes at 150-200 mm so that the intervening sections, “curls” which are not analysed, are smaller than the minimum size for a micrometastatic deposit. This generates a huge number of slides per node, particularly if the node is negative.

2.3.1 Sentinel node pathology protocol

There is little controversy about the histopathological procedure, it has been well described and is highly reliable with little deviation from the protocol described for melanoma and breast cancer. Table 2.4 outlines the pathology protocol described in studies of SNB in oral cancer.

Table 2-4 - Sentinel node pathology protocols reported in trials.

Author	**Pathological protocol**		
	Section – H&E	**Serial-step section (SSS)**	**Immunohistochemistry**
Stoeckli (2007[165])	2.5mm slices along long axis	At 150μm	Stained for H&E and pancytokeratin at each step.
Alkureishi([94]2009)	2mm through longest axis	Six slices at 150μm, third section stained by H&E	AE1/AE3 or MNF 116
Civantos (2010[166])	2-3mm from hilum to periphery	Not clear	4 slides from each section reviewed by central laboratory with pancytokeratin AE1/AE3. CK8/18, MNF 116
Chung (2015[167])	2mm through longest axis	At 250 μm	Six slides at each level using AE1/AE3
Agrawal (2015[168])	2mm along longest axis	“Institutional standard”	Reviewed by central laboratory with pancytokeratin AE1/AE3. CK8/18, MNF 116
Schilling(2016)[41]	Bisection or 2.5mm slices	Five slices at 150μm	AE1/AE3 looking for morphologically viable immunoreactive cells – confirmed by H&E of adjacent slice

The general pathology protocol is described in figure 2.8, based upon published guidelines[94] as well as ongoing work by the author in formulating best practice guidelines in conjunction with the National Institute of health and Care Excellence (NICE). A sentinel node biopsy is reported as positive when at least one node on one side of the neck contains viable carcinoma cells.

2.3.2 Pathological reporting of sentinel nodes and TNM staging

During the course of this work, guidance on pathological reporting and staging of OSCC was updated by the change from AJCC cancer staging manual 7th edition (TNM 7) published in 2010 [28] to the 8th edition (TNM 8)published in 2017[5]. Pathological sentinel node status is unchanged between the two versions with the exception of extranodal extension (ENE)[169] (Table 2.5).

Table 2-5 - Changes in pathological staging of sentinel nodes between AJCC 7th and 8th edition.

Nomenclature; Suffixes are used after the pN stage:
• (sn) to indicate sentinel node biopsy • (mi) to indicate micrometastases • (i+) to indicate ITCs
AJCC Cancer Staging manual 7th edition recognises the following staging:
• pN0(sn) for no sentinel node metastasis • pN1(sn) for no sentinel node metastasis • pN1(sn)(mi) for single ipsilateral node with micrometastasis • pN2(sn)(mi) for multiple ipsilateral node with micrometastasis • pN2c(sn)(mi) for contralateral or bilateral nodes with micrometastasis
AJCC Cancer Staging manual 8th edition recognises the following staging:
As above except in the case of extranodal extension (ENE) which will upgrade N status such that: • pN1(sn) with ENE will become pN2a(sn) • pN2(sn) with any node showing ENE will become pN3(sn) • pN2c(sn) with any node showing ENE will become pN3 (sn)

2.3.2.1 Isolated Tumour Cells (ITC)

In melanoma and merkel cell cancer the presence of ITC in the sentinel nodes, (sn)(i+), are considered a positive result but in breast cancer they are not. Due to lack of data there is currently no consensus on the significance of ITC in oral cancer, but emerging data indicates that the presence of ITCs impacts on the patient's prognosis[170]. For this reason and until further data is collected a result of (i+) i.e. viable tumour cells is followed by a completion neck dissection.

2.3.2.2 Tumour thickness

Up to 2017 tumour (T) staging categorisation was decided by largest tumour dimension and invasion of adjacent structures (Table 1.2). The TNM 8 introduced a new parameter of tumour depth to additionally stratify T stage (Table2.6).

Table 2-6 - Changes in AJCC cancer staging of oral cancer between 7th[171] and 8th edition[5].

Tumour (T) stage	**AJCC cancer staging manual 7th edition (TNM 7)**	**AJCC cancer staging manual 8th edition (TNM 8)**
T1	Tumor ≤2 cm	Tumor ≤2 cm, ≤5 mm depth of invasion (DOI)
T2	Tumor >2 cm but ≤4 cm	Tumor ≤2 cm, DOI >5 mm and ≤10 mm *or* tumor >2 cm but ≤4 cm, and ≤10 mm DOI
T3	Tumor >4 cm	Tumor >4 cm *or* any tumor >10 mm DOI

Patients are considered eligible for SNB if they are clinically staged T1-2[172], thus the addition of staging by depth will impact on the future treatment recommendation for patients upstaged to T3 by this parameter

alone – but only if it can be reliably predicted by pre-operative imaging or biopsy.

2.3.3 "On-Table" diagnosis of sentinel node metastasis

Rigorous pathological examination by serial sectioning outlined above takes up to one week to complete, thus the patient and clinician must wait for the diagnostic result before decision regarding definitive treatment can be established. Ideally the status of the sentinel node would be diagnosed whilst the patient remains under anaesthetic for the biopsy, and the decision to proceed with neck dissection decided in the same operation, this would also allow patients requiring microvascular reconstruction to undergo SNB as there would be no second procedure that could threaten the microvascular reconstruction. Some early adopters of SNB found they had good sensitivity (94%) using on-table frozen section (FS) to diagnose metastasis[173] but these results were not replicated in later larger studies[174-176] that showed FS had a sensitivity as low as 50% and concerns regarding processing the remaining node tissue have seen this approach fall out of favour.

Another possibility is intraoperative molecular sentinel node analysis by rapid processing methods including One Step Nucleic acid Amplification (OSNA), and quantitative Reverse Transcriptase – Polymerase Chain Reaction (qRT-PCR). OSNA based on automated isothermal amplification of cytokeratin CK-19 has been validated by 96% concordance with gold-standard histopathology in breast cancer[177], allowing on-table diagnosis within 20 minutes and a commercially available system (RD-100i OSNA system (Sysmex UK)) has

been recommended for on-table diagnosis in breast SNB by NICE[178]. OSNA based on CK-19 detection in lymph nodes also showed promise in OSCC[179, 180] however a study looking at the prevalence of expression of CK-19 within primary OSCC and corresponding metastatic nodes found that just 80% of nodal metastasis would be detected by this method[181] . Another more specific and sensitive target may be possible, but currently a commercial patent held by Sysmex Co. prevents further development of this technique.

2.4 Pathological analysis of sentinel nodes – summary

The pathological processing of sentinel nodes by serial sectioning is a logical and reliable process with little possibility of error if the correct nodes have been supplied to the laboratory. This thesis does not aim to test this stage of the sentinel node procedure. However, some post hoc analysis based on the TNM 8 will be considered to understand the effect this may have upon future studies.

2.5 Sentinel node biopsy technique and protocol development - Summary

Technical aspects of SNB technique have been appraised and based on this literature review key areas have been identified in which further research is required to define and refine sentinel node reliability and applicability every day clinical practice.

The most impactful potential advancement will be in moving the entire procedure into the operating room. Preliminary studies using fhSPECT would support this advance, but this innovative modality has only been tested as an adjunct to conventional imaging whereas it's full potential lies in sentinel node identification without the need for the patient to undergo lymphoscinctigraphy and SPECT/CT. If fhSPECT can be validated against these methods, many patients with deep body tumours such as larynx, lung, stomach, and intestine would be able to benefit from 'at risk' nodal identification and individualised staging. The hybrid tracer ICG-^{99m}Tc-Nanocoll is a simple modification of SN technique that can be immediately used in clinical practice. Its optical properties are superior for sentinel node identification compared to PVD in a number of patients with head and neck malignancy, but so far this advantage has not been widely recognised in the UK. Further work is needed to ascertain the additional benefit of fluorescence in order to justify the investment required to adapt the operating theatre and purchase equipment for NIR imaging compared to the low cost (naked-eye) detection of PVD.

Clearly the ability to pathologically stage the sentinel node in theatre is an ultimate aim but the development of this requires huge commercial investment and is some years away from use in routine clinical practice.

The first aim of this work is to test fhSPECT against established SNB technique in oral cancer, secondly to ascertain the benefit of fluorescence imaging in SNB in oral cancer, and finally to investigate the feasibility of applying SNB to new tumour groups.

Chapter 3 Blinded comparison of sentinel node localization in oral cancer by pre-operative lymphoscintigraphy and SPECT/CT with intraoperative localisation by freehand SPECT (fhSPECT).

3.1 Introduction

The complex nature of lymphatic drainage within the head and neck means that intraoperative gamma probe localisation of sentinel nodes is always informed by pre-operative imaging (lymphoscintigraphy and/or SPECT/CT). Freehand SPECT (fhSPECT) offers the possibility of real-time intraoperative lymphoscintographic mapping for sentinel node biopsy (SNB). The advantage of fhSPECT is the ability to build up a dynamic three-dimensional mapping of the radioactive 'hot-spots' within the region of interest, which will move with changes in the patient's position during surgery. It also allows for re-scanning during the procedure to reflect changes in the anatomy.

To date fhSPECT has been investigated as an adjunct to traditional sentinel node imaging rather than an independent localisation modality for SNB procedures. However, if fhSPECT proves accurate enough to localise sentinel nodes without the need for gantry-based outpatient imaging, the process could be moved to the operating theatre thus reducing the time and cost associated with the procedure.

This also opens up possibility to apply SNB to tumours such as tonsil and tongue base which are only accessible for injection with radiotracer once the patient is under general anaesthetic. Moreover, this system is markedly cheaper than the gantry based systems.

3.2 Study aim

This study aims to assess whether fhSPECT guided sentinel node biopsy can be accurately undertaken in patients with oral cancer by a surgeon who has been blinded to the results of pre-operative lymphoscintigraphy and SPECT/CT. The accuracy of the three imaging modalities (fhSPECT, lymphoscintigraphy and SPECT/CT) are measured by sentinel node identification rate, identification of positive sentinel nodes, and false negative rate. A secondary aim is to understand the optimum post injection window for identification of lymphatic drainage by fhSPECT, thus informing any future protocol that may rely on the intraoperative delivery of radiotracers.

3.3 Study population

Ethical permission was granted to recruit patients aged between 18 and 90 with newly diagnosed T1-T2 oral squamous cell carcinoma who were clinically staged N0 and were awaiting sentinel node biopsy. Clinical staging was undertaken by CT and MRI scan of the neck plus ultrasound fine needle aspiration cytology (FNAC) of any nodes that were clinically suspicious.

Exclusion criterion included any patients who were unable to give informed consent, who were pregnant or breast-feeding those who had undergone any previous neck surgery or radiotherapy and anyone in whom the use of a radiotracer was contraindicated.

Patients were identified via the head and neck outpatient clinic and the weekly head and neck Multi Disciplinary Meeting (MDM) at Guys Hospital, London. Patients deemed appropriate for inclusion by MDM discussion were approached by the author during outpatient clinic consultations, at least two weeks prior to undergoing surgery. It was at this time that the Patient Information Leaflet was discussed and given to the patient (Appendix A – Patient information leaflet).

In accordance with our ethical permission (Ref: 12/LO/1542), all patients were given the opportunity for further discussion about the study either in person or by telephone at least twenty-four hours after receiving the initial information. If following this consultation, patients were willing to enter into the study they were asked to sign informed consent documentation (Appendix B – Consent form), the recruitment and outcome of patients is shown in figure 3.1 (results section 3.6)

3.4 Materials and methods

Data collected at each stage of the procedure described below were contemporaneously recorded on a specifically designed proforma. Paper copies were anonymised and stored in the site file, held in a locked office in the head and neck department of Guys Hospital London. Data was transcribed into a password protected secure online database (Infoflex version 5, CIMS, UK) by the author and data was analysed using a combination of Infoflex v5 analysis module, Excel for Mac 2008 (version 12.3.2) and Statistical Package for the Social Sciences (SPSS IBM, version 24).

Images collected as part of the analysis were downloaded and stored on a password protected GSTT Trust PC, or kept on the DeclipseSPECT hard drive, along with each operative report.

3.4.1 Pre-operative Imaging

All patients presented first to the nuclear medicine department at Guy's Hospital to undergo imaging prior to surgery. Patients were assigned to receive the tracer on the afternoon before their operation (two-day protocol) or on the morning of surgery (one-day protocol) based on availability of the scanners in the nuclear medicine department. This pragmatic approach to scheduling was based on data showing that the success of the biopsy is not influenced by the time elapsed between the injection and surgery as long as the pathway is completed within 24 hours[136]. A member of the nuclear medicine and surgical team reviewed results of the imaging prior to theatre. One surgeon remained blinded to the results and was designated to lead the subsequent surgical procedure.

In order to test the ability of fhSPECT to identify immediate drainage, a subset of the study group underwent additional fhSPECT imaging following injection in the nuclear medicine department. This was only possible if the device was not being used in theatre at the time of injection.

The pre-operative sentinel node imaging adhered to a widely validated protocol[41, 94], which has also been previously reported in the literature by this department[182].

The tumour site was anaesthetised by topical anaesthetic spray (Xylocaine pump spray 10%, (Lidocaine 10mg/dose, AstraZeneca). Four submucosal injections of 99mTc-Nanocoll (GE Healthcare Ltd) were delivered by a nuclear medicine physician (GG) at equidistant points (12, 3, 6 and 9 O'clock position) 0.5mm from the margin of the tumour. The total effective dose was 40-80Mbq for a two day and 10-20 MBq for a one-day protocol.

Following injection, the patient rinsed their mouth with water to prevent artefact from swallowed tracer, and proceeded directly to dynamic lymphoscintigraphy. Patients were imaged in the supine position with the tumour side towards the camera or neutral position if midline.

Planar imaging was performed using a dual head gamma camera (e.cam, Siemens Healthcare, Munich Germany) using a low energy high-resolution collimator with 9.1mm resolution[183]. Dynamic images were taken in the anterior or oblique view (20 x 60s, 128 x128 matrix). Directly after dynamic imaging static images (120s or 300s, 256 x 256 matrix) were acquired. The position of the sentinel nodes were localised on the skin of the neck using a ^{57}Cobalt point-source marker and was marked on the skin, based on the static images of the head and neck

Following dynamic and static lymphoscintigraphy the patients progressed to SPECT/CT imaging using a dual-detector gamma camera with a mounted 2-

row multidetector CT scanner with intrinsic resolution of 3.8mm[184] (Symbia T, Siemens Healthcare). SPECT protocol used 128×128 matrix by180° in the anterior L-mode rotation with 3° angle step and 20–25 seconds per projection, in 8 iterations, using correction for attenuation and scatter. CT images were obtained by 130kV 17mAs 4.42-5mm slices and reconstructed to sagittal, axial, and coronal views. Co-registered SPECT/CT images were fused using E.soft 2007 application package (Siemens Healthcare) and any mis-registration was adjusted manually.

Table 3-1 - Nuclear medicine imaging protocol

Imaging Modality	**Imaging parameters**
Dynamic Lymphoscintigraphy	30-60 minutes continuous imaging (20 x 60s, 128 x128 matrix).
Static Lymphoscintigraphy	Anterior and oblique images (addition flood source image) (120s or 300s, 256 x 256 matrix)
SPECT-CT	128×128 matrix by180° CT: 130kV 17mAs 4.42-5mm slices

If undertaken, a post injection fhSPECT scan (by MM or CS) commenced five minutes after the injection had been completed. In these cases, the patient-tracking device was placed on the patient's forehead and the position marked on the skin using permanent marker pen and covered by a clear occlusive dressing (Tegaderm, 3M medical USA), allowing accurate relocation during surgery producing directly comparable scans. Both sides of the neck were scanned until clear images of radiation hotspots were seen or at least 2000 counts were obtained. The fhSPECT was completed within five minutes but if unsuccessful, no further delay was permitted, and the patient proceeded with

standard lymphoscintigraphy and SPECT/CT protocol described above, to minimize disruption to the standard care pathway.

Following review by the non-blinded surgeon, the position of all the identified sentinel nodes was recorded on a proforma.

3.4.2 Surgery

All surgery was undertaken at Guys Hospital, London by both MM and CS. One took the role of lead surgeon and was blinded to pre-operative imaging. Patients were admitted on the morning of surgery and consent reconfirmed. Intubation was via the nasotracheal approach. After photographing, all markings on the neck were removed by the non-blinded surgeon before the patient entered the operating room.

Anaesthetised patients were positioned supine, with head ring support and the 3D patient-tracking device secured to the forehead. The lead surgeon undertook an fhSPECT scan and the position of the identified sentinel nodes was marked on the neck with indelible pen, photographed, and recorded on the data collection sheet. An augmented reality image of the patient and 3D mode showing depth of the node from the probe tip was automatically recorded by the DeclipseSPECT and stored in the hard drive of the device.

The position of the nodes identified by fhSPECT informed the surgical approach and the results of the lymphoscintigraphy and SPECT/CT were withheld until the sentinel node procedure was completed to the satisfaction of the lead surgeon. The only planned exception to this was in the case of no

nodes being found by fhSPECT in which case the pre-operative imaging results were revealed at the beginning of the invasive procedure.

The tumour periphery was injected with Patent V blue dye (Guerin, Paris) 0.1mls by the same technique as the radiotracer.

Incision(s) in the neck were planned according to the established sentinel node position. Local anaesthetic was not used. Generally, a 2-3cm incision was placed in a skin crease closest to the node, or placed in a location convenient to access more than one nodal basin if required. The neck was opened in layers taking care to preserve neurovascular structures and to minimise bleeding. Once the target nodal basin was accessed the lead surgeon looked for blue staining. If no blue dye was seen the surgeon used the hand-held gamma probe to isolate the correct node. If the hot node could not be found in the basin using the gamma probe without navigation function a repeat fhSPECT scan was undertaken at this stage. All nodes were removed with a cuff of surrounding fat and the following information was recorded on a designated whiteboard in the operating room;

- Neck level from which the node was retrieved - When more than one node was removed from a neck level each was numbered according to the order in which they were removed e.g. left level IIa node 1 (L2a.1), left level IIa node 2.
- Size – maximum dimension measured in millimetres
- Colour - presence of optical tracer (Patent V Blue dye)

- Node scintigraphic count – the average gamma count obtained over ten seconds of recording once the node was excised and moved away from the patient.
- Bed scintigraphic count – average gamma count obtained over ten seconds with the probe tip placed within in the position that the node was excised. If the bed count remained high after all anticipated sentinel nodes were removed (based on fhSPECT) then another fhSPECT scan was undertaken
- Background count – the probe was held on the patient's chest pointing away from the injection site and mean gamma count obtained over ten seconds recorded.

All excised nodes were placed in separate pots containing 10% buffered formaldehyde solution and labelled according to neck level, number and gamma count. In accordance with convention[185], to be considered a sentinel node, the gamma count was a minimum of 10 times the background radiation and more than 10% of the count of the hottest node excised. Nodes that did not meet these criteria were labelled 'non-sentinel lymph node' and were submitted for routine H&E examination rather than serial sectioning.

Once this process was completed and before the neck incisions were closed, the results of the lymphoscintigraphy and SPECT/CT were revealed by the non-blinded surgeon. If there was discrepancy between the pre-operative imaging and the fhSPECT result (nodes not detected by fhSPECT) then the images were reviewed in full by both surgeons on a screen in theatre and a joint decision was made if further nodes required retrieval. Once all targeted

sentinel nodes had been removed, the neck was closed, and the primary tumour was excised, aiming for a pathologically confirmed clear margin of over 5mm. Sentinel nodes were transported to the histopathology laboratory at Guys Hospital on the same day as surgery and processed according to the protocol outlined in chapter 2.

3.4.3 Follow –up

Patients were reviewed on the ward, one day following surgery, to assess for any complications associated with the procedure. Complications were graded according the Clavien-Dindo classification[186]. Patients were reviewed in clinic one week after surgery to discuss the results of the sentinel node biopsy. If the biopsy proved positive for metastasis, a completion neck dissection was scheduled. The final nodal staging was based on combination of the SNB and the completion neck dissection. In accordance with UK guidelines post-operative radiotherapy was recommended to patients who had extracapsular spread or metastasis in more than one lymph node[187].

For this study patients were followed up in the head and neck clinic at Guy's Hospital for a minimum of 12 months after the procedure.

3.5 Recruitment and analysis plan

Descriptive statistics were used and as the study group underwent paired investigations, patients acted as their own control group. Sample size calculations showed that it would not be feasible to recruit enough patients in a single centre to show a statistical difference between the modalities. Based on previous unit activity and allowing for adequate follow up period it was decided to aim to recruit fifty patients into the study.

The primary outcome measure was the number of sentinel nodes correctly identified by fhSPECT, SPECT/CT and lymphoscintigraphy. The performance of each technique was compared using one-way repeated measures analysis of variance (ANOVA) analysis.

If an imaging modality showed a single hotspot where other modalities showed two or more separate areas this was considered to be a missed node by the modality. If two or more hot nodes were removed from an area that showed a single hotspot on all modalities (clustering) this was not considered a missed node.

The second outcome was the detection of positive sentinel nodes. The expected true positive rate in T1-T2 oral SCC is 23-34%.[41, 166, 188] In this group we expected 12-17 positive biopsies. To consider fhSPECT a safe option it should be able to detect all the positive nodes (SN+) that had been found by other methods i.e. 100% concordance between LSG or SPECT/CT and fhSPECT for all SN+ cases.

A false negative result was defined as any patient who developed an isolated recurrence in the neck after a negative SNB. The mean time to diagnosis of missed metastasis is 6 months following surgery[41], and all study patients were followed up for at least one year. The SENT multicentre trial showed that the false negative rate for SNB in oral cancer is 14%[41]. On this basis we would expect to find 2 -3 false negative cases in the study group cohort.

Univariate survival analysis models were built using Kaplan-Meier product-limit estimator for disease free survival (DFS). Table analysis on outcomes was performed using either chi-square or Fisher' exact to test significance, depending upon the distribution of the variable in question.

3.6 Results

Between November 2012 and November 2015 fifty patients were recruited to the study. Three patients undergoing SNB declined to enter the study and did not undergo navigation guided SNB (Figure 3.1, recruitment and flow diagram).

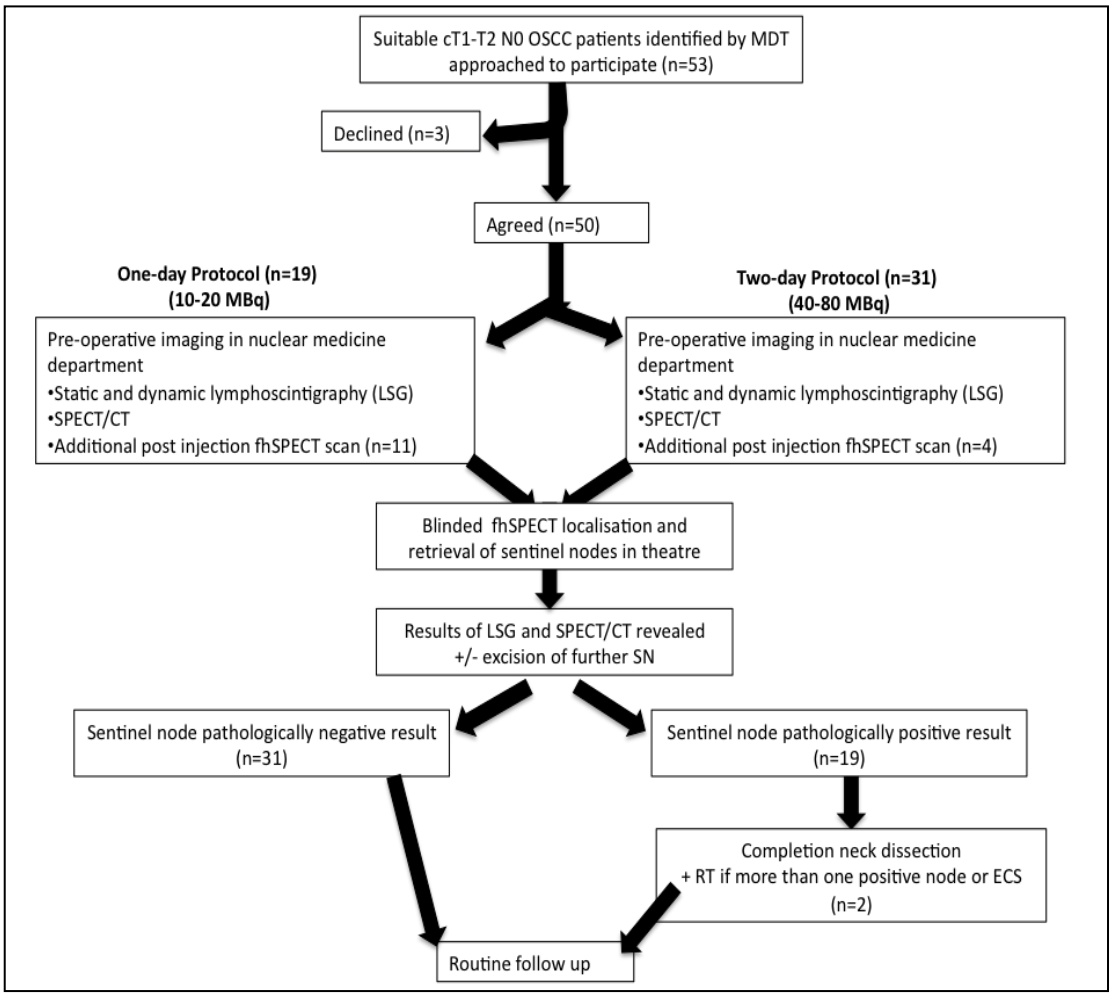


Figure 3-1 - Patient recruitment and flow diagram.

A total of 144 sentinel nodes were retrieved, an average of 2.88(+/-2.05) node per patient (range 1-8). The sentinel node biopsy was positive for metastasis in 19 patients (38%), in three cases two positive sentinel nodes were identified therefore 22 positive nodes were excised. Patient and tumour characteristics are shown in table 3.2

Six patients had midline tumours of which half drained to bilateral sentinel lymph nodes. Of the remaining patients with lateralised tumours, seven had bilateral sentinel nodes identified (7/44, 16%). In two cases positive sentinel

nodes were contralateral to the tumour (2/19) 10% of positive cases, both level 1b. None of the sentinel nodes were found below level III.

Table 3-2 Characteristics of patient and tumour.

	All patients (n=50)	Positive Sentinel node biopsy (n=19)	Effect of variable on sentinel node status
Male	(28/50) 56%	(10/28) 36%	p=0.7
Female	(22/50) 44%	(9/22) 41%	
Age (years median, standard deviation)	61.5 ± 12.08 (range 24-87)		p=0.61
Positive SNB	(19/50) 38%		
Negative SNB	(31/50) 62%		
Tumour location			
Tongue	(33/50) 66%	(15/33) 45%	p=0.3
Floor of mouth	(8/50) 16%	(2/8) 25%	
Lower alveolus	(3/50) 6%	(0/3) 0%	
Lower lip	(2/50) 4%	(1/2) 50%	
Retro-molar	(1/50) 2%	(1/1) 100%	
Buccal	(2/50) 4%	(0/2) 0%	
T stage (AJCC 7th Edition, 2010)			
T1	40 (80%)	12/40 (30%)	p=0.02
T2	10 (20%)	7/10 (70%)	
T stage (AJCC 8th Edition, 2016)			
T1	38 (76%)	9/38 (24%)	p=0.001
T2	9 (18%)	7/9 (78%)	
T3	3(6%)	3/3 (100%)	
N (sn) stage			
N1	(16/19) 84%		
N2b	(3/19) 16%		
Extracapsular spread			
Yes	(3/19) 16%		
No	(16/19) 84%		

Ten lymph nodes were excised during the SNB that contained weak gamma signal which did not meet the ex-vivo count criteria to be considered sentinel nodes. A further eight nodes were excised during the SNB procedure that contained no gamma signal. These non-sentinel nodes were examined histopathologically and all 18 were negative for metastasis.

Completion neck dissection was undertaken in all 19 patients with positive SNB. In two cases further positive nodes were found in the completion neck.

Post hoc analysis using the 2016 AJCC 8th edition cancer staging guidelines[189] upstaged three patients to T3, two that had been staged T1 and one T2 by the 2010 guidelines[28] . Table 3.2 shows the 8th edition guideline has a statistically significant correlation with sentinel node status (p-0.001), and that all the T3 cases were positive for metastasis.

3.6.1 Excised sentinel nodes

The location and pathological status of sentinel nodes that were excised are divided by anatomical site and represented in figures 3.2-3.7. These figures represent the total nodes submitted for serial sectioning having met intraopertive criteria (gamma count with/without blue dye) to be designated sentinel nodes. Review of all imaging modalities (fhSPECT, SPECT/CT, lymphoscintigraphy) was undertaken prior to submission to pathology ensuring no further hotspots remained in the neck, thus these nodes are considered the standard to which the pre-operative localisation modalities are compared.

Tongue tumours

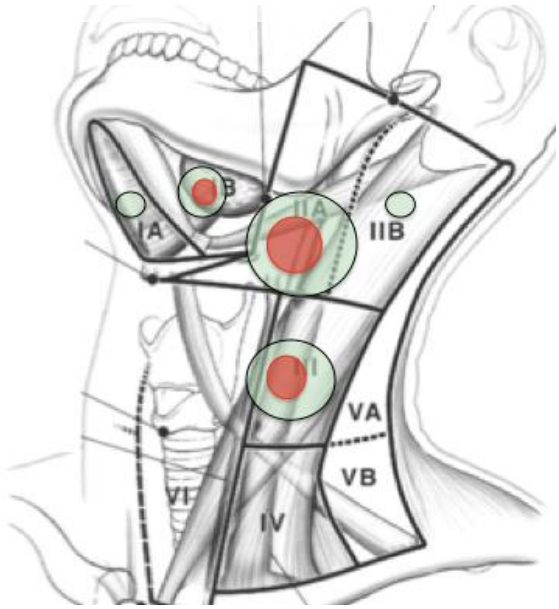


Figure 3-2 Tongue tumours

Number and location of sentinel nodes excised from primary tongue tumours (red= positive for metastasis, green = negative for metastasis)

	Negative Node	Positive Node
Facial	0	0
Level 1a	3	0
Level 1b	9	2
Level IIa	33	11
Level IIb	4	0
Level III	32	4
Level IV	0	0
Level V	0	0
Total	81	17

Floor of mouth

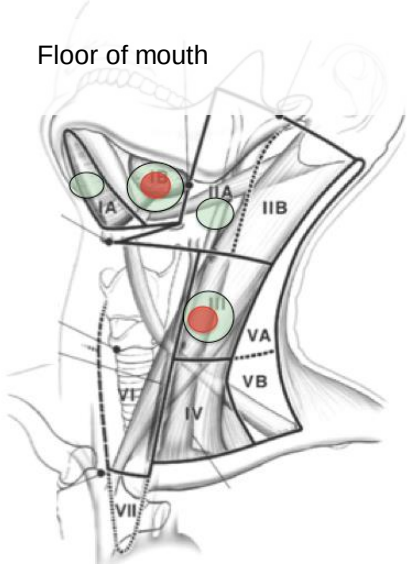
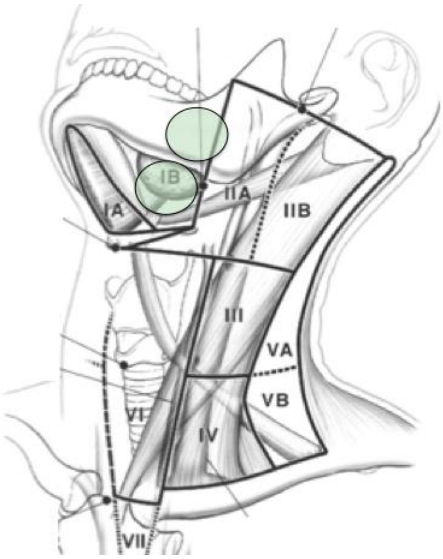


Figure 3-3 - Floor of mouth

Number and location of sentinel nodes excised from primary floor of mouth tumours.

	Negative Node	Positive Node
Facial	2	0
Level 1a	1	0
Level 1b	9	2
Level IIa	1	0
Level IIb	0	0
Level III	8	1
Level IV	0	0
Level V	0	0
Total	21	3

Lower alveolus

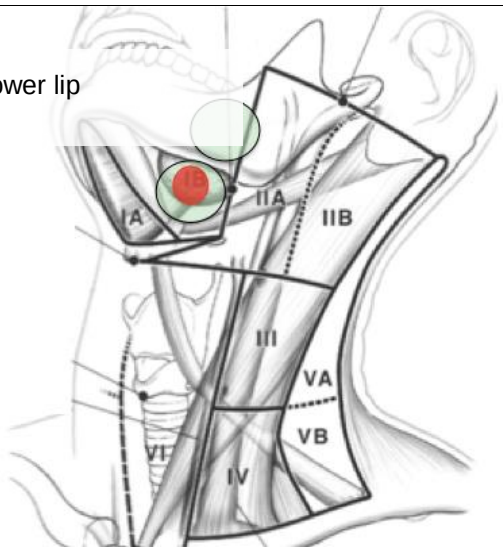


Number and location of sentinel nodes excised from primary tumours of the lower alveolus.

	Negative Node	Positive Node
Facial	3	0
Level 1a	0	0
Level 1b	5	0
Level IIa	0	0
Level IIb	0	0
Level III	0	0
Level IV	0	0
Level V	0	0
Total	8	0

Figure 3-4 Lower alveolus

Lower lip

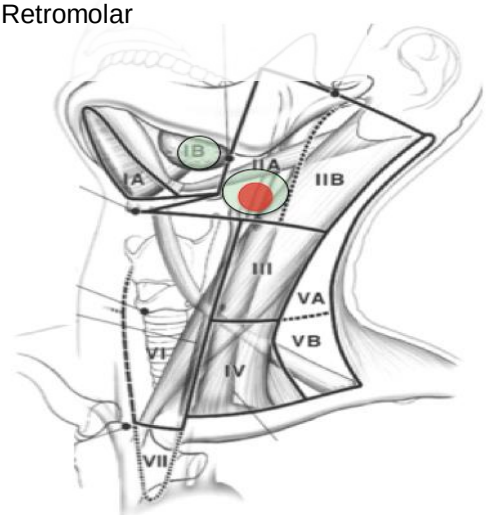


Number and location of sentinel nodes excised from primary tumours of the lower lip.

	Negative Node	Positive Node
Facial	2	0
Level 1a	0	0
Level 1b	4	1
Level IIa	0	0
Level IIb	0	0
Level III	0	0
Level IV	0	0
Level V	0	0
Total	6	1

Figure 3-5 - Lower lip

Retromolar

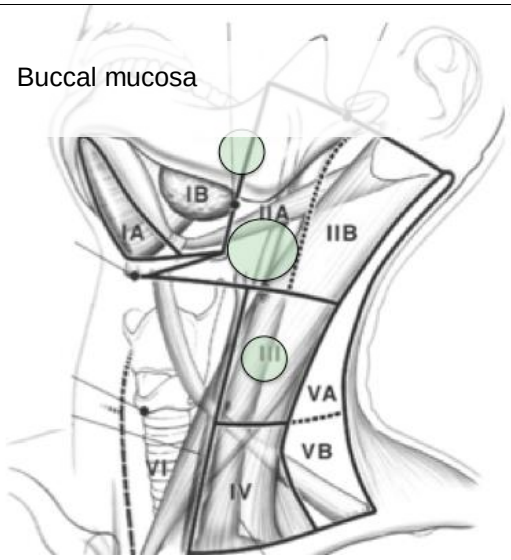


Number and location of sentinel nodes excised from primary tumour of the retromolar region.

	Negative Node	Positive Node
Facial	0	0
Level 1a	0	0
Level 1b	1	0
Level IIa	1	1
Level IIb	0	0
Level III	0	0
Level IV	0	0
Level V	0	0
Total	2	1

Figure 3-6 - Retromolar

Buccal mucosa



Number and location of sentinel nodes excised from primary buccal tumours.

	Negative Node	Positive Node
Facial	1	0
Level 1a	0	0
Level 1b	0	0
Level IIa	2	0
Level IIb	0	0
Level III	1	0
Level IV	0	0
Level V	0	0
Total	4	0

Figure 3-7 - Buccal mucosa

Figures 3.2 -3.7 Location of positive and negative sentinel nodes from all tumour sites.

3.6.2 Immediate fhSPECT scan

Fifteen patients underwent fhSPECT scan five minutes following injection of the radiotracer. The results of these were compared to the pre-operative fhSPECT scans which were taken in theatre just prior to the surgical procedure (range 3.5-18 hours post injection depending on if a one or two day protocol had been used).

In three cases the immediate fhSPECT scan showed no clear drainage to the neck. In a further two cases there was drainage seen to the neck but the signal could not be isolated to a discrete area. The remaining ten cases had hot nodal areas detected (Table3.3).

Table 3-3 - Cases that underwent immediate freehandSPECT (fhSPECT) scan following injection of radiotracer.
FOM= Floor of mouth. * denotes sentinel node that was found to contain metastasis.

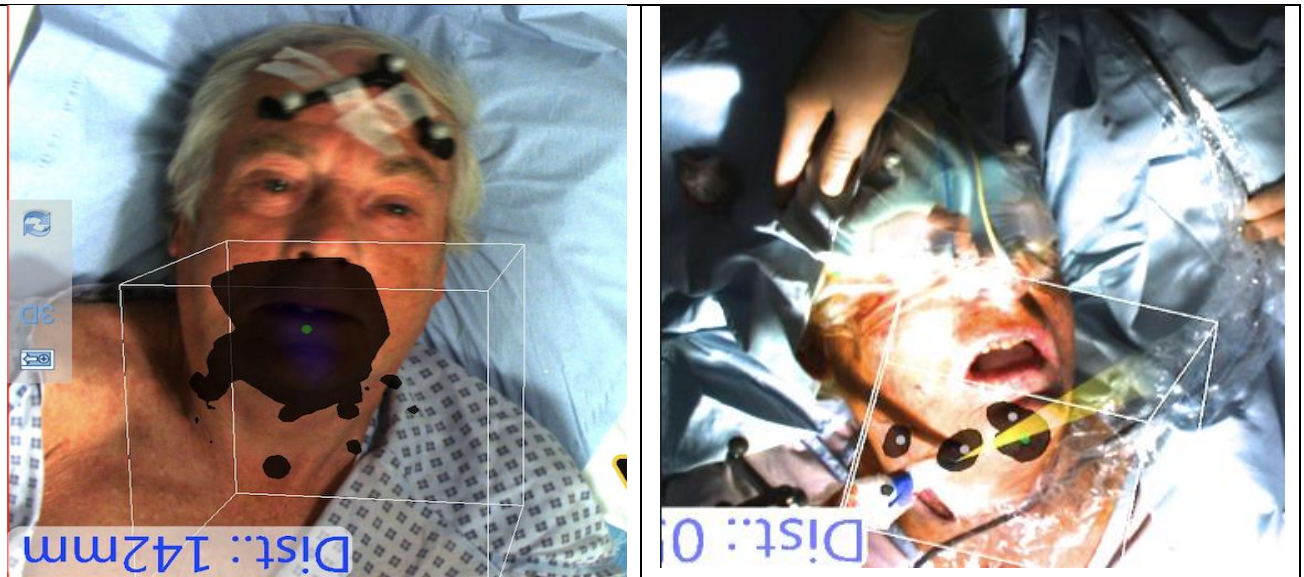
Case	Side of body	Tumour location	Dose of Nanocolloid (MBq)	One or two day protocol	Immediate fhSPECT result	SNB Result
1	Left	Tongue	20	One	Indeterminate drainage left neck	Positive
2	Left	FOM	20	One	Left and right submental and right IIa	Negative
3	Right	Tongue	20	One	Right level IIa	Negative
4	Right	Tongue	85	Two	Right level IIa and Right level III	Negative
5	Right	Tongue	20	One	Right level IIa	Positive
6	Left	Lower lip	20	One	Right and left Ib and left facial	Negative
7	Left	Tongue	33	One	Left level IIa *	Positive
8	Right	Tongue	19	One	Right level II/III*	Positive
9	Left	Tongue	17	One	No nodes found	Positive
10	Left	Tongue	20	One	Left Ib* Left IIa Right IIa	Positive
11	Left	FOM	20	One	No nodes found	Negative
12	Right	Lower lip	20	One	Left and right facial, Left IIa	Positive
13	Midline	FOM	54	Two	R submental, right facial, Right III	Negative
14	Midline	FOM	53	Two	Indeterminate drainage right and left neck	Positive
15	Left	Tongue	69	Two	No nodes found	Positive

Nine of the fifteen patients proved to have positive sentinel nodes by histopathological analysis (60%). The positive sentinel node was located by immediate fhSPECT in only three of these cases, thus the sensitivity for detection of positive sentinel nodes was 33% and the false negative rate 40%. Examples of cases are shown below (figures 3.8-3.10) these demonstrate characteristic high signal and scatter which is found at the tumour site immediately post injection and illustrate three scenarios that typically arise;

i) No discernable drainage is found on immediate fhSPECT scan with clear drainage shown on subsequent (pre-operative) fhSPECT scan. (5/15 cases)

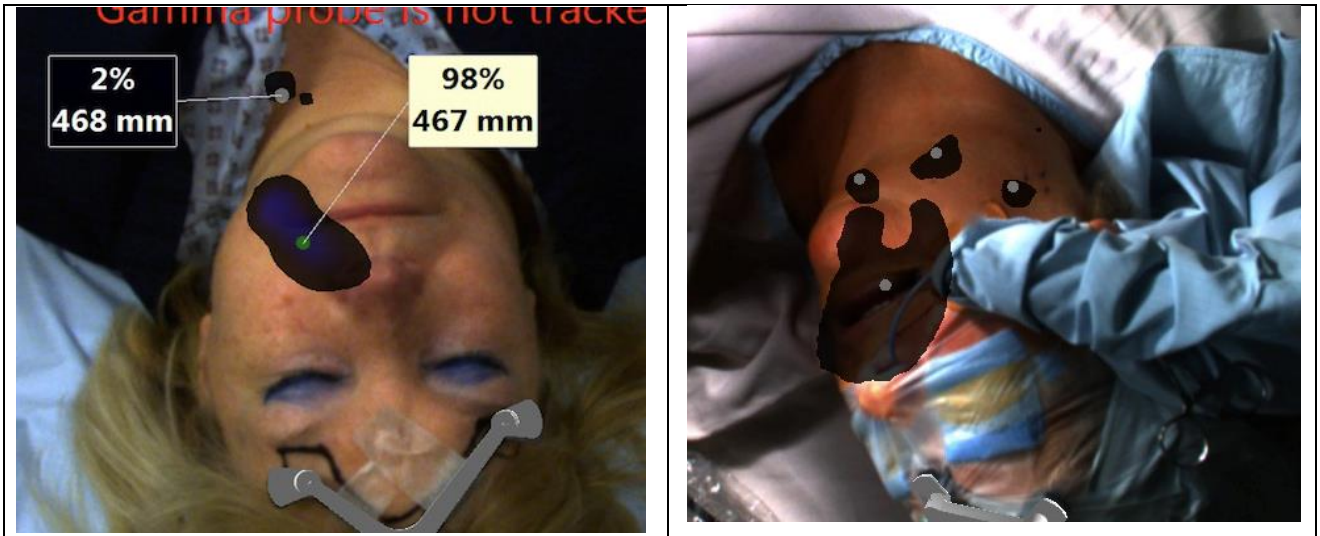
ii) Clear drainage seen on immediate fhSPECT scan, but additional drainage found on subsequent scans. (7/15 cases)

iii) Clear drainage seen on immediate scan with no additional hotspots found on subsequent scans. (3/15 cases)



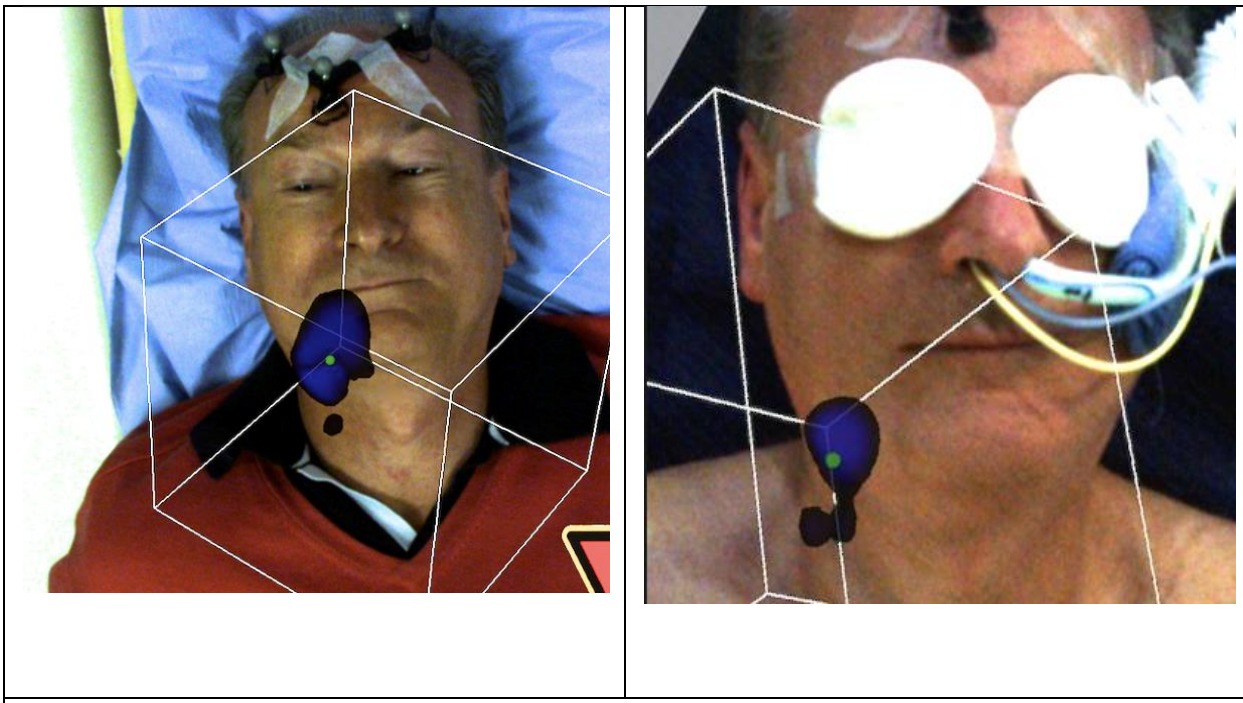
Case 14. Immediate fhSPECT scan (left) from midline floor of mouth tumour showing indeterminate drainage in both sides of the neck with high signal at the injection site. Pre-operative fhSPECT (right) at 17 hours post injection shows clear drainage to level I lymph nodes of which one contained metastatic deposits.

Figure 3-8 - Pattern one: No discernible drainage found on immediate fhSPECT scan with clear drainage shown on subsequent scans.



Case 7: Immediate fhSPECT (left) showing clear drainage from left lateral tongue (yellow box) to a single left level 2a node (black box) subsequently positive on pathological analysis. However pre-operative fhSPECT at five hours post injection found left level 3 and right level 2a nodes which were negative histlogically.

Figure 3-9 - Pattern two; Clear drainage seen on immediate fhSPECT scan, but further drainage found on subsequent scans.



Case 8. Immediate fhSPECT scan (left) showing drainage to right level 2a/3 lymph node. Pre-operative fhSPECT scan (right) at four hours post injection shows reduced signal at the injection site with increased signal at the level 2a/3 sentinel node but no additional hotspots.

Figure 3-10 - Pattern three: Clear drainage seen on immediate scan with no additional hotspots found on subsequent scan

Figures 3.8-3.10. Patterns of drainage found on immediate fhSPECT scan

3.6.3 Blinded fhSPECT compared to lymphoscintigraphy and SPECT/CT

Of the 144 sentinel nodes excised 95 were identified by lymphoscintigraphy, 122 by SPECT/CT and 125 by fhSPECT (Table 3.4). A sample case is shown in figure 3.11.

A one-way repeated measured analysis of variance (ANOVA) was conducted to evaluate the null hypothesis that there is no difference in the number of sentinel nodes localised by each modality. The results of the ANOVA indicate a significant effect, Wilks' Lambda $p<0.001$. Follow up comparisons indicated that pair-wise difference between lymphoscintigraphy and both SPECT/CT

and fhSPECT were significant (p=0.002) but comparison between SPECT/CT and fhSPECT were not (p=1.000).

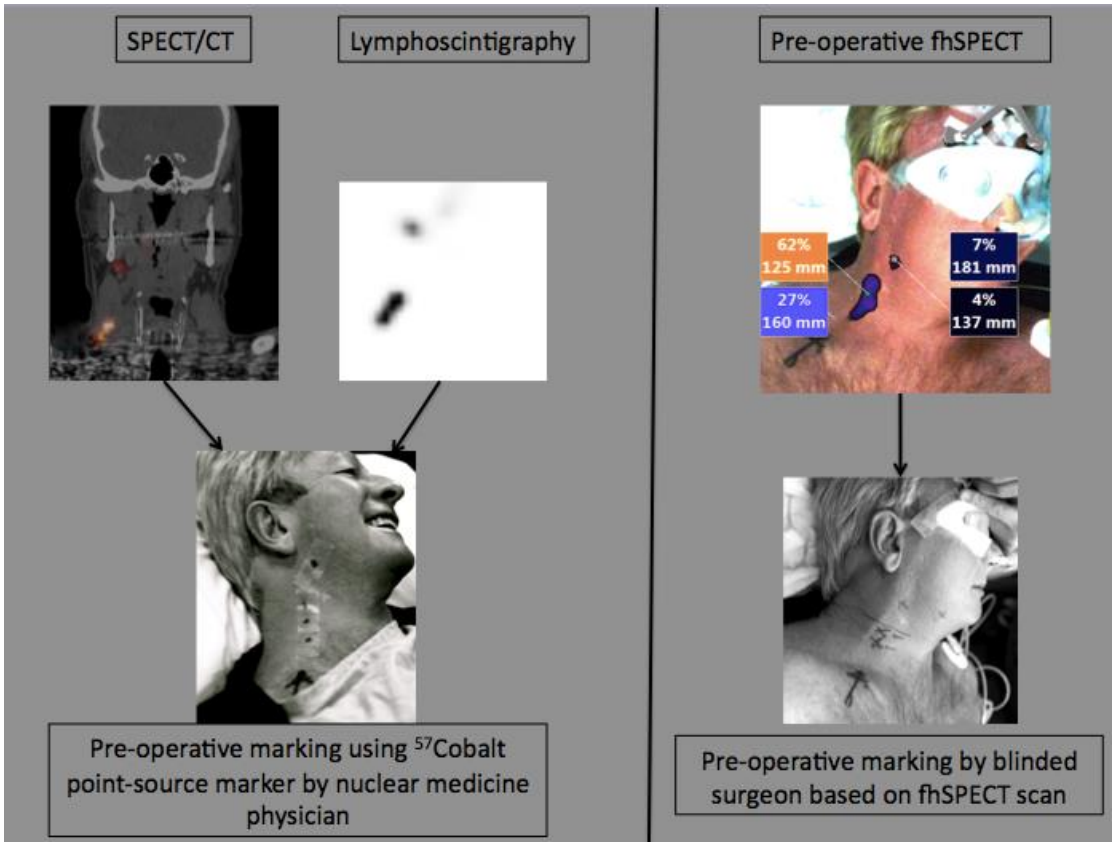


Figure 3-11 - Sentinel node identification by SPECT/CT and lymphoscintigraphy versus fhSPECT. All modalities found three sentinel nodes. Neck markings by nuclear medicine (left) and surgeon (right) can be compared (Nb. anterior mark 'x' by surgeon denotes the tumour site, which is masked in the nuclear medicine scans)

Table 3-4 Number of nodes found by each modality

	Total number of nodes	Mean number of nodes	Standard error	95% confidence interval	No drainage (n=50)
LSG	95	1.9	.149	1.5-2.2	4 (8%)
SPECT/CT	122	2.4	.184	2.3-3.0	1 (2%)
fhSPECT	135	2.5	.141	2.3-2.9	0

Lymphoscintigraphy failed to show any drainage in four patients. SPECT/CT showed no drainage in one patient and fhSPECT showed drainage in all patients. In two cases the surgical plan was changed after revealing the results of the SPECT/CT. In both cases the patient was rescanned by fhSPECT and further nodes were localised and excised. These nodes were negative for metastasis thus they are no considered a false negative result.

All modalities missed positive nodes in at least one patient (Table 3.5). The false negative rate for lymphoscintigraphy, SPECT/CT and fhSPECT was 26.3%, 15.8% and 5.3% respectively. If we consider the hypothetical situation that all the positive nodes missed were left until clinically apparent the overall neck control rate (NCR) for each modality in this cohort (fifty patients, three regional recurrences) would be 84%, 88% and 92% for LSG, SPECT/CT and fhSPECT.

Table 3-5 - Number of positive nodes found by each modality

	Missed positive cases (n=19)	False negative rate	Neck control rate
LSG	5	26.3%	84%
SPECT/CT	3	15.8%	88%
fhSPECT	1	5.3%	92%
Total (all modalities)	0	0%	94%

Freehand SPECT failed in to identify a positive node in one patient with a tongue tumour. The pre-operative fhSPECT scan identified a facial node and a level IIb node. Both pre-operative SPECT/CT and LSG found two hot areas in the left neck - level IIa and level III - of which the level IIa node was positive for metastasis.

3.6.4 Blue dye

At least one blue lymph node was found in 41/50 patients (82%), mean number of blue nodes was 1.8 (range 1-3). All blue nodes met gamma count criteria for sentinel nodes. Blue dye did not drain to the node containing metastasis in six of the nineteen SNB positive cases, a false negative rate of 32%.

3.6.5 Complications

There were minimal complications encountered following SNB. Only one patient had to return to theatre, the remaining six patients with complications had no change in their clinical course. Complications related to the sentinel node procedure are shown in table 3-6

Table 3-6 - Complications related to Sentinel Node Biopsy

Complication	Management/outcome	Clavien-Dindo Score
Wound infection	Responded to oral antibiotics	Grade 2 - Mild
Sialocoele	Aspirated in clinic twice	Grade 1 - Mild
Sialocoele	Aspirated once in clinic	Grade 1 - Mild
Sialocoele	Managed conservatively but re-admitted for observation at patient's request	Grade 1 - Mild
Marginal mandibular nerve weakness	Residual weakness (House-Brackmann score 2/5) at 18 months.	Grade 1 - Mild
Haematoma	Return to theatre for drainage	Grade 3b – Moderate
Haematoma	Managed conservatively	Grade 1 - Mild

3.6.6 Survival

Median follow up was 65 months (range 21-119 months). No false negative results were encountered in the follow up period. Five patients died from disease recurrence (three in the neck, two with distant metastasis), all were in the sentinel node positive group. Disease free survival for sentinel node positive versus sentinel node negative was significant (p<0.005, log-rank test figure 3.12).

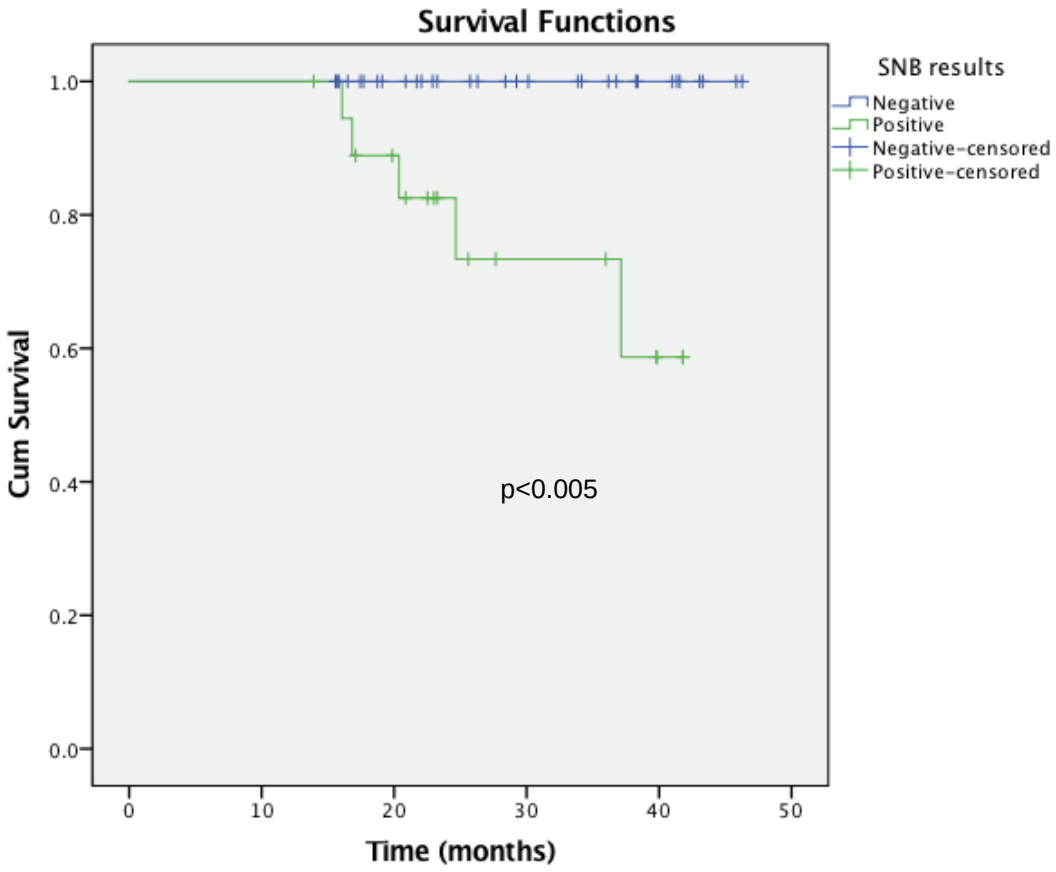


Figure 3-12 - Kaplan-Meier life table analysis showing disease free survival analysis for positive and negative sentinel node biopsy result

3.7 Discussion

The study group showed a slightly higher than expected rate of positive sentinel nodes (38%), of which a statistically significant proportion occurred in the T2 tumours ($n=10$, $p<0.05$). This increased the number of positive cases with which to test our hypothesis, but one could argue that T2 patients with a 70% risk of metastasis were disadvantaged by undergoing two procedures (SNB followed by END). These results are further accentuated by restaging using 2016 AJCC 8th edition cancer staging guidelines[189] in which three cases were upstaged to T3 by depth criteria, and thus would not have been offered SNB according to the eligibility criteria. All three of these cases had positive sentinel nodes.

Immediate fhSPECT was performed in 30% of the study group and provided new information in this field of investigation. The reason that not all patients could be scanned immediately post-injection was due to clashing scheduling of both surgery and injection. Often, more than one SNB cases would be scheduled per day therefore the DesclipseSPECT was in use in theatre at the time that the second patient was undergoing injection.

Nevertheless, the experiences gained showed that immediate fhSPECT is not reliable, with a false negative rate of 40%. This can be explained by the high level of signal at the injection site immediately after injection resulting in signal scatter and shine through effect masking signal tracking to the sentinel nodes.

This study protocol did not allow delay of more than ten minutes between injection of the tracer and commencement of lymphoscintigraphy. This is due

to historical concern about the risk of missing in-transit sentinel nodes. These are defined as nodes which the tracer completely passes through en-route to second echelon nodes and can escape detection if only late images are taken. In-transit nodes are recognised as rare occurrences and have been reported in melanoma but there are no reports of this situation occurring in oral cancer.

In this study the performance of fhSPECT, particularly in the immediate post injection period was unknown and so the study was designed to alter the standard imaging pathway as little as possible.

What has been shown clearly by these results is that lymphoscintigraphy performs poorly compared to both SPECT/CT and fhSPECT in the detection of positive and negative sentinel nodes ($p<0.005$). There was no additional benefit to performing LSG (i.e. it did not identify nodes missed by other modalities) thus opening up the possibility that LSG could be omitted allowing serial fhSPECT scans to be taken in the immediate period following injection. SPECT/CT is normally performed after the LSG protocol is finished, usually 90 minutes following injection. A future protocol could therefore be devised where patients are scanned every 10-15 minutes post injection followed by SPECT/CT at 90 minutes in order to assess the real-time tracking capabilities of fhSPECT.

Freehand SPECT showed excellent sentinel node identification when used pre-operatively, however it did miss a positive sentinel node in one case. In

this patient two hotspots had been identified in the left neck by all three modalities, LSG and SPECT/CT localised to level IIa and III whereas fhSPECT showed a facial node and a level IIB node. When the gamma probe was used independently of the navigation mode during the procedure, sentinel nodes were retrieved from level IIb and III. This suggests that there was a co-registration problem between the gamma probe and patient tracking device, leading to incorrect reconstruction of the gamma signal in three-dimensions. The DeclispeSPECT has two mechanisms to prevent errors of registration; 1) a simple calibration procedure of the gamma probe tracking device prior to the procedure and 2) a projected virtual reality image of the collimated beam which should coincide with video image of the probe tip during data collection.

In this case a potential explanation is that the head position (and thus the patient tracking device) was moved during the data collection. The tracking device is attached to the forehead and will map accurately when the head is rotated in a neutral position however if any lateral flexion of the neck is introduced the relationship between the nodal hotspots and patient tracking device is changed and could explain why the nodes appeared to have been shifted superiorly in relation to the actual position.

3.8 Conclusion

These data show that a surgeon who is naïve to the results of pre-operative sentinel node imaging can use freehand SPECT in the operating theatre to accurately locate sentinel lymph nodes. Freehand SPECT showed excellent sensitivity and a low false negative rate, but a higher detection of negative (possibly non-sentinel nodes). Data collected from immediate post injection fhSPECT is unreliable for SN node detection, suggesting that there is an as yet undefined optimum imaging window for this modality. It remains to be established if this imaging window coincides with a time frame that is compatible with intraoperative injection and sentinel node retrieval.

Chapter 4 Additional benefit of ICG-^{99m}Tc-Nanocoll in sentinel node localisation in patients with oral cancer. An exploratory phase II study.

4.1 Introduction

Optical tracers are used in sentinel node biopsy to improve the ease of localisation of sentinel nodes during tissue dissection. In surgery a collimated gamma probe can direct the surgeon to the area of highest signal intensity in the region identified by pre-operative imaging, but because of signal penetration and scatter in-vivo, the probe cannot determine which is the hot node in a cluster, nor when there are overlying nodes. This may lead to additional unnecessary tissue dissection (with subsequent scarring) and inadvertent removal of non-sentinel nodes. The current standard optical tracer is blue dye[94] which is injected separately to the radiotracer. The dye has different physical properties to the radiotracer and therefore the flow rate and retention within the node is not identical. Moreover, the dye stains the injection (tumour) site affecting the appreciation of tumour margins during surgery. Some surgeons avoid using blue dye for these reasons[41].

Indocyanine green (ICG) is a fluorescent green dye that can be non-covalently bound to preparations of albumin. This allows the formation of a multimodal tracer comprising a colloidal particle labelled with both nuclide (technetium-99) and fluorescent dye (ICG)[118]. Because the optical tracer is bound to the conventional tracer, the flow is theoretically identical. There is no

staining at the injection site as the fluorescent signal is not apparent unless tissues are viewed with a near infrared (NIR) camera. There is a risk of allergic reaction with ICG but it appears to be lower than with blue dye (0.0001% vs. 0.09% [99, 103]). The major disadvantage of ICG is that the detection of the signal can be attenuated by ambient light, which can be difficult to exclude in the operating theatre.

4.2 Study aim

This exploratory phase II study aimed to establish the additional benefit of the fluorescent signal in the multimodal tracer ICG-^{99m}Tc-Nanocoll in identifying sentinel nodes in patients with early oral cancer using a newly developed clinical spectral imager (CSI)[190].

The tracer was judged to have aided the SNB procedure in cases where:

i) Intraoperative fluorescence is detected through overlying tissue aiding localisation of sentinel nodes already identified by pre-operative imaging.

ii) Intraoperative fluorescence allows identification of sentinel nodes close to the injection site not detectable by gamma signal due to shine-through effect.

This study aimed to develop a reliable and repeatable intraoperative imaging protocol using the clinical spectral imaging device.

If an additional benefit of fluorescence guided SNB was established this would add to evidence supporting the use of fluorescence as the optical tracer of choice for SNB.

4.3 Endpoints and outcomes

The primary endpoint was the number of lymph nodes successfully identified by multimodal detection in a series of patients with cT1-2 NO oral cancer. We expected to find at least one hot and fluorescent node in each patient.
Secondary endpoints were the additional benefit of the fluorescent component of the multimodal tracer. These were defined as:

i) identification of fluorescent sentinel node at an early stage of the procedure as defined in section 4.7.6
ii) sentinel node identified by fluorescence alone (failure of gamma camera due to shine through effect)
iii) sentinel node localised on deeper dissection using fluorescence alone

Tumour identified on fluorescence because of low/absent gamma signal
Adverse events related to use of the tracer such as allergic reaction were also recorded.

4.4 Study set up and pre-clinical work

4.4.1 Regulatory approval

Ethical approval was granted for use of the blue dye +/- multimodal tracer as a non-CTIMP study (Clinical Trial of Investigational Medicinal Product) but second opinion was sought from the medicine health regulation authority (MHRA) as to whether this investigation should be registered as a drug trial. Because the multimodal tracer involved simple mixing of two licensed products with no pharmacological activity that are both excreted unmetabolised, it was advised that this study is considered equivalent to other trials using Nanocoll +/- blue dye and therefore not subject to regulations associated with CTIMP.

The multimodal tracer was new to Guy's and St Thomas' (GSTT) NHS trust therefore Trust approval was sought for its use. Evidence was presented to both the GSTT formulary committee and the Trust Risk and Quality (TRAQ) committee in November 2014. Both committees granted provisional approval for use of the multimodal tracer, ICG-^{99m}Tc-Nanocoll, for all patients undergoing sentinel node biopsy pending review of the first ten cases. A trust approved new procedure patient information leaflet was produced (Appendix C – PIS Multimodal tracer). Results of the first ten cases were presented to the TRAQ committee and subsequently full approval was granted to use ICG-^{99m}Tc-Nanocoll as the standard tracer for all tumour groups within GSTT NHS trust.

4.4.2 Risk assessment and training

The clinical spectral imager (CSI) was developed by collaboration between two CRUK Cancer Imaging centres, one based at Kings College London and the other at the University of Oxford. The design and development as well as pre-clinical and clinical validation of the system was published in 2014[190]. The CSI was assembled in-house thus exempt from CE marking requirements but underwent risk assessment by the Guy's and St Thomas' NHS trust clinical engineering and medical physics departments. Recommendations were made to reduce the risk of retinal damage from the emitted laser; patient's eyes to be covered during the procedure and staff working within 30cm of the device were advised to wear laser safety glasses.

The research team carried out additional steps to further improve safety; both MM and CS undertook a certified training course on the core knowledge of use of lasers. "Laser in use" signs were placed on the doors of the operating theatre when the CSI was in use. CS also ran a training session for the theatre staff to explain the use of the CSI.

4.4.3 Optimisation of fluorescence imaging

4.4.3.1 Pre-clinical optimisation of fluorescence imaging

Unpublished pre-clinical work was undertaken within the research group[191] to establish optimal preparation of the tracer, and optimal imaging conditions by the clinical spectral imager (CSI) in an animal tissue model. This work by Byrne et al. was based on publications describing the 'self-assembly' nature of the multimodal tracer[115]. A different method of assembly of the

compound by repeated cycles of heating was also investigated to ascertain if this increased the number of ICG molecules bound to Nanocoll, leading to improved intensity of the fluorescent signal. Results showed that there was no improvement in the fluorescence when the compound was heated compared to the self-assembly method.

The CSI allows six different settings for level of gain (amplification of signal) and eight for integration time (summation of signal over a specified period of time), resulting in 48 possible combinations. Changes in the settings affect camera sensitivity as well as image definition and brightness. Byrne conducted image optimisation studies using cuboids of porcine muscle injected with ICG, finding that optimal images were obtained by setting gain between x4 and x10 (possible range x2-10) and integration time between 0.4s and 0.5s (possible range 0.2s -4s). These data suggest a range that can be applied to the clinical setting, assuring a standardised quality of imaging. As porcine muscle has different light absorbance and auto fluorescence properties to human tissue, it was planned to confirm this image optimisation using tissue from the first case recruited into the study.

4.4.3.2 . Assessment of tumour autoflourescence

Baseline human tumour and non-tumour tissue was collected and imaged using the CSI as part of a separate and ongoing parallel study (Intra-operative GE-137 fluorescence imaging in breast cancer and oral cancer EudraCT Number: 2014-003554-13). The excitation and emission wavelengths of the CSI were set to optimal fluorescence characteristics of ICG (784 nm and >800 nm respectively). The maximum possible sensitivity to fluorescence

signal was achieved by adjusting the control unit settings to gain of x10 and integration time of 4 seconds. This confirmed that human OSCC tissue imaging by CSI with ambient light excluded at a distance of 20-30cm elicited no tissue autoflourescence in the desired range for ICG (Sample images Fig 4.1)

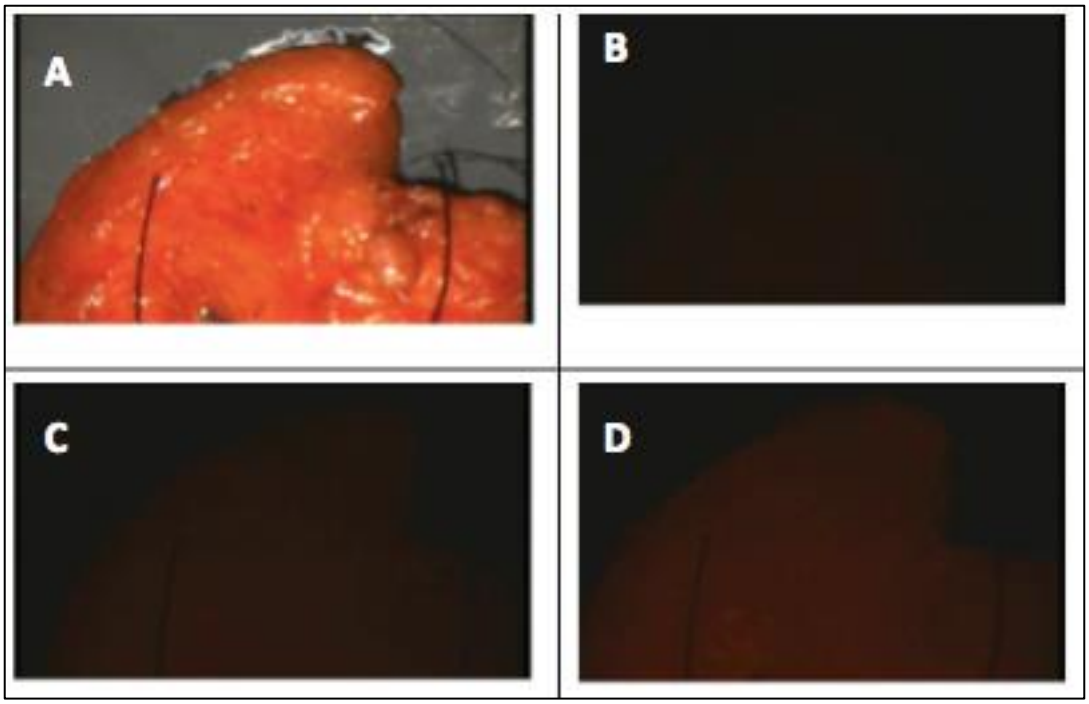


Figure 4-1 - Neck dissection tissue from EudraCT study 2014-003554-13 viewed under white light (WL)
Image A tissue viewed with near infrared light at 790nm wavelength at a variety of settings showing no tissue autofluorescence.
Image B - gain x4 integration time 40ms.
Image C - gain x8 integration time 0.5s.
Image D - gain x10 integration time 2.0s.

4.4.3.3 Quality assurance of fluorescence imaging

A reference phantom was constructed to calibrate the camera prior to each case. The phantom contained ICG solution diluted with water for injection, producing three different concentrations (Figure 4.2). The SBR was calculated under optimised conditions described in section 4.6.2 (distance 20cm, gain x

8, integration 0.5s, ambient light excluded), allowing detection of any drift in the sensitivity of the CSI over the study period.

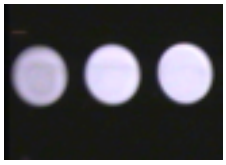


Figure 4-2 - Reference phantom of ICG solution- from left to right; 5.0, 10, 25 µM /ml

4.4.3.4 Blue dye fluorescence imaging

Patent V blue (PVB) dye (Guerin, Paris) exhibits fluorescence when excited by light just inside the visible spectrum (630-690nm). To confirm that there was no overlap between the fluorescent signals of ICG and PVB, luminance measurements were taken (as outlined in 4.5.2) from a node stained blue in a patient that had not received ICG. The CSI was set to excite at 630nm and then 784nm. This confirmed that there was weak fluorescence at 630nm and none at 784nm. The maximum luminance achieved by PVB at 630nm was 40% of that shown by ICG at 784nm under the same conditions (Fig 4.3 C).

As expected, there was no conflict between the fluorescence spectra of PVD and ICG. However, given that an endpoint of this study is the surgeon's subjective interpretation of the stage of surgery at which ICG emission was clinically useful, it was decided to omit blue dye to avoid influencing the surgeon. This would not preclude dual optical tracer use in future studies.

4.5 Material and methods

4.5.1 Study group and recruitment

Patients with newly diagnosed cT1-T2 N0 oral SCC were considered for this study. The eligibility criteria and the process of recruitment was identical to the process outlined in section 3.3. Additionally patients received a trust approved information leaflet about the multimodal tracer (Appendix C).

4.5.2 Preparation and injection of multimodal tracer

ICG powder (PULSION Medical Systems AG, Munich, Germany) was mixed with sterile water for injection (0.5% w/v, Ranbaxy UK) to achieve a concentration of 5mg/ml. From this solution 0.05 mL was taken (containing 0.25 mg of ICG) and added to 2 mL 99mTc-Nanocoll, giving a final concentration of ICG in the hybrid agent of 0.125 mg/mL. The mixed 99mTc-Nanocoll and ICG was gently agitated by hand and then left at room temperature for 30 minutes to allow association to take place. Once ready the tracer was drawn up into four separate tuberculin syringes each containing a volume of 0.1-0.2ml depending on if a one or two-day protocol was being undertaken. The final dose total of ICG was 0.05-0.1mg.

The tumour site was anaesthetised by topical spray (Xylocaine pump spray 10%, (Lidocaine 10mg/dose, AstraZeneca). Four submucosal injections of the light-green coloured multimodal tracer ICG-^{99m}Tc-Nanocoll were delivered by a nuclear medicine physician (GG) at equidistant points 0.5mm from the

margin of the tumour. The total effective dose of technitum-99m was 40-80Mbq for a two day and 10-20 MBq for a one-day protocol.

4.5.3 Pre-operative sentinel node localisation

Sentinel node imaging was undertaken by static and dynamic lymphoscintigraphy as well as SPECT/CT and fhSPECT. Prior to the operation the targeted sentinel nodes had been decided and location was marked on the neck. There was no blinding during this study. During surgery a hand held gamma probe (Crystal photonics GmBH, Germany) integrated into the declipseSPECT was used to locate the sentinel nodes.

4.5.4 Intraoperative set up of clinical spectral imager

The CSI unit was placed on a portable trolley and the camera head mounted on a custom-built clamp attached to a post elevated from the trolley. This ensured the camera could be easily moved in an out of the surgical field during the operation and eliminated position variation and movement artefact during image acquisition. The control panel and camera head tubing was covered by a transparent sterile drape (P3 medical, Bristol, UK) allowing the surgeon to adjust focus without decontamination of the surgical field. The CSI was connected to a laptop via the S-video out of the control unit. An analog to digital convertor USB Video & Audio Grabber (Winstars Technology Ltd), provided S-video to USB 2.0 interface linkage. Digital images were captured

using BlazeVideo HDAV Grabber software and stored in bitmap (.BMP) format. Images were analysed using ImageJ software (Fiji, National Institutes of Health, USA).

At the beginning of the procedure a white balance was taken with a surgical swab. A calibration image was taken using the reference phantom. Images then were recorded at pre-specified points during the operation. The image was first focused manually using the white light to obtain a crisp image. Ambient light in the theatre was minimized by using blackout blinds. Theatre lights were switched off during NIR image acquisition. Any LED screens that could not be switched off were covered temporarily with a reinforced surgical drape. Images were collected as described in section 4.5.6.

4.5.5 Surgery

All surgery was undertaken at Guys Hospital, London by MM. Capture of intraoperative images and ex-vivo fluorescence analysis using the CSI was undertaken by CS.

A standard approach to sentinel node biopsy was undertaken without blinding. Nodes were marked on the neck and a convenient incision was made. If more than one non-adjacent nodal area was explored separate incisions were made. No local anaesthetic was used. Near-infrared images were taken at predefined stages of the operation using the CSI as follows:

Stage 0. Through skin prior to incision

Stage 1. After skin had been raised by subplatysmal dissection

Stage 2. After unwrapping and retraction of adjacent muscle (e.g. digastric, sternocleidomastoid muscle) but prior to identification of the sentinel node by the naked eye.

Stage 3. After node localised by gamma count but prior to dissection from surrounding tissue (node may be visible to the naked eye).

Stage 4. Ex-vivo after excision (node visualised in black box to exclude ambient light)

Stage 5. Imaging of the bed from which the node has been excised to assess if fluorescent nodes remained.

After baseline (stage 0) imaging by the CSI, the incision was opened in layers down to the immediate subplatysmal plane. Following meticulous haemostasis, the surgical field was again viewed using the CSI. If no fluorescence was detected images were not recorded. Further dissection was directed according to gamma signal. This procedure was repeated for each plane of dissection until fluorescence detected or the node was excised. After excision all nodes were placed in a light excluding box on a background of blackened aluminium to allow calculation of maximum luminance.

If the fluorescent nodes were identified at an early stage of the procedure (0-3) no further images were taken to prevent unnecessary delay to the operation.

Data collected at each stage of the procedure were contemporaneously recorded on a specifically designed proforma. Paper copies were anonymised

and stored in the site file, held in a locked office in the head and neck department of Guys Hospital London. The surgeon also dictated a report of the operation immediately following the procedure specifically referring to the helpfulness of the fluorescence. Data was transcribed into a password protected secure online database (Infoflex version 5) by CS and data was analysed using a combination of Infoflex v5 analysis module, Excel for Mac 2008 (version 12.3.2) and Statistical Package for the Social Sciences (SPSS IBM, version 24).

Images collected as part of the analysis were downloaded in bitmap format and stored in on a password protected external hard drive.

4.5.6 Optimisation of Intra-operative fluorescence imaging

Image optimisation analysis was undertaken on tissue from the first study case. Images were taken using all 48 setting combinations at a distance of 20cm inside a black box that completely excluded ambient light. Mean luminance was calculated by drawing region of interest boundary around the fluorescent sentinel lymph node as well as a similarly sized piece of surrounding adipose tissue. Signal to background ratio (SBR) was calculated by dividing the mean nodal luminance by the background mean (adipose tissue) giving a value in arbitrary units. Sample images are shown in Figure 4.3 where optimal imaging (SBR 1.4) is shown in image 4.3C using gain amplification x8 and an integration time of 0.5 seconds. Figure 4.3B demonstrates under exposure (SBR 1.2) whereas in 4.3D (SBR 1.080) the image is over exposed.

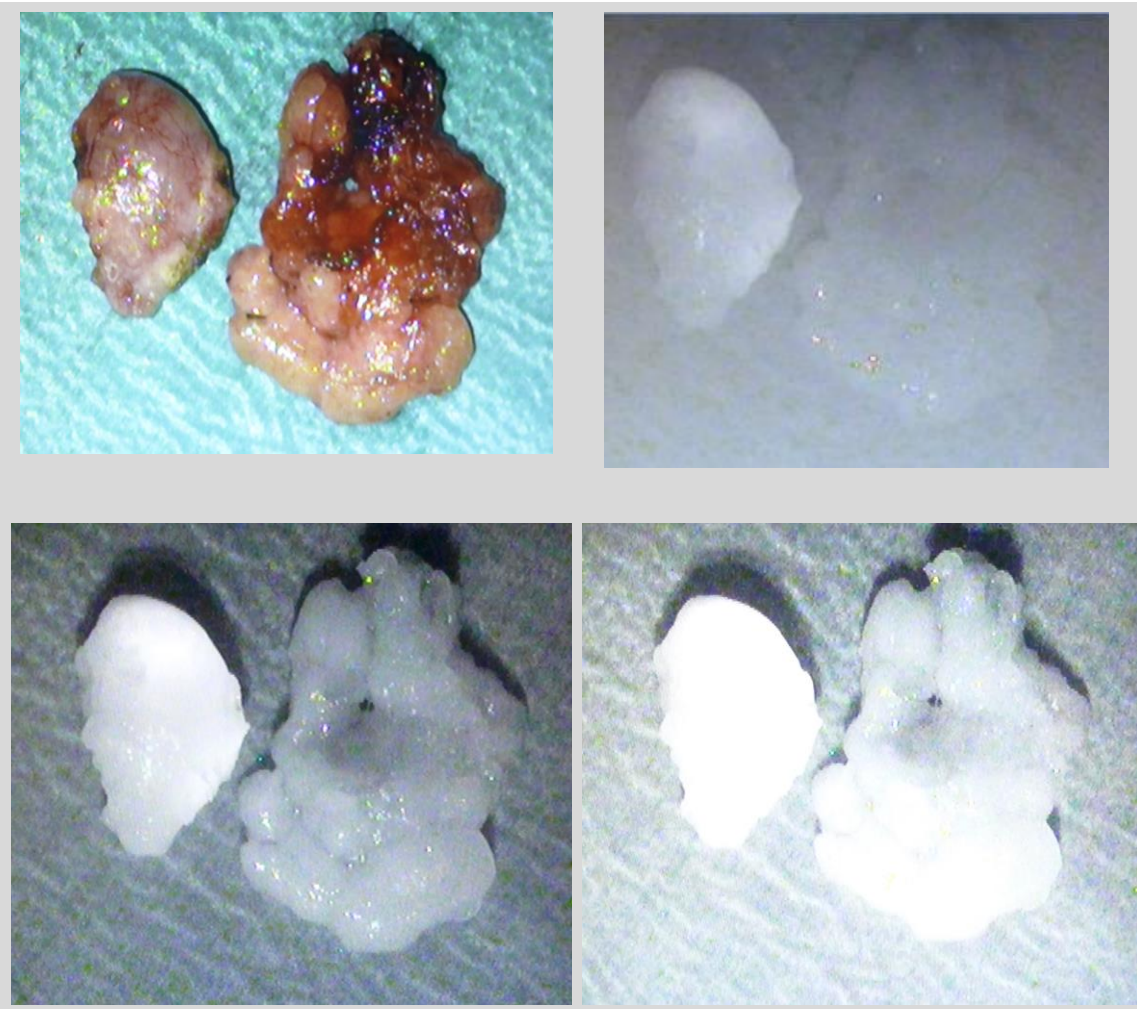


Figure 4-3 - (A-D) Fluorescently labelled sentinel node (left,) designated as signal region of interest (ROI) and surrounding fat tissue (right) designated background ROI.

Tissues viewed under different light settings by the near infrared clinical spectral imager.

A. White light only
B. Excitation at 784nm gain setting x4, integration 40ms. Signal to background ratio (SBR) 1.2.
C. Excitation at 784nm gain setting x 8, integration of 0.5 s. SBR 1.4.
D Excitation at 784nm gain setting x 10 , integration 2.0 s, SBR 1.08

4.5.7 Follow up

Patients were reviewed on the ward one day following surgery to assess for any complications associated with the procedure. Complications were graded

according the Clavien-Dindo classification[186]. Patients were reviewed in clinic one week after surgery to discuss the results of the sentinel node biopsy. If the biopsy proved positive for metastasis a completion neck dissection was scheduled. The final nodal staging was based on combination of the SNB and the completion neck dissection. Post-operative radiotherapy was offered to patients who had extracapsular spread or metastasis in more than one lymph node.

4.6 Results

Between November 2014 and June 2016 twenty-seven patients were enrolled in this study. Characteristics are shown in table 4.1. In the group 41% had positive sentinel nodes, of whom 40% had T1 tumours. Two patients had metastasis with extracapsular spread. At a mean 13 months follow up (range 4-23) all but one of the patients were alive and free of disease. One patient with a pT2N1(sn) tumour of the tongue died from lung metastasis without local or regional recurrence.

Table 4-1 - Characteristics of patients in the tumour group

Variable	**All patients (n=27)**	**Positive Sentinel node biopsy (n= 11)**
Male	16	5/15 (33.3%)
Female	11	6/11 (55%)
Age (years median, standard deviation)	63 (range 24-87)	
Positive SNB	11 (41%)	
Negative SNB	16 (59%)	
T1	20 (74%)	8/20 (40%)
T2	7 (26%)	3/7 (43%)
Tumour location		
Tongue	19 (70%)	7/19 (37%)
Floor of mouth	5 (18.5%)	2/5 (40%)
Lower alveolus	1 (3.7%)	0/1
Lower lip	1 (3.7%)	1/1 (100%)
Buccal mucosa	1 (3.7%)	1/1 (100%)

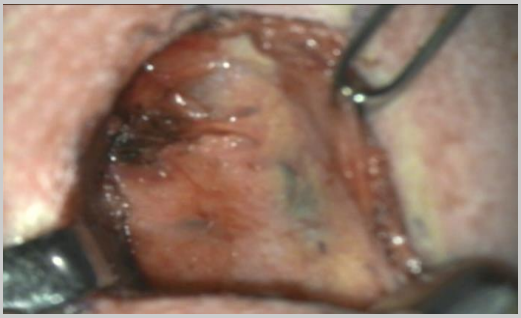
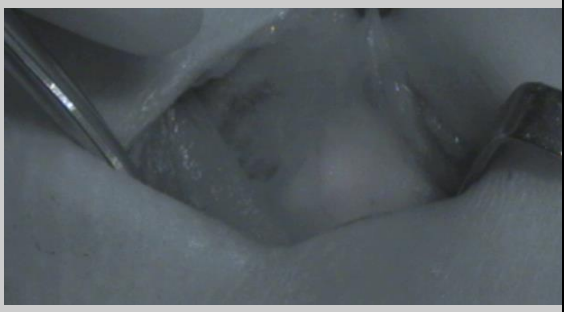
4.6.1 Fluorescence detection of sentinel nodes – primary endpoint

A total of 84 sentinel nodes were removed giving an average of 3.11 nodes per patient (range 1-7). Thirteen positive sentinel nodes were removed from eleven patients. The primary endpoint was met in all patients with least one fluorescent node detected. All positive sentinel nodes were fluorescent. An average of 2.61 fluorescent nodes were identified per patient (range 1-6). A total of twelve non-sentinel nodes were excised, five of these were hot but did not meet cut-off criteria to be judged a sentinel node. Only two non-sentinel nodes were fluorescent, in both cases (Number 6 and 7 in table 4.2) there was one unusually hot sentinel node with counts per second (CPS) >3000 causing some of the excised but still high CPS nodes to be down-graded to second echelon by the 10% rule.

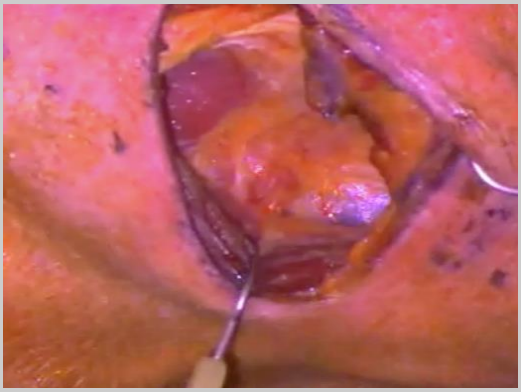
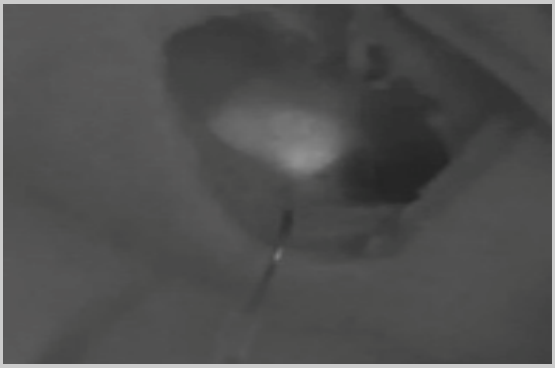
4.6.2 Near infrared imaging results - secondary endpoints

No transcutaneous fluorescent signal was found prior to incision (Stage 0 imaging) Figure 4.4 shows different intraoperative stages at which fluorescence was seen.

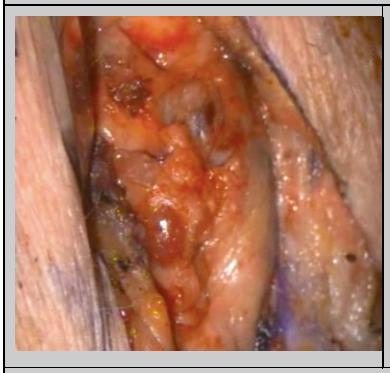
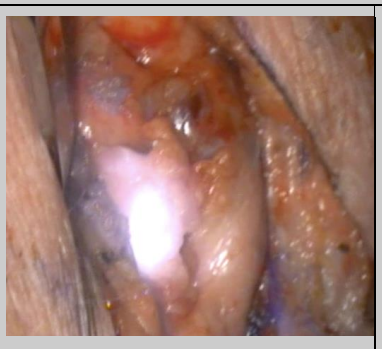
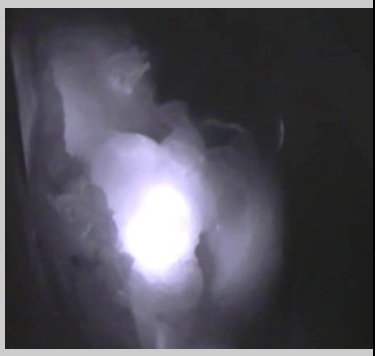
Stage 1. After skin flaps raised in subplatysmal plane. Image shows weak fluorescent signal anterior to muscle.

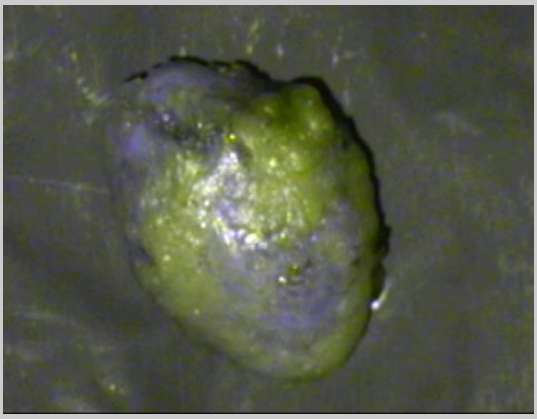
Stage 2. After unwrapping and retraction of adjacent muscle. Image shows moderate fluorescent signal deep to the retracted muscle.

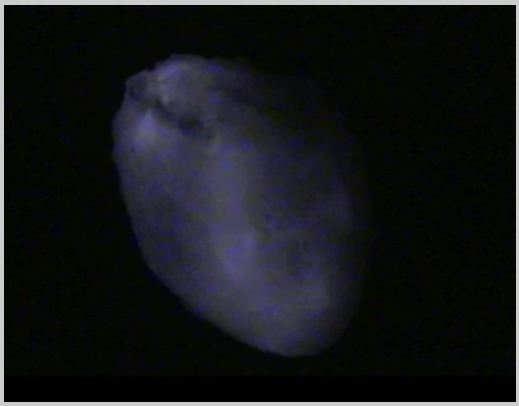



Stage 3. After node localised by gamma count but prior to dissection from surrounding tissue. Left image shows a node visible to the naked eye. Middle image demonstrate excellent signal, with white light setting allowing normal tissue visualisation. Right image shows same node viewed by NIR only.

Stage 4. Ex-vivo. Left image shows excised node under white light only, imaged in a black box (ambient light excluded). Right image show NIR only, with weak patchy signal found.





Stage 5. Imaging of resection bed. Left and right images show region from which a fluorescent node was removed (indicated by tip of forceps). No further signal is seen.

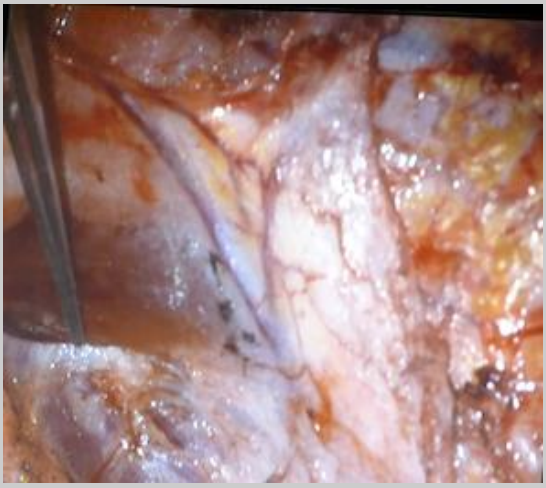


Figure 4-4 - Fluorescence imaging results obtained at stage 1-5. Findings in each case are shown in table 4.2

Case	Tumour location	Sentinel nodes by gamma signal (neck level, node number, (counts per second))	Fluorescent sentinel nodes (neck level, node number (maximum luminance))	Stage of operation fluorescence detectable and Surgeon comment	Fluorescence additional benefit?
1	Left (L) Oral tongue	L2a(805) L3(920)	L2a (201) L3 (220)	Stage 2 *Good signal*	Yes
2	Right (R) Oral tongue	R3.1 (263) R3.2(144), R2a (130)	R3.1 (80) R3.2 (63) R2a (71)	Stage 3 *Poor signal, faint with all lights dimmed*	No
3	Left Oral tongue	L3.1 (208), L3.2(84), L3.3 (148), L1b (232)	L3.2 (43), L3.3 (16), L1b (90)	Stage 4 *Fluoresced outside neck only*	No
4	Right Oral tongue	R2a(685)	R2a (208)	Stage 1 *Excellent signal, fluoresced through overlying fat*	Yes
5	Left Oral tongue	R3 (528), L3 (413), R2a.1(58), R1b (415), Submental (279),L1b(154), L2a (187)	R3 (111), L3 (79), R2a (12), r1b.1 (55)submental (14), L1b (10) L2a (10)	Stage 3 *Reasonable signal*	No
6	Left Oral tongue	L2a.1(8814), L2a.2(354), L1b(1500) R1b(3777)	L2a.2 (250) L2.a.2(93) L1b (80) R1b (80)	Stage 3 &4 *Brightest node was hottest node but other nodes seen only inside black box*	No
7	Left Floor of mouth	L1b(3810) R1b(117)	R1b (80) L1b (65)	Stage 3 *Reasonable signal*	No
8	Left Oral tongue	L3.1(239), L3.2(40), L2a(191), L2a.2(171), L2b(47)	L3.1 (101) L2a.1 (19) L2a.2(12)	Stage 3 *Hottest nodes were moderately flourescent*	No
9	Oral tongue	R3.1 (876), R3.2(496), R3.3(286)	R3.1 (196) R3.2 (201) R3.3 (160)	Stage 2 *Excellent signal, fluoresced through overlying fat*	Yes
10	Oral tongue	R2a.1(835), R2a.2(1087) R2a.3(1605), R2a.4(901)	R2a.1 (202) R2a.2 (260) R2a.3 (240)	Stage 2 *Good signal 3/4 hot nodes*	Yes
11	Lower lip	R Prefacial(578)R post facial (82), R3 (297), L1b(772), L2a(575) L3(618)	Prefacial (120) L1b (188),	Stage 3 *Reasonable signal*	No
12	Oral tongue	L2b.1(449) L2b.2(170), L3(148)	L2b.1 (86), L3 (12)	Stage 3 *Good signal*	No

13	Oral tongue	L2a.1(421),L2a.2(18), Submental(481), R1b(540), L1b(60), R3.1(396), R3.2(697)	L2a.1 (56) R1b (260) R3.1 (34) R3.2 (59)	Stage 5 *Fluoresecence very bright in level 1b right side, subsequently hot ex-vivo two other level 1 node not very fluorescent but hot ex-vivo*	Yes
14	Floor of mouth	L3(120), L1b.1 (23), L1b.2(49)	Need to check 143	Stage 3 *Reasonable signal*	No
15	Floor of mouth	R1b.1(123), R1b.2(130)	R1b.1 (89) R1b.2 (114)	Stage 4 *Fluorescent signal mainly ex-vivo*	No
16	Oral tongue	R2a.1(171) R2a.2(59)	R2a.2 (113) R2a.2 (70)	Stage 3 *Good signal*	No
17	Oral tongue	L2a(456), L3(150)	L2a (203)	Stage 2 *Very strong signal from one node* (subsequently positive)	Yes
18	Oral tongue	L2a.1(219) L2a.2(2054) L2a.3(365)	L2a.1 (92) L2a.2 (170)	Stage 3 *Moderate signal*	No
19	Oral tongue	L2b(87), L1(85)	L2b(180), L1(196)	Stage 2 *Good signal helping node localisation*	Yes
20	Buccal mucosa	R2b(-), Facial 1 (-) Facial 2 (-), !a(-)	Facial 1 (80) Facial 2 (71)	Stage 2 *Vey low gamma signal making node localisation difficult. In this case the fluorescence helped because the gamma signal did not help in finding nodes seen on scans.*	Yes
21	Lower alveolus	R facial (35) R1b(41) 1a(-)	Rfacial R1b (55)	Stage 4 *No comment made*	No
22	Oral tongue	L2a(626) L3.1(32) L3.2(-) L3.3(22)	L2a (154)	Stage 4 *Hottest node fluoresent*	No
23	Floor of mouth	1a(48) L1b(321) R1b(89) L3(-)	1a (22) L1b (56) R1b (12)	Stage 4 *Fluoresence ex-vivo only*	No
24	Oral tongue	R2a.1(176) R2a.2(100), R2a.3(-) 1a(114)	R2a.1 (166) R2a.2 (55)	Stage 4 *No comment made*	
25	Floor of mouth	1a(-) R1b(36) L1b(471)R2a(-) L2a.1(287) L2a.2(205)	R1b L1b L2a.1 L2a.2 (poor quality of recording)	Stage 3&4 Submental nodes only in vivo but remainder	No

				week fluorescence out of body	
26	Oral tongue	L2a.1 (2376) L2a.2(366) 1a (1515)R1b(1000)	L2a.1(214) L2a.2(242) 1a (134) R1b(280)) R2a(88)	Stage 2 &3 *Differential signal highest from submental and right 1b*	Yes
27	Oral tongue	L2.1 L2.2 L3 (gamma signal not legible)	L2.1 (190) L2.2 (201) L3 (18)	Stage 3 *Helped for level 2 nodes not level 3*	Yes

Table 4-2 Results of multimodal SNB using ICG-Tc99m Nanocoll.
Colour identifies the status of the nodes: red = positive for metastasis, orange = non-sentinel nodes, green = only identified by fluorescence in-vivo. Maximum luminance is given in arbitrary units. Surgeon comments are given in italics.

4.6.3 Additional benefit of ICG

Table 4.2 shows that fluorescent signal was of additional benefit over and above conventional gamma imaging and met the secondary end points in ten cases (37%). In just one case the fluorescent signal was detected at stage 1 imaging, but fluorescence mostly helped in localizing the sentinel nodes during deeper dissection (stage 2 imaging in 7 out of ten). In one case fluorescence detected a node that had been missed due to shine through effect (Case 13) and in another (Case 20) fluorescence guided the surgery because the gamma signal was unusually low.

These data show that cases in which fluorescence helped in locating the node during surgery had a maximum luminance of >200, these cases also showed the strongest gamma signal. To investigate this further univariate analysis was undertaken using Pearson's correlation which showed a significant relationship (p=0.351). Scatter graph plot of gamma signal against luminance was constructed and simple regression analysis produced a correlation coefficient (R^2) of 0.301. When fluorescent nodes with gamma signal above 1000 CPS were excluded (10/63, 16%), the R^2 increased to 0.4 (Fig. 4.4). The true estimation of correlation may be limited by the boundaries of 16-bit grey scale of PNG images.

4.7 Discussion

We have demonstrated that all patients recruited in this study had tumour draining nodes identified using fluorescent labelling, and importantly that all

metastatic nodes were fluorescent. Furthermore, fluorescence provided additional benefit in over one-third of patients (37%), where nodes were not identified accurately on gamma detection alone. Fluorescence guided SNB has the potential to improve intraoperative identification of sentinel nodes. By highlighting the position of nodes before they become visible under white light, operating time and tissue dissection could both be reduced. However, in open surgery the interference of ambient light can significantly interfere with intra operative appreciation of the signal thus adding a level of complexity to the use of this technique. Consideration was given to measuring the variability of the luminance between patients and the correlation with the intensity of the gamma signal in order to optimise the fluorescence signal. There is however, huge variability between the gamma signal intensity between patients presumably as a result of minor changes in injection technique, fluid compartment pressures and flow as well as tissue mobility and patient activity following injection.

PVB was not used in this study because of the possibility that it would influence the operator's appreciation of the fluorescent signal, thus we have no direct comparison of the performance of the blue dye. However, chapter three in this thesis describes a study using the same edibility criteria in which PVB was used as the optical tracer. These data showed that at least one blue lymph node was found in 41/50 patients (82%), but in six of the nineteen SNB positive cases the sentinel node was not blue. Thus the sensitivity and FNR of PVB compared to ICG-^{99m}Tc-Nanocoll in these two studies is 32% vs. 0%, and 68% vs. 100%. These figures would support the hypothesis that

fluorescence imaging by hybrid tracer is superior to optical imaging with PVB. However, we have also shown that there is no interference between the optical properties of the two dyes and they could be used together, although aside from the ability to see blue staining without special equipment it is difficult to see the advantage of PVB.

Following these results, all further work was undertaken with the multimodal tracer ICG-^{99m}Tc-Nanocoll and PVB was no longer used as an optical tracer.

Chapter 5 Feasibility study for the development of sentinel node biopsy in salivary gland cancer

5.1 Introduction

Surgical management of early stage salivary gland malignancy, especially of the parotid gland, is complex with a variety of options for the both the primary site and the neck. A number of factors are considered prior to surgery; tumour size, histological subtype, and grade. These parameters give some indication about the propensity of the tumour to metastasise (occult metastasis are reported in 17% of low grade compared to 25% of high grade T1/2 parotid tumours[49]), but the pattern of lymphatic flow particularly in relation to intra- and periglandular nodes has not been well characterized. Thus the extent of both gland excision and cervical nodal resection is not clear. Such decisions have significant implication for the patient, for example the incidence of facial nerve paresis and paralysis following total parotidectomy compared to superficial lobectomy is 15%vs. 6.8% and 4.4% vs. 0.08%[192] respectively.

Despite Ernest Gould's 1960 report of sentinel node biopsy in salivary gland disease[63] there has been little advance in the topic over the last 50 years. Gould's series consisted of 28 patients with parotid tumours in whom he sent the 'angular node' for frozen section analysis in order to decide if radical neck dissection was necessary. There were major limitations to his approach - Gould did not use any tracer to map the lymphatic flow but assumed the sentinel node would be the same in each case, and on final histopathology 20 of the 28 cases were found to be benign tumours. In total three of the malignant cases had positive sentinel nodes. Despite these flaws his

approach served as the foundation for the development of SNB in other areas of the body but use in salivary malignancy stalled.

In 2006 a case series of six patients with parotid carcinoma was reported[193]. The authors used 99mTc- labelled nanocolloid (50MBq) injected at 8 sites around the tumour. A sentinel node was identified on static lymphoscintigraphy within ten minutes of injection in all patients. All underwent SNB and concomitant level II-IV or V neck dissection depending upon the location of the sentinel node. Positive sentinel nodes were found in two patients and in one there was a false negative result. In this case the false negative result was attributed to an intra glandular metastatic node disrupting drainage. Lymphoscintigraphy images from this study show that the entire gland was hot (Figure 5.1), presumably due the large number of peri-tumoral injections given. This combined with the poor anatomical detail gleaned from static lymphoscintigraphy would certainly preclude the identification of intra- and periglandular nodes, thus reducing the sensitivity of the technique significantly.

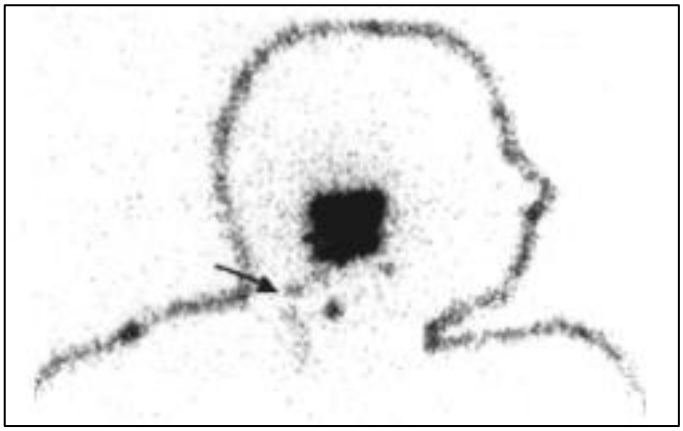


Figure 5-1 - Right anterior oblique lymphoscintigram 10 minutes post injection in a patient with salivary duct carcinoma in the right parotid gland.
Image taken from Starek et al. 2006[193]

Currently there is only one other publication that describes the use of SNB for primary salivary malignancy [194]. This case report describes use of SNB in a minor salivary gland tumour but the injection and imaging technique is not reported.

There is clearly a need to be able to map the at-risk lymph nodes from salivary tumours both in the gland itself and the neck and tailor surgery accordingly. There is a real prospect that the extent of surgery can be reduced with a significant reduction in risk of surgical morbidity to the patient. Recent developments in sentinel node biopsy technology should allow improved localization of intra- and extraglandular nodes, allowing wider introduction of SNB in the management of salivary gland malignancy.

5.2 Study aim

This study aims to develop a modern SNB protocol for use in salivary gland malignancy. The developed protocol should be able to reliably identify both intra and extra-glandular lymphatic drainage. The result of the SNB will be validated against concurrent resection +/- nodal dissection, the extent of which has been decided by MDT consensus. In this validation study the SNB is not used to decide if neck dissection is necessary, in contrast to studies presented in chapters 3 and 4 of this thesis.

5.2.1 Endpoints and outcomes

Salivary gland malignancies are rare tumours, but this study aims to recruit the largest series of salivary patients to undergo SNB to date. For initial validation it was felt recruitment of ten patients would be feasible.

The primary endpoint is identification of a sentinel node in all cases. Furthermore we would expect to be able to preoperatively identify intra- and periglandular sentinel nodes.

The secondary endpoint is the false negative rate compared to concurrent nodal resection if undertaken.

Information generated from this study should allow development of a protocol for a prospective multicentre study of SNB in salivary tumours.

5.3 Study population and recruitment

Ethical approval was obtained to recruit patients aged between 18 and 90 with newly diagnosed T1-T2 primary malignancy of salivary origin who were clinically staged N0 were approached for consent. Eligible patients presented with a lump and no associated complaint such as pain, numbness, tumour fixity or muscle weakness. Tumour diagnosis was confirmed by ultrasound fine needle aspiration cytology (FNAC) or punch biopsy and reviewed by a specialist head and neck pathologist. Clinical staging was undertaken by CT and MRI scan of the neck plus FNAC of any nodes that were clinically suspicious.

Exclusion criterion included patients who were unable to give informed consent, pregnant or breast-feeding, those whom had undergone any previous neck surgery or radiotherapy and anyone whom had a history of allergy to contrast agents.

Patients were identified via the head and neck outpatient clinic and the weekly head and neck Multi Disciplinary Team Meeting (MDM) at Guys Hospital, London. Patients deemed appropriate for inclusion by MDM discussion were approached by the author during outpatient clinic consultations at least two weeks prior to undergoing surgery. It was at this time that the Patient Information Leaflet specifically explaining the role of sentinel node biopsy in salivary gland cancer was discussed and given to the patient (Appendix D – Patient information leaflet salivary cancer), in addition from 2014 patients were given the information leaflet about the multimodal tracer (Appendix C).

All patients were given the opportunity for further discussion about the study either in person or by telephone at least twenty-four hours after receiving the initial information. If following this consultation, patients were interested in entering into the study they were asked to sign a consent form (Appendix B), Three copies of the consent form were completed: one for the patient; one for the notes and one for the site file.

5.4 Materials and methods

5.4.1 Radiotracer injection technique

The initial protocol was based on the two-day technique successfully employed in oral squamous cell carcinoma, where four peritumoural injections of Tc99m-nanocoll are delivered at a dose of 40-80Mbq. From November 2014 patients received the multimodal tracer ICG-Tc99m-Nanocoll.

In the case of parotid tumours, the injections were given under ultrasound scan (USS) guidance. The overlying skin was prepared using a topical alcohol wipe (Steret, Medlock Medical) and allowed to dry. The tumour was reviewed by ultrasound imaging and the overlying skin was marked with pen at the intended site(s) of injection. A 23G (blue) needle 25mm in length was inserted under ultrasound visualisation so that the tip was lying within the gland adjacent to the tumour. The needle was connected to a pre-filled syringe containing the radiotracer in which an air bubble had been deliberately incorporated to ensure the tracer passed through the dead-space of the needle. Injection was undertaken under ultrasound visualisation as assurance that the needle position had not moved outside the gland. Pressure was placed on the area with a clean swab whilst the needle was withdrawn to prevent backflow of the tracer through the needle tract.

The imaging protocol was reviewed after every two cases by the research team to decide if improvements could be made. It was agreed that there

would be no increase in the tracer dose above 80MBq nor any additional scanning undertaken.

5.4.2 Pre-operative imaging

Immediately following injection of the radiotracer patients underwent static and dynamic lymphoscintigraphy followed by SPECT/CT as described in section 3.4.1. Lymph node position was marked on the neck using indelible pen and covered with an occlusive tegaderm^{TM} (3M, USA) dressing.

5.4.3 Surgery

All surgery was at performed by MM at Guys Hospital, London. Node position was confirmed in the operating theatre by fhSPECT scan using the DeclipseSPECT system (Surgiceye, GmBH Germany), there was no blinding in this study. If the multimodal tracer (ICG^{99m}Tc-Nanocoll) was used the near infrared imaging system was used when the surgeon needed help in locating the sentinel nodes during surgery.

5.4.3.1 Parotid gland surgery

Surgery was performed under general anaesthetic with nerve monitoring (NIM-Response® 3.0, Medtronic, USA). Procedures performed ranged from partial superficial to radical parotidectomy. If intraparotid sentinel nodes were identified, these were excised and sent as separate specimens to the main tumour.

5.4.3.2 Sublingual gland surgery

Sublingual tumours were excised by wide excision the gland with removal of the lingual plate of the mandible en-bloc. The primary site was reconstructed with a free flap based on the radial artery of the non-dominant hand.

5.4.3.3 Minor salivary gland surgery

Minor gland tumours were excised by wide excision and reconstructed with free flap if required.

5.4.3.4 Sentinel Node Biopsy surgery

Sentinel node excision was undertaken alongside the planned surgery. When neck dissection was performed the sentinel node(s) were identified within the specimen ex-vivo by fhSPECT +/- fluorescence imaging. These sentinel nodes were marked with a stitched plastic ring and sent along with fhSPECT or fluorescence images to the pathologist to ensure that serial sectioning was performed on the correct nodes.

If sentinel nodes were located in an area outside the planned operative field, these were accessed through the same incision wherever possible.

If the sentinel nodes were located in regions that were difficult to access or located outside the usual surgical field, then the nodes were not removed to avoid additional surgical morbidity to the patient.

5.4.3.5 Neck dissection surgery

The requirement and extent of neck dissection was decided during the MDM. If neck dissection was not recommended the patient was still considered eligible for sentinel node biopsy but would not undergo concurrent validation neck dissection.

5.4.4 Pathology

Sentinel nodes underwent serial sectioning according to the protocol described in section (2.3). However, at stage 3 anti-pan cytokeratin antibody (AE1/AE3) was supplemented by further immunostaining if indicated by the tumour subtype.

If a node could not be macroscopically identified and dissected from within an area of tissue that was registering a high gamma signal ex-vivo then the entire tissue was submitted for serial sectioning.

5.5 Results

Between 2012 and 2016 ten patients with primary salivary malignancy were recruited, of which eight were parotid tumours. The characteristics of the patient and tumours are shown in table 5.1.

Table 5-1 - Characteristics of patients/and tumours with primary salivary malignancy recruited between 2012 and 2016

Tumour location	Diagnostic FNAC /Biopsy	Surgery to primary	Neck Dissection	Final Tumour Pathology	Pathological Staging	Post operative radiotherapy (y/n)
Parotid	Salivary malignancy, unspecified	Partial superficial parotidectomy	None	Carcinoma ex-pleomorphic adenoma	T2N0M0	Yes
Parotid	Low grade Mucoepidermoid carcinoma	Partial superficial parotidectomy	None	Low grade Mucoepidermoid carcinoma	T1N0M0	No
Parotid	Salivary malignancy, unspecified	Total conservative parotidectomy	Selective level I-IV	Epithelial-myoepithelial carcinoma	T2N0M0	Offered but declined by patient.
Parotid	Adenoid cystic carcinoma	Superficial parotidectomy	Selective level II and III	Intermediate grade Mucoepidermoid	T2N1(sn)M0	Yes
Sublingual	Mucoepidermoid carcinoma	Wide excision FOM and RFFF reconstruction	Selective level I-IV	Intermediate grade Mucoepidermoid	T1N0M0	Offered but declined by patient.
Sublingual	Low grade adenocarcinoma	Wide excision FOM and RFFF reconstruction	Selective level I-IV	Adenoid cystic carcinoma	T1N1(sn)M0	Yes
Parotid	Adenoid cystic carcinoma	Radical parotidectomy and ALT reconstruction	Selective level I-IV	Adenoid cystic carcinoma	T3N0M0	Yes
Parotid	Acinic Cell carcinoma	Total conservative parotidectomy	Selective level I-IV	Adenoid cystic carcinoma	T2N0M0	Yes
Parotid	Epithelial-myoepithelial carcinoma	Superficial parotidectomy	Selective level Ib-III	Epithelial-myoepithelial carcinoma	T2N0M0	Yes
Minor Salivary gland (palate)	Adenoid cystic carcinoma	Hemimaxillectomy and FFF reconstruction	No	Adenoid cystic carcinoma	T2N0M0	Yes

One patient was upstaged to T3 intraoperatively due to gross extension through the gland capsule. Frozen sections of the facial nerve were also sent intraoperatively showing extensive perinueral spread. All but one of the patients were offered post-operative radiotherapy

(PORT). In two cases this was because the tumour excision margins were close and in the remainder recommendation was based on histological tumour character or node positivity.

5.5.1 Imaging results and protocol development

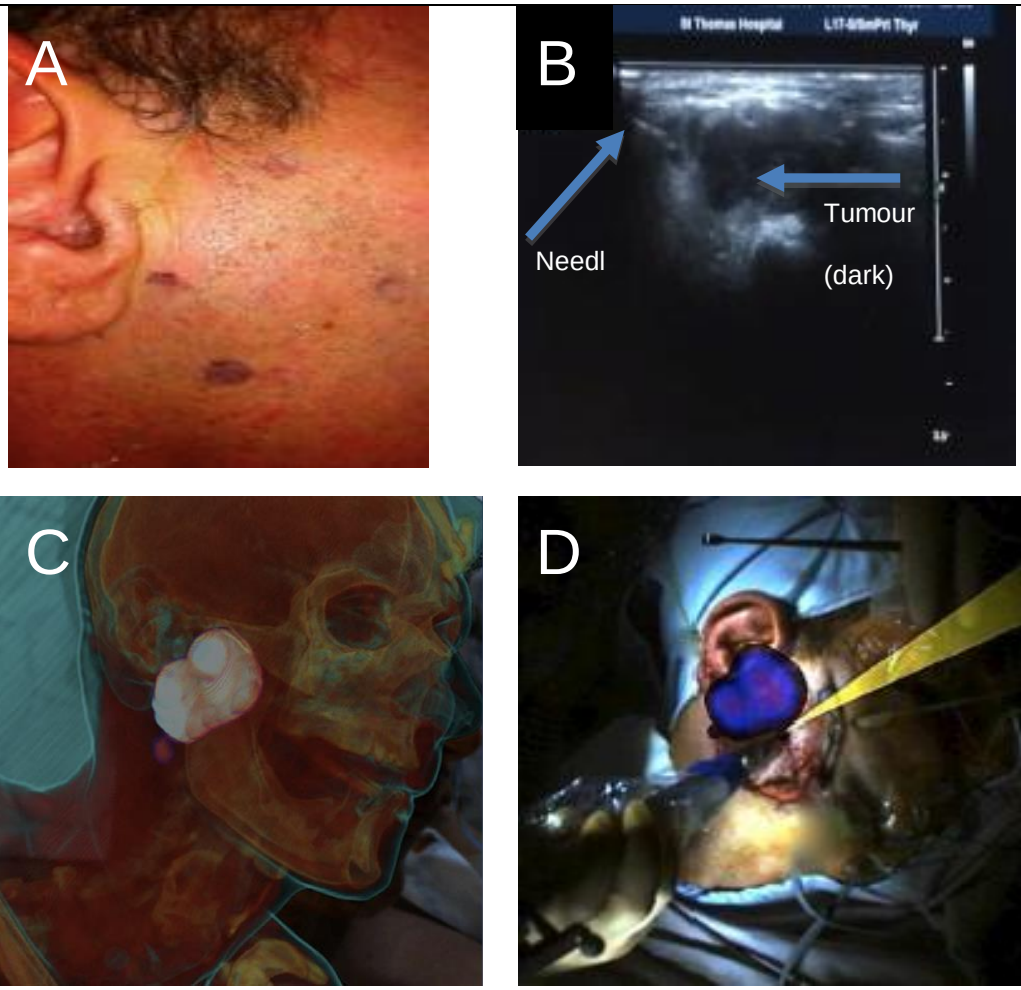
All patients had sentinel nodes identified and in two cases these contained metastatic deposits. There were no false negative results. Table 5.2 describes the injection technique and imaging findings for each case.

Table 5-2 - Sentinel node biopsy findings in salivary gland tumours.
LSG = lymphoscintigraphy, SPECT/CT = single-photon emission computed tomography, fhSPECT = freehand SPECT, ICG = Indocyanine green. Y/N indicated if the sentinel node was identified by the method

Case	Injection method	Node	Location	LSG	SPECT/ CT	fhSPECT	Node size(mm)	ICG	Gamma (cps)	SNB result
1.Parotid	4 x peritumoural	SN1	RIIb	N	Y	Y	12	N/A	195	Negative
		SN2	RIIb	N	N	Y	20	N/A	20	Negative
		SN3	Periglandular (preauricular)	N	Y	Y	8	N/A	135	Negative
2.Parotid	4 x peritumoural	SN1	Intraparotid (superficial lobe)	N	N	Y	2	N/A	699	Negative
		SN2	Intraparotid (superficial lobe)	N	N	Y	4	N/A	249	Negative
3.Parotid	Single intratumoural	SN1	RIIa	Y	Y	Y	18	N/A	83	Negative
4.Parotid	Single intratumoural	SN1	LIIa	Y	Y	Y	25	Yes	91	Positive
		SN2	LIIa	N	Y	Y	38	Yes	38	Negative
5. Sublingual	Single intratumoural	SN1	LIII	N	Y	Y	18	Yes	45	Negative
		SN2	LIb	N	Y	Y	8	Yes	51	Negative
		SN3	LIIa	Y	Y	Y	12	Yes	39	Negative
6. Sublingual	Single intratumoural	SN1	R Ib	Y	Y	Y	11	No	185	Positive
		SN2	L Ib	N	Y	Y	8	No	349	Negative
		SN3	L Ib	N	Y	Y	8	No	138	Negative
		SN4	LIb	N	Y	Y	12	No	110	Negative
		SN5	LIIa	N	Y	Y	12	No	56	Negative
7.Parotid	Single intratumoural	SN1	Intraparotid (parotid tail)	N	N	Y	20	Very weak	5	Negative
		SN2	RIIa	N	Y	Y	8	Very weak	5	Negative
8.Parotid	Single intratumoural	SN1	RIIa	Y	Y	Y	19	Yes	74	Negative
		SN2	RIII	Y	Y	Y	14	Yes	14	Negative
9.Parotid	Single intratumoural	SN1	Periglandular (pre-auricular)	N	N	Y	10	No	27	Negative
		SN2	Periglandular	N	N	Y	6	Yes	10	Negative
		SN3	Intraparotid (superficial lobe)	N	N	Y	10	Weak	12	Negative
10.Minor salivary gland (palate)	Single intratumoural	SN1	Left post-facial	Y	Y	Y	10	Y	45	Negative
		SN2	LIIa	Y	Y	Y	10	N	5	Negative
		SN3	RIIa	Y	Y	Y	10	N	5	Negative
		SN4	Left parapharyngeal	N	Y	N	Not sampled			

The first two cases were injected by peritumoural method (4 separate injections) under ultrasound guidance. In both cases immediate lymphoscintigraphy showed that the entire gland was hot and no sentinel nodes could be identified. SPECT/CT proved more useful than LSG in the first case, identifying a level IIb sentinel node, which was confirmed the following day by preoperative fhSPECT and excised (Fig.5.1). An additional node in the same neck level as well as a small node on the superio-posterior facial surface of the gland (pre-auricular periglandular) was identified by gamma count intraoperatively.

In the second case however both LSG and SPECT/CT failed to show any drainage. At surgery the next day however, when the total gamma signal within the gland had decreased, fhSPECT was able to clearly identify an intraparotid hotspot (Fig 5.2). This area was excised separately to the tumour, guided by a strong gamma signal, but it was not possible to identify a discrete lymph node within the surrounding parotid tissue (no optical tracer had been used). Thus, a small sample of parotid tissue measuring 8x11mm was submitted for serial sectioning. Within the specimen two lymph nodes were found measuring just 2mm and 4mm in maximum dimension, both negative for metastasis.



A. Surface marking of peritumoural injection.
B. Ultrasound scan showing injection of tracer around the tumour within the parotid gland.
C. Rendered reconstruction of SPECT/CT scan showing sentinel node in level IIb
D. Intraoperative fhSPECT locating level IIb nodes.

Figure 5-2 (A-D). Sentinel node biopsy parotid tumour case 1.

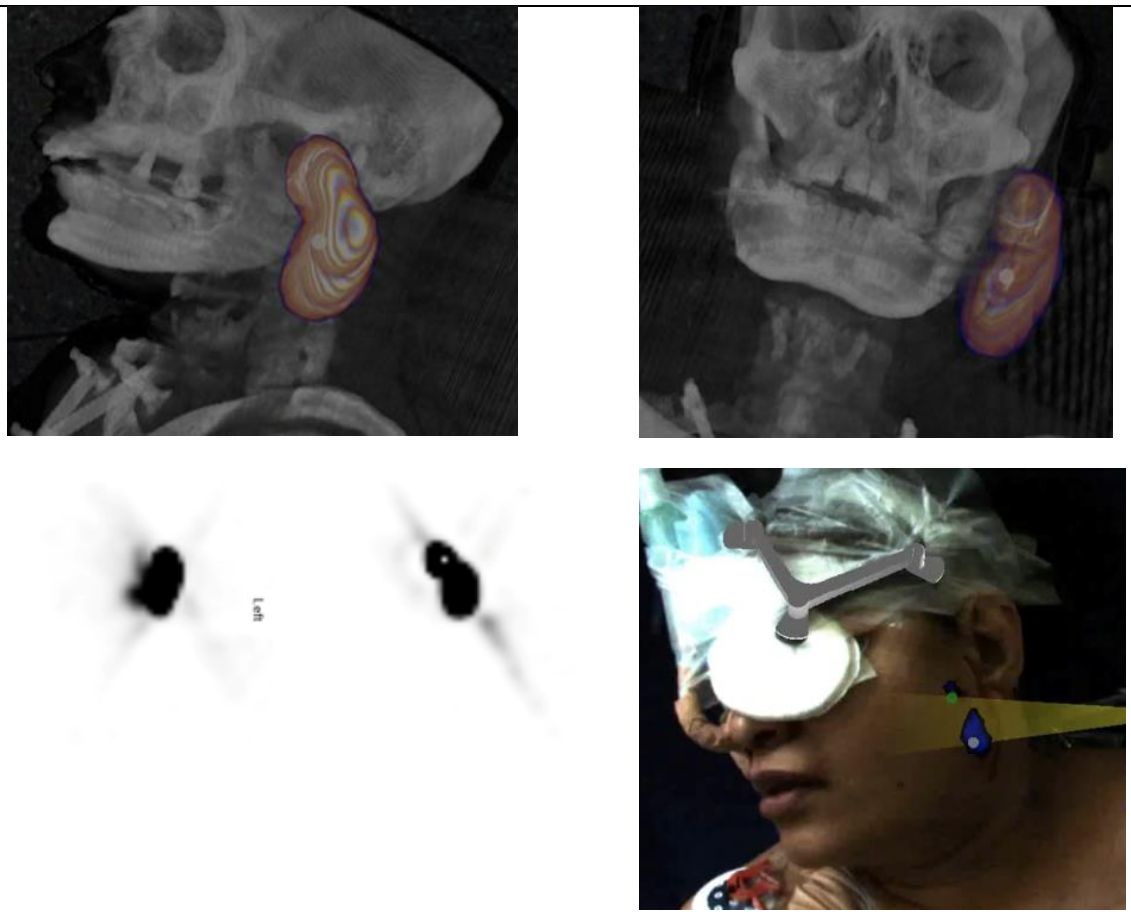


Figure 5-3 - (A-D) Sentinel node biopsy parotid tumour case 2.

A. Lateral and
B. Anterior-posterior (AP) rendered SPECT/CT image showing complete filling of the parotid gland.
C. Lateral (left) and AP (right) lymphoscintigraphy showing complete filling of the gland and no drainage.
D. D. Pre-operative freehand SPECT (fhSPECT) one day post injection showing tumour (inferior) and intraparotid sentinel node (superior, contained two small lymph nodes)

Following review of these cases it was decided that the very high signal seen within the entirety of the gland immediately after injection was likely to affect the ability to identify sentinel nodes on lymphoscintigraphy especially intraglandular sentinel nodes. Thus a change in protocol was instituted to see if reliable drainage could be found by single intra-tumoral injection. The total tracer dose was injected under ultrasound guidance with the needle placed

into the centre of the tumour. This change in protocol allowed first identification of a sentinel node by lymphoscintigraphy (Fig 5.4). In addition the gland did not show such intense signal allowing better delineation of the sentinel node and the procedure was more comfortable for the patient. Following this all patients underwent single injection only.

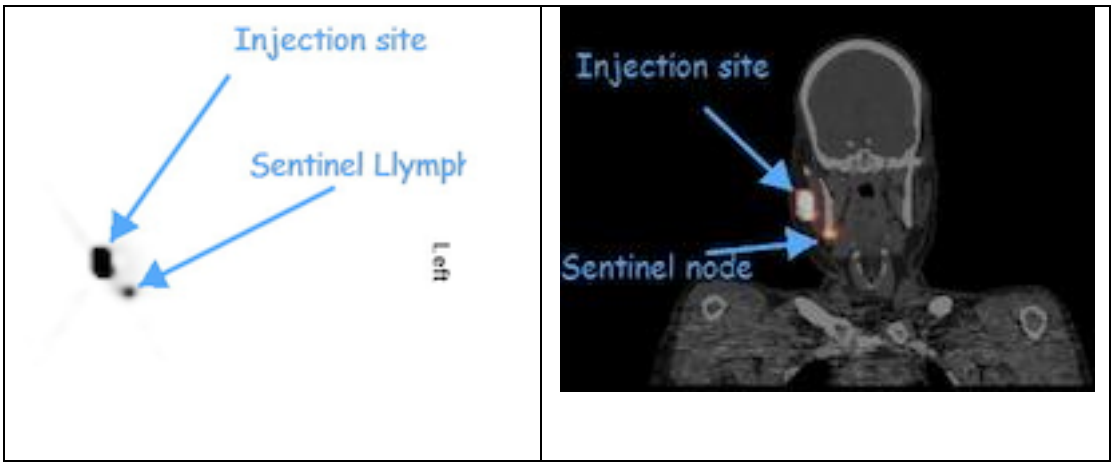


Figure 5-4 - Intratumoral injection of Tc99m-Nanocoll, showing drainage to a level IIa lymph node on lymphoscintigraphy(left) and SPECT/CT (right)

5.5.2 Parotid drainage

Fifteen sentinel nodes were located in the eight patients with parotid tumours. Seven of the sentinel nodes were found in a peri- or intraglandular location. Periglandular nodes were found in the pre-auricular tissue overlying the gland capsule. All the intraglandular nodes were within the superficial lobe of the gland. Pattern of drainage is shown in figure 5.5.

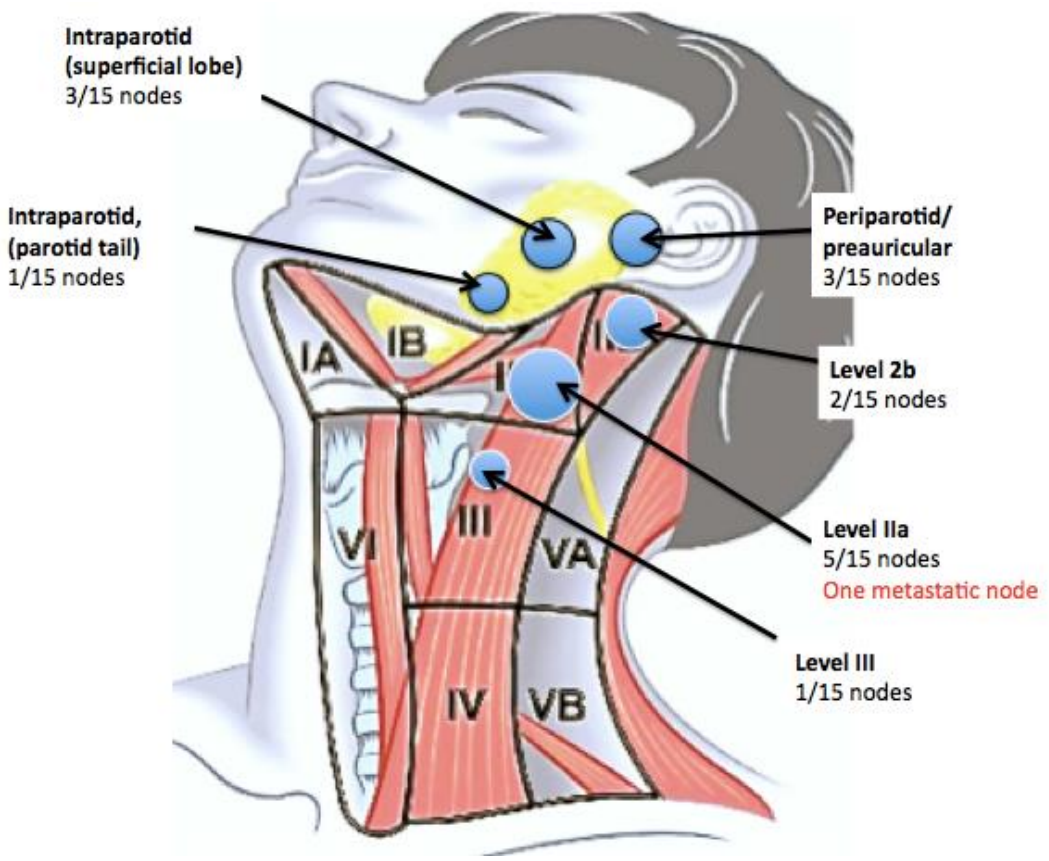


Figure 5-5 - Drainage pattern from parotid tumours

Lymphoscintigraphy performed poorly in localising sentinel nodes from parotid tumours, identifying just three of fifteen nodes. SPECT/CT found eight of fifteen nodes pre-operatively but neither SPECT/CT nor LSG were able to show peri- or intraglandular nodes. Fluorescence was used in four patients and fluorescent nodes were identified in all these cases although in half the signal was low and the fluorescence was only appreciated once the tissue had been excised. Freehand SPECT seemed performed well, however it had the advantage that it was the only imaging modality used on day two of the protocol, and therefore shine through effect was reduced. It is possible that SPECT/CT or LSG performed on the second day would perform better than on day one

Just one patient had metastatic disease; the positive node was detected by SNB at level IIa. In this case no additional intraparotid metastatic nodes were found during routine histopathology.

5.5.3 Sublingual gland drainage

Two patients with sublingual tumours underwent SNB. In both cases injection was delivered under direct vision into palpable tumours of the floor of mouth. Level Ib was the most common location for sentinel nodes (figure 5.6). One metastatic node was located in the contralateral level Ib.

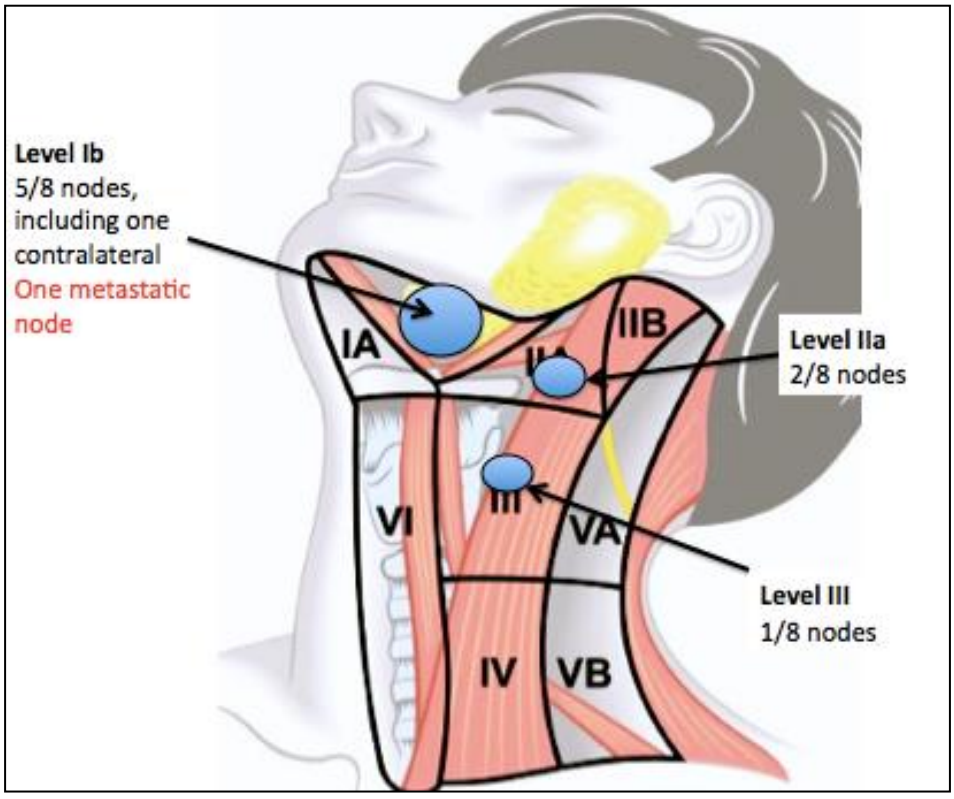


Figure 5-6 - Drainage from sublingual gland tumours

5.5.4 Minor salivary gland drainage

Just one patient with a minor salivary gland tumour was included in this study. The tumour was located in the left posterior palate and was injection under direct vision. Sentinel nodes were localised to left post facial and parapharyngeal as well as bilateral level IIa. Figure 5.7 shows the location of the parapharyngeal node on SPECT/CT. It was not possible to locate this node by fhSPECT because of limitations in obtaining multidimensional gamma counts, this was the only node in the series that fhSPECT failed to localize. This parapharyngeal node was outside the normal field of dissection and therefore not excised.

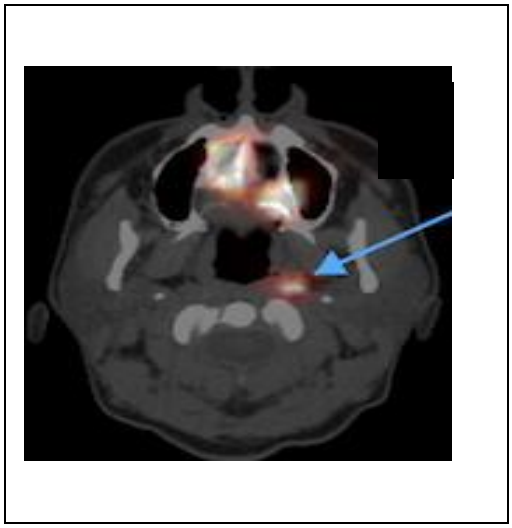


Figure 5-7 - SPECT/CT of left palate tumour showing injection site anteriorly and left parapharyngeal node (arrow)

5.6 Pathology Results

All ten patients were correctly diagnosed with salivary malignancy by pre-operative fine needle aspiration, but the histopathological subtype was changed by definitive pathology in five cases, reflecting the difficulty of characterising these tumours by cellular pathology. Two metastatic sentinel nodes were found with no additional metastatic nodes in the nodal dissection. The immunostain used in stage three of the serial sectioning protocol (chapter 2), are shown in table 5.4

Table 5-3 - Immunostain for salivary tumour histological subtype.

Salivary tumour histological subtype	**Immunostain**	**Imunohistochemical target**
Acinic cell	AE1/AE3	"Pancytokeratin" marker for CK types 1 - 8, 10, 14 - 16 and 19
Adenoid cystic	CK-7	Cytokeratin 7
Epithelial myoepithelial	P63	"Myoepithelial marker "
Mucoepidermoid carcinoma	AE1/AE3	"Pancytokeratin" marker for CK types 1 - 8, 10, 14 - 16 and 19

Seven patients had contiguous neck dissection with an average of 23 nodes removed per patient (range 15-66). These specimens were assessed by routine H&E examination, which do not show any additional metastatic nodes.

5.7 Discussion

These data show that sentinel node biopsy is a viable procedure in salivary gland malignancy. The study aim of 100% sentinel node identification rate as well as localisation of intra-and periglandular lymph nodes was achieved.

The advantage is the introduction of personalised care with management tailored to the individual. This is important particularly in parotid cancer for there is the prospect that total parotidectomy with its attendant risk of injury to the facial nerve can be replaced with a more minimally invasive procedure. Futhermore sentinel node biopsy can direct the need and extent of neck dissection, as with oral cancer.

However, this is early work and represents the largest cohort of salivary gland cancer patients to undergo SNB to date. Further validation studies are required, and due to the rarity of these cancers this will need mutlicentre if not multinational collaboration. This work has refined a protocol that can be immediately transferred into subsequent phase II/III trials.

Further aim of this work is to use the refinements test here to extend the role of SNB into other tumour groups.

Chapter 6 First experiences in extending the role of SNB in the head and neck and beyond

6.1 Introduction

This thesis aimed to improve the SNB technique and extend its role. Two new technologies have been evaluated with good results, and a new tumour group investigated. To finish this work and open up new channels of research, these techniques were applied to more challenging tumours. For the first time in the UK SNB in thyroid, laryngeal and prostatic cancer have been performed. This chapter discusses our initial experiences with particular focus on the challenges encountered.

6.2 Sentinel Node Biopsy in Thyroid Cancer

6.2.1 Background

Sentinel node biopsy has the potential to accurately stage the neck in thyroid tumours, thereby ending the uncertainty surgeons currently face in deciding the extent of neck dissection required. The test has promise with meta-analysis of 24 studies showing the technique found an occult metastasis rate of 42.9% in differentiated thyroid tumours[195]. The false negative rate was 0-16% depending upon the protocol used, and the best method of performing SNB is not agreed.

Wiseman et al. reviewed the protocol of ten published studies[196]. Seven out of ten studies used blue dye alone whilst the other three used radiotracer +/- blue dye. It was noted that the blue dye had a propensity to stain the parathyroid glands, which are similar in appearance to nodes and in some cases leading to their inadvertent removal and subsequent hypocalcaemia in 12%. This result is not surprising if you consider that injection of blue dye is a well-known technique for the intraoperative localisation of aberrant parathyroid glands[197-199].

Le et al. performed a prospective study of SNB in 39 patients with thyroid cancer[200]. Sentinel nodes could be detected ten minutes after intratumoural injection with 20MBq of radiotracer in 38 cases. The sensitivity of SNB when compared to the neck dissection specimen was 88%. The incidence of permanent hypoparathyroidism was 3.1% suggesting that the radiotracer is more accurate in differentiating lymph nodes from parathyroid glands than blue dye alone.

The use of SPECT/CT in these cases seems to improve the detection of nodes in the lateral compartment of the neck, showing drainage in up to 50% of patients in two recent studies[201, 202].

The aim of this pilot study was to trial the use of multimodal tracer ICG-Tc99m Nanocoll and fhSPECT imaging in thyroid cancer SNB in order to develop techniques that could be applied to a validation study.

6.2.2 Method

Ethical approval was granted to recruit patients with differentiated thyroid cancer who were due to undergo total or partial thyroidectomy with removal of the central or lateral lymph nodes. Patients were offered SNB alongside their surgery (patient information sheet – Appendix E).

Up to 24 hours prior to surgery consented patients underwent single intra-tumoural ultrasound guided injection of 10-40Mbq ICG-^{99m}Tc-Nanocoll followed by lymphoscintigraphy and SPECT/CT as described in section 3.4.1. If possible immediate fhSPECT was undertaken prior to nuclear medicine imaging.

During surgery the sentinel nodes were identified using a combination of fhSPECT (declipseSPECT) and NIR fluorescence imaging (section 4.5.6). The tumour excision and planned nodal resection completed the operation. Nodes that were outside traditional nodal resection fields were not removed.

6.2.3 Results

Four patients with FNA proven differentiated thyroid tumours undertook SNB alongside standard surgery. Three had a two-day protocol and received 40MBq ICG-^{99m}Tc-Nanocoll with one undergoing same day injection at a dose of 14MBq. Imaging and pathology results are reported in table 6.1.

Two patients underwent immediate fhSPECT which yielded no useful results. SPECT/CT did identify sentinel nodes in all patients, although unexpectedly this did not tally with the intraoperatively identified nodes in all cases. In two cases hot and fluorescent sentinel nodes were identified lying on the surface

of the thyroid gland (periglandular node example shown in image 6.2) close to the injection site. Neither had been found on pre-operative imaging and in one case the node contained a metastatic deposit.

Table 6-1 – Imaging and pathology results of sentinel node biopsy in thyroid cancer

Tumour histology and location	**Immediate fhSPECT**	**LSG result**	**SPECT/CT result**	**Sentinel nodes identified at surgery** (SN status)	**Neck dissection pathology**
Right lobe papillary carcinoma	Nil seen (scatter)	No drainage	Right level IIa Paratracheal (not taken)	Pre-cricoid (negative) Anterior thyroid (negative) Right level IIa (positive)	Right neck dissection with positive nodes in level I
Right lobe, possible intrathoracic metastasis	Not done	Left level IV node	Left level IV	Left level IV (negative) Periglandular (positive) Right level IIa (negative)	Further positive nodes, thoracic and left neck
Right lobe papillary carcinoma	Not done	No drainage	Right level IV	Right level IV (negative)	Multifocal papillary tumour with multiple metastatic nodes in right neck.
Left lobe follicular carcinoma	Nil seen (scatter)	Left level 2	Left level IIa	Periglandular (negative) Level IIa (negative)	Bilateral neck dissection, positive node level IV

A total of nine sentinel nodes were excised, two of which were positive for metastasis. In both cases additional positive nodes were removed during concurrent neck dissection. Two cases with negative sentinel nodes were found to have multiple positive nodes in the neck dissection, giving a false negative rate of 50%. Sample images are shown in figures 6.1-3

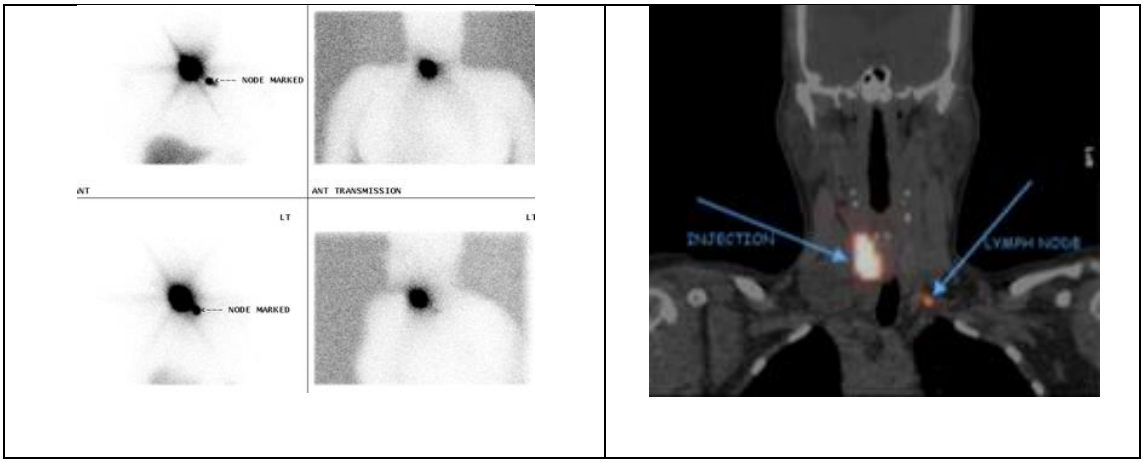


Figure 6-1 Lymphoscintigraphy (left) and SPECT/CT images (right) from injection of 40MBq ICG-99mTc-Nanocoll into tumour of the left lobe of thyroid.

Both scans show drainage to contralateral level IV node.

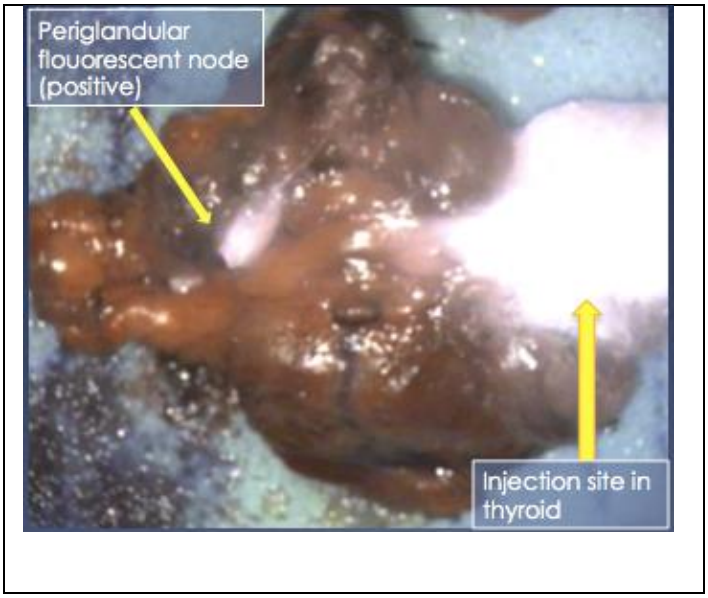


Figure 6-2 - Intraoperative white light/NIR imaging showing periglandular fluorescent node and channel near to the injection site.

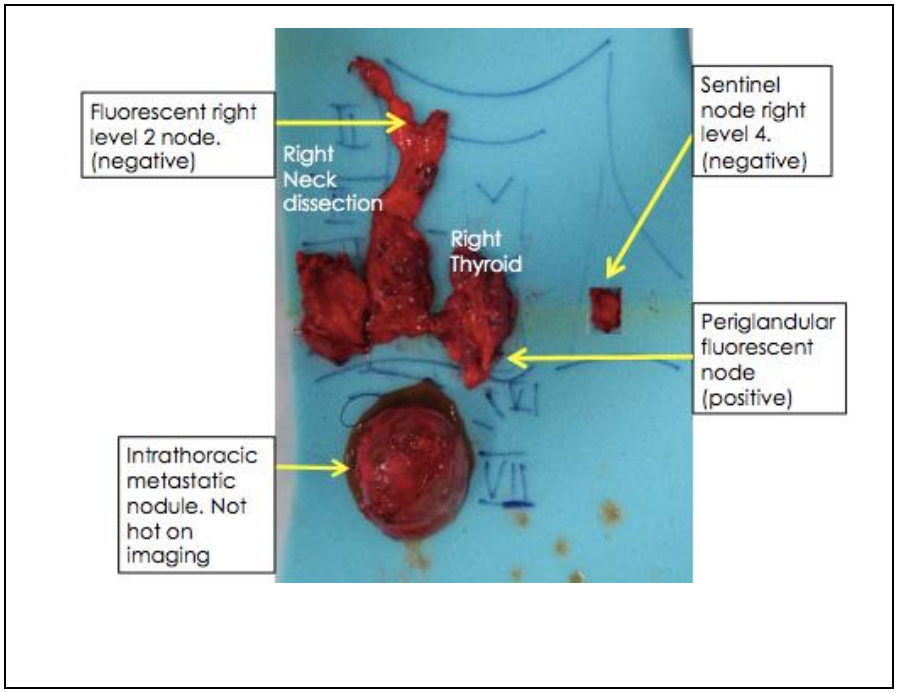


Figure 6-3 - Excised specimen (right hemithryroidectomy, right lateral neck dissection, left level IV sentinel node and intrathoracic metastatic deposit orientated anatomically.

All patients received post–operative radioiodine therapy and were alive and free of disease at latest follow up. No patients had permanent hypocalcaemia.

6.2.4 Discussion - sentinel node biopsy in thyroid cancer

These preliminary results show that more refinement is needed before SNB can be applied to thyroid cancer. In this small series the drainage was fickle and appeared to change between the nuclear medicine scans and surgery. The reliability of the technique may have been affected by the patient selection. SNB is a test for patients that are clinically N0, but in this study validation against concurrent nodal resection was planned. Surgeons were reluctant to recruit patients without at least borderline suspicion of involved nodes. All four recruited patients had radiologically suspicious nodes pre-

operatively but equivocal FNA. In the final pathology all patients has multiple metastatic nodes in the neck. Sentinel nodes were found in every case but were falsely negative in two patients, both had multiple highly cystic metastasis. It is possible that the metastatic process being well underway undermined the validity of the SNB test.

Our imaging did show clearly in two patients a node on the surface of the gland (Figure 6.2) close to the injection site, which has not been previously reported in the sentinel node literature. In one patient this node contained metastasis but in the other the sentinel node was falsely negative suggesting that it is not a constant first echelon node, but certainly this warrants further attention in any future work.

6.3 Sentinel node biopsy in Laryngeal cancer

6.3.1 Background

The usefulness of SNB in laryngeal tumours is extremely pertinent as there is little consensus on the management of the N0 neck[203, 204]. Because the larynx is a midline anatomical structure some surgeons elect to clear nodes from both sides of the neck even if the tumours is lateralised[58, 203, 205]. Some however will clear just one side, but only for the smallest of tumours because laryngeal cancers appear to have the highest occult metastasis rate (over 30%) out of all the head and neck tumours[206]. SNB has two major advantages at this site. Firstly, advances in surgical techniques now allow for some primary laryngeal tumours to be resected via a minimally invasive transoral approach (e.g. TORS, Trans Oral Robotic Surgery)[207, 208], but

the advantage of minimal surgery is lost where concomitant neck dissection increases the surgical morbidity. Secondly uni- or bilateral elective neck dissection is common with advanced laryngeal tumours or those failed cases first treated by chemo-radiotherapy[209, 210]. In such circumstances the potential reduction in morbidity if neck dissection can be avoided are even greater than in the virgin neck[209].

There are published data on the feasibility of SNB in laryngeal tumours, which show excellent sensitivity of the technique with both open or transoral resection of the tumour. Lawson et al [211]published a prospective case series of 29 patients with supraglottic tumours staged by SNB and concomitant neck dissection. Radiolabelled nanocolloid was injected peripherally and also into the tumour under direct vision at the start of surgery. The tracer was allowed a short time to flow into the lymphatic system before the primary tumour was resected. This reduced the intense radioactive shine from the primary tumour that could obscure the much weaker accumulation of the tracer in the SNs of the neck. The sentinel nodes were then located via hand held gamma probe and excised before the neck dissection was under taken. When compared to the completed neck dissections the sensitivity of SNB was 100%, specificity was 78% (there was one further positive node found in one patient although this case also had +ve SNB) and negative predictive value 100%.

A retrospective analysis of 20 patients with laryngopharyngeal tumours staged by SNB as part of a larger case series of combined head and neck tumours[212] also showed that in 100% of patients the SNs were identifiable. When the SN analysis (by either frozen section of H&E staining) was

compared to the neck dissection or subsequent nodal recurrence, the false negative rate was 6.9%.

The technique was further expanded to include patients that had undergone previous treatment (surgery/radiotherapy) for a neck malignancy. In 2013 Flach et al.[213] reported their findings in 13 patients with primary laryngeal tumours compared to 6 patients with previously treated head and neck squamous cell carcinoma. They found that sentinel nodes were identifiable in 92.3% of primary tumour patients compared to just 16.7% of those that had been previously treated. The sensitivity and NPV were 80% and 87.5% respectively.

Data would suggest that SNB in primary laryngeal tumours is a reliable technique. There is however a range of sensitivity reported (80-100%) and we believe that a combination of intraoperative fhSPECT and multimodal tracer ICG-^{99m}Tc-Nanocoll will improve the ease of identification of the sentinel nodes.

6.3.2 Method

Ethical permission was granted to recruit patients with laryngeal cancer who were undergoing partial or total laryngectomy alongside unilateral or bilateral elective nodal clearance. Eligible patients were offered SNB alongside their surgery (Appendix F –PIS Laryngeal cancer).

As these tumours are not amenable to injection in the outpatient setting the tracer was injected peritumourally by laryngoscopy at the beginning of the

surgical procedure. No nuclear medicine scans were undertaken, and the sentinel nodes were solely located by intraoperative fhSPECT and NIR fluorescence imaging.

6.3.3 Results

One patient with a midline cT4aN0M0 laryngeal SCC consented to undergo SNB alongside primary surgical management by laryngectomy and bilateral neck dissection. A total of 40 MBq of ICG-^{99m}Tc-Nanocoll was injected in four divided doses (Figure 6.4a). Immediate fhSPECT scan showed drainage to two left sided sentinel nodes (Figure 6.4 b, left IIa and III). The specimen was resected en-bloc and re-scanned ex-vivo by fhSPECT (Figure 6.4c) and NIR imaging showing the same hotspots.

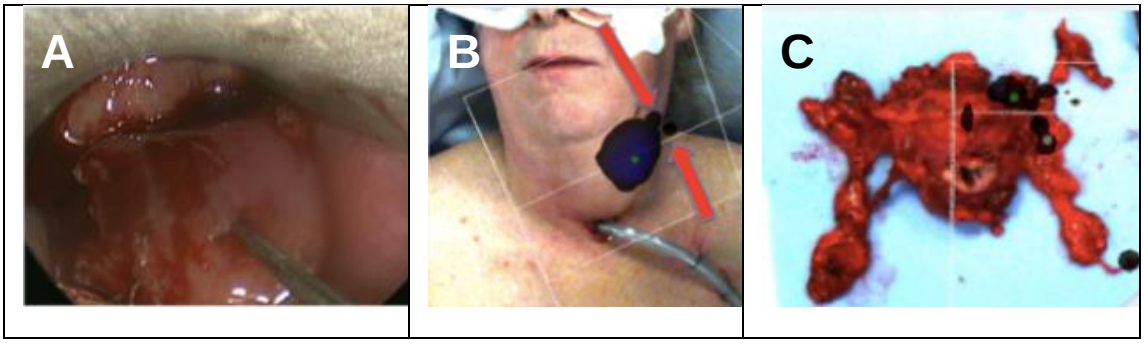


Figure 6-4 - UK first Laryngeal SNB.

A. Injection of tracer during laryngoscopy.
B. B immediate fhSPECT scan showing two hotspots in the left neck (shown by two red arrows).
C. C Laryngectomy and bilateral neck dissection specimen by fhSPECT showing two hot spots in the left neck.

Final staging was pT4aN1M0 with a single positive node found in the right neck, thus the SNB was falsely negative. This patient went on to have post-operative chemoradiotherapy and was alive and disease free at last follow up.

6.3.4 Discussion

Here we present initial experience with SNB in laryngeal cancer. For the first time sentinel node imaging was undertaken solely in theatre. In this instance the tracer did not flow to the sentinel node. This was a large tumour and it was difficult to identify the margins to target the peritumoural injections. This may have resulted in uneven distribution of the tracer and non-representative drainage. It is not clear if it necessary to inject the tracer peritumourally, and it would be advantageous if reliable drainage could be traced following single intratumoural injection. Further work is needed to validate this proposed technique.

What has been shown in this case is that tumour drainage can be mapped in real time intraoperatively by fhSPECT. This encouraging finding supports the move of the procedure from the nuclear medicine department and into the theatre, opening up the potential applications of SNB.

6.4 Sentinel node biopsy in Prostate cancer

In order to challenge the reliability of the techniques we have explored, and to test the general applicability to new tumour groups we found a non- head and neck tumour in which SNB had potential application.

6.4.1 Background

The European Association of Urology guidelines[214] suggest that pelvic lymph node dissection (PLND) should be undertaken alongside radical

prostatectomy if the risk of metastasis is >7%. Pelvic nodal dissection (PLND) remains the gold standard staging tool but increases surgical time and morbidity [215, 216]. Furthermore, there is debate about the extent of the nodal dissection. The dilemma stands between a higher false negative rate with limited PLND (where up to 50% of metastasis would be missed[217, 218]) compared to an increased morbidity from extended PLND[215]. It is into this gap that sentinel lymph node biopsy neatly fits by directing the surgeon just to the at-risk lymph nodes, and this is reflected in the fact that there are good published data series of over 2000 prostate cancer patients that have been staged by SNB[219, 220]

6.4.2 Method

Ethical approval was obtained prior to recruiting a high-risk patient (Gleason 3+3) with informed consent. The patient was offered SNB alongside robotic assisted prostatectomy and pelvic lymph node dissection. On the morning of surgery the patient underwent transanal ultrasound guided injection of radiotracer. 100MBq of Tc99m labelled nanocolloid was delivered into each lobe of the prostate. The patient underwent static as well as dynamic lymphoscintigraphy followed by SPECT/CT. During surgery sentinel nodes were identified by fhSPECT, dissected from the rest of the lymph node specimen and sent for separate histopathological assessment according to a sentinel node protocol (serial step sectioning, H&E and cytokeratin staining).

6.4.3 Results

Two sentinel nodes were identified on lymphoscintigraphy within 20 minutes of tracer injection (Figure 6.5a). Sentinel nodes were located in the left obdurator and internal iliac lymph nodes. Second echelon nodes were seen in the pre-sacral and para-aortic region (Figure 6.5b and 6.5c).

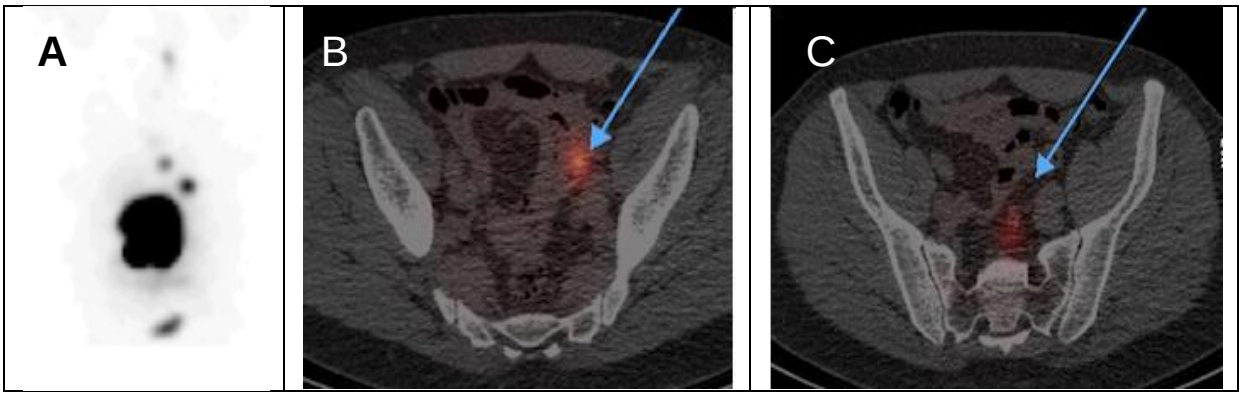


Figure 6-5
A: Planar lymphoscintigraphy anterior view showing injection site with two sentinel nodes superiorly and a small amount of tracer in the bladder below.
B SPECT/CT showing left oburator node
C Internal iliac node.

Sentinel nodes were subsequently identified within the lymph node resected lymph node packets and imaged ex-vivo by fhSPCT (Figure 6.6). Pathological examination of the sentinel nodes revealed no metastasis and the rest of the nodal resection was also pN0. There were no complications associated with the procedure.

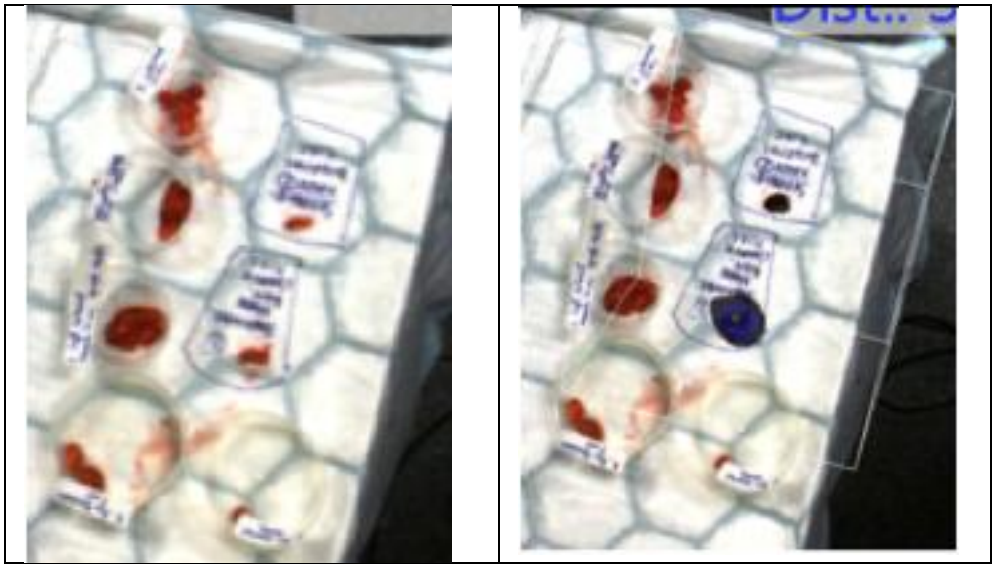


Figure 6-6 - A. Pelvic node dissection packets divided by anatomical region, sentinel nodes were dissected separately and shown in the two squared outlines. B same tissue viewed by fhSPECT showing both sentinel nodes have radioactivity (blue).
No other nodes were hot.

6.4.4 Discussion

Our technique demonstrates the feasibility of providing SNB for patients with high-risk disease. Draining sentinel nodes were identified within a timely fashion, and with validation could allow for one step intraoperative radionuclide injection and sentinel node identification.

Although this is the first reported case in the UK the technique is well described in the literature. In 2009 Holl et al[219] published a validation study of 2020 patients that underwent SNB and showed an SN detection rate of 98% and a false negative rate of just 6%. Sixty-nine percent of men in this trial had SNB as the sole staging tool, and in 7.2% positive SNs were found outside the field that would normally be dissected even for ePLND. In 2013 Winter et al. [220]published their series on 1229 patients that underwent SNB as part of an open radical prostatectomy. They compared the detection of metastatic nodes by SNB to expected levels based on the EAU predictive

nomogram. They found that 15% of metastasis would have been missed if treatment had been given according to the EAU guidelines. The SNB technique has been validated in robot assisted laparoscopic prostatectomy (RALP)[221] and is complimentary to this minimally invasive and patient tailored surgical approach.

6.5 Chapter Summary

The cases discussed in this chapter represent the first step in translating the validated SNB technique into new areas. These results highlight some of the technical difficulties that will be encountered in establishing SNB in new tumour groups. They also bolster evidence that SNB can be tweaked to suit the physiology and anatomy of the tumour. Once the optimum protocol has been elucidated for each tumour group, the technique can be more widely established.

Final conclusions regarding the overall project will be discussed in the next chapter.

Chapter 7 Final conclusions and plan for future work

This body of work has delved into a number of new areas in sentinel node biopsy in head and neck cancer. Traditional sentinel node imaging (lymphoscintigraphy and SPECT/CT) has been directly tested against intraoperative imaging by freehandSPECT, the first time that this has been done in any tumour group. The intraoperative system worked well, with an excellent false negative rate of just 5.3%. This study showed that lymphoscintigraphy performs particularly poorly in sentinel node detection, and SPECT/CT although more accurate than LSG does not appear to give an advantage over fhSPECT. This has huge implications for the future standard for SNB. If the imaging can be transferred to theatre then much of the cost and time associated with the procedure can be reduced. The culmination of this project did look at one case where solely intraoperative imaging was used. In this patient with laryngeal cancer sentinel nodes were identified but proved to be falsely negative. This was probably because of difficulty in accurately injecting the tracer. Furthermore our data looking at the accuracy of immediate fhSPECT in oral cancer showed that the optimum timing of injection and scanning are not yet fully understood. The next area of work will be to look at refining the injection and scanning technique in patients without nuclear medicine scans. Ideally deep and difficult to access tumours could be mapped using a single intratumoural injection as we demonstrated with salivary gland SNB. An ideal model would be tonsil tumours where the patient could undergo injection on two separate occasions - one by peritumoural and one by intratumoural method. Scans (SPECT/CT and fhSPCET) should be taken both times to see if the drainage by both methods is identical. To assess the optimum imaging window

serial fhSPECT scans could be undertaken. For fhSPECT to be most useful, accurate mapping would need to be possible within 10 minutes of injection in order to minimise disruption in the surgical flow, whether this is possible is yet to be confirmed.

Multimodal detection of sentinel nodes using ICG-^{99m}Tc-Nanocoll was successfully performed. In this series of oral cancer patients, the fluorescence aided detection in over one-third of cases. The specificity of ICG was vastly superior to control data looking at reliability of patent blue dye (PVB). Thus, confirming that PVB can be dispensed with as an optical tracer. Most of the fluorescent nodes however were discovered at a later stage of the surgery, once a significant amount of tissue dissection had been performed. Ideally the fluorescence would be visible through the skin, which was reported in other case series. This may be due to patient selection, tracer formulation and imaging protocol/equipment. There are a number of commercially available fluorescence imaging systems that could be investigated in the future. Ideally the development of multimodal probe that could incorporate gamma and fluoresence detection would be advantageous.

Salivary tumours are a new area for SNB, particularly with these improved methods. Here we have shown that SNB is feasible and reliable. This protocol will pave the way for future work in salivary malignancy in the form of prospective multicentre studies.

Our finding have been extrapolated to other new tumour groups – thryoid, layrnx and prostate with promising initial results.

On critical reflection this body of work has fulfilled its aim to investigate novel techniques in sentinel node biopsy and to provide evidence to support their incorporation into day-to-day clinical practice. The hope is that following this

work, the possibilities for SNB have been truly expanded meaning that many patients around the world will be able to benefit from personalised staging in the treatment of their cancer.

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Appendix A. Patient information sheet

Guy's and St Thomas' NHS
NHS Foundation Trust

Short title of project

New Intra Operative Sentinel Node Biopsy Procedure (Mouth)

Full title

Adaption and validation of a 3D navigation system that tracks the intraoperative injection of radiolabelled colloid to the Sentinel node.

INTRODUCTION

The commonest way cancer cells spread are by tiny lymphatic channels that drain fluid from the cancer to lymph nodes around the tumour. The lymph nodes act as a form of filter and catch the tumour cells. The spread is not random but goes first to a single node then later on to other nodes. This first node is called the SENTINEL NODE. If the SENTINEL NODE is free of tumour you can safely assume all the other surrounding lymph nodes are also free of tumour. The spread of cancer cells to the local lymph nodes is so important that it is customary when removing the main cancer to remove the surrounding lymph nodes at the same time. This can greatly increase the magnitude of surgery and the side effects that come from this surgery.

This situation has now changed and in some tumours (breast and skin), lymph nodes are sampled for cancer rather than all the lymph nodes being removed. At the moment this type of surgery is limited to certain cancers, as the technology had not been developed for other types of cancer. We believe there is a reliable technique to identify the SENTINEL NODE in the type of cancer that you have been diagnosed with.

We now have at Guy's an intra operative 3D tracking system that will allow us to identify the SENTINEL NODE in the operating theatre. The node can then be removed and checked for cancer.
At present the accepted standard of care in the UK for your disease is to remove the mouth tumour and the surrounding lymph nodes. But at Guy's Head and Neck cancer centre we lead a European Trial where is was established that the sentinel node biopsy was safe when used in the management of early mouth cancer and in addition saved about 75% of patients a neck dissection. This is not going to change; you are going to be offered the choice of adopting the conventional UK approach (tumour removal and neck dissection) or the current standard of care at Guy's (tumour removal and sentinel node biopsy). Once you have made your choice, what we propose is at the time of your surgery we identify the SENTINEL NODE draining your tumour by using both conventional imaging by camera and CT scan before surgery and again by the new 3D sentinel node imaging system during the operation. We use the same radiotracer as is used in breast cancer, which has been safely given to hundreds of thousands of patients. This is injected around the tumour just before surgery. The tracer will then flow to the SENTINEL NODE . We will check where the sentinel nodes are by using our normal test which involves a gamma camera to watch for the appearance of radioactive tracer as it flows in the sentinel node and then perform a SPECTCT (the camera will move around your body in the nuclear medicine department) which will ensure that the site of the sentinel node is found prior to surgery. We can detect the sentinel node at surgery using our 3D navigation system. Your SENTINEL NODE will then be removed before carrying on with the standard operation.

Approximately 150 patients with a similar disease as you will be involved in this study. Before you decide to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the information carefully and discuss it with friends, relatives and your

GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. This leaflet will explain the procedure, tell you how it is performed and will answer some of the questions you may have.

What is the purpose of the Study?
The aim of the study is to see if a new 3d Navigation technique can help us to reliably identify the SENTINEL NODE in people with early cancers.

Why Have I been chosen?
You have been recently diagnosed with an early mouth cancer and are due to undergo surgery for this disease. Your surgery will entail removal of the tumour and in addition either all the lymph nodes around the tumour or just the sentinel nodes depending on which approach you choose. The surgery you receive will not be changed, however during the operation we would like to identify the SENTINEL NODE using the technology we have described above. We are asking only suitable patients with early cancer to take part in this study.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you do decide to take part you are still free to withdraw at any time and without giving a reason. If you decide not to take part it will in no way affect the care you receive.

What will happen to me if I take part?
The injection of a very low dose of radiotracer around the tumour will be undertaken after the mouth has been numbed with anaesthetic spray. The amount of radioactivity from this tracer is lower than is used in routine CT scans and is completely removed by the body. Less than 1ml of tracer is injected. You will then be laid on a bed and a camera will watch the spread of tracer from your mouth into your neck. You do not have to do anything. Once we know in which direction the tracer is draining we can get a much more accurate image by using a SPECT/CT. Again there is nothing for you to do but lie on a bed while the picture is captured.

You will then move to the operating theatre where you will be put to sleep in the normal way. When you are asleep some blue dye is injected around the tumour once again and this also travels to the sentinel node and helps the surgeon identify the right sentinel node which ideally is both radioactive and blue. In addition we will use the 3D tracking system before we start surgery to identify the position of the sentinel node with a third imaging system. All sentinel nodes identified will be removed for carefully examined by our pathologists. The rest of your operation will be completed in the standard way. The sentinel node biopsy will not affect the success of your operation in any way.

What do I have to do?
You do not need to do anything special as part of this study. You will recover from your operation in the normal way.

What is the procedure that is being tested?
The procedure is a 3d Navigation tool called Surgiceye, which allows us to visualise the SENTINEL NODE through the skin. It can be used alongside other equipment that may be used during your operation. Surgiceye has been validated for use in other parts of the world but we are the first NHS Hospital in the UK to be able to use it.

What are the side effects of taking part?
Sentinel node biopsy has been performed for several years, and there are very few side effects associated with it. These are:
1. Blue dye may stain the urine. We inject blue dye into the tissues around your tumour, and since the dye is removed by your kidneys, for about one day after your operation, your urine will be stained blue.
2. Hypersensitivity. There have been a few reports of people being allergic to the blue dye and very occasionally to the nanocoll. This is more common in people who suffer allergies to other things. If you tend to suffer from allergies, please let us know.

3. There is radiation used during the procedure. The dose is low and equivalent to 2 months of natural background radiation or a lifetime cancer risk of 1 in 50,000 (compare this to a natural cancer risk of 1 in 4). This additional risk is low

.

What are the possible disadvantages and risks of taking part?
Sentinel node biopsy has been shown to be very safe and has a very low complication rate. Because we are using a new technique the operation may take slightly longer but this will only be a short time compared to the total surgery. If the SENTINEL NODE is not found within a short time we will not continue to look for it but we will proceed directly with your planned operation.

What are the possible benefits to taking part?
If you agree to take part in this research project it may not help you directly. You are planned to receive the current standard of care for your tumour.

We expect most of the SENTINEL NODES will be found in the area that your surgeon already plans to operate in, however we know from some published data that about 10% of the time the SENTINEL NODES are found in another area of the neck. If this happens to you then you may have another small incision on the side of the neck to remove the SENTINEL NODE. The benefit of this is that we can detect cancer spread, which would have been missed by the standard treatment.

Because the cure rate for your type of cancer is extremely good our overall aim is to eventually reduce the amount of surgery patients such as yourself will need. We are trying to refine our treatment regimes so that patients will get exactly the treatment needed to eradicate the cancer and nothing more or less. In that respect your participation in this study will be helping future generations of cancer sufferers

What happens when the research study stops?
After you have undergone the surgery you will get your results in the normal way. This will be to decide about further treatment for the cancer (e.g radiotherapy treatment). All cancer patients are followed-up in the outpatient clinic for a few years. Your participation in this study will not change the treatment you receive nor the follow up in clinic.

Will my taking part in this study be kept confidential?
All information that is collected about you during the course of the research will be kept strictly confidential.

What will happen to the results of the research study?
We intend to publish our results in the medical literature and if we do so your anonymity will be maintained throughout.

Who is organising and funding the research?
The research is being undertaken Guys Hospital and its affiliated centres as part of its Academic Health Sciences Centre status.

Who has reviewed the study?

The study protocol has been reviewed and approved by the local research ethics committee, which is made up of both doctors and lay members.

What if there is a problem?

Questions and Concerns – If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Please contact: Principle Investigator Professor Mark McGurk: mark.mcgurk@kcl.ac.uk : 02071884348

Complaints – If you have a complaint, you should talk to your research doctor who will do their best to answer your questions. If you remain unhappy, you may be able to make a formal complaint through the NHS complaints procedure. Details can be obtained through the Guy's and St Thomas' Patient Advisory Liaison Service (PALS) on 0207 1888188, address: PALS, KIC, Ground floor, north wing, St Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH .

Harm – This trial is co-sponsored by King's College London and Guy's and St Thomas' NHS Foundation Trust. The sponsors will at all times maintain adequate insurance in relation to the study independently. Kings College London, through its own professional indemnity (Clinical Trials) and no fault compensation and the Trust having a duty of care to patients via NHS indemnity cover, in respect of

any claims arising as a result of clinical negligence by its employees, brought by or on behalf of a study patient.

FURTHER INFORMATION

If you or your family have any questions or require any further information, please contact Professor McGurk 02071884348

Many thanks for reading this information leaflet.

Appendix B. Consent Form

Study number:_____

Patient Hospital Number for this trial:_____

CONSENT FORM

Validation of 3D navigation during intra operative Sentinel Node Biopsy in respect to head and neck, urological, breast and gynaecological cancers. An Observational Guy's Study

1. I confirm that I have read and understood the information sheet dated 14/9/12 Version 2.1 for the above study and have had the opportunity to ask questions.

2. I understand that my participation is entirely voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.
3. I understand that sections of any medical notes may be looked at by responsible individuals undertaking and supervising the study where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.
4. I consent to all the specimens removed at surgery to be used by the teams involved in the study for research on sentinel nodes and other related genetic/laboratory investigations.

5. I consent to take part in the study.

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Name of Researcher	Date	Signature
_____	_____	_____
Name of person taking consent (If different from researcher)	Date	Signature

Appendix C. Patient information sheet multimodal tracer

Guy's and St Thomas' NHS
NHS Foundation Trust

PATIENT INFORMATION SHEET

New Intra Operative Sentinel Node Biopsy Procedure

Adaption of a tracer for identification of sentinel lymph nodes in early cancer

The commonest way cancer cells spread are by tiny lymphatic channels that drain fluid from the cancer to lymph nodes around the tumour. The lymph nodes act as a form of filter and catch the tumour cells. The spread is not random but goes first to a single node then later on to other nodes. This first node is called the SENTINEL NODE. If the SENTINEL NODE is free of tumour you can safely assume all the other surrounding lymph nodes are also free of tumour.

At Guys and St Thomas' NHS trust we are introducing an innovative type of tracer to identify the SENTINEL NODE. This tracer can be used to highlight the SENTINEL NODE which can then be removed during your operation.

The new tracer is a mixture of a standard radiotracer (which is routinely used for sentinel node biopsy) and a fluorescent green dye (called indocyanine green, ICG). The radiotracer has been safely given to hundreds of thousands of patients with only a tiny risk of an allergic reaction to the protein (nanocoll) injected. The new technique we are introducing is the addition of fluorescent green dye to the tracer, which means that the SENTINEL NODE will glow green under certain lights. This will make it easier to find the correct SENTINEL NODE at surgery.

The combination of fluorescent green dye and radiotracer has been used in this country in a very small number of cases only, but it has been used in other parts of Europe in over 500 patients and the results have been good. The fluorescent dye has been used for other medical conditions (such as assessment of blood flow through parts of the body) since the 1950s and has a very good safety record. The mixture of radiotracer and green dye has been carefully examined by chemists in the laboratory as well as in animal studies to make sure that there are no undesirable effects when these two compounds are given together.

The addition of the fluorescent dye to the tracer does not alter the operation you will be having, but because this is a relatively new technique we would like to ask your permission to use the fluorescence alongside the standard treatment you will receive.

Do I have to have the new tracer?

It is up to you to decide whether or not to have the new tracer. If you do decide not to you will have the standard tracer and this will in no way affect the care you receive.

What will happen to me if I have the new tracer?

The radiotracer and fluorescent dye will be mixed together and injected in exactly the same way as the standard tracer. Normally patients have four very tiny injections around the tumour. Less than 1ml of tracer is injected. Following this there may be some scans required but this depends on the type of tumour you are having surgery for – this will be exactly the same no matter which tracer is used. The only difference is during your operation. If you have the new tracer the surgeon can use a special light (near infrared wavelength) which will cause the fluorescent tracer to glow brightly and should make it more easy to find the SENTINEL NODE.

What do I have to do?

You do not need to do anything special. You will recover from your operation in the normal way. We will check with you the day following the surgery if you have noticed any side effects that could be related to the tracer

What are the possible side effects of the new tracer?

1. The green dye that we inject fluoresces under certain lights. This means that if the injection is given in a visible part of the body that this will glow under near-infrared light. Similarly small amounts are passed in the urine and this can glow also. All these changes are temporary as the dye is removed from the body completely within a couple of days.

2.Hypersensitivity. There have been a few reports of people being allergic to the fluorescent dye. This is more common in people who suffer allergies to other things and people. If you tend to suffer from allergies, please let us know. The risk of an allergic reaction has been estimated as less than one in ten thousand.

Who has approved this tracer?

The new tracer has been reviewed and approved by Guys and St Thomas' NHS trust formulary committee as well as by the trust Clinical Governance Committee.

Many thanks for reading this information leaflet.

Appendix D. Patient information sheet salivary cancer

Guy's and St Thomas' NHS
NHS Foundation Trust

New Intra Operative Sentinel Node Biopsy Procedure (Salivary)

Full title

Adaption and validation of a 3D navigation system that tracks the intraoperative injection of radiolabelled colloid to the Sentinel node.

INTRODUCTION

The commonest way cancer cells spread are by tiny lymphatic channels that drain fluid from the cancer to lymph nodes around the tumour. The lymph nodes act as a form of filter and catch the tumour cells. The spread is not random but goes first to a single node then later on to other nodes. This first node is called the SENTINEL NODE. If the SENTINEL NODE is free of tumour you can safely assume all the other surrounding lymph nodes are also free of tumour. The spread of cancer cells to the local lymph nodes is so important that it is customary when removing the main cancer to remove the surrounding lymph nodes at the same time. This can greatly increase the magnitude of surgery and the side effects that come from this surgery.

This situation has now changed and in some tumours (breast and skin), lymph nodes are sampled for cancer rather than all the lymph nodes being removed. At the moment this type of surgery is limited to certain cancers, as the technology had not been developed for other types of cancer. We believe there is a reliable technique to identify the SENTINEL NODE in the type of cancer that you have been diagnosed with.

We now have at Guy's an intra operative 3D tracking system that will allow us to identify the SENTINEL NODE in the operating theatre. The node can then be removed and checked for cancer.

At present the accepted standard of care for your disease is to remove the salivary gland tumour and the surrounding lymph nodes. This is not going to change; you are going to get standard treatment. What we propose is at the time of your surgery we identify the SENTINEL NODE draining your tumour and remove it. We use the same radiotracer as is used in breast cancer, which has been safely given to hundreds of thousands of patients. This is injected into the tumour. The tracer will then flow to the SENTINEL NODE. We will check where the sentinel nodes are using our normal test using a gamma camera to watch for the appearance of radioactive tracer in the sentinel node and perform a SPECTCT (the camera will move around your body in the nuclear medicine department) this will ensure that the site of the sentinel node is found prior to surgery. We can detect the sentinel node at surgery using our 3D navigation system. Your SENTINEL NODE will then be removed before carrying on with the standard operation.

Approximately 150 patients with a similar disease as you will be involved in this study. Before you decide to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take

time to decide whether or not you wish to take part. This leaflet will explain the procedure, tell you how it is performed and will answer some of the questions you may have.

What is the purpose of the Study?
The aim of the study is to see if a new 3d Navigation technique can help us to reliably identify the SENTINEL NODE in people with early cancers.

Why Have I been chosen?
You have been recently diagnosed with an early salivary gland cancer and are due to undergo surgery for this. Your surgery will entail removal of the lymph nodes around the tumour. The surgery you receive will not be changed, however during the operation we would like to identify the SENTINEL NODE using the technology we have described above. We are asking only suitable patients with early cancer to take part in this study.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you do decide to take part you are still free to withdraw at any time and without giving a reason. If you decide not to take part it will in no way affect the care you receive.

What will happen to me if I take part?
All procedures related to this study will be undertaken whilst you are under general anaesthetic. We estimate this will add about ten to fifteen minutes to your operation time. A very low dose of a radioactive tracer and a blue dye will be injected around the tumour within 24 hours of your operation. The amount of radioactivity from this tracer is lower than is used in routine CT scans and is completely removed by the body. Using these methods to guide us we will identify the SENTINEL NODE, which will be removed and carefully examined by our pathologists. The rest of your operation will be completed in the standard way. The sentinel node biopsy will not affect the success of your operation in any way.

What do I have to do?
You do not need to do anything special as part of this study. You will recover from your operation in the normal way.

What is the procedure that is being tested?
The procedure is a 3d Navigation tool called Surgiceye, which allows us to visualise the SENTINEL NODE through the skin. It can be used alongside other equipment that may be used during your operation. Surgiceye has been validated for use in other parts of the world but we are the first NHS Hospital in the UK to be able to use it.

What are the side effects of taking part?
Sentinel node biopsy has been performed for several years, and there are very few side effects associated with it. These are:

1. Blue dye may stain the urine. We inject blue dye into the tissues around your tumour, and since the dye is removed by your kidneys, for about one day after your operation, your urine will be stained blue.
2. Hypersensitivity. There have been a few reports of people being allergic to the blue dye and nanocoll. This is more common in people who suffer allergies to other things. If you tend to suffer from allergies, please let us know.
3. There is radiation used during the procedure. The dose is low and equivalent to 1 year of natural background radiation or a lifetime cancer risk of 1 in 7000 (compare this to a natural cancer risk of 1 in 4). This additional risk is low.

What are the possible disadvantages and risks of taking part?
Sentinel node biopsy has been shown to be very safe and has a very low complication rate. Because we are using a new technique the operation may take slightly longer but this will only be a short time compared to the total surgery. If the SENTINEL NODE is not found within a short time we will not continue to look for it but we will proceed directly with your planned operation.

What are the possible benefits to taking part?
If you agree to take part in this research project it may not help you directly. You are planned to receive the current standard of care for your tumour.
We expect most of the SENTINEL NODES will be found in the area that your surgeon already plans to operate in, however we know from some published data that about 10% of the time the SENTINEL

NODES are found in another area of the neck. If this happens to you then you may have another small incision on the side of the neck to remove the SENTINEL NODE. The benefit of this is that we can detect cancer spread, which would have been missed by the standard treatment.
Because the cure rate for your type of cancer is extremely good our overall aim is to eventually reduce the amount of surgery patients such as yourself will need. We are trying to refine our treatment regimes so that patients will get exactly the treatment needed to eradicate the cancer and nothing more or less. In that respect your participation in this study will be helping future generations of cancer sufferers

What happens when the research study stops?
After you have undergone the surgery you will get your results in the normal way. This will be to decide about further treatment for the cancer (e.g radiotherapy treatment). All cancer patients are followed-up in the outpatient clinic for a few years. Your participation in this study will not change the treatment you receive nor the follow up in clinic.

Will my taking part in this study be kept confidential?
All information that is collected about you during the course of the research will be kept strictly confidential.

What will happen to the results of the research study?
We intend to publish our results in the medical literature and if we do so your anonymity will be maintained throughout.

Who is organising and funding the research?
The research is being undertaken Guys Hospital and its affiliated centres as part of its Academic Health Sciences Centre status.

Who has reviewed the study?

The study protocol has been reviewed and approved by the local research ethics committee, which is made up of both doctors and lay members.

What if there is a problem?

Questions and Concerns – If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Please contact: Principle Investigator Professor Mark McGurk: mark.mcgurk@kcl.ac.uk : 02071884348

Complaints – If you have a complaint, you should talk to your research doctor who will do their best to answer your questions. If you remain unhappy, you may be able to make a formal complaint through the NHS complaints procedure. Details can be obtained through the Guy's and St Thomas' Patient Advisory Liaison Service (PALS) on 0207 1888188, address: PALS, KIC, Ground floor, north wing, St Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH .

Harm – This trial is co-sponsored by King's College London and Guy's and St Thomas' NHS Foundation Trust. The sponsors will at all times maintain adequate insurance in relation to the study independently. Kings College London, through its own professional indemnity (Clinical Trials) and no fault compensation and the Trust having a duty of care to patients via NHS indemnity cover, in respect of any claims arising as a result of clinical negligence by its employees, brought by or on behalf of a study patient.

FURTHER INFORMATION

If you or your family have any questions or require any further information, please contact
Professor McGurk 02071884348

Many thanks for reading this information leaflet

Appendix E. Patient information sheet thyroid cancer

Short title of project

New Intra Operative Sentinel Node Biopsy Procedure (Thyroid)

Full title
Adaption and validation of a 3D navigation system that tracks the intraoperative injection of radiolabelled colloid to the Sentinel node.

INTRODUCTION

The commonest way cancer cells spread are by tiny lymphatic channels that drain fluid from the cancer to lymph nodes around the tumour. The lymph nodes act as a form of filter and catch the tumour cells. The spread is not random but goes first to a single node then later on to other nodes. This first node is called the SENTINEL NODE. If the SENTINEL NODE is free of tumour you can safely assume all the other surrounding lymph nodes are also free of tumour. The spread of cancer cells to the local lymph nodes is so important that it is customary when removing the main cancer to remove the surrounding lymph nodes at the same time. This can greatly increase the magnitude of surgery and the side effects that come from this surgery.

This situation has now changed and in some tumours (breast and skin), lymph nodes are sampled for cancer rather than all the lymph nodes being removed. At the moment this type of surgery is limited to certain cancers, as the technology had not been developed for other types of cancer. We believe there is a reliable technique to identify the SENTINEL NODE in the type of cancer that you have been diagnosed with.

We now have at Guy's an intra operative 3D tracking system that will allow us to identify the SENTINEL NODE in the operating theatre. The node can then be removed and checked for cancer.

At present the accepted standard of care for your disease is to remove the thyroid tumour and the surrounding lymph nodes. This is not going to change; you are going to get standard treatment. What we propose is at the time of your surgery we identify the SENTINEL NODE draining your tumour and remove it. We use the same radiotracer as is used in breast cancer, which has been safely given to hundreds of thousands of patients. This is injected into the tumour. The tracer will then flow to the SENTINEL NODE. We will check where the sentinel nodes are using our normal test using a gamma camera to watch for the appearance of radioactive tracer in the sentinel node and perform a SPECTCT (the camera will move around your body in the nuclear medicine department) this will ensure that the site of the sentinel node is found prior to surgery. We can detect the sentinel node at surgery using our 3D navigation system. Your SENTINEL NODE will then be removed before carrying on with the standard operation.

Approximately 150 patients with a similar disease as you will be involved in this study. Before you decide to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. This leaflet will explain the procedure, tell you how it is performed and will answer some of the questions you may have.

What is the purpose of the Study?
The aim of the study is to see if a new 3d Navigation technique can help us to reliably identify the SENTINEL NODE in people with early cancers.

Why Have I been chosen?
You have been recently diagnosed with an early thyroid cancer and are due to undergo surgery for this. Your surgery will entail removal of the lymph nodes around the tumour. The surgery you receive will not be changed, however during the operation we would like to identify the SENTINEL NODE using the technology we have described above. We are asking only suitable patients with early cancer to take part in this study.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you do decide to take part you are still free to withdraw at any time and without giving a reason. If you decide not to take part it will in no way affect the care you receive.

What will happen to me if I take part?
All procedures related to this study will be undertaken whilst you are under general anaesthetic. We estimate this will add about ten to fifteen minutes to your operation time. A very low dose of a radioactive tracer and a blue dye will be injected around the tumour during your operation. The amount of radioactivity from this tracer is lower than is used in routine CT scans and is completely removed by the body. Using these methods to guide us we will identify the SENTINEL NODE, which will be removed and carefully examined by our pathologists. The rest of your operation will be completed in the standard way. The sentinel node biopsy will not affect the success of your operation in any way.

What do I have to do?
You do not need to do anything special as part of this study. You will recover from your operation in the normal way.

What is the procedure that is being tested?
The procedure is a 3d Navigation tool called Surgiceye, which allows us to visualise the SENTINEL NODE through the skin. It can be used alongside other equipment that may be used during your operation. Surgiceye has been validated for use in other parts of the world but we are the first NHS Hospital in the UK to be able to use it.

What are the side effects of taking part?
Sentinel node biopsy has been performed for several years, and there are very few side effects associated with it. These are:

4. Blue dye may stain the urine. We inject blue dye into the tissues around your tumour, and since the dye is removed by your kidneys, for about one day after your operation, your urine will be stained blue.
5. Hypersensitivity. There have been a few reports of people being allergic to the blue dye and very rarely with nanocoll. This is more common in people who suffer allergies to other things. If you tend to suffer from allergies, please let us know.
6. There is radiation used during the procedure. The dose is low and equivalent to 1 year of natural background radiation or a lifetime cancer risk of 1 in 7000 (compare this to a natural cancer risk of 1 in 4). This additional risk is low.

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What are the possible disadvantages and risks of taking part?
Sentinel node biopsy has been shown to be very safe and has a very low complication rate. Because we are using a new technique the operation may take slightly longer but this will only be a short time compared to the total surgery. If the SENTINEL NODE is not found within a short time we will not continue to look for it but we will proceed directly with your planned operation.

What are the possible benefits to taking part?
If you agree to take part in this research project it may not help you directly. You are planned to receive the current standard of care for your tumour.

We expect most of the SENTINEL NODES will be found in the area that your surgeon already plans to operate in, however we know from some published data that about 10% of the time the SENTINEL NODES are found in another area of the neck. If this happens to you then you may have another small incision on the side of the neck to remove the SENTINEL NODE. The benefit of this is that we can detect cancer spread, which would have been missed by the standard treatment.

Because the cure rate for your type of cancer is extremely good our overall aim is to eventually reduce the amount of surgery patients such as yourself will need. We are trying to refine our treatment regimes so

that patients will get exactly the treatment needed to eradicate the cancer and nothing more or less. In that respect your participation in this study will be helping future generations of cancer sufferers

What happens when the research study stops?
After you have undergone the surgery you will get your results in the normal way. This will be to decide about further treatment for the cancer (e.g iodine treatment). All cancer patients are followed-up in the outpatient clinic for a few years. Your participation in this study will not change the treatment you receive nor the follow up in clinic.

Will my taking part in this study be kept confidential?
All information that is collected about you during the course of the research will be kept strictly confidential.

What will happen to the results of the research study?
We intend to publish our results in the medical literature and if we do so your anonymity will be maintained throughout.

Who is organising and funding the research?
The research is being undertaken Guys Hospital and its affiliated centres as part of its Academic Health Sciences Centre status.

Who has reviewed the study?

The study protocol has been reviewed and approved by the local research ethics committee, which is made up of both doctors and lay members.

What if there is a problem?

Questions and Concerns – If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. Please contact: Principle Investigator Professor Mark McGurk: mark.mcgurk@kcl.ac.uk : 02071884348

Complaints – If you have a complaint, you should talk to your research doctor who will do their best to answer your questions. If you remain unhappy, you may be able to make a formal complaint through the NHS complaints procedure. Details can be obtained through the Guy's and St Thomas' Patient Advisory Liaison Service (PALS) on 0207 1888188, address: PALS, KIC, Ground floor, north wing, St Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH .

Harm – This trial is co-sponsored by King's College London and Guy's and St Thomas' NHS Foundation Trust. The sponsors will at all times maintain adequate insurance in relation to the study independently. Kings College London, through its own professional indemnity (Clinical Trials) and no fault compensation and the Trust having a duty of care to patients via NHS indemnity cover, in respect of any claims arising as a result of clinical negligence by its employees, brought by or on behalf of a study patient.

FURTHER INFORMATION

If you or your family have any questions or require any further information, please contact
Professor McGurk 02071884348

Many thanks for reading this information leaflet.

Appendix F. Patient information sheet larynx cancer

Guy's and St Thomas' NHS
NHS Foundation Trust

Short title of project

New Intra Operative Sentinel Node Biopsy Procedure (Larynx)

Full title

Adaption and validation of a 3D navigation system that tracks the intraoperative injection of radiolabelled colloid to the Sentinel node.

INTRODUCTION

The commonest way cancer cells spread are by tiny lymphatic channels that drain fluid from the cancer to lymph nodes around the tumour. The lymph nodes act as a form of filter and catch the tumour cells. The spread is not random but goes first to a single node then later on to other nodes. This first node is called the SENTINEL NODE. If the SENTINEL NODE is free of tumour you can safely assume all the other surrounding lymph nodes are also free of tumour. The spread of cancer cells to the local lymph nodes is so important that it is customary when removing the main cancer to remove the surrounding lymph nodes at the same time. This can greatly increase the magnitude of surgery and the side effects that come from this surgery.

This situation has now changed and in some tumours (breast and skin), lymph nodes are sampled for cancer rather than all the lymph nodes being removed. At the moment this type of surgery is limited to certain cancers, as the technology had not been developed for other types of cancer. We believe there is a reliable technique to identify the SENTINEL NODE in the type of cancer that you have been diagnosed with.

We now have at Guy's an intra operative 3D tracking system that will allow us to identify the SENTINEL NODE in the operating theatre. The node can then be removed and checked for cancer.

At present the accepted standard of care for your disease is to remove the larynx tumour and the surrounding lymph nodes. This is not going to change; you are going to get standard treatment. What we propose is at the time of your surgery we identify the SENTINEL NODE draining your tumour and remove it. We use the same radiotracer as is used in breast cancer, which has been safely given to hundreds of thousands of patients. This is injected into the tumour whilst your are asleep. The tracer will then flow to the SENTINEL NODE and we can detect it using our 3D navigation system. Your SENTINEL NODE will then be removed before carrying on with the standard operation.

Approximately 150 patients with a similar disease as you will be involved in this study. Before you decide to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take

time to decide whether or not you wish to take part. This leaflet will explain the procedure, tell you how it is performed and will answer some of the questions you may have.

What is the purpose of the Study?
The aim of the study is to see if a new 3d Navigation technique can help us to reliably identify the SENTINEL NODE in people with early cancers.

Why Have I been chosen?
You have been recently diagnosed with an early larynx cancer and are due to undergo surgery for this. Your surgery will entail removal of the lymph nodes around the tumour. The surgery you receive will not be changed, however during the operation we would like to identify the SENTINEL NODE using the technology we have described above. We are asking only suitable patients with early cancer to take part in this study.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be asked to sign a consent form. If you do decide to take part you are still free to withdraw at any time and without giving a reason. If you decide not to take part it will in no way affect the care you receive.

What will happen to me if I take part?
All procedures related to this study will be undertaken whilst you are under general anaesthetic. We estimate this will add about ten to fifteen minutes to your operation time. A very low dose of a radioactive tracer and a blue dye will be injected around the tumour during your operation. The amount of radioactivity from this tracer is lower than is used in routine CT scans and is completely removed by the body. Using these methods to guide us we will identify the SENTINEL NODE, which will be removed and carefully examined by our pathologists. The rest of your operation will be completed in the standard way. The sentinel node biopsy will not affect the success of your operation in any way.

What do I have to do?
You do not need to do anything special as part of this study. You will recover from your operation in the normal way.

What is the procedure that is being tested?
The procedure is a 3d Navigation tool called Surgiceye, which allows us to visualise the SENTINEL NODE through the skin. It can be used alongside other equipment that may be used during your operation. Surgiceye has been validated for use in other parts of the world but we are the first NHS Hospital in the UK to be able to use it.

What are the side effects of taking part?
Sentinel node biopsy has been performed for several years, and there are very few side effects associated with it. These are:

7. Blue dye may stain the urine. We inject blue dye into the tissues around your tumour, and since the dye is removed by your kidneys, for about one day after your operation, your urine will be stained blue.
8. Hypersensitivity. There have been a few reports of people being allergic to the blue dye and very occasionally to the nanocoll. This is more common in people who suffer allergies to other things. If you tend to suffer from allergies, please let us know.
9. There is radiation used during the procedure. The dose is low and equivalent to 2 months of natural background radiation or a lifetime cancer risk of 1 in 50,000 (compare this to a natural cancer risk of 1 in 4). This additional risk is low.

What are the possible disadvantages and risks of taking part?
Sentinel node biopsy has been shown to be very safe and has a very low complication rate. Because we are using a new technique the operation may take slightly longer but this will only be a short time compared to the total surgery. If the SENTINEL NODE is not found within a short time we will not continue to look for it but we will proceed directly with your planned operation.

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What happens when the research study stops?
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