

The Sentinel Node

In

Surgical Oncology

Mohammad Reza Safaei-Keshtgar
BSc, MB, BS, FRCSI, FRCS(Gen)

University of London

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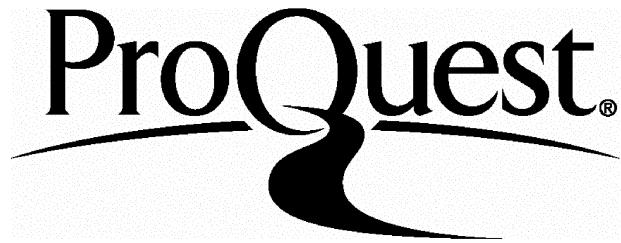
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**To my parents, who are the
best for their care
and support**

**To my wife for her sacrifices,
support and
encouragement**

**To my children,
Soroosh, Asma and Safoora
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*With them the Seed of Wisdom did I sow,
And with my own hand labour'd it to grow:
And this was all the Harvest that I reap'd-
"I came like Water, and like Wind I go."*

Omar Khayyam

ABSTRACT

This thesis is composed of nine chapters. Chapter-1 reviews the milestones in surgical management of breast cancer over the last century with special emphasis on axillary management. The role of axillary lymph node dissection (ALND) in staging, loco-regional control and survival of breast cancer patients is discussed. Limitations of imaging modalities in diagnosing the histological status of the axillary lymph node are also presented.

Chapter-2 deals with the sentinel lymph node (SLN) concept and its importance as a staging procedure in surgical oncology. A historical review of the advances in this field is presented. The structure and function of lymph node is also outlined in this section. Technical issues including the radiopharmaceutical and particle size, the injection dose and volume, the injection technique, imaging technique, types of gamma detection probes, design of gamma camera, detection techniques of sentinel node during surgery are presented in this section.

Chapter-3 describes the patients and methods used including inclusion and exclusion criteria, injection and imaging protocols, image processing and display and surgical detection technique and histological analysis of the sentinel node. The results of sentinel node biopsy in 101 breast cancer patients are presented and discussed. Data on dynamic and static imaging, detection rate, sensitivity and specificity of the technique as well as positive and negative predictive values are presented. Pitfalls related to the technique of SLN detection and localization and lessons learnt from these are described in detail in this section of the thesis.

Chapter-4 deals with the importance of a reliable intraoperative tool to determine the histological status of the sentinel node. The study of the role of intra-operative touch imprint cytology and optical biopsy in determining the histological status of the SLN is presented.

Chapter-5 describes a new injection technique used for the delivery of radiopharmaceutical for sentinel node biopsy. This technique involves the use of a needle-free and pain free injection system.

Chapter-6 deals with the experience gained in sentinel node biopsy in other areas of surgical oncology. It includes malignant melanoma, penile carcinoma, anal carcinoma, squamous carcinoma of head and neck and colo-rectal carcinoma.

Chapter-7 presents the results and experience gained on the radiation safety aspects of sentinel node biopsy. We analyse the radiation dose to the patient, staff and report on the radiation waste generated as a result of SLNB procedure and present some guidelines with regards to safe use of radioactivity.

Chapter-8 presents the cost issues in sentinel node biopsy and preliminary data are presented.

Chapter-9 presents the concluding remarks and future directions. It is concluded that the concept of sentinel node biopsy is valid in the management of patients with early breast carcinoma and malignant melanoma. Whether SLNB is ready to replace conventional ALND in breast cancer remains unclear. Although SLN biopsy is now a reliable staging investigation the data on regional control and long term survival is lacking.

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European Journal of Surgical Oncology 1999; 25(6): 658-659

CLAIMS OF ORIGINALITY

The following are original work and findings:

1. A new injection technique for painless delivery of radiopharmaceutical for sentinel node biopsy using needle-free injection system.
2. Intra-operative diagnosis of the histological status of sentinel node using optical biopsy technique and imprint cytology.
3. Autoradiography of the sentinel lymph node to study pattern of distribution of radiocolloid within the SLN.
4. Electron microscopy of sentinel node.
5. Radiation safety issues in particular measurement of radioactive waste generated during surgery and radiation dose to patients after intradermal administration of radiotracer.
6. Langers axillary arch in association with sentinel node in breast carcinoma and the potential difficulties that it can pose during surgical excision.
7. Sentinel node biopsy in anal carcinoma is for the first time described.
8. Study of particle kinetics and design of optimal imaging protocol, as a result of performing dynamic imaging in all patients who underwent SLNB for breast cancer.
9. Preliminary analysis of cost implications of sentinel node biopsy as compared with axillary node dissection.

ABBREVIATIONS

ALND	Axillary Lymph Node Dissection
ANN	Artificial Neural Networks
AL-PR	Artificial-intelligence pattern-recognition
AP	Antero-Posterior
ARSAC	Administration of Radioactive Substances Advisory Committee
CEA	Carcino Embryonic Antigen
CT	Computed Tomography
DCSI	Ductal Carcinoma in Situ
ELND	Elective Lymph Node Dissection
GDP	Gamma Detection Probe
FNAC	Fine Needle Aspiration Cytology
H & E	Haematoxylin and Eosin
HCA	Hierarchical Cluster Analysis
ICRP	International Commission on Radiological Protection
ID	Invasive Ductal Carcinoma
i.d.	Injection Dose
IHC	Immunohistochemistry
IL	Invasive Lobular Carcinoma
KBq	Kilo Bequerel
KeV	Kilo Electronvolts
lat	Lateral
LCIS	Lobular Carcinoma in Situ
LEGP	Low Energy General Purpose
LEHR	Low Energy High Resolution

LFOV	Large field of view
LiF	Lithium Fluoride
LIQ	Lower inner quadrant
LOQ	Lower outer quadrant
LOS	Length of stay
MBq	Mega Bequerel
MIBI	Methoxy Isobutyl Isonitrile
MIRD	Medical International Radiation Dose
mGy	Milli Gray
min	minute
ml	milliliter
mm	millimeter
MRI	Magnetic Resonance Imaging
mSV	Micro-Sievert
n	Number
NRPB	National Radiological Protection Board
PET	Positron Emission Tomography
p.i.	Post injection
POPUMET	Protection of the Patient Undergoing Medical Examination or Treatment
RCR	Royal College of Radiology
RPA	Radiation Protection Advisor
RT-PCR	Reverse transcriptase-polymerase chain reaction
SCC	Squamous Cell Carcinoma
SLND	Selective Lymph Node Dissection
SPET	Single-Photon Emission Tomography

TIC	Touch Imprint Cytology
TLD	Thermoluminescent Dosimeter
TLND	Therapeutic Lymph Node Dissection
UIQ	Upper inner quadrant
UOQ	Upper outer quadrant
VLLW	Very low level waste
57Co	57-Cobalt
99mTc	99m-Technetium
WLE	Wide local excision

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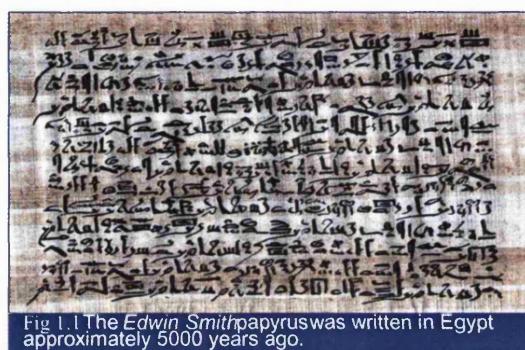
Chapter-1

A HISTORICAL PERSPECTIVE OF BREAST CANCER MANAGEMENT. AIMS OF THESIS

1.1 Historical perspective

....'If thou examinest a man (person) having bulging tumours of his breast and thou findest that swellings have spread over his breast; if thou puttest thy hand upon his breast upon these tumours, and thou findest them very cool, there being no fever at all herein when thy hand touches him; they have no granulations, they form no fluid, they do not generate secretions of fluid and they are bulging to thy hand. Thou shouldst say concerning him. There is no treatment'.

The *Edwin Smith* papyrus was written in Egypt approximately 5000 years ago. It was translated by J H Breasted¹ in 1930. It has the first description of the application of surgery to the treatment of illness. In fact it seems to be the first objective document concerning human illness. The above translation indicates that the ancient Egyptians distinguished inflammatory mastitis from carcinoma of breast. Some even go further and suggest that the above passage described locally advanced breast cancer perhaps with satellite nodules and demonstrated the wisdom of the unknown author in recognizing the futility of treatment².



In or around 400 BC, Hippocrates³ suggested the theory of humoral imbalances for the development of cancer but was unable to describe the nature of these imbalances. He also wrote 'It is better to give no treatment in cases of hidden cancer (referring to non-ulcerating carcinoma); treatment causes speedy death, but to omit treatment is to prolong life'. Approximately 500 years later Celsus⁴ made probably the first attempt to classify and stage breast carcinoma. He recommended four stages of breast cancer: (1) early malignancy (2) cancer without ulcer (3) ulcerating cancer (4) fungating cancer.

This was followed by Galen's theory of *Melancholia*⁴ (excess accumulation of black bile) as the cause of breast cancer in approximately 200 AD. In support of his view, Galen suggested that women clear themselves of black bile during their monthly periods and therefore after menopause they are not cleansed. He used this argument to explain the increased incidence of breast cancer in the fifth and sixth decade of life and suggested repeated bleeding (venesection) as a means of reducing the *Melancholia*.

Thomas Bartholin (1616-1680) was the first person to described the lymphatic system and suggested that the tumours were coagulems of lymph developing proximal to a blockage of lymphatic vessels. He suggested that both the humoral and mechanical theories of oncogenesis coexist and lead to tumour development⁴.

Gaspar Aselius, in 1627, for the first time demonstrated lacteals which he found in a recently fed dog. The similarity between the chyle of these lacteals and milk encouraged other seventeenth century observers to seek direct links between the lacteals and the lymphatics of the breast in order to explain lactation. In 1654 Pecquet saw the milk-laden lymphatics of a lactating bitch

enter the thorax. But he mistook the direction of the lymph flow and suggested: '*let him learn what path there is for chyle, unmixed with blood, to the breasts. Let him learn also that there is in the living body, just as in the sky, a milky way*⁵.

Cruikshank in 1786 first described the lymphatics of the human breast. He wrote: "... *two sets of absorbents, one accompanying the external thoracic artery and vein and the other the internal thoracic (mammary) vein. The external absorbents arise from the nipple and from the external part of the mamma, from the integuments and the tubuli lactiferi. They run outwards towards the axilla and sometimes pass through small glands halfway between the nipple and the axilla*⁵.

James Symes, then professor of surgery in Edinburgh, is credited for being the first person to make the association between involved axillary nodes and poor prognosis. In 1842 he wrote: ' The result of an operation for carcinoma when the glands are affected is almost always unsatisfactory however perfectly they may seem to be taken away. The reason for this is probably that the glands do not take part in the disease unless the system is strongly disposed to it'⁶.

In 1867, Moore⁷ of the Middlesex Hospital in London proposed that complete removal of the breast (total mastectomy) is the treatment of choice for lesions in any area of the breast. In 1880, Samuel Gross⁸, recommended that under the most favourable circumstances, namely when the tumour was small and there was no palpable axillary lymph nodes, the appropriate procedure was the removal of the entire breast with overlying skin, dissection of pectoral fascia and axillary lymph node dissection. Kuster⁹ in 1883 also recommended routine axillary clearance in all breast cancer cases.

In 1883, Sappey¹⁰, demonstrated a subareolar lymphatic plexus into which the subdermal and paranchymal lymphatics drained by using mercury injections into a lactating breast. He emphasized that the predominant lymphatic drainage was to the axilla and specifically denied that any lymph vessels left the posterior surface of the breast or that any leaving the breast reached the internal mammary chain.

It was Rudolf Virchow (1821-1902) who suggested that cancers arose from normal cells in reaction to abnormal stimuli, a singularly modern viewpoint⁴. Yet paradoxically it was Virchow who promoted the view of the centrifugal spread of cancer along the lymphatics by cellular proliferation. Virchow's viewpoint became dominant and contributed to the evolution of the radical mastectomy.

The introduction of the classical radical mastectomy in the latter part of the nineteenth century is credited to William Stewart Halsted¹¹. Halsted emphasised the role of lymphatic permeation as a major mechanism of tumour dissemination in breast cancer but failed to appreciate the role of haematogenic spread. It was assumed that a cancer spreads in continuity from its origin in columns of malignant cells. These passed along the lymphatic channels until they were arrested temporarily in the first group of regional lymph nodes, which were thought to act as filter. It was further assumed that when the filtration capacity of these lymph nodes was exhausted they acted as a nidus for tertiary spread to more distant lymph nodes and then via the fascial planes to the skeleton and the vital organs. Halsted even suggested that there was no skeletal involvement unless there was an overlying cutaneous metastasis with the skeleton being involved as a result of continuity from the original growth via

the skin metastasis. With this concept, it seemed reasonable to assume that radical en bloc surgery would cure more patients than local excision of the breast alone. Halsted proposed that this radical surgical approach to breast cancer management was the key to successful treatment of the disease. He reported local and regional recurrence rates of 6% and 16%, respectively. This seemed like a major therapeutic achievement, yet were no reports on survival benefit.

Gross of Philadelphia⁸ reported a 9% ten year survival in a series of patients treated with simple mastectomy alone. Lewis and Rinehoffs¹² from the John Hopkins Hospital published the results of patients treated by radical mastectomy and reported only a 12% ten year survival. Haagensen and Stout¹³ also critically analysed the data on 640 patients who had undergone radical mastectomy over a 9 year period and discovered that only 36% of the patients were alive after five years. There was no doubt that the radical operation reduced the incidence of local recurrence, but it was becoming clear that there was no improvement on survival rate

In 1927, Sampson Handley of the Middlesex Hospital reported on the clinical significance of the internal mammary lymph node. In 1948 Patey¹⁴ described a less radical approach to surgery of primary breast cancer. He introduced a modified radical mastectomy as a less mutilating operation with similar outcome as radical mastectomy. The pectoralis major was preserved but the extent of axillary surgery remained the same.

In 1965 Devitt¹⁵ suggested that , in breast cancer: “axillary lymph node metastases are an expression of a bad prognosis rather than a determinant”.

In 1960's, the Halstedian viewpoint was replaced by new concepts. Bernard Fisher¹⁶ suggested a new biological hypothesis. He perceived breast cancer as a systemic disease at the time of diagnosis and variations in loco-regional treatment would not improve the overall survival. He explicitly stated that "biological rather than anatomical factors would explain why certain lymph nodes contain metastasis and others do not. Regional lymph node do not provide barrier to tumour cell spread. The blood and lymphatic system are so unified insofar as tumour spread is concerned, that there can be no orderly pattern of tumour cell dissemination based on mechanical considerations". It was also suggested that the time and rate of systemic spread would depend not only on the innate aggressiveness of the tumour but the immunocompetence of the host. In presenting the long-term data from the National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol B-04¹⁷, it was demonstrated that patient survival was identical whether ALND was performed or not. The therapeutic consequences of this paradigm shift was to minimise the importance of loco/regional therapy and to concentrate on systemic approaches directed to the treatment of micrometastases. Breast conservation surgery was to come into focus and radical operations were progressively discontinued.

Although breast conservation had been advocated by some in the years preceding the second world war, it wasn't until the late 1960's and early 1970's that proper randomised controlled trials were launched. These ultimately demonstrated that a combination of local surgery and radiotherapy could achieve similar results to those obtained with radical and modified radical mastectomies, although total axillary clearance remained an integral part of this procedure, too¹⁸.

It is understandable therefore that over the years, a less invasive approach to axillary management has been sought for staging purposes. Palpation is notoriously unreliable in staging the axilla with sensitivity and specificity data of less than 70%^{21,22}. Other methods have been evaluated to determine the axillary nodal status. These include lymphoscintigraphy²³, CT scanning²⁴, ultrasound scanning²⁵, ^{99m}Technetium-Sestamibi²⁶, positron emission tomography F-DG PET^{27,28}, and MRI scanning. There is no evidence that any of these imaging modalities can reliably reflect the true status of the axillary lymph nodes.

1.2 Axillary Surgery

The management of the axilla in breast cancer patients has been the subject of intense debate and controversy. Despite a tendency towards a conservative approach for the surgical treatment of primary breast carcinoma, the complete axillary lymph node dissection (ALND) has remained an integral part of breast cancer management for over a century. This is because the histological status of the axillary lymph node is still considered the single most important prognostic indicator in breast cancer patients. A lack of imaging techniques or a minimally invasive procedure to determine the status of the axillary lymph node with acceptable accuracy has been the main reason for continuing to perform ALND. It is ironic that the extent, morbidity and cost of the staging procedure (ALND) is greater than the surgical treatment of the primary tumour.

Recent epidemiological studies indicate that breast cancers are smaller in size at presentation and have less likelihood of lymph node involvement than in the past. This could be as a result of widespread use of mammographic screening and increased patient's general awareness²⁹⁻³⁰. In T1/T2 tumours up to 70% of patients have a negative axillary dissection³¹ and more than 50% of

these node negative patients will develop a morbidity related to ALND^{30,32}. Histological examination of axillary lymph nodes is the most reliable technique to determine the axillary nodal status in breast carcinoma but the extent of axillary dissection is controversial. This varies from axillary sampling to complete axillary nodal dissection (ALND). A well performed level III axillary clearance provides for excellent tumour control and a recurrence rate of less than 2%³³. Nevertheless there are still potential complications associated with this procedure. These include post-operative pain, seroma formation, limitation of shoulder movements, paresthesia and numbness of upper arm, inadvertent damage to neuro-vascular structures and the distressing complication of lymphoedema. Moreover, the hospital stay is prolonged which has its cost implications.

Single axillary node biopsy has been tried by Davis et al³⁴ with a failure rate of 42%. Locker et al³⁵ performed triple node biopsy (axillary, apical and internal mammary) but the recurrence rate was 21%. The technique of axillary sampling involving dissection of axillary tail and adjacent tissues containing central axillary node group is often inadequate³⁶. Steel et al³⁷ in a randomised trial of 417 patients with operable breast cancer, suggested that excision of at least four nodes from lower axillary fat pad was as accurate as ALND but Kissen et al³⁸ reported that axillary sampling failed to identify axillary nodal metastasis in 8% of patients and in another 10% of cases there were no identifiable lymph nodes.

1.3 Prognostic importance of ALND

The axillary lymph node status is the most important prognostic indicator in breast cancer and the presence of axillary metastasis is associated with a

reduced overall and disease free survival^{39,40}. The number of involved axillary nodes also has a prognostic significance^{39,41}. The results of a national survey by the American College of Surgeons³⁹ indicate a 72% survival and 19% recurrence rate in the absence of any axillary nodal disease. These data change to 63% and 33% in the presence of 1 positive lymph node and to 52% and 44% in the presence of 4 positive lymph nodes, respectively.

1.4 Therapeutic value of ALND

There is substantial evidence that ALND provides for good local disease control in the axilla with local recurrence rates of 2% or less^{33,41,42}. Control of local disease within the axilla is essential since axillary recurrence is difficult to treat, can be exceedingly unpleasant to the patient and contribute to a significant reduction in the quality of life. The NSABP trial protocol B-04¹⁷ concluded that failure to treat involved axillary nodes is not associated with worse survival outcome. The CRC trial for early breast cancer⁴³ also reported no survival difference between patients treated with simple mastectomy alone (with radiotherapy later if there was disease recurrence) and those treated with mastectomy and immediate post-operative radiotherapy despite a highly significant increase in recurrence rate in the first group. Some authors have taken an opposing view and argued that the local disease control may translate in improved overall survival⁴⁴⁻⁴⁷ and others have suggested that ineffective axillary treatment may result in survival disadvantage⁴⁸⁻⁵⁰. McMaster et al⁵¹ argues that this potential survival improvement as a result of ALND must be considered carefully before this procedure is abandoned in favour of a less invasive technique.

1.5 Axillary External Beam Radiotherapy

Many studies have demonstrated that radical axillary radiotherapy is as effective as ALND in preventing axillary node recurrence and authors have questioned the therapeutic value of ALND⁵²⁻⁵⁴. Nevertheless two commonly mentioned complications of radiotherapy, although rare, are brachial plexus neuropathy and lymphoedema. Oslen et al⁵⁴, studied 161 recurrence-free patients for this complication with a median follow-up of 50 months. They reported that 5% of the patients receiving radiotherapy developed a disabling radiation induced brachial plexopathy and 9% developed mild symptoms. It seems that this complication is directly related to the radiotherapy technique and with special attention to treatment fields and dosimetry, this problem can be avoided. We prefer ALND to axillary irradiation in our surgical practice because although brachial plexus neuropathy is rare, it is a devastating and untreatable complication.

1.6 Adjuvant Systemic Therapy

Although axillary staging in breast cancer can help in rational decision making about adjuvant therapy, such therapy decisions can also be made without reference to lymph nodal status. Currently, adjuvant systemic therapy in the form of cytotoxic chemotherapy or hormonal therapy alone or in combination is recommended for most invasive breast cancers. The recent world overview of Early Breast Cancer Trialists' Collaborative Group⁵⁵ on adjuvant polychemotherapy indicates that the proportional risk reduction for recurrence and mortality appears to be the same for node negative and node positive breast cancer patients. Nevertheless in terms of 10 year survival, the absolute benefit is 7% for those patients with node negative disease and 11% for those

with node positive disease. It also indicates that adjuvant polychemotherapy is associated with a more modest improvement in long-term survival and reduction in local recurrence rate of 2-3% in those aged 50-69. This indicates that age alone should not be a barrier to the use of adjuvant polychemotherapy (up to the age of 70). These findings were irrespective of oestrogen receptor status or whether adjuvant tamoxifen was given or not. Moreover the recent world overview on adjuvant tamoxifen for early breast cancer⁵⁶ indicates that the proportional mortality reduction is similar for node positive and node negative women although the absolute benefit is higher for node positive patients. In the trials of adjuvant tamoxifen therapy for 5 years; the absolute improvements in 10-year survival were 10.9% (SD 2.5) for node positive and 5.6% for node negative patients (SD 1.3). In the context of proportional risk reduction from adjuvant tamoxifen therapy, it is suggested that the fundamental parameter is the oestrogen receptor status of the primary tumour and not whether the patient is young or old, with or without nodal involvement or receiving chemotherapy or not⁵⁶. In the light of these findings one can strongly argue that knowledge of the nodal status is not important in deciding whether adjuvant systemic therapy is required or not, although it may help in choosing the type of such therapy. Yet there are growing indications that the number of involved axillary lymph nodes does affect the type of adjuvant chemotherapy that patient receives⁵⁷ although this is NOT yet evidence based. Presence of unfavourable primary tumour characteristics such as large size, lympho-vascular permeation, poor nuclear grade, peri-neural invasion, S-phase using flow cytometry, overexpression of oncogene, absence of oestrogen receptor, highly proliferative indices, tumour aneuploidy and presence of c-erbB2 or Cathepsin D⁵⁸⁻⁶⁵ may be of equal value

in decision making regarding administration of adjuvant chemotherapy irrespective of axillary nodal status.

1.7 Axillary management in small breast cancer

In a study performed by Silverstein et al²⁹, in 1031 patients with breast carcinoma, three parameters were analysed: axillary node positivity, disease free survival and breast cancer specific survival. The authors divided the patients into six subgroups according to tumour size (T category). These included Tis (DCIS), T1a, T1b, T1c, T2 and T3. It was noted that the nodal positivity for DCIS was 0%; T1a 3%; T1b 17%; T1c 32%; T2 44%; T3 60%. There was reduction in the disease-free and breast cancer specific survival with every increment in T value. The authors make a case for elimination of routine ALND in patients with T1a breast carcinoma as only 3% are likely to be positive. “How can we justify 100 node dissections in an attempt to find three patients with positive nodes to treat with chemotherapy, one of whom, at most, will be helped. The cost of such procedure was estimated to be in a region of 1 million US dollars⁶⁶. Table-1.1 summarises the results of four studies with a total number of 4937 patients analysed to determine the frequency of lymph node positivity according to the tumour size⁶⁷.

Specific histological types of breast cancers with very good prognosis (e.g. tubular, papillary and colloid) are another group which have an extremely low rate of axillary lymph node metastasis and would not benefit from routine ALND and adjuvant systemic therapy.

Author	Group	T1a size	T1b (<0.5 cm)	T1c (0.5-1.0 cm)	T2 (1.0-2.0 cm)	T2 (2.0-5.0 cm)
Silverstein	1031	3	17	32	44	
McGee	3077	12	23	33	54	
Giuliano	259	10	13	30	-	
Cady	570	-	17	31	44	
Weighted Average	4937	7%	19%	32%	51%	

Table-1.1 Frequency of tumour positive lymph nodes in breast cancer related to tumour size

1.8 Sentinel node concept

Lymphatic mapping and selective lymphadenectomy is an attractive approach in the management of patients with breast carcinoma. This approach aims to stratify patients for appropriate surgery without submitting the majority of those without lymph node metastases to an unnecessary regional lymph node dissection.

Table-1.2 summarises the theoretical basis for sentinel lymph node concept.

Theoretical basis for SLN concept
Lymph flow is orderly and predictable
Tumour cells disseminate sequentially
The sentinel node is the first node encountered by tumour cells
Sentinel node status predicts distant basin status
Patients present with earlier stage of disease
Basin involvement is less frequent
Surgery can be targeted to appropriate population

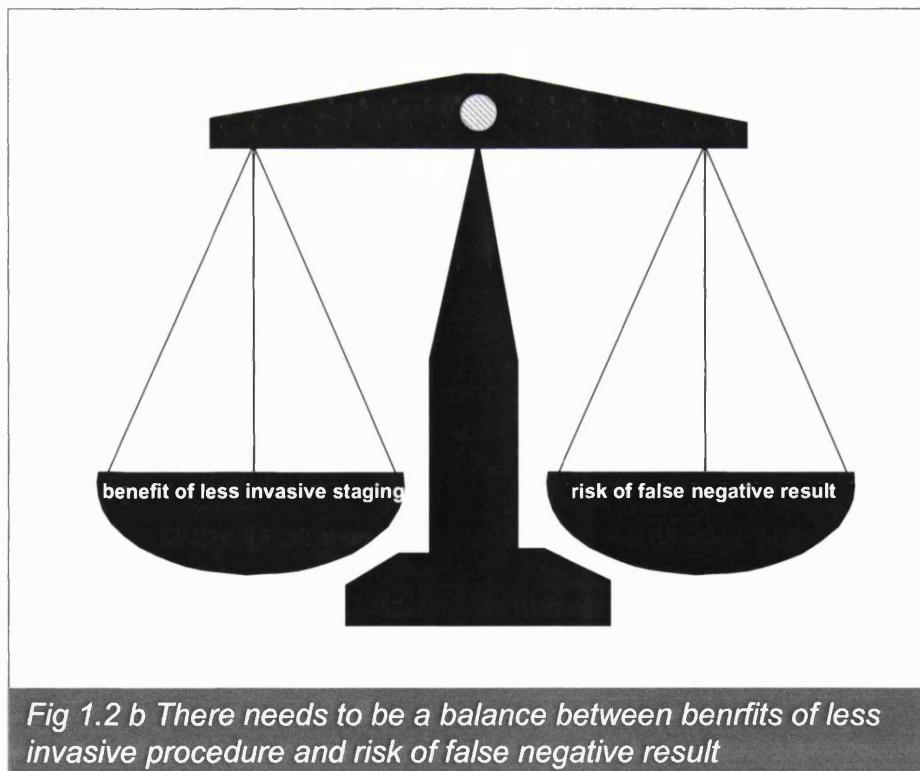
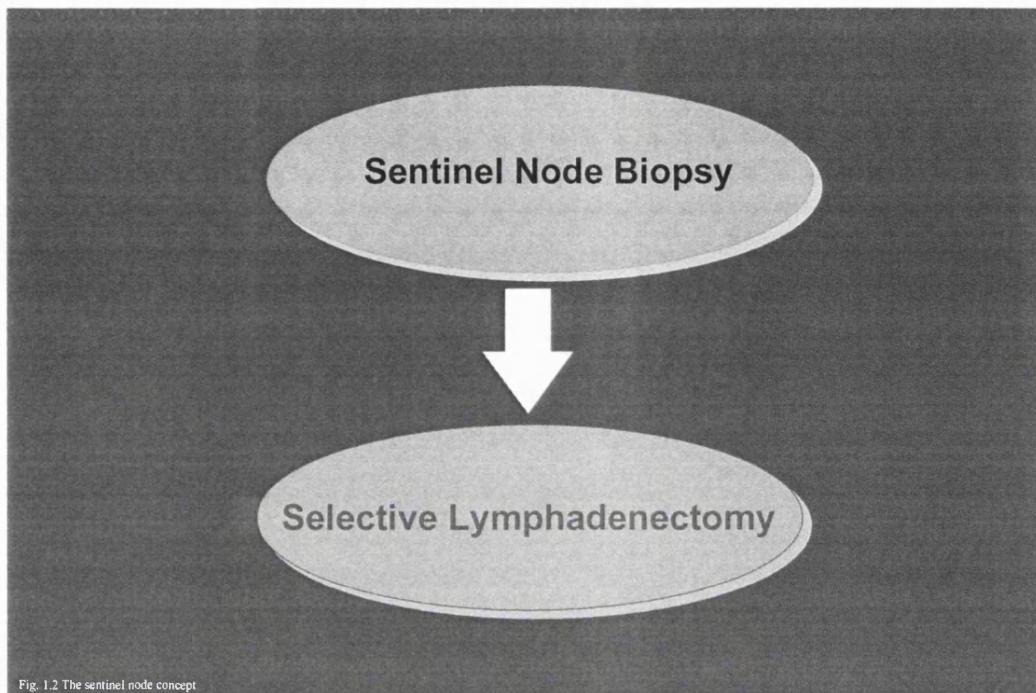
Table-1.2

The sentinel node is the lymph node at greatest risk of harbouring metastatic deposits. Retrieving this node requires a concerted effort from nuclear medicine physician, surgeon and pathologist. This approach is minimally invasive and appears to allow the same information to be gathered as with axillary node dissection but with less morbidity.

This concept was introduced by the pioneering work of Donald L. Morton and colleagues from the John Wayne Cancer Center in Santa Monica. He proposed the concept of "lymphatic mapping with sentinel lymph node biopsy" in the management of patients with malignant melanoma and the first paper was published in 1992⁷⁰. The definition that was offered for sentinel lymph node by Morton and co-workers was: 'any lymph node which receives direct drainage from a tumour'. The concept was based on the hypothesis that lymph flow is orderly and predictable and that tumour cells disseminate sequentially.

If the SLN concept is proven to be true, it will enable surgeons to perform 'selective lymphadenectomy' which is a new approach in cancer staging (fig 1.2). On the other hand it is important to appreciate that the SLN biopsy is only a diagnostic test. Before it is introduced into routine clinical use, its role and accuracy must be established.

The critical issue in sentinel node biopsy is the false negative rate⁵¹. Presently ALND is associated with a false negative rate of up to 3%⁷³⁻⁷⁴. What remains difficult is to determine what is an accepted false negative rate. "At what stage does the benefit of a less invasive staging investigation outweigh the risk of false negative result?"⁵¹.



1.9 Aims of this Thesis

The publication of Veronesi et al⁷⁵ in the Lancet in 1997 generated significant attention. In the UK, at the time of beginning of this project, no data were available, in peer reviewed literature, on the accuracy and reliability of the SLN biopsy as a staging procedure in breast cancer.

The aims of this thesis are to test the following hypotheses:

1. The histological status of the SLN is a true predictor of the axillary lymph node status in patients with breast carcinoma.
2. The SLN concept is valid in other areas of Surgical Oncology including malignant melanoma, colorectal carcinoma, anal, penile and head and neck carcinoma.
3. Touch imprint cytology is a reliable intra-operative diagnostic tool in determining the histological status of the sentinel lymph node.
4. Optical biopsy can be used as a reliable intra-operative diagnostic tool to establish the histological status of the sentinel lymph node.
5. Injection of the radiopharmaceutical with the needle free injection system leads to successful localization of the SLN.
6. Radiation risk to patients and staff groups involved in all aspects of the SLN technique is minimal.
7. SLN biopsy in breast carcinoma is associated with minimal morbidity and is cost-effective.

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Chapter-2

SENTINEL NODE CONCEPT

2.1 Introduction

Lymphatic mapping and sentinel node biopsy (SLNB) is one of the most important recent developments in surgical oncology. This approach aims to stratify patients for appropriate surgery without submitting the majority of those without lymph node metastases to an unnecessary regional lymph node dissection.

A review of the literature reveals that this concept was described previously. In 1959 Ernest Gould¹ presented a paper at the twelfth Annual Symposium of the James Ewing Society (predecessor of the Society of Surgical Oncology), entitled "Observations on a *sentinel node* in cancer of parotid". Based on twenty eight patients who were studied over an eight year period, routine excision of 'angular node' for intra-operative frozen section was recommended. "The histology of this sentinel node may guide the surgeon in his decision regarding radical neck dissection in continuity with parotidectomy". The significance of this message was not appreciated at that time.

Three years later, in 1963, Oliver Cope² referred to the 'Delphian node' as a node that will 'foretell the nature of disease process' affecting a nearby organ. In 1977, the Urologist, Ramon Cabanas³ was the first person to introduce the SLNB concept as a staging procedure in penile carcinoma. He proposed that squamous cell carcinoma of penis initially drains to a particular lymph node in the groin that is always in the exact position around the superficial epigastric vein. He confirmed this sentinel lymph node (SLN) to be the first site of metastasis and recommended routine bilateral SLNB as a logical approach in the treatment

of penile cancer. Block groin dissection would follow only after a positive SLNB. The reliability of Cabanas approach however, was limited by the relatively crude localization technique. He injected contrast media into the dorsal lymphatics of the penis. He then proceeded with a pelvic X-ray and the first visualised lymph node was designated as the sentinel lymph node. Despite the initially interesting results, Cabanas' concept fell into oblivion.

This was followed by the pioneering work of Donald L. Morton and Alistair Cochran⁴ from the John Wayne Cancer Center in Santa Monica. In the late 1980's he proposed the concept of "lymphatic mapping with sentinel lymph node biopsy" in the management of patients with malignant melanoma. His landmark article was published in 1992.

Morton and co-workers defined the sentinel node as: 'the initial lymph node upon which the primary tumor drains'. In other words, the sentinel node (first-tier node) is the lymph node which is in the direct drainage pathway of the primary tumour. The concept was based on the hypothesis that lymph flow is orderly and predictable and that tumour cells disseminate sequentially. As the first lymph node to meet the tumour cells has the highest chance of harboring the metastatic disease, its histological status would predict the status of the remainder of the lymphatic basin. This concept provided a new opportunity to stratify patients for appropriate surgical intervention. By suggesting that other lymph nodes would become involved in a later phase, Halsted's theory of *sequential tumour dissemination* was revived⁵.

Applying existing technology, Morton and co-workers developed a practical approach to identify the sentinel lymph node. After preliminary animal studies using Patent Blue-V and Isosulfan Blue dye, the technique was tested in a

clinical setting in patients with malignant melanoma. They injected vital blue dye intradermally to stain the lymphatics, followed by a careful surgical exploration of the regional basin. In 1993 Alex and associates⁶ injected radionuclide (^{99m}Tc-sulphur colloid) and introduced the technique of gamma probe guided surgery. Following the success of the sentinel node concept in melanoma, it was applied to patients with breast cancer.

There has been immense interest in SLNB judging by the many publications in the peer-reviewed literature. Significant attention is given to this subject in editorials in major medical journals⁷⁻⁹. The reports are almost uniformly enthusiastic about the potential of this technique and even surgical guidelines have been published for sentinel lymph node detection in carcinoma breast. Proponents of the sentinel node technique argue that by pre-selecting a lymph node that is most likely involved with metastatic deposit, the pathologist can be guided to perform a more detailed histological analysis and the likelihood of detecting micrometastatic disease is greatly increased¹⁰. Despite all this enthusiasm there are significant differences in practice, which involve almost all aspects of the technology involved (table-2.1).

Interestingly, and in spite of these differences, reported data is, in general, most encouraging. It seems relevant to review the subject of the detection of the sentinel lymph node and discuss the present areas of uncertainty.

Technical Issues	
What probe?	
What tracer?	
What injection site?	
Single or multiple injection sites?	
Large or small volume of injectate?	
Massage or no massage of injection site?	
What dosage?	
Which mode of detection is preferable?	<ul style="list-style-type: none"> -Probe detection only -Probe detection and imaging -Patent blue dye alone or in combination with probe detection -Sentinel lymph node detection only -Sentinel lymph node detection and lymphoscintigraphy
Which form of imaging is best?	<ul style="list-style-type: none"> -Dynamic imaging -Early imaging -Early and late imaging
Which is the most appropriate site of injection?	<ul style="list-style-type: none"> -Intra-tumour injection -Peri-tumour injection -Sub-dermal injection -Sub-cutaneous injection
What pathological evidence is required?	<ul style="list-style-type: none"> -Frozen section histology -Imprint cytology -Haematoxylin and eosin staining -Serial sectioning -Cytokeratin immunohistochemistry (e.g. MNF116) -Polymerase chain reaction

Table 2.1: Technique and methodologies in sentinel node detection and imaging

2.2 Technical Consideration

There are several techniques for the detection of the sentinel lymph node, different radiopharmaceuticals are also available for injection, there is controversy as to the injection site, practice varies from single to multiple injections, it varies considerably with large or small volumes of injectate used and there is also considerable variation in the amount of radioactivity given to patients. From a detection point of view there are those groups, which advocate external probe detection only! Others combine external probe detection with radionuclide imaging with a gamma camera. There are groups, which have still advocated the use of patent blue dye alone or indeed in combination with probe detection. There are also groups who have aimed at the detection of the sentinel lymph node alone whilst others have attempted to combine detection of the sentinel node with the detection of all lymph nodes in the appropriate lymph basin. There is significant discussion as to the minimal amount of histopathological evidence, which is required to be obtained from the sentinel node.

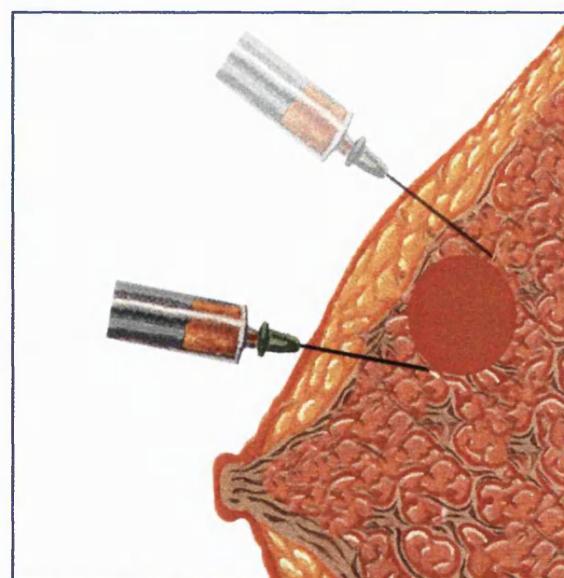
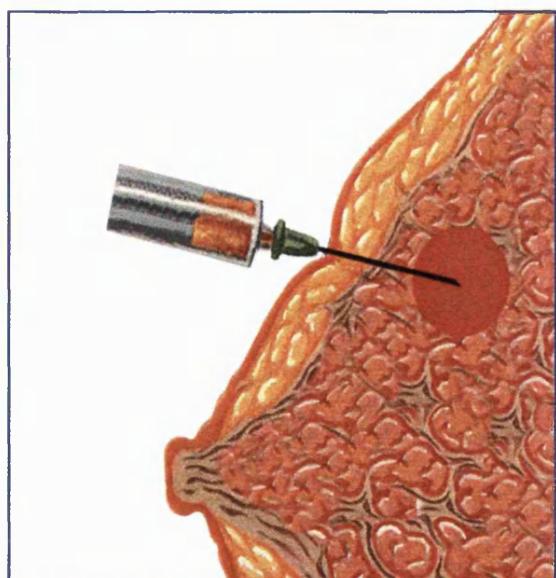
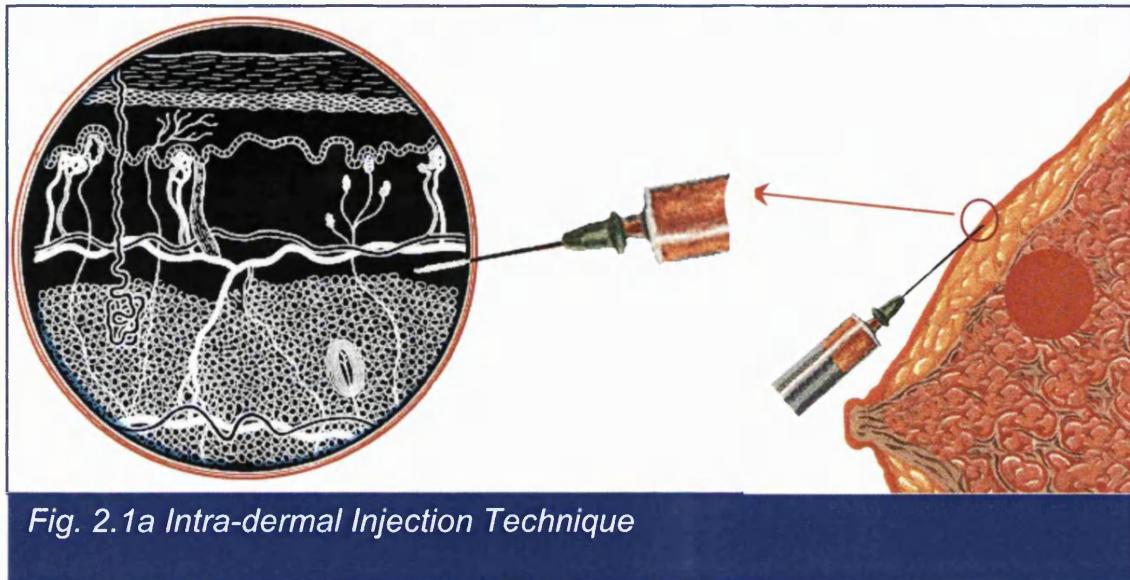
It is astonishing that groups have used such varying techniques in terms of the delivery of an appropriate radiopharmaceutical to the area of interest to be investigated. It is well known from the literature that lymphatic tissue is most prevalent in the peripheral layer of the skin, such that an intra-dermal injection will deliver the tracer to an area rich in lymph in contrast to sub-cutaneous tissue, which has fewer lymphatic vessels (fig.2.1a). It is also well known that the direct injection of the tracer into a tumour will have as a consequence the administration of an indicator into a high pressure system which is not physiological (fig.2.1b). Moreover there is an unavoidable concern that this may

lead to increased seeding of malignant cells from the needle track as a result of puncture of the tumour. It would therefore seem appropriate to only pursue a direct intra-tumour injection if there are clear and overriding advantages to other techniques. There is scant evidence in the literature for this.

A peri-tumour administration of tracer, which is one of the commonly practised techniques, will deliver the radiopharmaceutical into a relatively rich area of lymphatics. The migration of colloidal particle is much slower than seen with a subdermal technique and there is a need for a larger volume of the injectate and higher dosage of radiopharmaceutical to be administered (fig. 2.1c).

It is also clear from the literature that as the sophistication of the methods used to gather pathological evidence from sentinel lymph node increases, so does the sensitivity of detection of micrometastases. The significance of detecting a few if not single micrometastases in a lymph node remains unknown.

From a legal point of view, radiation protection legislation differs significantly from country to country but in general, a radiopharmaceutical needs to be licensed before it is made available for routine use. The tracers used in Europe for lymphoscintigraphy and sentinel lymph node detection were mostly developed in the early 1970's and were aimed at the imaging of the reticuloendothelial system of the liver and spleen and bone marrow. The properties and overall characteristics of these tracers have therefore not been optimised, in general, for sentinel lymph node detection. There is a significant variability in the particle size of these tracers and



ultimately, if this approach is to succeed, an appropriate licensed product will be required .

Different results have been obtained in respect to specific patient groups. It is now evident from the literature that poorer results in the detection of the sentinel lymph node are clearly obtained when all lesions are included, when multicentric lesions are investigated, when patients are investigated who have already undergone a surgical procedure. A refinement of the appropriate indications and patient selection is in progress with the ongoing trials. Similar comments can be made for the selection of sentinel lymph node detection in patients with melanoma.

2.3 The lymphatic system

In the last 40 years, a significant body of knowledge has accumulated about the lymphatic system, its dynamics and circulation. Figures 2.2 and 2.3 show a diagrammatic representation of a lymph vessel and its relationship with the surrounding milieu. It can be seen that lymph vessels are larger than the surrounding capillaries, that they are end or terminal vessels with lymph flowing in a single direction determined by valves which ensure the uni-directionality of this flow.

Individual lymphatic capillaries are kept in the cellular tissue matrix by so-called anchoring filaments which will distend if the surrounding environment is distended (for example by the administration of a certain volume of a substance). Particulate substances of appropriate size when delivered to the interstitial fluid can traverse the lymphatic capillary endothelium and hence be removed by the lymphatic system towards the first lymph draining nodes.

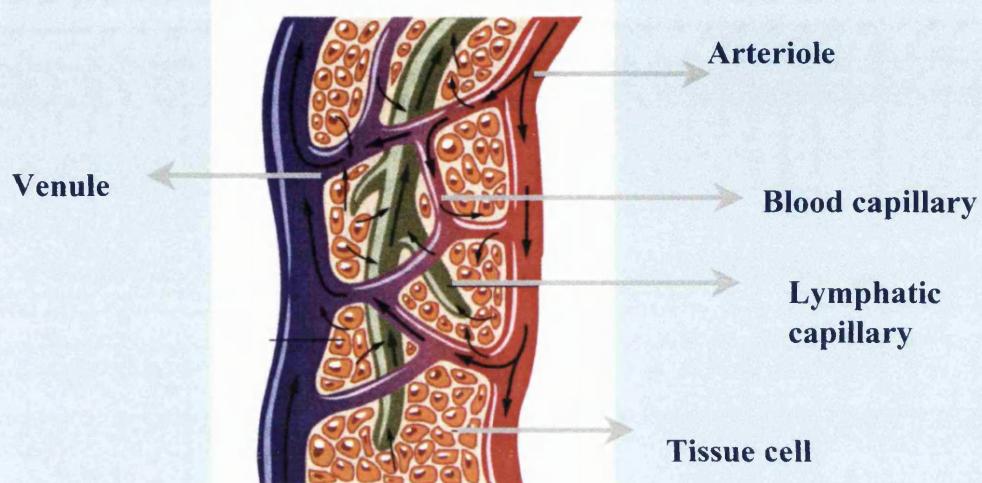


Fig 2.2 Schematic representation of lymphatic and blood vessels

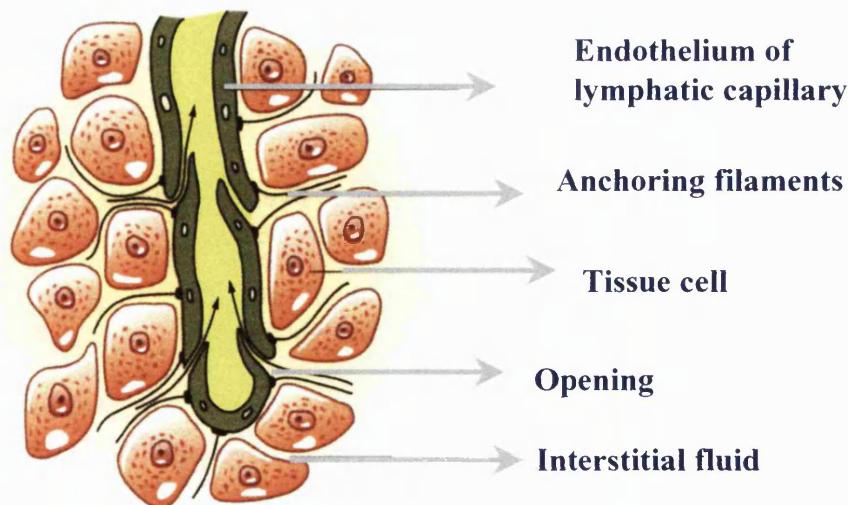


Fig. 2.3 A representation of a lymphatic capillary

Approximately 90-95% of lymph is formed by capillary filtration as fluid leaks out of capillaries into the interstitial space and is not directly reabsorbed into the venous capillaries¹¹. The other 5-10% is produced by cells during aerobic metabolism.

Approximately three litres of lymph flow into the circulation per day, equivalent to 120 mls of lymph flow per hour at rest. Lymphatic flow can increase with exercise by a factor of 10 to 30 and lymphatic channels are seen to contract and relax every 2-3 minutes. Table 2.2 summarises factors that affect lymphatic flow.

Factors affecting lymph flow
Movement of tissues
Tissue compliance and temperature
Intrinsic lymphatic pump
Gravity
Chemical and humoral agents
Inflammation
Increased interstitial pressure
Venous occlusion

Table-2.2

There is still uncertainty and contradiction regarding the exact anatomical distribution and functional pathways of lymphatic flow from the breast. The mammary gland is developmentally derived from the ectoderm¹² and the dermal and parenchymal lymphatics of breast meet at the sub-areolar lymphatic

plexus¹³ and from here one to two main lymphatic trunks drain towards the axilla. Sappey's illustration of one or two large collecting lymph trunks originating from the subareolar lymphatic plexus (fig. 2.4) has been confirmed by other investigators using direct lymphangiographic techniques^{14,15}.

The first demonstration of lymphatics by intra-lymphatic administration of Ethidol is attributed to Kinmonth¹⁶ (1952). The first lymph autoradiography, carried out in rabbits is attributed to Sherman and Ter-Pogossian¹⁷ (1953). These authors first demonstrated the concentration of radioactive colloid gold following an interstitial injection. At the Middlesex Hospital in 1954 Handley and Thackray, investigating the lymph spread in patients with carcinoma of the breast, already noted in a series of 150 patients that 10% of these were free of axillary disease. In the same year Turner-Warrick¹⁸ performed one of the first lymphoscintigraphic studies accompanied by blue dye lymph drainage visualisation, publishing his findings in the Lancet in 1955. Grant¹³ also described an intricate system of closely-related cutaneous and parenchymal pathways, which are responsible for lymphatic drainage of breast.

Hultborn et al¹⁹ in 1955 performed a lymphoscintigraphic study investigating the drainage of lymph from the breast to the supplying basins. These authors showed that most of the breast lymph would drain to the axilla in these patients. Other studies support the fact that the principal route of regional drainage is predominantly towards the axillary nodes^{20,21}. All quadrants also show some lymph drainage to internal mammary nodes, but the proportion has been estimated to be very small²⁰. Regardless of tumour location within the breast, early dissemination will very likely involve the axillary nodes. While there are undoubtedly several alternative lymph drainage routes, these secondary

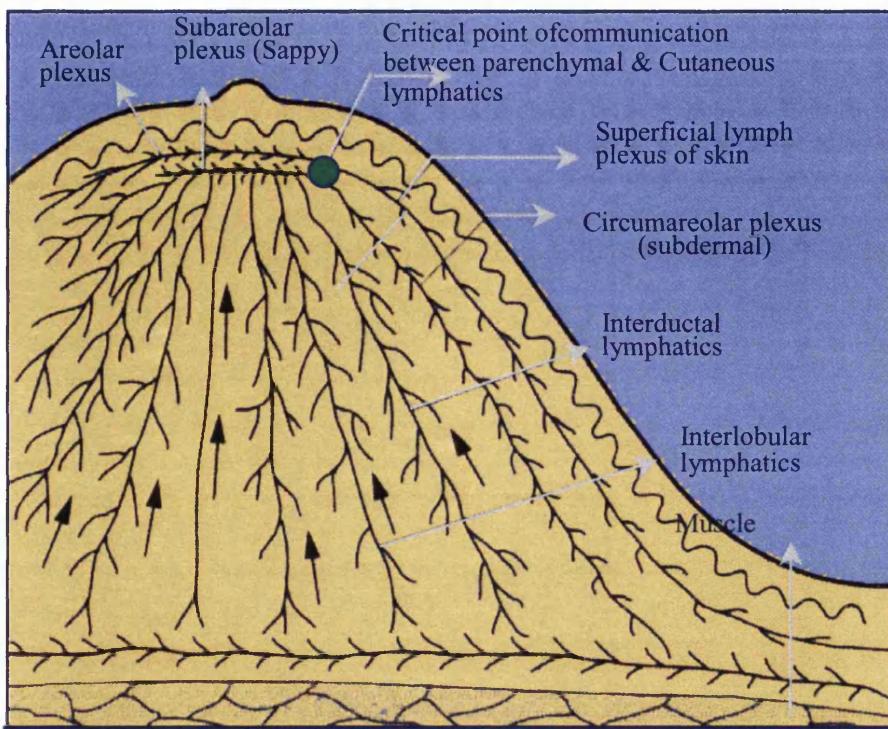


Fig. 2.4a According to Sappy, all lymph from breast drains into subareolar plexus

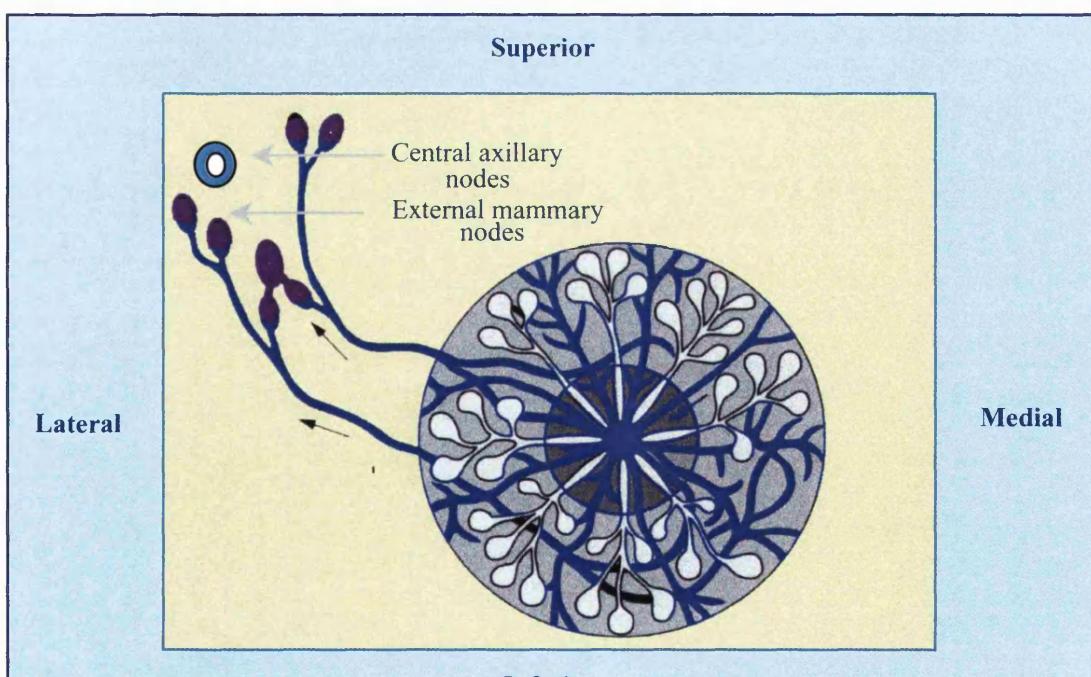


Fig. 2.4b Demonstration of critical point of communication between parenchymal and cutaneous lymphatics

pathways only assume clinical significance in advanced states of lymphatic spread.

Uren et al²² investigated the pattern of lymphatic drainage in a group of 34 patients with breast cancer. Drainage exclusive to the axillary nodes was found in 58% of all cases, to the axillary and internal mammary node chain in 19.4% of cases, to the axilla plus internal mammary node plus sub-clavicular node chain in 13% of cases, to the axilla and intra-clavicular node chain in 3.2% of cases and in 6.4% of cases preferential drainage was seen in the internal mammary node. It is important to underline that this study was carried out with an interstitial administration of a very small particle (few nm) sized antimony sulphide colloid. A technique with multiple injections was used, surrounding the breast mass. In this sense the technique was optimised for lymphoscintigraphy but clearly not optimised for the detection of the sentinel lymph node. As the years passed by, almost every lymphatic basin and region was demonstrated by the technique of lymphoscintigraphy and much useful information was recorded in the literature, with a variety of injection techniques and tracers (table-2.3).

As far as internal mammary node is concerned clinical studies have shown that internal mammary node involvement is uncommon when axillary nodes test negative; the greater the extent of axillary involvement, the greater the likelihood of internal mammary metastases²³.

There are still uncertainties surrounding tumour cell kinetics and the mechanisms initiating tumour cell dissemination.

Injection Site	Lymph node groups
Dorsum of foot	Femoral, inguinal, external iliac, para-aortic
Dorsum of hand	Epitrochlear, axillary, supraclavicular
Mammary, periareolar	Axillary, supraclavicular, upper parasternal
Chest wall	Axillary, supraclavicular, upper parasternal
Subcostal post. rectus sheath	Diaphragmatic, parasternal, internal mammary
Buccal mucosa	Jugular
Orbit	Deep cervical
Larynx	Parapharyngeal, superior/ inferior jugular
Hepatic capsule	Right parasternal, mediastinal
Splenic capsule	Splenic hilar
Lower oesophagus	Mid-mediastinal, Coeliac, upper peri-aortic
Gastric cardia	Coeliac, upper peri-aortic
Peritoneal cavity	Anterior mediastinal
Vulva	Inguinal, external iliac
Rectal	Sup. haemorrhoidal, inf. Mesenteric, perirectal
Intraprostatic	Peri-prostatic, internal iliac

Table-2.3 Lymphatic regions demonstrated by lymphoscintigraphy (from Ege GN. Lymphoscintigraphy in oncology. In Henkin et al., eds. Nuclear Medicine, vol II. St. Louis: Mosby; 1996:1505-1523)

While direct permeation of lymphatics by tumour can occur, it has long been clear that 'tumour embolism' represents the major mode of spread. Entrance of tumour emboli to the lymph vessels is gained through patent junctions between endothelial lining cells, which may be opened by traction on anchoring filaments due to an increase in interstitial fluid volume. It is possible for tumour emboli to

become trapped in superficial lymph vessels leading to the development of satellite and in-transit metastases, so typical of malignant melanoma. After gaining access to the lymph node, tumour cells are arrested in the subcapsular sinus, where tumour growth continues (fig. 2.5).

There are many collateral lymph vessels leaving a specific tributary region and these anastomose freely during their course towards the draining nodes. This provides for alternative lymph routes, although they only become accessible once distal obstruction leads to incompetence of the valves. In this way, the spread of tumour may become retrograde and thus appear to be unpredictable²⁴. Gilchrist²⁵ clearly illustrated this process by injecting coloured particle suspensions into afferent lymph vessels. Increasing pressure eventually caused re-routing of the lymph flow through proximal collateral channels to reach retrograde nodes.

The concept originally expressed by Bartels²⁶ in 1909 that the only way for lymph to reach blood is through at least one lymph node is challenged by Fisher²⁷. He proposed that tumour cell emboli, in addition to being carried to the regional lymph node, may bypass such node and gain access directly to the thoracic duct and systemic circulation (fig. 2.6). He also challenged Virchow's theory of 'lymph nodes are effective barrier to tumour cells' and believed to the contrary²⁷.

As far as the existence and anatomical location of direct lymphatico-venous communications is concerned, there is still uncertainty. Rusznyak²⁸ has summarised most of the reported evidence and concluded that, although lymphatico-venous communications may be demonstrated experimentally,

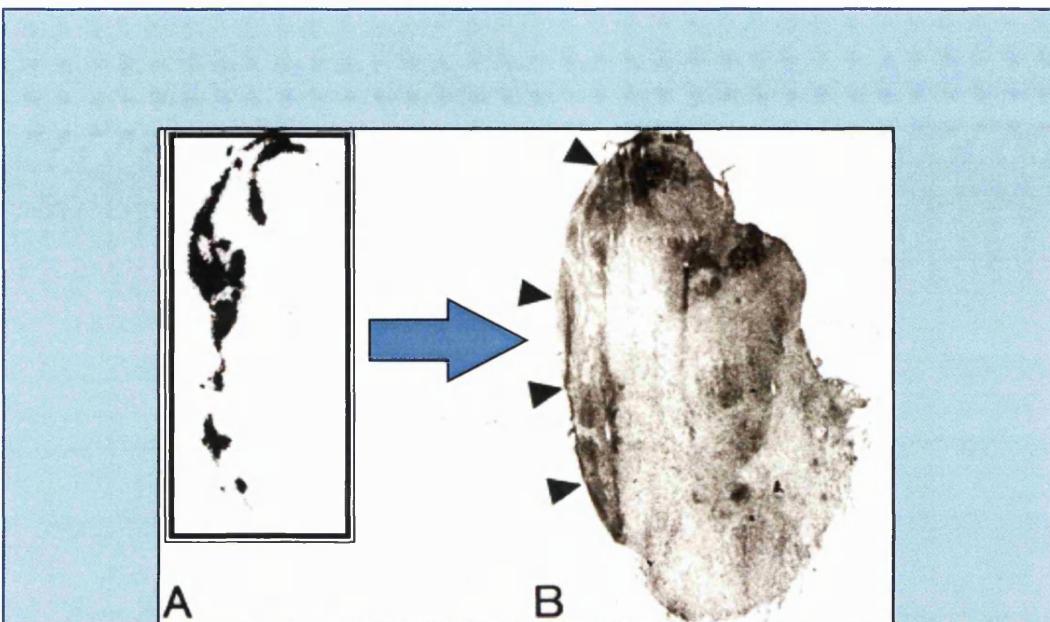


Fig.2.5. Autoradiograph of a sentinel node showing the distribution of radioactive particles along the subcapsular sinus.

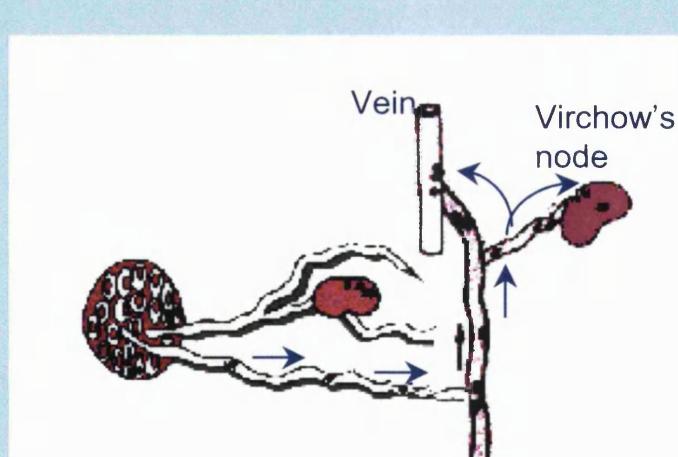


Fig.2.6 Fisher theory of direct access of tumour cell emboli, to the thoracic duct and systemic circulation, bypassing the lymph nodes

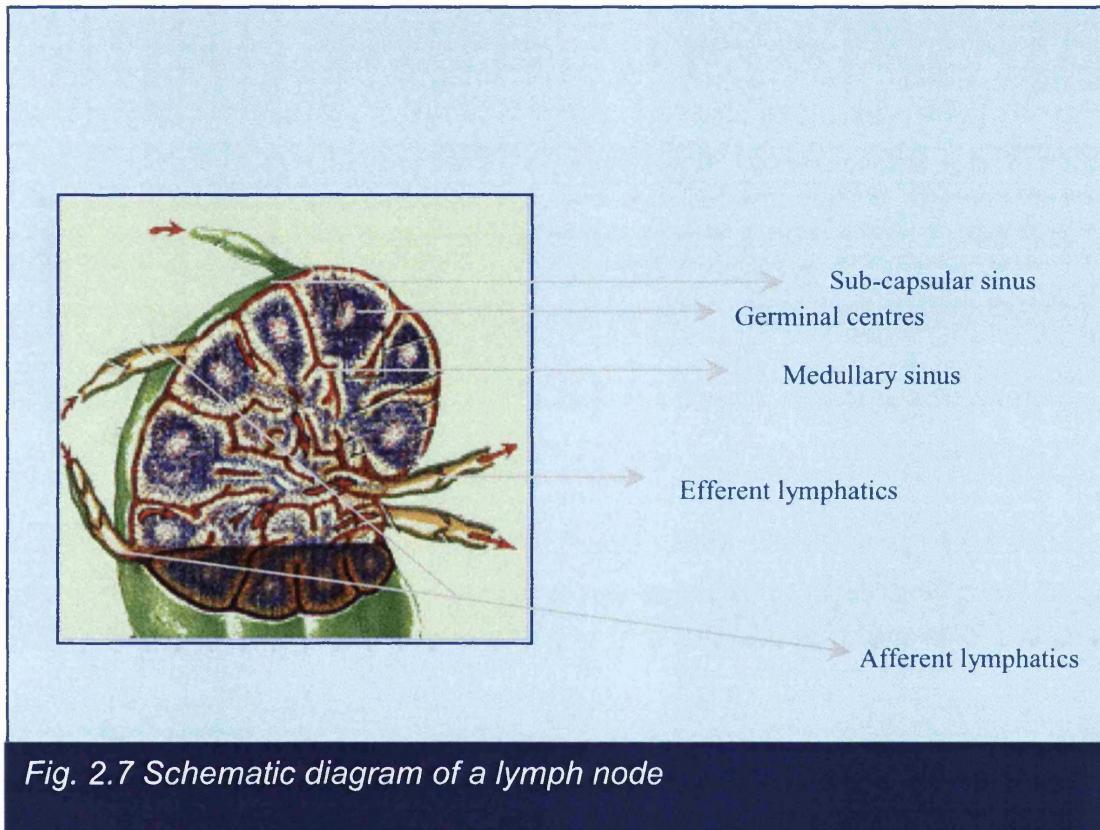
they are functionally negligible under normal circumstances. Such communications have never been demonstrated in dermal or mammary regional lymphatics. The existence of similar direct lymph-venous communications within lymph nodes themselves, proposed by Pressman²⁹, has also been challenged³⁰.

The fact that there are conflicting views in the literature about tumour dissemination suggest that this subject is not well understood at present. From the existing data it can be presumed that, until pathological congestion arises, there is a sequential progression of tumour cells passing via lymphatic vessels to the primary draining lymph node.

2.4 Lymph node anatomy

A lymph node is a discrete structure composed of dense collections of lymphocytes, plasma cells and macrophages surrounded by a capsule of mature collagen. Afferent lymphatic channels enter the cortex of the node through the capsule, draining lymph into the subcapsular sinus (fig. 2.7).

The sinuses have many fine reticulin fibres traversing their lumen which trap particulate matter passing through. Cortical sinuses arise from the subcapsular sinus and pass towards the medulla of the node where they become known as medullary sinuses. These eventually coalesce to form the efferent lymphatic channels through which lymph leaves the node and passes centrally, eventually to enter the thoracic duct.



The subcapsular sinus has only a partial lining of endothelium, as do the cortical sinuses, which means that the cells of the cortex and medulla have direct contact with the lymph fluid contained in the sinuses. Thus the lymphocytes, plasma cells, macrophages and histiocytes can be thought of as partially lining the sinuses. As these sinuses pass towards the medulla, macrophages become the most prominent cells lining their walls²³. These macrophages are sometimes referred to as sinus histiocytes.

Fibrous trabeculae within a lymph node organise the lymphocytes into cortical follicles and also act as the scaffolding for the subscapular and cortical sinuses.

2.5 Radiopharmaceuticals

A radiopharmaceutical is a specific compound which is labelled with a small amount of a radionuclide in order to allow this product to be detected externally after administration to a patient. Both the substance to be labelled and the radioactive nuclide are usually in such small amounts that there is no associated pharmacological effect.

The term 'tracer' is also often used in respect to a radiopharmaceutical. This is because the chemical quantities which are labelled are in the order of a millionth of a gram. External detection of the signal emitted by the radiopharmaceutical allows the tracer to be recorded either by an imaging device, such as the Anger gamma camera or by a probe (such as for the per operative detection of the sentinel node).

With the publication in 1965 by Garzom et al³¹ of a first colloid labelled with the widely available radionuclide, Technetium-99m, a new era for

lymphoscintigraphy began. Tc-99m is the most commonly used radionuclide in routine nuclear medicine applications. It is universally available at a very economic price.

Tc-99m is produced indirectly either by the neutron irradiation of Molybdenum-98 or as a fission product of Uranium-235. A Tc-99m generator is supplied by a variety of commercial manufacturers and is most often supplied on a once a week basis. In the larger radiopharmacies many hundreds of individual doses can be prepared for individual patient use. Table-2.4 summarises the physical properties of Tc-99m.

Physical properties of Tc-99m	
Half life of radioactive decay	6.02 h
Energy of gamma ray emission	140 keV
Beta particle emission	None
Soft tissue thickness to reduce gamma rays to 50%	46mm
Thickness of lead to reduce gamma rays to 50%	0.17 mm

Table-2.4

2.5.1 Radiopharmaceuticals for lymph node detection and lymphoscintigraphy

Colloidal Gold-198 was the first tracer to be used for lymphoscintigraphy. It has a good and uniform particle size range of about 5 nm. It is no longer in use in view of

its excessive radiation dose delivered to the target organs. There are other labelled colloids available for the purpose of lymphoscintigraphy.

The term colloids is usually reserved for particles sizes below 300-400 nanometers. The term microaggregate is reserved for particle sizes of 500 to 3000 nm and the term macroaggregate for particle sizes greater than 5000 nm.

Table-2.5 summarises some of the commonly used radiopharmaceutical.

Agent	Manufacturer	Particle Size		% of particle
		nm		
Nanocoll	Sorin Biomedica Diagnostics, Italy	<80	95	
		80-100	4	
		>100	1	
Albu-Res	Sorin Biomedica Diagnostics, Italy	200-1000	90	
Nanocis	CIS bio International France	3-15		
Microlite	DuPont, USA	10		
Sulphur Colloid	CIS-US	<100	15-20	
		100-600	70-80	
		700-5,000	2-4	
		>5000	0.5-5%	
Antimony Sulfide Colloid	3-30			

Table-2.5 Particle size and particle size distribution of commonly used $Tc-99m$ labelled radiopharmaceutical agents

It is important to remember that these compounds were optimised for the purpose of the visualisation of the reticuloendothelial system in man. This was

at a time when neither diagnostic ultrasound nor computed x-ray tomography were available and well before the sentinel node concept was introduced.

The labelled colloids must be prepared in a reproducible manner, be and remain stable once labelled, have a reasonable shelf life and have a well defined particle size range (table-2.6). It is important to appreciate that different particle sizes will clearly influence the methodology and results to be gained.

Ideal Colloid
Licensed product
Narrow particle size range
99m Tc label
Stable on storage
Lymph channel transport
Rapid transport
SLN retention
Stable in blood (no shrinkage or growth)

Table 2.6

The particles need to be larger than 0.005 nm in size, to gain access into the lymphatic system. This is because smaller particles may penetrate or leak into the capillary membrane and therefore do not enter the lymphatic channels³² (Table-2.7).

When administered in the intercellular space, smaller particles will migrate much more rapidly from the injection site. This requires rapid monitoring, both for imaging and external probe detection.

Colloids

Few nm	Exchange through blood capillaries
Tens of nm	Absorbed into lymph capillaries
Hundreds of nm	Trapped in interstitial space
Large particles -	Do not migrate

Table-2.7

Larger particle sizes will migrate much more slowly and will require late imaging records and external probe detection. At present, there is no clear consensus as to an optimal particle size for the detection of the sentinel lymph node.

The particles move through the lymphatic system by rhythmical contraction and relaxation of smooth muscles that surround lymphatic vessel. Factors that increase lymphatic flow include muscular activity, respiratory movements and massage of the area. Anaesthesia may decrease lymphatic flow³³.

The trapping mechanism of particles by lymph nodes has always been considered as a result of macrophage activity and phagocytosis. Whilst particle size is relevant, so are the particle numbers and the charge of the particle. When in touch with sera particles may change in size and this appears to happen with sulphur colloid particles and less so with albumin particles. Temperature, pH and length of the manufacturing process all influence the distribution of the particle sizes to be obtained. In an experiment performed by Alazraki et al³⁴, they found major changes in the labelling yield of sulphur colloid particles. When a kit was heated for 10 minutes and allowed to cool for 5 minutes, a higher radiochemical purity was obtained for the end product. The

use of a reduced heating time procedure resulted in a significant increase in the percentage of particles smaller than 400nm. With increased heating time a significant decrease in the percentage of smaller sized particles is seen. Alazraki and associates recommend for sulphur colloid the use of a 3 minute versus a 5 minute heating protocol.

A number of attempts were made to define the optimal characteristics of radiocolloid uptake in lymph nodes. A widely quoted study by Strand and Persson³⁵ investigated these characteristics in a rabbit animal model where tracers were injected subcutaneously and bilaterally below the xiphoid process of the rabbit. In general, the smaller the particle size the higher the colloid uptake in parasternal lymph nodes. The study was then extended by the same group who concluded that the optimum particle size for interstitial lymphoscintigraphy is of the order of tens of nanometres.

In a study by Kaplan and associates³⁶, two Technetium-99m labelled radiopharmaceuticals for lymphoscintigraphy were compared in man (stannous phytate and antimony sulphide). The authors concluded that antimony sulphide was to be preferred since it showed a greater number of internal mammary lymph nodes in patients with breast cancer. Again in this study a sub-costal injection of the radiocolloid was given below the xiphisternum.

It is necessary to emphasise that most literature which describes the distribution of these labelled colloids in animal experiments or in man referred to data obtained with injection techniques not optimised for the detection of the sentinel lymph node. So whilst there is a significant amount of literature which discusses the impact of injection techniques, particle size and the visualisation

of lymph node chains, specific studies for the investigation of these factors in the detection of the sentinel lymph node, are still needed. It is clear that in the 1970s and 1980s the aim of lymphoscintigraphy was to visualise the majority of lymph nodes in a particular lymph basin for staging purposes but also for delineation of lymph flow patterns and an improved definition of a radiotherapy field. (table-2.8, 2.9).

Indications for lymphoscintigraphy
Tumour staging
Definition of radiotherapy fields
Evaluation of lymphoedema vs venous oedema
Evaluation of drainage patterns (leak)
Lymphangiectasia
Chyle stasis

Table-2.8

Indication for sentinel node detection
Surgical management of :
Melanoma
Breast cancer
Colorectal cancer
Head and neck cancer
Penile cancer
Other

Table-2.9

In Table-2.10 data is compiled from the literature in respect to various radiocolloids and uptake in parasternal lymph nodes. The data shows the large variation in particle size and uptakes, at 2 and 4 hours post sub-costal administration. Recent *in vivo* comparisons in man tell a different story and require reflection.

Product	Proprietary name	Size nm	Stability	uptake % (2h)	Uptake % (5h)
mμAA	Microlite	10	Constant	1.5	1.5
μAA	Albucoll	70	Constant	0.3	0.5
Sb ₂ S ₃	Labelaid	45	Constant	1.1	1.7
(Sn)S	Hepato	90	Constant	0.3	0.4
(Re)S		360,60	Constant	0.3	1.3
μAA	AlbuRES	250	Constant	0.6	0.7
Sulphur	In-house	600	Variable	0.2	-

Table-2.10 Properties of various radiocolloids and their uptake in parasternal nodes

Paganelli et al³⁷ investigated three different colloidal sizes in a significant number of patients with carcinoma breast and determined the number of sentinel lymph nodes detected. The tracer was given sub-dermally and the methodology optimised for sentinel node imaging and detection. Figure 2.8 and the table-2.11 summarise the main findings. From this it can be seen that the tracers with smaller particle sizes led to the visualisation of a greater number of lymph nodes, in fact rendering more difficult the imaging and the external detection of the sentinel node. Best results were obtained with the larger particle size colloids.

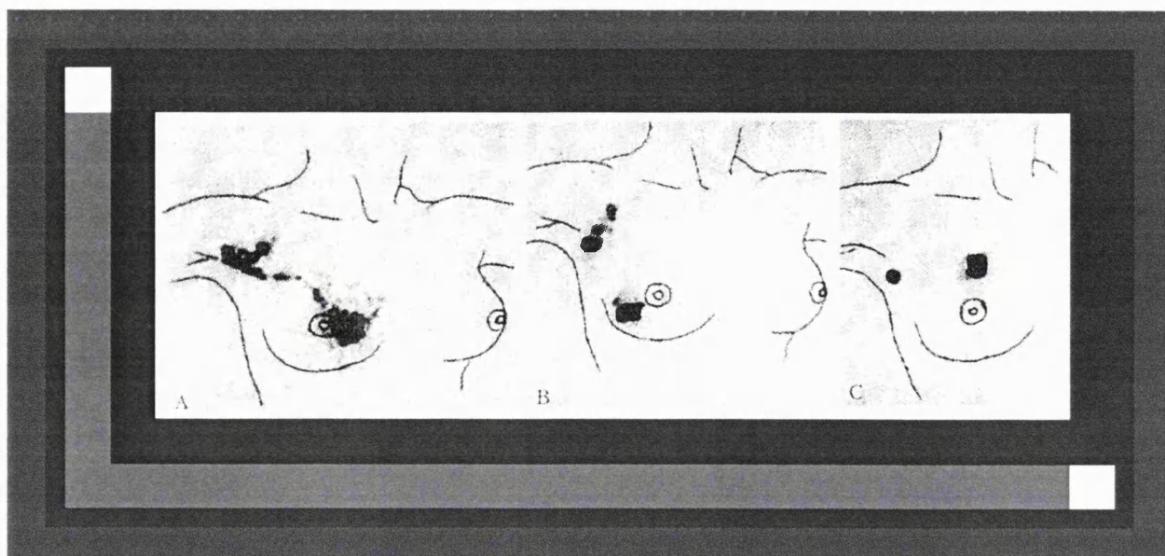


Fig. 2.8 The relationship between colloid particle size and number of sentinel nodes imaged.

Size of Tracer (nm)	Positive Patients	No. of Nodes
<50	29/30	1-5
<80	26/30	1-4
>200<1000	155/155	1-2

Table-2.11 The smaller the particles, the greater the number of nodes imaged.

In a study published in 1998 by Glass et al³⁸, the kinetics of three lymphoscintigraphic agents were investigated in patients with cutaneous melanoma. Whilst all agents were passed through a similar sized filter (200 nm) it became clear that for appropriate sentinel lymph node imaging, detection at 30 minutes is more appropriate than late imaging. Having passed the three agents through a similar sized filter, there were no significant differences in the quality of nodal visualisation and the half times of washout of tracer from these nodes.

From own observations it is also clear that with certain types of preparations, particle sizes may vary considerably from batch to batch. In recent experiments, we found in between batch variations for the same preparation from the same

manufacturer, of the order of factors of 4. It is vital that batch quality control of the preparations must be available in order to reduce these variations which will clearly impact on practice and results.

It is to be expected that manufacturers will optimise new kits for the non invasive detection of the sentinel node, with improved stability of the preparations. The interest generated by these new clinical applications of the radioactive tracer method may justify the effort and cost which will be necessary to achieve the desired outcome.

In view of the inherent difficulties in keeping a stable particle size it may well be that other types of particulate compounds might be developed - for example Tc-99m labelled liposomes could represent a step forward in this field. Recent work with liposomes suggests that progress is being made in this area as well. Tc-99m liposomes with an average size of greater than 100nm migrate somewhat less from the injection site than liposomes with an average size.

In a brief summary then, it can be stated that there is a need for identifying an ideal colloid for sentinel lymph node visualisation, which is clearly different from a particulate tracer which is optimised for the visualisation of all lymph nodes. It will be important that the range of particle sizes is known, that the product is stable on storage and when reconstituted, that it is labelled with Technetium-99m and that for the purpose of sentinel lymph node detection it contains particles in the range of 80-200 nm in size.

In fact the study by Paganelli³⁴ where the authors quote a particle size range between 200-1000 nm is likely to have been conducted with a range of particles not exceeding 400 nm (personal observations).

2.7 The Gamma Camera

The gamma camera was developed by Hal Anger³⁹ in 1958. Although the design principles he first established still remain, in the past 40 years , a steady number of refinements have been introduced by many workers. In the early 1980s, camera manufacturers integrated a computer system into the gamma camera which allowed the image data to be stored digitally for later processing and display. This development, together with improvements in the performance of the imaging detector which was able to view the organ to be imaged in 360 degrees, led to the development of the cross-sectional imaging technique single-photon emission tomography (SPET).

For the purpose of sentinel node imaging, a single-headed planar gamma camera with its associated computer system is adequate to fulfill all technical requirements. Therefore the technique of sentinel node imaging is clearly placed within the technical capability of every clinical nuclear medicine department.

2.7.1 Design Principles of the Gamma Camera

A large thin scintillation crystal is situated in the front face of the gamma camera. As soon as the crystal is impacted by an incoming gamma ray, a minute burst of visible light energy is generated within the crystal structure. It is for this reason that the Anger gamma camera is often referred to as a scintillation camera.

Figure 2.9 illustrates the operation of a modern digital gamma camera system. Gamma rays pass through a honeycomb structure of the collimator which is mounted in front of the detector. These only allow the rays that are traveling perpendicular to the camera face to get through to the scintillation crystal and reject the scattered radiation. This improves the spatial resolution of the acquired data. A hexagonal array of typically 50 - 70 discrete photo-multiplier tubes are held in a fixed assembly at the back of the crystal, and when summed together their outputs record the pattern of light generated by the scintillation process. Signals from those photomultipliers registering a significant response to the detected gamma ray are processed by specialized hardware and software to determine the precise location of the gamma ray impact as x and y coordinates. These are then digitized and are directly stored to the computer to contribute to the formation of a scintigraphic image.

The acquired image is usually formed over a specified time interval, and consists of many individual detected gamma ray events whose overall pattern of distribution reflects the two-dimensional distribution of activity viewed by the detector.

Each gamma camera utilizes a range of collimators individually designed to optimize either detector sensitivity or spatial resolution by varying degrees, and constructed to be inter-changeable components of the detector.

The gamma camera computer is usually integral to the camera system and provides an interface through which the acquisition of digital image data may be controlled by the operator. Image data are stored as a two-dimensional pixel matrix of detected gamma events, and may be collected in a number of

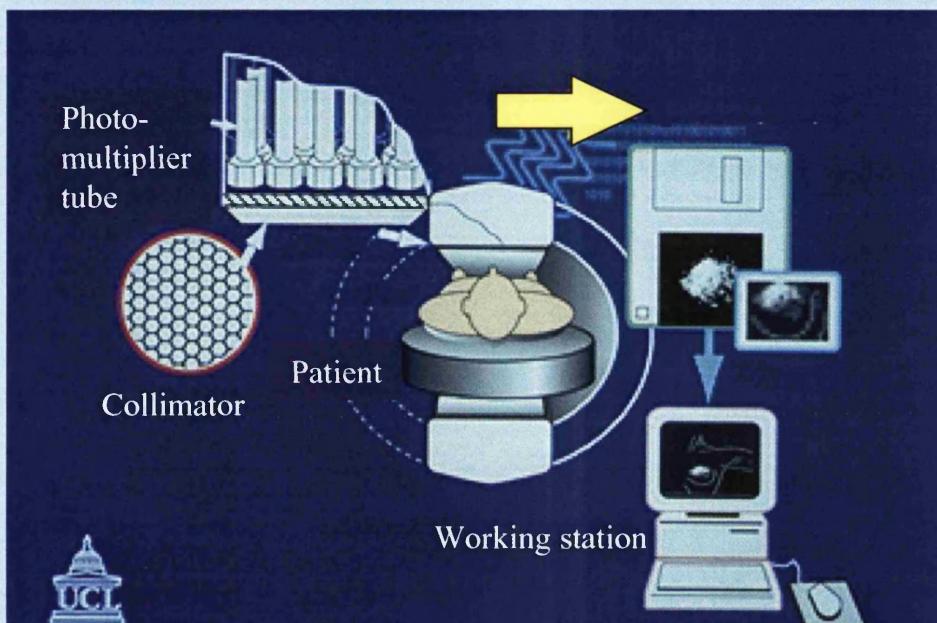


Fig. 2.9 Schematic representation of the operation of a digital gamma camera .

formats, e.g. as a single-frame "static" projection or as a continuous dynamic sequence of short time frames at a specified projection.

Whole-body scans may be generated by moving the integrated patient couch with respect to the detector(s) at a specified constant speed along the length of the patient, whilst acquiring a single, scanned view of the distribution of tracer throughout the body. Whole- or part-body scanned images are useful in interpreting lymphatic drainage from melanomas, particularly of the lower limb (fig 2.10).

Review of a sequence of dynamic frames in cine form allows the underlying time dependency of tracer uptake to be clearly visualized for the structures imaged.

A computer program also allows analysis of dynamic data to gain information about particle kinetics through the generation of time-activity curve data from the region of interest (fig. 2.11).

Early imaging, commencing immediately after administration of the tracer, will give a rapid indication of the progress of the radiocolloid from the administered site. It will also allow for an early warning, should the tracer fail to migrate. Additionally, specific individual images may be added together, subtracted from each other and otherwise manipulated for optimal display.

It is possible to obtain sentinel node images with very high resolution, as the tracer activity is often superficially located with minimal overlying tissue.



*Fig. 2.10
Whole- or part-body scanned images are useful in interpreting lymphatic drainage from melanomas, particularly of the lower limb*

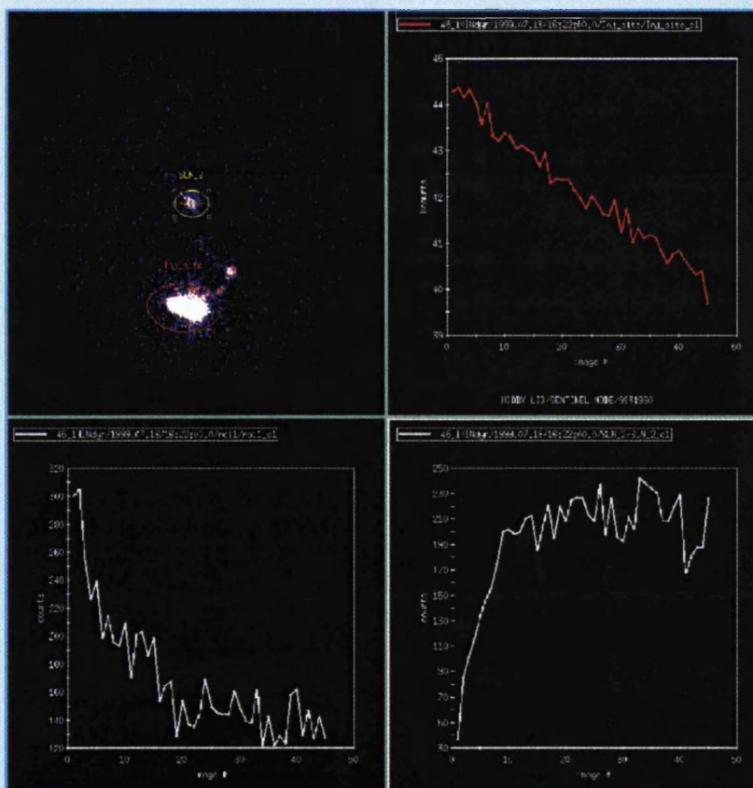


Fig.2.11 Dynamic data analysis. Generation of time activity curve.

This may be exploited by using a high-resolution collimator, a fine image matrix (optimally 256 x 256) and careful patient positioning to minimize distance between the source of activity and the detector face. This is achieved by ensuring that the camera face is parallel to the surface that is being imaged. Therefore in breast lymphoscintigraphy, we acquire an anterior oblique image at about 30 degree angle rather than anterior projection. Through these strategies individual lymphatic ducts and their interrelationship are made visible, and detection of sentinel nodes lying very close to the injection site is enhanced.

2.8 The Gamma-detection probe (GDP)

The idea of using a hand-held probe for the localization of radioactive tissue was first reported by William G. Myers in 1960⁴⁰. The first reported intraoperative use of the probe was in 1984⁴¹ as a part of the detection of CEA-producing tumours with radiolabelled monoclonal antibodies.

External probes are non-imaging detectors which can be optimised for intraoperative use. They hence allow pick up of a specific signal from a focused site within the body where the greatest concentration of tracer is encountered. With the renewed interest in sentinel lymph node detection, probe technology has changed rapidly over the last few years, mainly in order to optimise portability, ease of use and signal detection.

The design of the probe is based on the use of a semiconductor detector in a hand-held instrument. We use the Neoprobe 1500 probe (Dublin, Ohio) for our study (fig 2.12).

The probe uses a built-in 12-mm cadmium telluride detector, together with a preamplifier, and has the ability to detect gamma rays from a radionuclide labeled tracer. Its small size allows for easy transport and used during surgery .

The gamma photons detected by the crystal are converted into both a digital numerical display and an auditory signal. The efficacy of detection is the ratio between the area of the detector and the area of the sphere of radiation. The ratio between these two increases proportionately to the square of the distance between them according to the inverse square law (fig. 2.13).

The probe, through visual and auditory signals, guides the surgeon to target the area of increased activity through the shortest route and therefore minimize the extent of dissection and tissue disruption.

In determining the most appropriate probe the following figures of merit are often considered:

Sensitivity: number of counts detected per unit of time per area detector.

Spatial Resolution: the minimal distance between two signals which can be separated with sufficient statistical certainty.

Energy resolution: the ability of the probe to distinguish degraded from non-degraded radiation.

Collimation : the ability of the detector to pick up a signal from a circumscribed volume of tissue to be investigated. It is important that the probe has good collimation and side shielding (fig 2.14)

Other features includes the overall ergonomics and design of the probe, the facility of peri-operative use, facilities for use in a sterile environment, cost, etc.

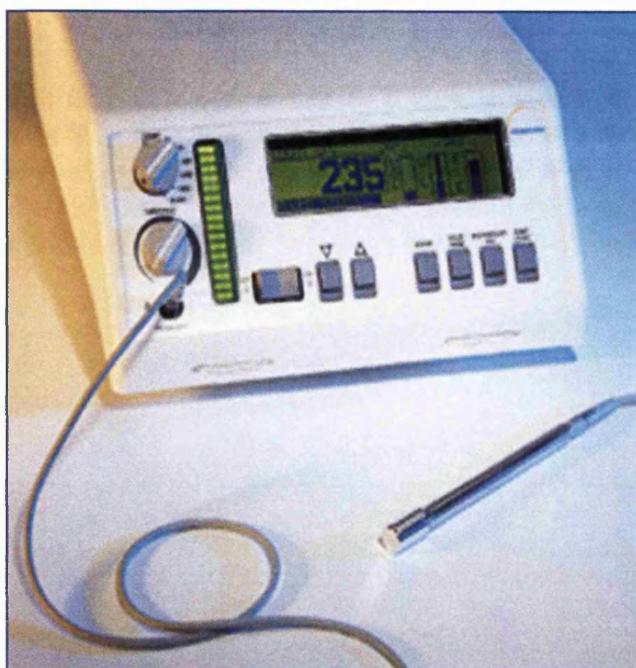
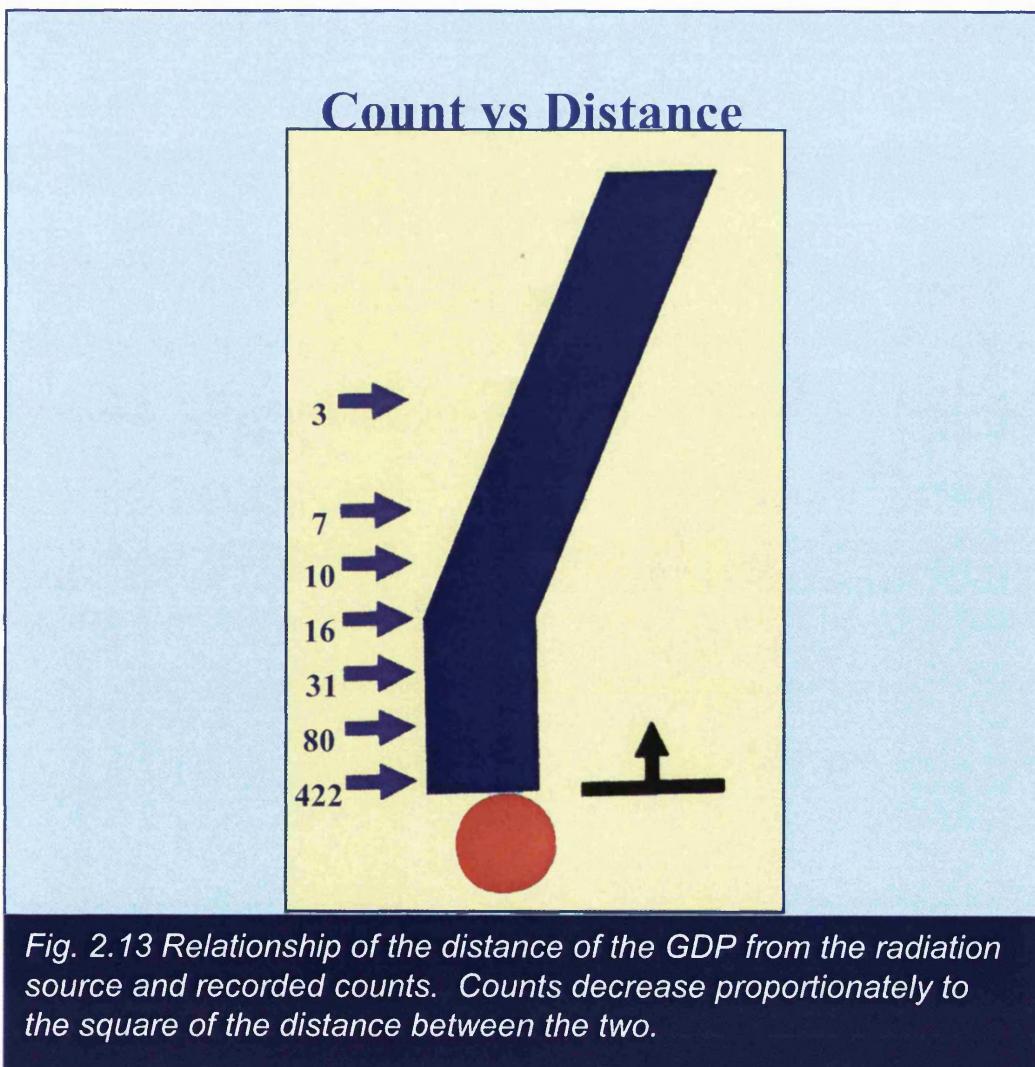


Fig. 2.12
Neoprobe 1500



This gamma detector has a special "squelch" mode facility which calculates the mean count of a given point (5-s count), calculates the standard deviation (square root) and starts to emit a sound only when the count is 3 standard deviations above the mean count denoting significantly higher radiation.

2.8.1 Principles of probe guided surgery

The surgical technique is based on careful survey of the area in question. Slow scanning is mandatory. One principle, which is the basic point of this technology, is the so called three-point counting principle. At first an in-vivo count is done, the active tissue is then excised and then an ex-vivo count is performed to verify that the right tissue was excised. The bed of resection is then probed again to confirm that no radioactive tissue was left behind. This residual activity measurement confirms the completeness of excision. It is also important to use external collimator in situations where there is a high background radiation activity as in upper outer quadrant lesions, where there is a close proximity between the injection site and the SLN (fig 2.15). A recent paper published in 1998 by Tiourina et al⁴², provides for a review of the main figures of merit of a number of probes available for sentinel node detection. It is noteworthy that the main figures of merit between a number of probes varies significantly and may vary by a factor of 4. The transmission of signal through the shielding surrounding the detector may vary by factors as great as 40 and that the detection sensitivity of the probes in air or water may also vary by a factor of the order of 20!

The Need for Good Collimation

Injection Site

Detector

Sentinel Node



Side shielding reduces side penetration

$^{99m}\text{Tc TVL} = 0.9 \text{ mm lead}$

need $> 1000 \times$

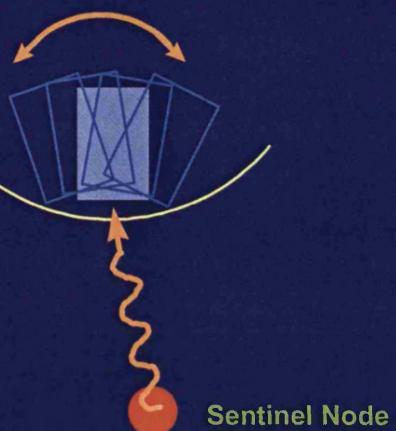


Fig. 2.14 Need for good collimation and side shielding

'Line of Sight' Localisation Strategy

- The detector is moved through a Cone
- line of maximum count - rate is established
- dissection continued along this line
- procedure is repeated as resection is advanced
- line of approach is modified as necessary

Requires good spatial resolution
and detector sensitivity



Optimal Detector Alignment

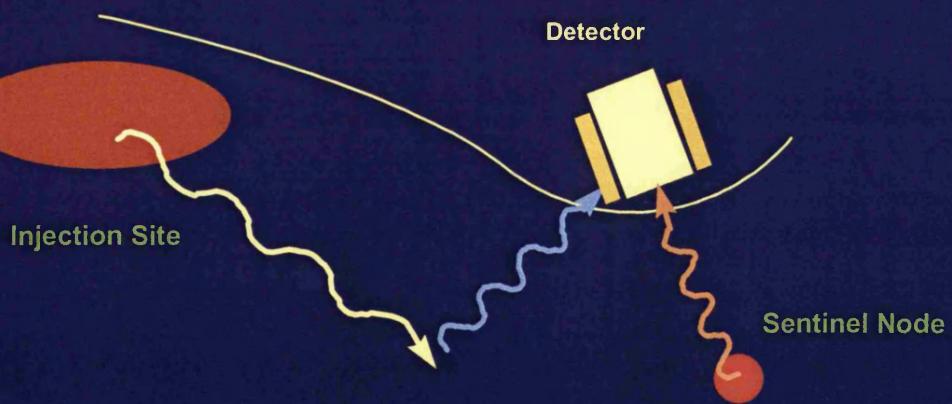


Fig. 2.15 Principles of probe guided surgery

2.9 Carcinoma Breast

Breast cancer remains the commonest malignancy in women, with 570 000 new cases in the world each year. It comprises 18% of all female cancers. In the United Kingdom, the age standardised incidence and mortality is one of the highest in the world. The incidence among women aged 50 approaches two per 1000 women per year, and the disease is the single commonest cause of death among women aged 40-50 which accounts for about one fifth of all deaths in this age group⁴³.

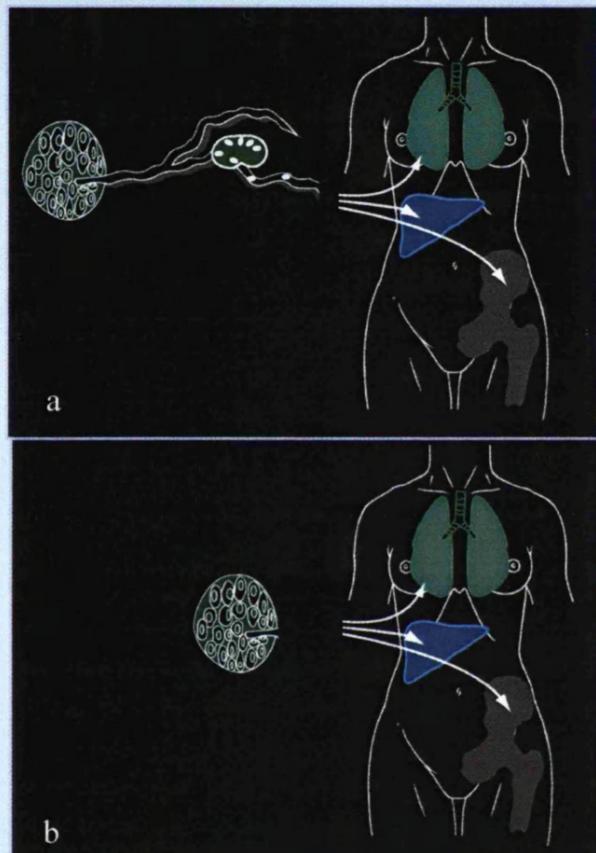
In patients with invasive breast cancer, the histological status of the axillary lymph node remains the most powerful predictor of recurrence and survival. The presence of nodal metastases decreases five-year survival by approximately 40 percent, as compared with that among patients who are free of nodal disease^{44,45}. It is also evident beyond any doubt that post-operative adjuvant chemotherapy significantly reduces the risk of distant disease and dissemination with positive impact on survival. For all these reasons the knowledge of the true status of axillary nodes is important for the appropriate management of these patients.

It is assumed that the first regional lymph node which drains lymph from a primary tumour is the first node to receive the seeding of lymph borne metastatic cells. A survey of the literature does appear to indicate that tumour cells disseminate fairly sequentially and that the so-called “skip metastases” are only rarely encountered. A study by Veronesi et al⁴⁶ have closely looked at this issue. They studied 1446 cases of breast cancer in which 839 patients showed evidence of nodal disease. The first level was the site of metastases in 828, the

second level in 364 and the third in 187. When a single lymph node was involved, it was nearly always at the first level. In only 11 cases (1.3%), were the second and/or third levels invaded without metastases at the first level. Therefore, the percentage of cases with skip metastases was very low. The authors argue that although the first level metastatic seeding is highly predictive of spread to second and third level, they still recommend total axillary dissection for the purpose of proper staging and adequate loco-regional control and adjuvant treatment. They conclude that the spread of breast cancer to the axilla follows a regular pattern; the first level is invaded first, whilst in most cases, the second and third levels are involved only when the first is substantially affected.

There are two fundamental concepts which, however, are still cause for much controversy (fig 2.16). It has been stated that the sentinel lymph node concept is too Halstedian in nature⁸. Tumours do not evolve and disseminate into local, regional and distant metastases in an orderly fashion. At the time of patient presentation, most tumour biologists would lead us to believe that tumours have seeded distally at the time of clinical presentation or manifestation. Whilst this debate persists, it is fair to expect that advocates of sentinel lymph node scintigraphy and detection need to remain aware of the possible pitfalls in this approach.

From a surgical management point of view, however, it is also clear that a significant number of patients who undergo axillary lymph node clearance do endure pain and cost and significant complications, including paresthesias, lymphedema, seroma,



*Fig.2.16 A representation of the metastatic spread of the breast cancer:
a, orderly progression
b, random progression*

infection, and limitation of shoulder motion with no apparent benefit. Between 60-70% of patients referred to a modern practice, will have completely negative axillary lymph node exploration. Between 3-12% of patients do develop limiting lymphoedema with the associated morbidity. The additional costs of the surgical exploration of the axilla must also be taken into account.

The sentinel node technique has the advantage of being a less invasive procedure and potentially with less morbidity. In a randomised study performed by Schrenk et al⁴⁷, the postoperative morbidity after SLNB was compared with that of ALND. There were 35 patients in each group. The parameters that were carefully measured included arm circumference, subjective lymphedema, pain, numbness, effect on arm strength and mobility, and stiffness). Patient characteristics were comparable between the two groups. Postoperative follow-up was 15.4 months (range, 4-28 months) in the SN group and 17.0 months (range, 4-28 months) in the ALND group. Following axillary dissection, patients showed a significant increase in upper and forearm circumference of the operated arm compared with the SLN patients, as well as a significantly higher rate of subjective lymphedema, pain, numbness, and motion restriction. No difference between the two groups was found regarding arm stiffness or arm strength, nor did the type of surgery affect daily living. The authors conclude that the SLNB is associated with negligible morbidity compared with ALND.

On the other hand, an important study by Turner et al⁴⁸ provides ample evidence and further validates the sentinel lymph node hypothesis for breast carcinoma. If the sentinel node is non involved, as judged by H&E staining and immunohistochemistry, then the probability of non sentinel lymph node

involvement (analysed in the same manner) is less than 0.1%. The true false negative rate was 0.97% (1 patient in 103).

Albertini¹⁰ in 1996 (62 patients) reported on a 92% successful localisation rate for the sentinel lymph node, the absence of skip metastases and that in 67% of all patients with a positive sentinel lymph node, this was the only site of disease. He stated that "the beauty of lymphatic mapping is that it allows the surgeon to give the pathologist one or two sentinel lymph nodes to perform a more detailed examination". Veronesi⁴⁹ reporting in the Lancet in 1997 investigated 163 patients, reported a 97.5% accurate prediction of the lymph node status. In 95% of patients there was concordance between the negative sentinel lymph node and the negative status of the axillary nodes.

Borgstein et al⁵⁰ reporting in 1998 on 130 patients, demonstrated the sentinel lymph node in 89% of all cases, with a significant failure rate in patients submitted to previous excision biopsy (36%) with a small failure rate (4%) when the tumour was palpable in situ. The biopsy of the sentinel lymph node was 98% accurate for the prediction of nodal metastases. Cox et al⁵¹ reporting in 1998 on 466 patients, showed successful identification of the sentinel lymph node in 94% of all cases.

It must be remembered that the sentinel-lymph-node biopsy is a diagnostic test used to determine the status of regional lymph nodes. As with any diagnostic test, it is important to establish the sensitivity, specificity, positive and negative predictive values, overall accuracy, and false negative and false positive rates of the procedure before it can be accepted as clinically useful and valid.

In a recent combined analysis by McMasters and co-workers⁵², in 1385 patients with breast carcinoma, the overall sensitivity was 94% with 100% specificity and 6.2% false negative rate when the sentinel node histology was compared with the axillary node dissection specimen. The overall accuracy reported in this combined analysis was 98% with a positive predictive value of 100% and negative predictive value of 97% (table-2.13).

No. of patients	Pts with SLN identified	Technique	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Overall accuracy (%)	SLN only positive node (%)	False negative rate (%)
1385	1198	All	94	100	100	97	98	48	6.2

Table-2.13

Another meta-analysis by Miltenburg et al⁵³ (11 studies and 912 patients) emphasized the successful identification and concordance rate of SLN histology with that of ALND. The SLN technique was successful in over 97% of patients if certain techniques and inclusion criteria are used. They also concluded that SLNB reflects the histological status of the axilla in 97% of cases and has a 5% false negative rate (table-2.14).

N	Successful SLN identification (%)	Concordance of SLNB and ALND (%)	False negative rate (%)
912	762 (83.6%)	747 (98.0%)	15/296 (5.1%)

Table-2.14

The result of this meta-analysis is in keeping with that of McMasters et al. It is clear that data, in several significantly large sample studies, give credence to the sentinel lymph node concept in breast cancer!

The critical issue in SLNB is the false negative rate as this can lead to inadequate treatment decision. There is a false negative rate of 2-3%, associated with ALND^{52,54}. We need to balance the advantage of a less invasive staging investigation against the risk of a false negative rate. A false negative biopsy can lead to incorrect staging of cancer and inadequate adjuvant treatment decisions. SLNB can be a useful method of nodal staging, if it can be performed with a similarly low rate of false negative results.

Although these results are promising, there is still a need to take a cautious approach in implementing this technique in routine clinical use. In particular in the light of recent publication of Braun et al⁵⁵ in the New England Journal of Medicine where they analysed the effect of cytokeratin-positive cells in the bone marrow on survival of patients with breast cancer. The authors obtained bone marrow aspirates from the iliac crests of 552 patients with breast cancer and found that in 199 patients (36%) there was evidence of occult metastases based on cytokeratin staining. What is worrying is the fact that they did not find any correlation between lymph node histological status and presence of micrometastases in the bone marrow. These patients were followed for a period of four years and patients who had occult metastatic cells in their bone marrow had worse prognosis than the group without micrometastasis.

Whether SLNB is ready to replace conventional ALND in breast cancer is therefore remains unclear. As far as staging is concerned, there is enough

evidence in the literature to support that this technique is a reliable staging investigation. As far as regional control and long term survival is concerned, we simply do not know the answer at present. These issues strongly point to the need for large multi-centre trials which would ultimately determine the appropriate clinical indications of this methodology and its impact on the surgical management of patients presenting with carcinoma breast. Fortunately such trials are well on the way in the United Kingdom (ALMANAC trial) and in the United States (NSABP-32) with primary endpoints of survival, long term regional control and morbidity.

2.10 Malignant melanoma

Approximately 80 million cases in Europe have been reported. The incidence appears to be increasing, with a 5 year survival heavily dependent on tumour thickness. For tumours of less than 1.5mm thickness and without metastases, survival is greater than 90%, for tumours greater than 4mm thickness, survival drops to 50% and even further to 10% when patients present with distant metastases. Most melanomas are seen to be superficial (65%), can present as nodular (25%), as lentigo maligna (5%) and as acral lentiginous in a further 5% of cases.

There is a very significant experience of sentinel lymph node detection in melanoma. Compiled by Kapteijn (table 2.15) it can be seen that many studies have been carried out since the early studies by Morton⁴.

A review article published by Singluff⁵⁶ in 1994 on the surgical management of regional lymph nodes in 4,682 patients offers interesting background information. Although lymphoscintigraphy in this particular analysis was carried

Author (year)	NO. of patients	Breslow thickness	Identific- ation of sentinel node	Method (BD/GDP)	Metastases (% patients)	False negative rate
Morton (1992)	223	CS-I*	82	BD	21	1
Morton (1994)	72	68:>0.65 mm 2:<0.65 mm 2: unknown	90	BD	15	0
Lingham (1994)	25	Mean: 3.75 mm (range 1.5-8.1)	100	BD	27	0
Thompson (1995)	228	111:>1.5 mm 7:<1.5 mm	87	BD	23	0
Krag (1995)	121	109:>0.75<4 mm 11:>4mm 1 unknown	98	GDP: 77 BD+GDP: 44	12	0
Albertini (1996)	106	Mean: 2.24mm (>0.75mm)	96	GDP+BD	15	0
Mudun (1996)	25	3:<1.5 mm 18:>1.5<4 4:>4mm	100	GDP	24	Not given
Karakousis (1995)	55	>1mm	93	BD	24	Not given
Kapteijn (1995)	110	>1mm	99.5	GDP+BD	23	2.7

Table 2.15 Results of sentinel node biopsy in melanoma . *CS-I= Clinical Stage-1 BD= Blue Dye, GDP: Gamma Detection Probe (Data compiled by Kapteijn BAE, Biopsy of the sentinel node in melanoma, penile carcinoma and breast carcinoma. ACADEMISCH PROEFSCHRIFT, the Netherlands Cancer Institute 1997; 20.

out in only a minority of patients, the authors already stated that lymphoscintigraphy is probably the best way to identify basins draining a specific area of the skin. It was also stated that the number of basins identified by this methodology may overestimate the number of basins in which nodal metastases acquire clinical relevance. Clearly the concept of the sentinel lymph node was to emerge. In the same year Reintgen et al⁵⁷ (in 1994) published an important study on the orderly progression of melanoma and nodal metastases. 42 patients were investigated of which 34 had histologically negative sentinel nodes, with the remainder of the nodes in the basin also being negative. No skip metastases were documented. The authors concluded that nodal metastases from cutaneous melanoma are not random events. Sentinel lymph nodes in the lymphatic basins could be mapped and identified individually, and these have been shown to contain first evidence of melanoma metastases. The authors concluded that this information could be used to revolutionise melanoma care.

Albertini⁵⁸ in 1996 investigated 106 consecutive patients comparing the sentinel lymph node detection technique with vital blue dye mapping. Whilst the blue dye staining identified 69.5% of sentinel nodes, radioisotope detection allowed for the identification of 83.5% of sentinel nodes. When combined both techniques detected 96% of the sentinel lymph nodes.

Leong⁵⁹ in 1997 investigated 163 patients. There was a 98% successful rate of detection of the sentinel lymph node, 18.4% frequency of microscopic metastatic melanoma involving the sentinel lymph node and 27.3% of sentinel lymph nodes were detected in the absence of blue dye! The authors concluded

that gamma probe guided resection would minimise the extent of lymph node dissection.

A study by Joseph et al⁶⁰ in 1998 looked at 83 melanoma patients with a positive sentinel lymph node, from 600 patients with stage I-II disease. The sentinel lymph node was positive in 30% of patients with tumour thickness greater than 4mm, positive in 18% of patients with tumour thickness between 1.5mm-4mm, positive in 7% of patients with tumour thickness between 1.0-1.5mm and 0% positivity for sentinel lymph nodes of tumours less than 0.76mm in thickness.

Cascinelli⁶¹ publishing in the Lancet in 1998 confirmed the inefficacy of elective regional node dissection.

Although the concept of SLNB is validated in malignant melanoma, there is currently no evidence that incorporating sentinel node biopsy in the routine management of melanoma improves survival or regional tumour control.

2.11 Training

Training is an important factor in the success of the procedure as there is no doubt that the procedure is operator dependent and there is a definite learning curve. This point is illustrated in a multi-center study by Krag et al⁶² in which 11 surgeons from different centers reported success rate of identifying the sentinel node ranging from 79-98%. More importantly false negative rate varied from zero to 28.6% .

Bass et al⁶³ have looked at this issue in detail and recommend that each surgeon is required to perform at least 30 procedures of SLN biopsy followed by completion ALND. Adequate audit and quality assurance needs to be in place.

2.12 Summary

A vast amount of data in peer reviewed literature has now emerged in respect to the detection of the sentinel lymph node and its impact on the surgical management of cancer. Whilst most data has been collated from patients suffering from carcinoma breast and carcinoma of the skin (melanoma), an increasing interest is shown in the application of this technique in other areas of surgical oncology (head and neck cancer, colorectal cancer and penile and vulvar cancer).

It seems that tumour dissemination to the regional lymph nodes, at least in breast carcinoma and malignant melanoma, is not a random event. So far it can be stated that the detection of the sentinel lymph node is highly accurate at least in the context of carcinoma breast and melanoma, that there is a rapidly increasing database population, that training programmes relevant to multidisciplinary teams need to be developed for this methodology, with emphasis on standardisation of tracer and techniques, improved design of detector technology and appropriate patient selection and education. There is clear scope for improvement of the technology involved and the refinement in the protocols to be pursued.

Before SLNB is accepted as a standard of care, we need to await the outcome of multicentre trials.

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Chapter-3

TECHNIQUE OF SENTINEL NODE LOCALISATION AND BIOPSY IN BREAST CARCINOMA

3.1 Study protocol

Initial work at our Institution started in 1997. After visiting two leading European Centers in this field, (i.e. European Institute of Oncology in Milan and the Netherlands Cancer Institute in Amsterdam) and a period of intensive training, we designed and proposed our study protocol. Ethics Committee and ARSAC approval was obtained. Our patient selection criteria was as follows:

3.1.1 *Eligibility criteria*

- I. All patients with proven invasive breast carcinoma (T1,T2 tumour) diagnosed on “Triple assessment” i.e. clinical examination, imaging (mammogram and ultrasound) and tissue diagnosis (cytology, core-cut biopsy), where surgical treatment would involve removal of the primary tumour and axillary dissection.
- II. Non-palpable invasive breast cancer were also eligible.

3.1.2 *Exclusion criteria*

- I. Pregnant and lactating women.
- II. Patients with previous breast or axillary surgery on the same site.
- III. Patients with multi-focal/multi-centric carcinomas of breast.
- IV. Patients with clinically involved axillary lymph node(s).

It seems that the success rate of sentinel node localization is lower in patients with previous breast surgery¹ although some studies report that the false negative rate is not affected².

Multifocal tumours are likely to involve more than one lymphatic trunk from the

mammary gland to the axillary nodes giving rise to a false negative result^{3,4}. Two out of four false negative cases in a study performed by Veronesi et al⁵ had multifocal tumour and other groups have also reported false negative results because of multifocality of the primary breast cancer⁶.

It is important to exclude patients with clinical involvement of axillary lymph nodes. This is one of the potential pitfalls in the sentinel node localization² and can lead to a false negative result. This is likely to be due to a change in the lymphatic flow, if the sentinel node is replaced with the metastatic carcinoma and is mechanically blocked and non-functional; as a result of the flow changes, the colloid bypasses the sentinel node and moves on to a non-sentinel lymph node. This point is clearly demonstrated in the clinical case presentation in the 'Pitfalls' section of this chapter.

For recruitment, at the time of initial diagnosis, patients were informed about the sentinel node biopsy project and a leaflet explaining the procedure, was given. In the subsequent visit, patients had an opportunity to ask any questions about the study. Informed consent was subsequently obtained from all patients included in the trial.

3.2 Injection Technique

We were faced with the question of which injection technique to choose. There were three reported injection techniques at the time. The commonly practiced peri-tumour injection technique was described by Krag et al⁷. The radiocolloid is injected around the tumour into the breast parenchyma (fig.2.1a). Due to a

relatively scantier lymphatic supply of the breast parenchyma as compared to the skin^{2,8}, the tracer migration is slower than as achieve with a subdermal injection. Moreover a larger volume and dosage of the injectate and multiple injections are required for this technique to be successful. Krag et al⁷ proposed an optimal volume of 4-8 ml in four 1-2 ml aliquots. In this setting, dynamic imaging is less useful since one is less likely to see the lymphatic tract. Imaging is commonly performed 1-2 hours after the injection is administered.

The other injection technique is intra-tumour injection (fig 2.1b), which is not widely used and it appears that at present only one center is practicing this technique⁹. Concerns are expressed with regards to direct injection into a high pressure tumour environment and also theoretical worries about tumour dissemination and needle tract seedling.

The third injection technique makes use of a subdermal (intradermal) injection of the tracer (fig 2.1c). This technique was first described by the Veronesi and co-workers⁵. The term subdermal and intradermal injections are used interchangeably in the literature. From the strict histological point of view, intradermal injection is the correct terminology as the injectate is delivered into a rich lymphatic plexus of the reticular dermis within the dermal layer of the skin. The principle behind this technique is that the mammary gland is developmentally derived from the ectoderm. It is postulated that there is a critical point of communication between dermal and parenchymal lymphatics at the sub-areolar lymphatic plexus¹⁰ and from here limited number of lymphatic trunks drain towards the axilla. Sappey's illustration of one or two large collecting lymph trunks originating from the subareolar lymphatic plexus (fig.2.4)

has been confirmed by other investigators using direct lymphangiographic techniques^{11,12}.

Borgstein and co-workers¹³ validated this hypothesis. They injected the radioactive colloid into the breast parenchyma in a peritumoural fashion in 33 consecutive females with invasive breast cancer and in the operating room, the blue dye was injected intradermally. There was a 100% concordance in delineation of the sentinel node with intradermal blue dye and intramammary ^{99m}Tc-labelled albumin. The location of the primary tumour and the tumour size seemed not to alter the success rate of the procedure. The advantage of subdermal technique is that it is more predictable and fast with a high success rate in localizing the sentinel lymph node. This technique is criticized for low depiction rate of internal mammary nodes.

We chose the intradermal injection technique for our patients in this trial. A limited number of patients expressed their desire to participate in the trial but were needle phobic. In this group we used for the first time, a needle free injection system for the delivery of the radiopharmaceutical.

3.2.1 The Intra-dermal Injection procedure

3.2.1.1 Preparation for injection

It is important to check the total dose and the volume of ^{99m}Tc-colloidal albumin (fig.3.1). It is good radiation safety practice to be ready to inject before removing the syringe from its lead case and a syringe lead shield may also be used for this purpose. The injection time and the dose and volume needs to be recorded carefully.

3.2.1.2 Positioning the patient

The patient is positioned in a supine position with the arm to the side. The tumour is palpated and the overlying skin is carefully marked.

3.2.1.3 Administration of the radiopharmaceutical

The tracer dose of 10 -15 MBq of Tc-99m labeled Nanocoll is injected intradermally into the skin overlying the tumour. The injection volume of 0.2 ml and 25 G needle is used to administer the tracer. Prior to injection, 0.2 ml of air is drawn in to the syringe behind the radioactive colloid. The main purpose of this is to ensure that no radiocolloid is left in the needle after the injection, as the volume of the injectate is small. This also safeguards against the spillage of the radiocolloid which may cause a contamination artifact after withdrawal of the needle. The other advantage of an air bubble in the syringe is that it helps to disperse the colloid in the subdermal space. Fig 3.2 clearly demonstrates the first point. In this case, air was not drawn in the syringe and on withdrawing the plunger after the injection was given, approximately half of the injection dose was left in the syringe.

The syringe is held at an angle of 10-20 degrees to the horizontal plane and the skin overlying the tumour is punctured using a 25 G needle (fig 3.3). The injection is delivered in to the dermal lymphatic plexus and this is confirmed by a raised bleb at the injection site. After the injection is given, cotton wool is applied over the injection site and to prevent back flow of the injected colloid and skin contamination, the injection site is sealed using a small adhesive plaster. The patient is asked to massage over the injection site for a minute with a fresh cotton wool.

It is important to avoid spillage of the radioactive tracer, as this can cause a contamination artifact on the lymphoscintigraphy which can mimic the sentinel node. This point is illustrated in the section on pitfalls (section 3.9).

It is the responsibility of the physician administering the radiopharmaceutical to ensure the proper disposal of the waste.

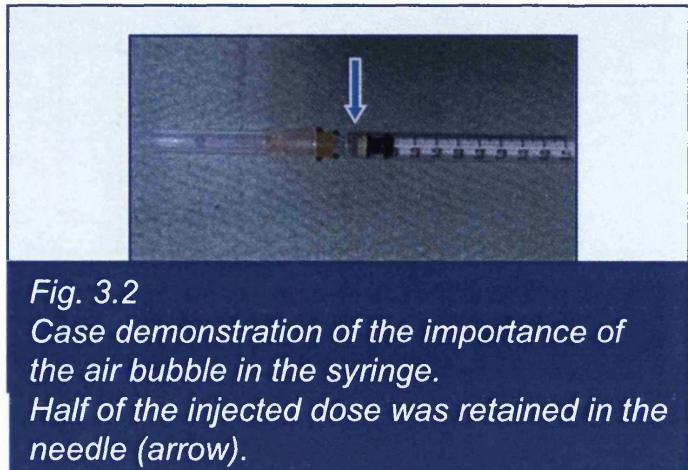
3.3 Injection Technique in non-palpable breast cancer

With widespread use of screening mammography and increased patient's awareness, increasingly higher numbers of non-palpable screen detected breast carcinomas are seen. These lesions by virtue of representing early breast carcinoma, are the most suitable for the sentinel node biopsy. A definitive diagnosis of invasive breast carcinoma is mandatory before considering these patients for the sentinel node biopsy.

For non-palpable lesions that are detected by mammogram or ultrasound scan, the overlying skin can be marked, and the injection can be delivered intradermally at the marked site with the technique described earlier (fig 3.4).



*Fig. 3.1
Preparation for injection*



*Fig. 3.2
Case demonstration of the importance of
the air bubble in the syringe.
Half of the injected dose was retained in the
needle (arrow).*

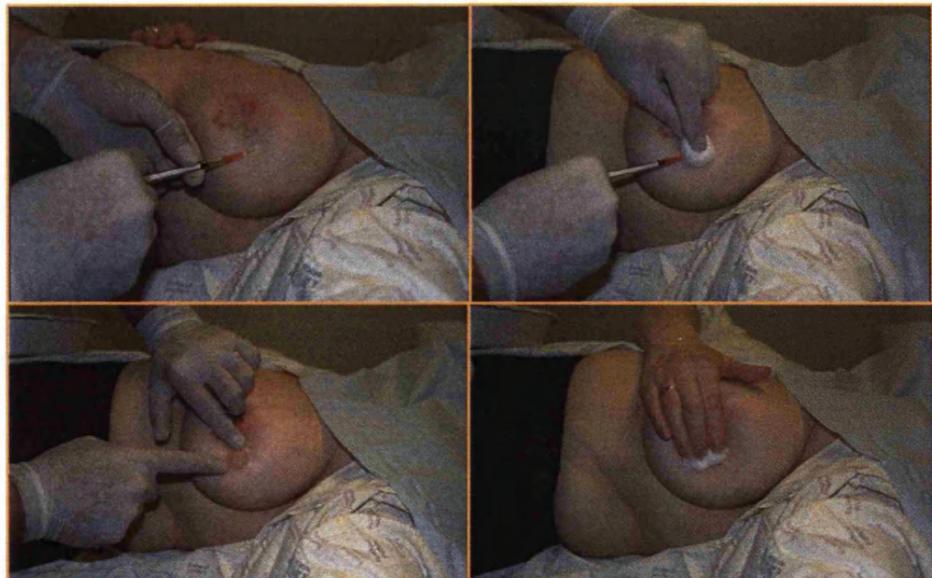


Fig. 3.3 Intradermal injection technique.

3.4 Administration of Radionuclide with Needle-Free Syringe

We were prompted to evaluate the feasibility of the administration of the radionuclide with a needle-free injection system as we encountered a patient with breast cancer who wished to undergo SLNB and was needle phobic.

Needle phobia is a recognised entity and there have been reported cases of patients' refusal to undergo SLN localisation because of it¹⁴.

Since injectate delivery is via a micro-orifice in the J-Tip device at high velocity, it is virtually pain-free¹⁵. To administer the radiopharmaceutical the syringe is applied firmly and perpendicular to the skin surface (fig. 3.5). The new technique of the delivery of radiopharmaceutical for sentinel node localization in surgical oncology is presented in chapter 4 of this thesis.

3.5 Lymphoscintigraphy

The purposes of lymphoscintigraphy are to determine the number of lymph nodes that are in a direct drainage pathway of the primary tumour, to differentiate these first-tier nodes from subsequent nodes and to locate sentinel nodes which present in unusual anatomical locations. It is also suggested that preoperative lymphoscintigraphy is a good predictor of success of SLNB in breast carcinoma.

It should be emphasized that the radiopharmaceuticals used for SLN detection are not tumour seeking agents, but rather 'lymph node seeking' agents. The radiotracers are accumulated in lymph nodes by macrophages, independently from the presence or absence of metastatic involvement. A "positive"



Fig. 3.4

Needle localisation of non-palpable breast carcinoma

a, under ultrasound guidance b, mammographic localisation



Fig. 3.5 a, Needle-free syringe

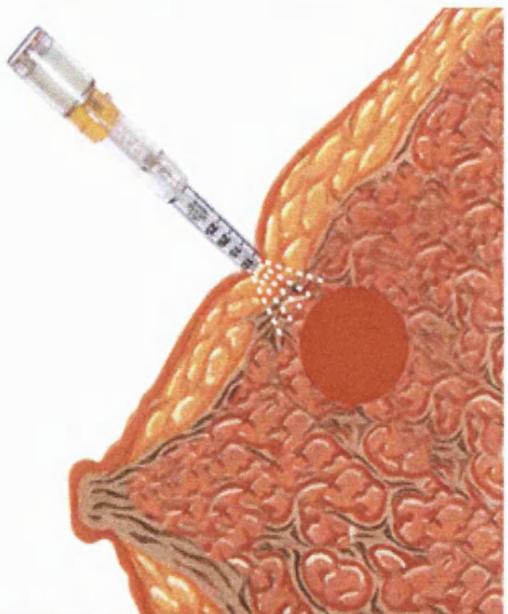


Fig. 3.5 b, Delivery of radiopharmaceutical in breast cancer

lymphoscintigram does not imply that the node is involved with metastatic tumour, it simply suggests that the SLN has been successfully identified. In fact a heavily invaded node may not accumulate the tracer and may remain undetected leading to a false negative result. This point is illustrated in the pitfall section of this chapter.

Several authors have stressed the crucial role of imaging in melanoma patients¹⁷⁻¹⁹.

Pts	Tracer	Dose (MBq)	Volume (ml)	Injection site	SLN identified (%)	
Borgstein	130	NC	40	4	NA	89
Uren	34	AC	20	0.3	PT	91
Veronesi	163	NC	7	0.2	SD	98
Gill	36	AC	NA	NA	PT	83
Reuhl	73	NC	54	0.5	PT	85
Schneebaum	15	RC	60	NA	PT	87
Sandrucci	37*	NC	26	0.8	PT	97
O'Hea 6	60	SC	11	4	PT	75
De Vries	48	NC	60	0.2	IT	79
Roumen	83	NC	60	2	PT	80
Van der Ent	60	NC	370	4	PT	98

Table-3.1 SLN identification rate with various techniques

SC, ^{99m}Tc-sulfur colloid, NC, ^{99m}Tc-nanocolloid, AC, ^{99m}Tc-antimony sulphide colloid; RC, ^{99m}Tc-rhenium colloid; PT, peri-tumoral injection; IT, intra-tumoral injection; SD, sub-dermal injection; NA, data not available. * seven patients could not be evaluated.

Although lymphoscintigraphy in breast cancer has been questioned²⁰, knowledge of lymphatic drainage patterns has been found to predict difficulties in identifying the sentinel node and to assist in minimizing a false-negative biopsy¹⁹.

A significant additional advantage conferred upon imaging is the capability to demonstrate potentially undetectable nodes when using the probe alone. This includes those sentinel nodes sited very close to, or actually underlying, the injection site²¹, sentinel nodes located away from the expected lymph node basins and finally those sentinel nodes situated very deep to the skin surface and/or demonstrating very low levels of tracer uptake.

3.6 Sentinel Node Imaging Technique in Breast Cancer

At our Institution every patient who is scheduled for SLNB, undergoes preoperative lymphoscintigraphy. Figure-3.6 illustrates experiments that we conducted to optimize our imaging protocol before recruiting patients into our trial.

Our practice has evolved, and has been further refined, over the course of the first 30 or so patients studied. We have modified and improved detailed aspects of the protocol in response to the technical problems encountered during initial studies.

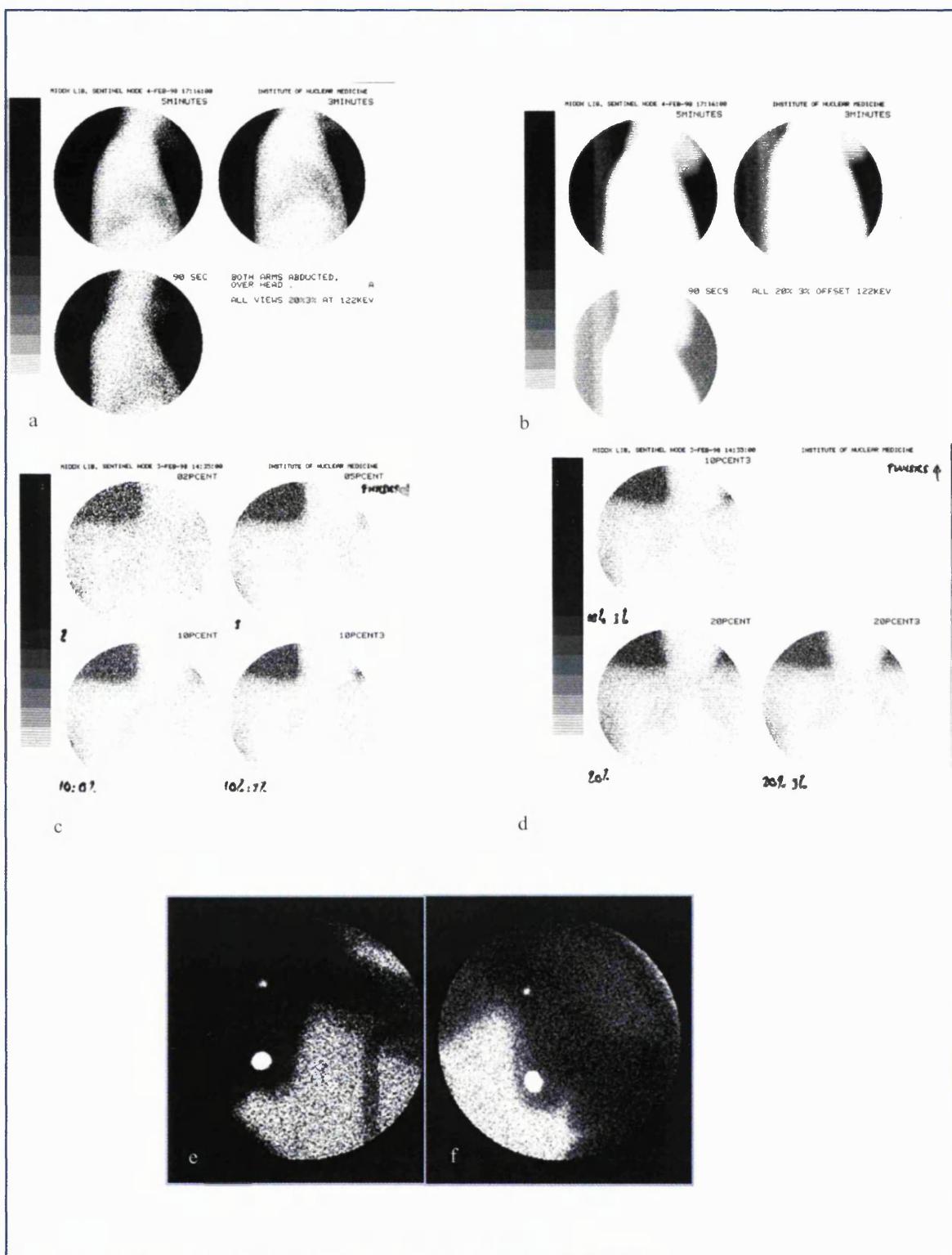


Fig. 3.6 Optimization of imaging. a, Imaging in supine position with both arms abducted over head with transmission energy window of 122 KeV:20% width (lateral view) b, arms by the side with the same setting, note the lung fields are not visualised (lateral view) c, transmission energy window set at 122 KeV 10% width (anterior view)d, transmission energy window set at 122 KeV 20% width (anterior view) d, imaging with the patient leaning forward in a hanging breast position (lateral view)

3.6.1 Imaging protocol

A single-headed gamma camera is suitable for the acquisition of all sentinel node image data. We use a low energy high-resolution collimator (LEHR) to achieve maximal resolution of the lymphatic structures and a large field of view (LFOV) detector - for full coverage of all those nodal basins that are likely to drain tracer from the injection site.

Our imaging protocol comprises of three phases:

1. Initial rapid dynamic imaging sequence performed immediately after administration of tracer. We perform dynamic imaging to study kinetics of radiotracer after injection and also to establish whether or not dynamic imaging is required in SLNB for breast cancer
2. A set of static images acquired at the completion of dynamic acquisition.
3. Delayed imaging at no less than 12 hours after administration of radio-nuclide.

3.6.2 Patient positioning

The patient is positioned for imaging before the tracer is injected to ensure minimum delay between injection and start of the initial dynamic study. The imaging is performed in the supine position with the arm on the affected side abducted at approximately 90°, which is identical to the position on the operating table (fig. 3.7). Patients are asked to remove their bra and all metallic objects before imaging is commenced. They are also asked to empty their bladder to avoid problems during imaging.

Early images should be acquired in the relevant anterior oblique projection,

with the detector orientated at approximately 30° anteriorly. In this angle, the camera face is parallel to the chest wall and axilla. The exact angle should be recorded and reproduced for all later acquisitions. It is important to ensure that the detector field of view covers the thorax from the mid-line to the lateral surface, and fully encloses the axillary region. The field of view should reach axially from the costal margin to the sternal notch, extending to the supraclavicular fossa. The contours of the shoulder should also be included as a landmark for transmission imaging, if this is possible.

In tumours located in the upper outer quadrant of breast, the injection site may overshadow the sentinel node. We therefore retract the breast medially and downwards and tape it in position to avoid this problem (fig.3.8).

3.6.3 Acquisition of Early Dynamic and Static Image Data

The dynamic sequence is commenced immediately after injection. We collect data into a 256x256 pixel matrix with 10 second framing for 15 minutes, followed by 1 minute framing for a further 30 minutes. Immediately after completion of dynamic acquisition, 5 minute 256x256 word mode static image is acquired with the patient in the same position. This generates a static image of high technical quality approximately 45 minutes after the tracer is injected. By administering one intradermal injection of a small volume of tracer, we have not found it necessary to shield the injection site with a lead mask.

With the help of electronic marking facilities, we mark the position of the nipple on the static image (fig. 3.9). We have found that use of a radioactive point source



Fig. 3.7 Patient position during imaging (anterior oblique view)

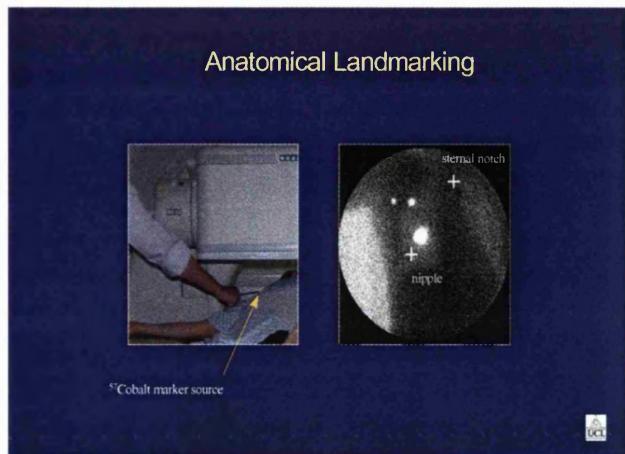


Fig. 3.9 Using the electronic marking Facility to mark position of nipple

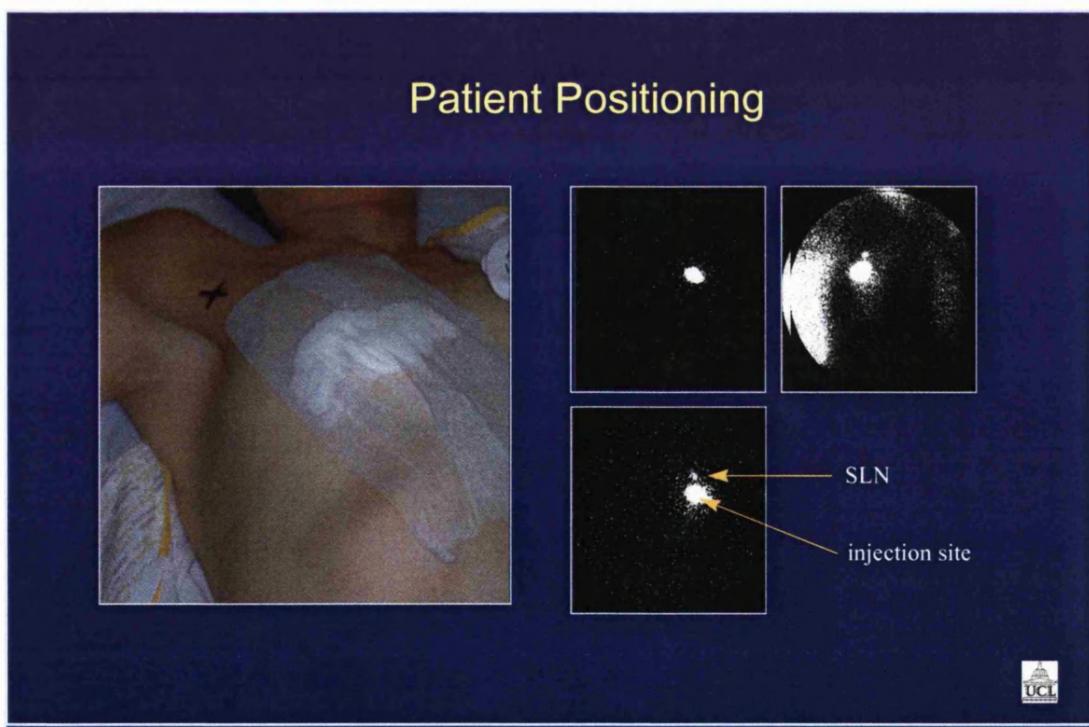


Fig. 3.8 Taping the breast onto the chest medially and inferiorly to prevent overshadowing of the sentinel node from the injection site

marker can introduce unacceptable ambiguity when interpreting the image at a later stage, as the resulting detail may be easily confused with a site of genuine physiological tracer uptake if it is not meticulously documented.

If the sentinel node is demonstrated at this stage, a transmission view (256x256 word mode) is acquired for two minutes, with the patient remaining in the same position. This adds anatomical landmarks to the data and makes the study easier to interpret. This is obtained by placing a ⁵⁷Cobalt flood source immediately beneath the patient, encompassing the field of view and orientated parallel to the camera face (fig.3.10).

An energy window appropriate to the flood source radionuclide is selected for the gamma camera. The transmission image should not be used for diagnostic purposes but is acquired as a valuable tool for adding anatomical landmarks. Acquisition of a transmission image should render the lung fields visible for the majority of patients (fig.3.11).

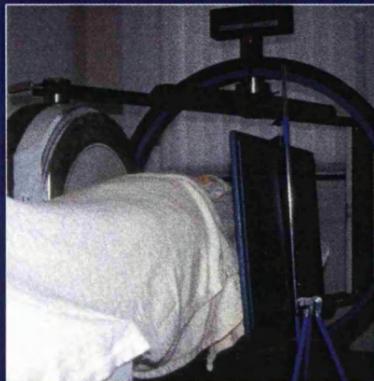
A flexible, radioactive line source affixed to the skin can be helpful in delineating the anterior-posterior outline of the breast, if a flood source is not available. This may be conveniently achieved using a commercially available 57-Cobalt filled line source (fig.3.12).

Most gamma camera computer systems incorporate software permitting the operator to add together two count-normalised images. The maximum count density for the sentinel node in the emission image and the mean count density for a representative region of unattenuated transmission source are compared,

Transmission Imaging



Anterior Oblique Image



Lateral Image

Fig 3.10

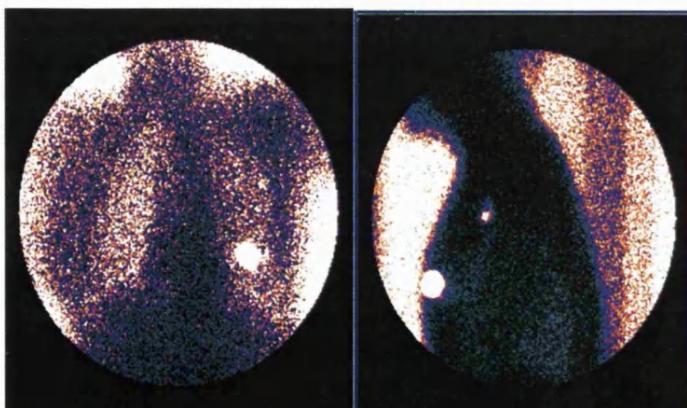


Fig 3.11 Lung fields visible as an additional anatomical landmark



Fig 3.12 57-Cobalt filled line source

weighted and used to add a weighted, 'count-normalised' emission image to the associated transmission image, thereby generating a 'composite study' (3.13). It is important to ensure that the patient is positioned identically for the acquisition of both sets of data and does not move throughout the procedure.

At this stage the position of SLN is marked on the skin surface immediately overlying the site of tracer uptake. This assists in planning the incision for surgery and is easily achieved by using a ⁵⁷Cobalt point source marker and the gamma camera in persistence mode. The marker source is placed directly onto the skin surface and progressively advanced towards the site of tracer uptake until its own image superimposes upon that of the node. Once the exact site of uptake is located the overlying skin is identified by accurate marking with a water-resistant permanent marker (3.14).

This is followed by the acquisition of a lateral image. This helps to determine the depth of the node beneath the anterior skin surface. In upper outer quadrant lesions this image may also reveal an undetected sentinel node if it has been obscured by activity from the injection site (fig. 3.15).

For optimal image quality the patient is positioned with the arm on the affected side over-abducted until a comfortable position is found. This removes the arm as a source of attenuation between the sentinel node and the detector and minimizes the distance between collimator face and source of activity, thereby improving image resolution.

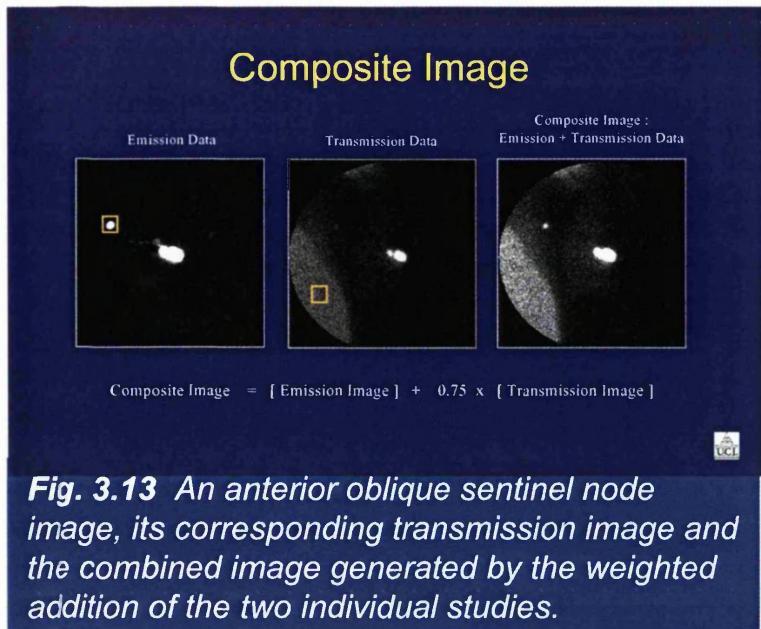


Fig. 3.13 An anterior oblique sentinel node image, its corresponding transmission image and the combined image generated by the weighted addition of the two individual studies.

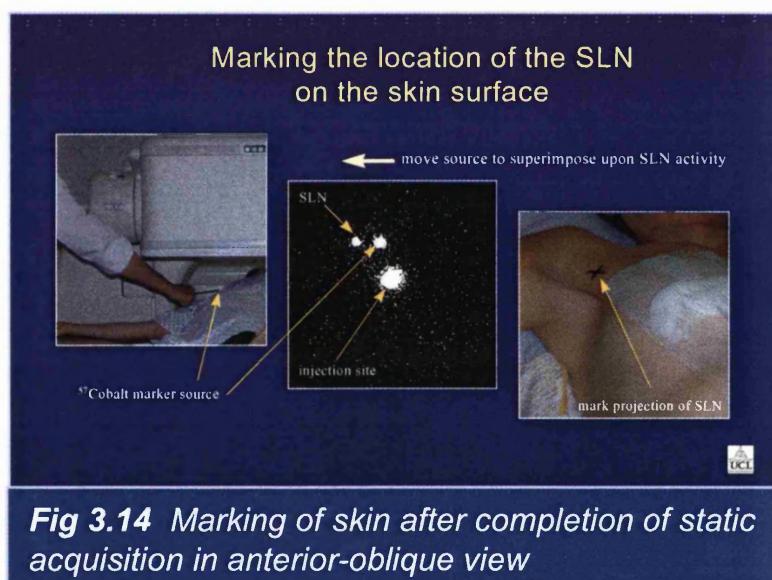


Fig 3.14 Marking of skin after completion of static acquisition in anterior-oblique view

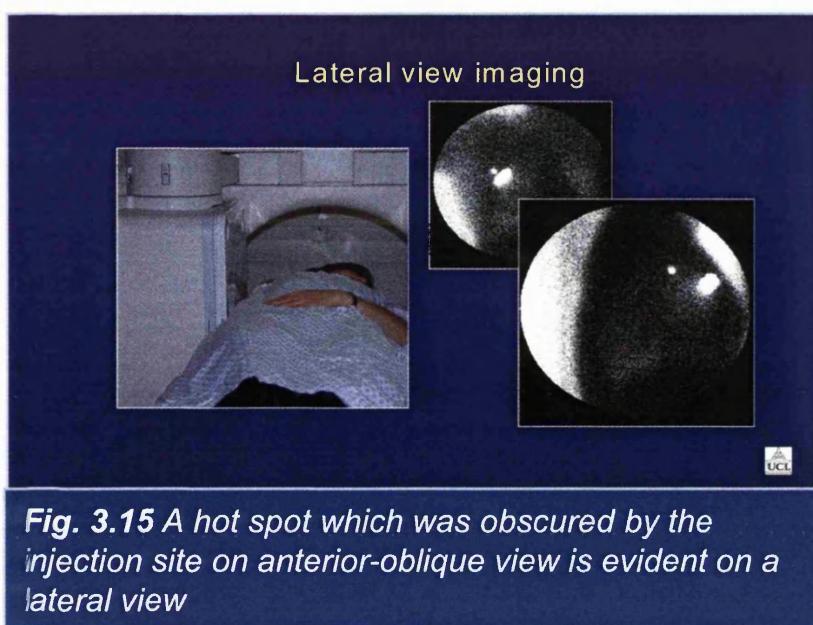


Fig. 3.15 A hot spot which was obscured by the injection site on anterior-oblique view is evident on a lateral view

The field of view of the detector should extend from the cricoid to the costal margin. A five minute 256x256 word mode static image is acquired in this position. Again an electronic nipple marker is included at this stage. With the patient remaining in the same position, a two minute transmission image is also acquired in the same manner as described before, reproducing the previous positioning and imaging conditions.

3.6.4 Acquisition of Late Static Image Data

Delayed anterior-oblique and lateral static emission and transmission images (12-18 hours after injection) are acquired as described for five minutes each, reproducing the previous positioning and imaging conditions.

3.6.5 Image Processing and Display

The dynamic study is subsequently reviewed in cine form to allow the underlying time-dependency of tracer uptake to be clearly visualised. Selected frames may then be extracted for individual display to best illustrate the migration of tracer, either as single frames or as the sum of a short sequence of consecutive frames. As a standard display 2 frames are chosen from the initial 15 minutes of dynamic acquisition (10 sec frames) and two from the subsequent 30 minutes acquisition (1min frames).

Each static emission image is displayed alongside its corresponding combined emission and transmission image, with any anatomical landmarks superimposed, e.g. a marker for the nipple. A comparison of equivalent anterior oblique and lateral images at differing time points will assist in the interpretation of tracer flow through the sentinel node(s).

3.7 The Surgical Technique in Breast Cancer

The surgical techniques employed in the intraoperative detection of sentinel node have varied significantly. These range from blue dye lymphatic mapping alone to probe guided surgery alone or in combination with blue dye technique. Giuliano et al²² performed lymphatic mapping by using isosulfan blue vital dye which was injected in a peritumoural fashion into the breast parenchyma on 174 patients. A success rate of 65% and a sensitivity of 75% was reported. The same authors²³ subsequently report a success rate of 99% with further experience in detection of the SLN using the blue dye lymphatic mapping only. In another study by Flett et al²⁴ the authors report a success rate of 82% and sensitivity of 83% in 68 consecutive patients with breast cancer using blue dye only. These reports suggest that although the SLN biopsy with blue dye lymphatic mapping is achievable with acceptable success rate and sensitivity, a significant training element comes into place²³. The other limitation of this technique is that localization of sentinel nodes in lymphatic basins other than the axillary basin is not possible^{8,25,26}. The timing of the injection of the blue dye is crucial for the success of the procedure. If injected too early, there would be extensive blue staining of lymph nodes in the nodal basin making the task of sentinel node localization impossible. On the other hand if the injection is administered too late, successful localization may fail due to the inability of the dye to reach the sentinel node caused by inadvertent disruption of the lymphatic channels during dissection.

Knowledge of the location of the sentinel node before surgical exposure is what differentiates probe guided surgery and blue dye lymphatic mapping.

The technique of probe guided surgery for intra-operative detection of SLN in breast carcinoma was introduced by Krag and associates²⁰ after their initial success in staging patients with melanoma^{27,28}. The success rate of this technique was 82%. Another important advantage of probe-guided surgery is the fact that complete excision of the sentinel nodes can be verified by directing the probe into the wound to measure residual tracer activity.

In a study performed by Albertini and associates¹, combining the blue dye and probe guided surgery in 62 patients, a success rate of 92% was reported with 100% accuracy in predicting the axillary node status. These authors concluded that the addition of gamma detection probe increased the success rate from 73% to 92% as in 12 patients, blue dye did not appear in the lymph nodes but focal hot spots were detected by probe. A higher detection rate of 98% is reported by Veronesi and co-workers⁵ with a false negative rate of 5.4%, after intradermal injection of colloidal albumin and probe guided surgery.

There are several reports that indicate that the combination of blue dye lymphatic mapping and probe guided sentinel node localization is complementary^{2,6,29}. The overall success of sentinel node localization is maximized and the incidence of false negative results are reduced when both techniques are used together. This is because a probe will give the surgeon a sense of direction and allows detection of non-visible nodes due to their radioactive content, whilst the blue dye helps as a visual guide when the node is exposed. Combination of both techniques may also accelerate the learning curve of each method used in isolation. It has also been our experience that additional information from pre-operative lymphoscintigraphy is very helpful in predicting the success of the sentinel node biopsy.

Table-3.2 summarizes the success rate of various detection techniques and clearly demonstrates higher success rate of combination of pre-operative lymphoscintigraphy, probe guided surgery and blue dye lymphatic mapping.

In our trial, we used both dye and radionuclide techniques for the intra-operative detection of the sentinel lymph node.

3.7.1 Intra-operative Sentinel Node Detection Technique

Once the patient is anesthetized and prepared for the operative procedure, the probe and its cable are covered with suitable sterile plastic tubing for extra safety to avoid contamination. The probe's display unit is placed in front of the surgeon. It is essential to ensure that the probe is in a good working order before commencing the operation. We also ensure that the probe is well secured during operation to avoid its accidental fall since operating probes are very sensitive to trauma and costly to replace (fig.3.16).

3.7.2 Injection of Patent Blue Dye

We inject 1-2 ml of patent blue dye subdermally into the skin overlying the primary tumour (fig.3.17). It is best to use a syringe with a luer lock, which avoids any accidental spillage of the blue dye during its administration under pressure. It is good practice to inform the anaesthetist about the patent blue dye injection, as occasionally total body blue coloration is noted, and this may cause confusion with hypercapnia or pulmonary embolism³⁰. The complication of an anaphylactic reaction as a result of blue dye administration is very rare must be borne in mind²⁹. We also warn patients about blue discolouration of urine as a result of blue dye administration and occasional tattooing of the skin (fig.3.18). Occasionally a blue tract is visualized after administration of the blue dye (fig. 3.19).

Success rate

Technique	No. of patients	No. of studies	weighted average
Blue dye	667	8	76.3%
Probe	635	6	91.5%
Dye & probe	104	2	91.3%
Imaging & probe	508	5	87.8%
Imaging, probe & dye	649	5	93.8%

Alazraki & Nieweg et al



Table-3.2 Success rate of various detection technique

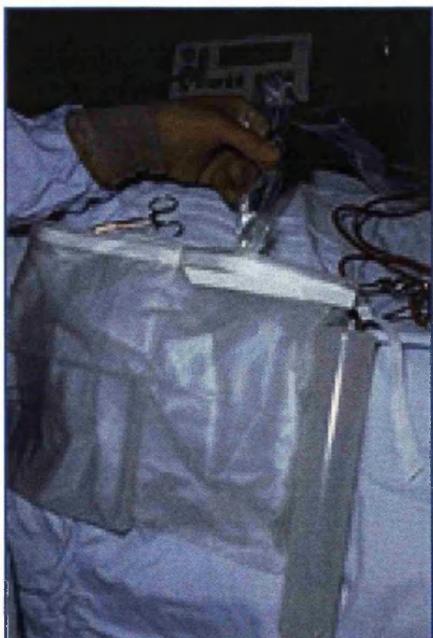


Fig 3.17 Injection of patent Blue Dye

Fig 3.16 Gamma probe is well Secured during operation



Fig 3.18 Blue discolouration of urine and faint tattooing of skin as a result of Patent Blue dye injection



Fig 3.19 Blue lymphatic tract visible after injection of Patent Blue dye. This Corresponds to the lymphoscintigram which shows the same pathway

Before the incision is made all the blue stained swabs are removed from the operation field and if the gloves are stained, these are changed. This is to avoid any misunderstanding as a result of inadvertent staining of axillary tissue during dissection. Sometimes the lymphatic duct can be visualized through the intact skin.

3.7.3 Determination of the site of incision

It has been our observation that whilst it is helpful to mark the skin under the gamma camera, it is not always accurate, as this is performed only in one plane. We verify the location of the sentinel node before the incision is made (fig. 3.20).

The gamma detection probe (Neoprobe 1500) is applied over the axilla, around the skin mark. The probe is moved slowly over the skin to find the center of the hot spot with maximum signal. This is confirmed by a high pitch audio signal from the probe and a high radiation count as compared to the background activity.

3.7.4 Measurement of the background activity

Background radiation activity is measured by pointing the probe away from the injection site. In most cases we perform this by applying the probe around the sternal notch area and assuring that it is held at a 90 degree angle to the body (fig 3.20).

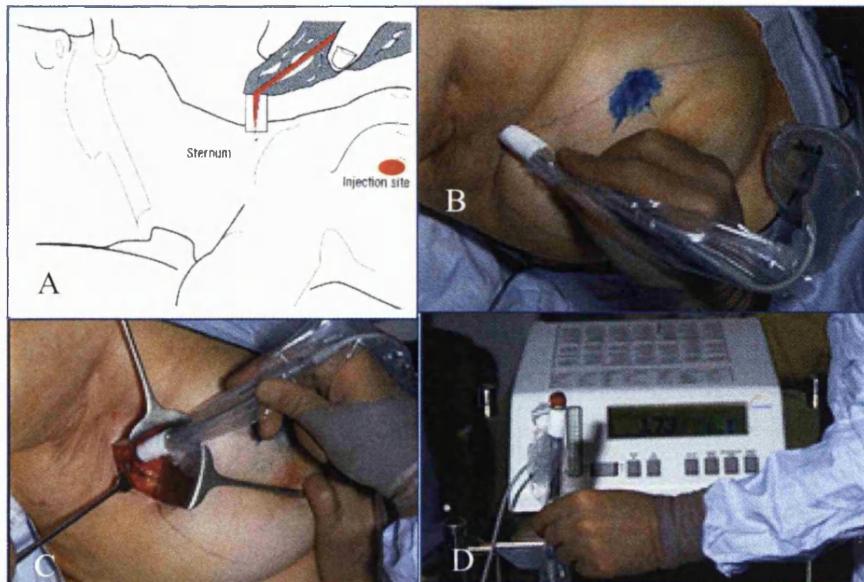


Fig 3.20 Intra-operative detection of the sentinel lymph node. A, measurement of background activity B, determination of the site of incision C, establishing the line of sight D, Confirmation of ex-vivo radioactivity.

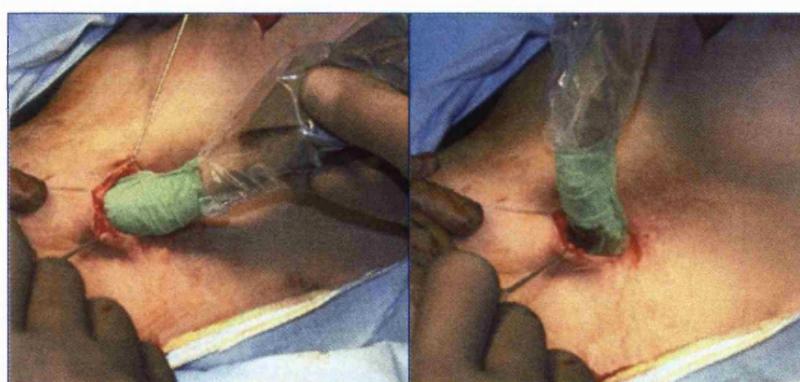


Fig 3.21 Measurement of residual activity after SLN biopsy

3.7.5 Probe guided surgery

The gamma detection probe gives the surgeon a sense of direction. Krag and associates describe this as establishing the “line -of-sight”. In this way the dissection is not blind and the surgeon determines the shortest route to the sentinel node. As a result tissue disruption is minimal. It is important to avoid pointing the probe towards the injection site as this will artificially raise the radiation count. The audio signal's pitch increases as the sentinel node is approached and this helps the surgeon to accurately locate the hot spot without needing to survey the control unit (fig 3.20).

3.7.6 Excision of the sentinel node

The sentinel node is approached by a blunt dissection with special attention to haemostasis. If a blue stained lymphatic duct is encountered during dissection, care is taken not to transect it as this will lead to leakage of the blue dye and blue discoloration of the axillary tissue.

After the sentinel node is localized by the probe, additional visual assistance from the blue dye can also help, the radioactivity of the node is recorded by the probe as “in vivo counts/sec”. The radioactive node is excised and the “ex-vivo” count is measured. To avoid any interference from the background radioactivity of surrounding areas, the “ex-vivo” count is best done by applying the node over the probe which is faced upwards (fig 3.20). The sentinel node is labeled separately and sent fresh to the lab for further analysis.

3.7.7 Verification of sentinel node excision

It is very important to confirm the complete removal of the radioactive nodes. This is achieved by re-applying the probe into the wound. A careful measurement of the residual activity is done. It is important to angle the probe in all directions to ensure that there is no residual sentinel lymph node. This is one of the clear advantages of radiocolloid guided surgery (fig.3.21). Krag and associates advocate removal of radioactive nodes until the background activity at the bed of the sentinel node resection site is reduced to less than 10% of that of the most radioactive resected sentinel node.

3.7.8 Completion lymphadenectomy

After the sentinel node is excised, the standard axillary node dissection is performed. This is because we are at present still validating the predictive value of sentinel node biopsy in determining the status of the axillary lymphatic basin.

3.8 Pitfalls of Sentinel Node Detection in Breast Cancer

3.8.1 Spillage of radiopharmaceutical and contamination artifact

Contamination of the injection site by spread of tracer is possible as the radiopharmaceutical is absorbed onto the cotton wool swab used after injection. This is dispersed over the neighbouring skin during massage by the inappropriate further use of this swab (fig.3.22). Careful attention to the injection technique can prevent this problem. The skin at the injection site should be immediately covered by a small adhesive plaster to prevent leakage of tracer from the puncture site. The cotton wool swab used at injection should

be discarded immediately afterwards, and a new swab used to massage the skin.

Contamination artefacts may also occur after the spillage of the radiotracer onto the couch (fig.3.23) or patient's clothing (fig.3.24). This can mimic a focal hot spot and can potentially be confused with a sentinel node. Extra care during injection can overcome this problem. It is also very important to discard the radioactive waste immediately after the injection. Any contaminated clothing or bedding should be removed and replaced.

3.8.2 Spillage of radiopharmaceuticals after injection with the J-tip syringe

Extra care needs to be taken during injection of radiocolloid with the J-Tip needle free injection system. As the driving force in this syringe is compressed carbon dioxide in a cartridge, the operator has no control over the delivery of radiotracer after pressing the trigger button.

Contamination due to spillage can be a major drawback of this device, if it is not used properly (fig 3.25). It is important to hold the syringe perpendicular to the skin surface and to apply a gentle pressure. It is also very important to warn the patient about the hissing sound which is heard as soon as the trigger button is pressed. Inadvertent movement of the patient can lead to spillage. Ideally injection should not be delivered near the camera face. It is also good practice to cover the remainder of the breast with an absorbent sheet.



Fig 3.22 Contamination of adjacent skin during massage of the site

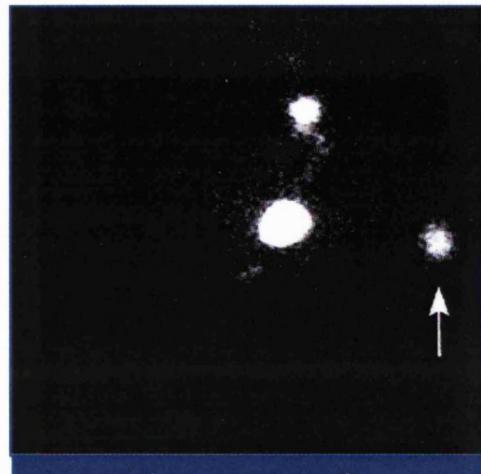


Fig 3.23 Contamination of imaging couch due to a minor spill of tracer

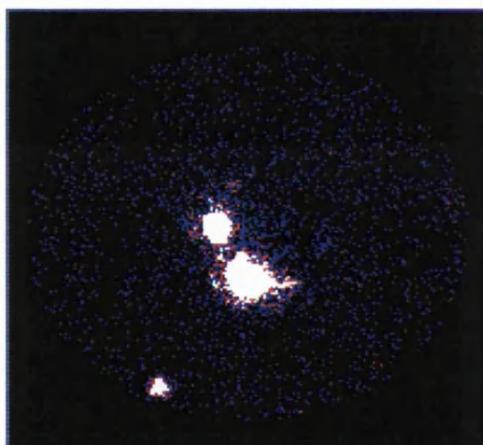


Fig 3.24 Contamination of the patient's gown, due to a minor spill of tracer.

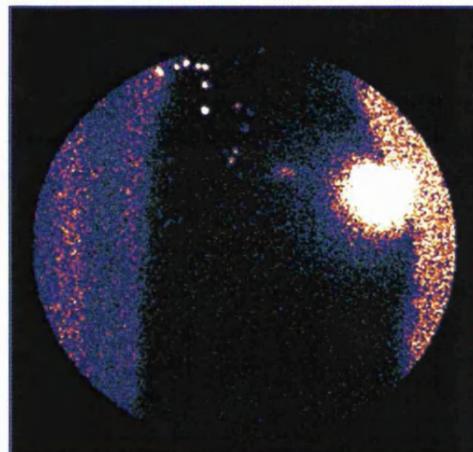


Fig 3.25 Contamination artefact after injection with needle-free injection device

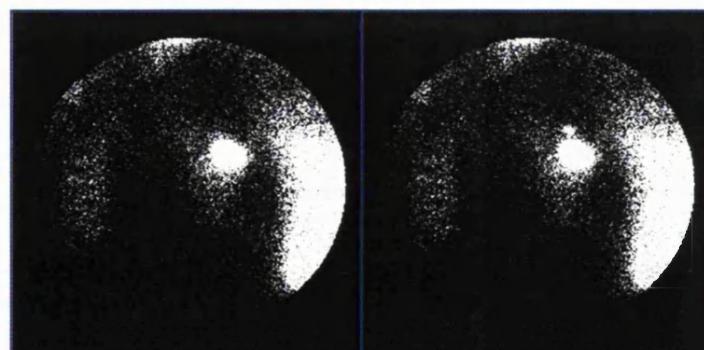


Fig 3.26 Shine through phenomenon during imaging. Breast retraction reveals the hot spot.

3.8.3 Pitfalls of imaging and detection

3.8.3.1 *Upper Outer Quadrant Lesions*

Upper outer quadrant lesions can be difficult during imaging and gamma probe localization³⁰. This is due to the close proximity of the injection site to the sentinel lymph node leading to radiation scattered from the injection site reaching the detector. This is termed as the 'shine through phenomenon'. In a multicenter validation study by Krag et al²¹, all false negative results occurred in patients who had the primary carcinoma in the lateral half of the breast. Figure-3.26 illustrates the shine through phenomenon during imaging. To overcome this problem, it is important to retract the breast downwards and medially and tape it into position. Lateral view imaging is also very important.

Proximity of the injection site to the sentinel node can also cause problem during intra-operative detection with the probe. To overcome this, the following is helpful:

1. Angling of the probe away from the injection site.
2. Use of additional collimation to reduce the scattered radiation and background activity.
3. Sometimes it may be helpful to excise the primary lesion prior to the sentinel node biopsy to remove the more active source of radioactivity from the proximity of the sentinel node.

3.8.4 Extensive infiltration by metastatic carcinoma

A preserved functional capacity of the lymph node for nodal uptake of the radioactive colloid is critical for successful localization². Nodal uptake is

progressively reduced if there is extensive infiltration with metastatic carcinoma. This is a cause for worry as it can lead to a false negative sentinel node biopsy and is one of the most important potential pitfalls. In a study by Borgstein and associates, in four patients the less radioactive non-sentinel node contained extensive tumor infiltration as compared with hot sentinel nodes which were not involved with carcinoma in two of these patients. They recommend careful scrutiny of the lymphoscintigraphic images to overcome this potential pitfall. It is also very important to select patients appropriately and exclude those with clinical evidence of lymph node involvement.

We also encountered one patient during the learning phase of our trial which illustrates these points and is now reported.

3.8.4.1 Case report

A 54 year old overweight man presented with a central lump in his left breast. On examination there was a 4 cm firm to hard mass which was located in the retroareolar region. The tumour was clinically tethered to the pectoral fascia. Examination of axilla was very difficult due to the patient's obesity. Triple assessment was diagnostic of breast carcinoma.

A pre-operative Tc-99m - MIBI (methoxy isobutyl isonitrile) scan showed uptake at the site of primary tumour as well as some uptake in the axilla (fig 3.27). On lymphoscintigraphy, despite administering the radiocolloid intradermally, there was exclusive drainage to the internal mammary chain and there was no uptake in the axilla (fig3.28).

During surgical exploration of the axilla, a blue tract leading to a large mass of lymph node was evident. There was no blue discoloration of the node (fig.3.29)

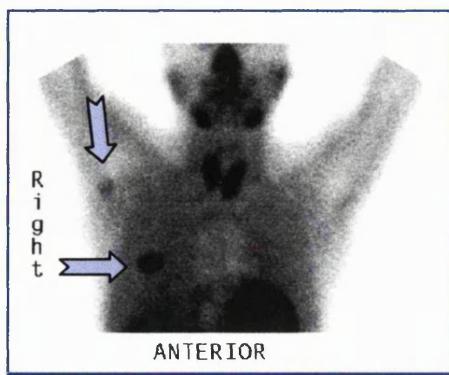


Fig 3.27 MIBI scan showing uptake at the site of primary tumour and in the axilla (arrows)

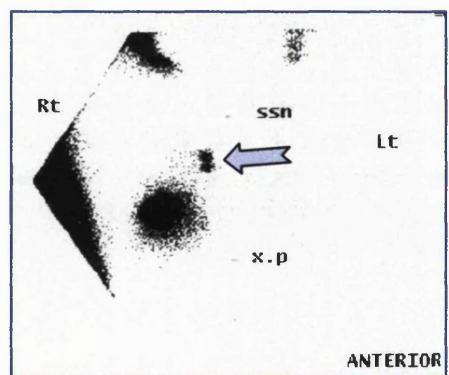


Fig 3.28 Imaging shows exclusive drainage into the internal mammary chain (arrow)

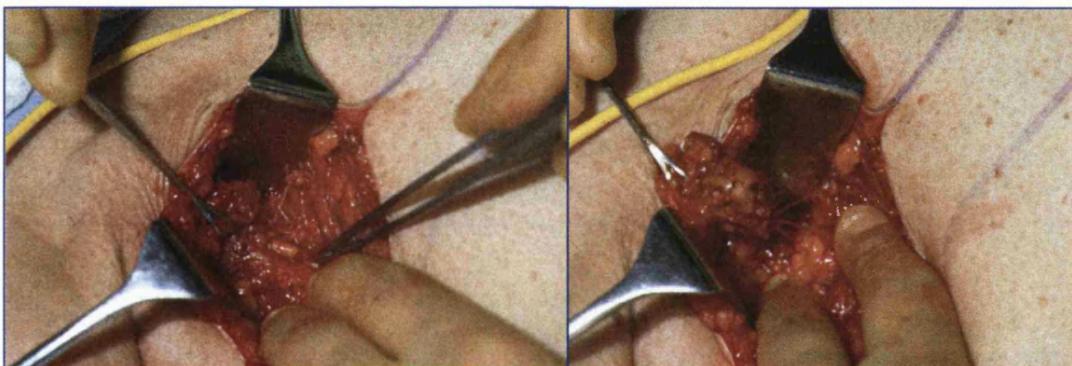


Fig 3.29 Intra-operative findings: a mass of lymph nodes within the axilla, no blue discolouration of the tract or the lymph nodes noted.



Fig 3.30 Ex-vivo radiation count measurement does not show any activity.

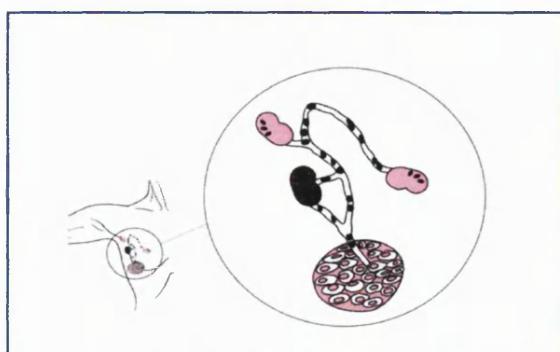


Fig 3.31 Complete replacement of the SLN with metastases can lead to a false negative result.

and on applying the probe, no radiation count was recorded. Ex-vivo radiation count failed to record any activity (fig.3.30). On histological analysis, the lymph node was completely replaced with metastatic carcinoma with evidence of extra nodal spread.

This case illustrates reduced functional capacity of the lymph node due to complete replacement with tumour. This leads to mechanical obstruction to afferent lymphatic channels which in turn leads to changes in the direction of flow and opening up of alternate lymphatic drainage routes (fig 3.31). Internal mammary drainage after intradermal injection of tracer is very rare. In this case, the drainage was re-routed to the internal mammary chain because of mechanical obstruction of the sentinel node. This case highlights the importance of patient selection in order to minimize the false negative result.

3.8.5 Reduced functional capacity due to fatty degeneration of the SLN

The other reason for reduced functional capacity of lymph node is fatty degeneration of the axillary lymph node. We have observed this condition in elderly patients (fig 3.30). Tracer uptake by the lymph node is reduced which in turn makes the probe localization difficult. Additionally, it may pose difficulty in preparing frozen sections for histological analysis. Additional use of blue dye and careful interpretation of lymphoscintigraphic scans may help to overcome this problem.

3.8.6 Intra-mammary Sentinel Node

Intramammary lymph nodes are increasingly reported since sentinel node biopsy in breast cancer has been introduced (3.31).

Pre-operative lymphoscintigraphy is an important pre-operative investigation for its detection.

3.8.7 Radioactive Nipple Marker

Use of a radioactive nipple marker, without clear documentation as to its exact location may give rise to uncertainty when interpreting the image data (fig.3.32). It is therefore important to use the gamma camera's electronic anatomical marking facility if this is available.

3.8.8 Residual uptake of tracer due to immediately preceding radionuclide scan

Figure 3.33 shows a lymphoscintigram in a patient who had a bone scan 2 days prior to admission for the sentinel node biopsy. There is retention of ^{99m}Tc -labelled phosphonate (HDP). There is also some uptake of colloidal tracer in the liver which is due to trapping by the reticuloendothelial cells.

Skeletal retention of phosphonate tracers in bone is normally significant, and therefore if at all possible a period of not less than three days should elapse before sentinel node mapping is performed. This would ensure that visualisation of the sentinel node is not confused with uptake of phosphonate tracer within the anterior ribs or sternum.

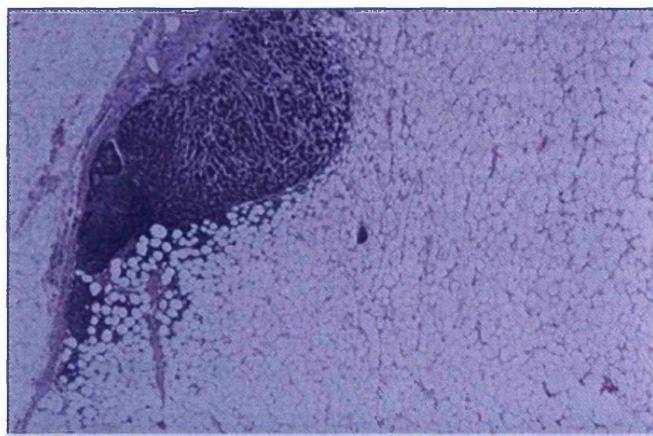


Fig 3.30 Reduced functional capacity of the SLN due to fatty degeneration.

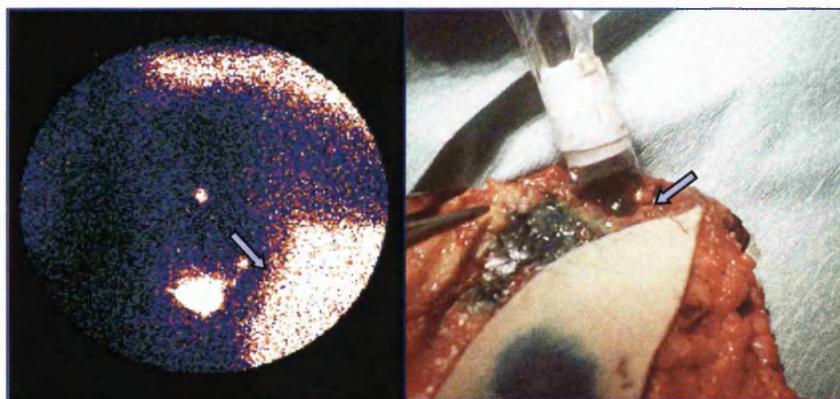


Fig 3.31 Intramammary lymph node (arrow).

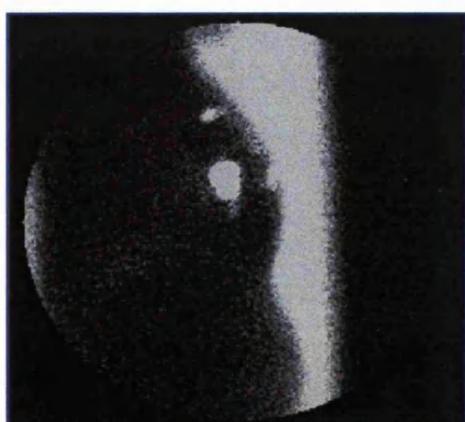


Fig 3.32 Radioactive nipple marker can mimic a sentinel node.

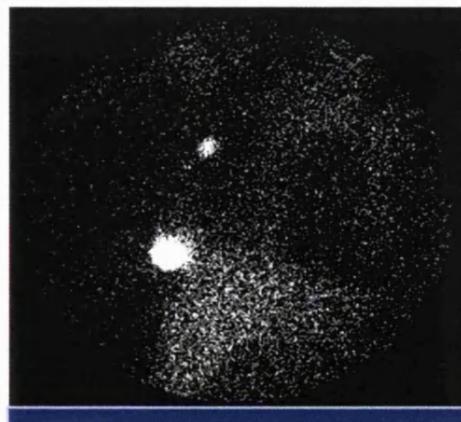


Fig 3.33 Residual uptake of tracer due to an immediately preceding isotope bone scan

3.8.9 Langers Axillary Arch and Sentinel Node

Langer's axillary arch is a relatively rare anatomical variation of the latissimus dorsi muscle insertion. The length varies from 7-10 cm in length and 5-15 mm in breadth. It crosses the axilla in front of the axillary neurovascular bundle to join the inferior surface of the tendon of the pectoralis, coracobrachialis or the fascia over the biceps muscle³⁴ (fig.3.34).

The clinical importance of this condition in axillary lymph node dissection (ALND) and lymphoedema as well as latissimus dorsi flap reconstruction has previously been described^{33,34}. Axillary vein obstruction in association with this condition has also been reported³⁵. We encountered two patients with the Langer's axillary arch during sentinel lymph node (SLN) biopsy.

3.8.9.1 Case reports

Case 1

A 43 year old female presented with a 5 months history of a lump in the right breast. On examination there was an ill-defined mass in the upper inner quadrant of the right breast. There were no palpable axillary lymph nodes. Triple assessment confirmed the diagnosis of breast carcinoma. She was scheduled for quadrantectomy, SLN biopsy and ALND. Standard lymphoscintigraphy was performed a day prior to surgery. Anterior and lateral data acquisition was obtained which showed an unusually high SLN in the axilla. This finding was more prominent on the lateral view (fig. 3.35). At operation it was very easy to localise the SLN (fig.3.36) using a combination of patent

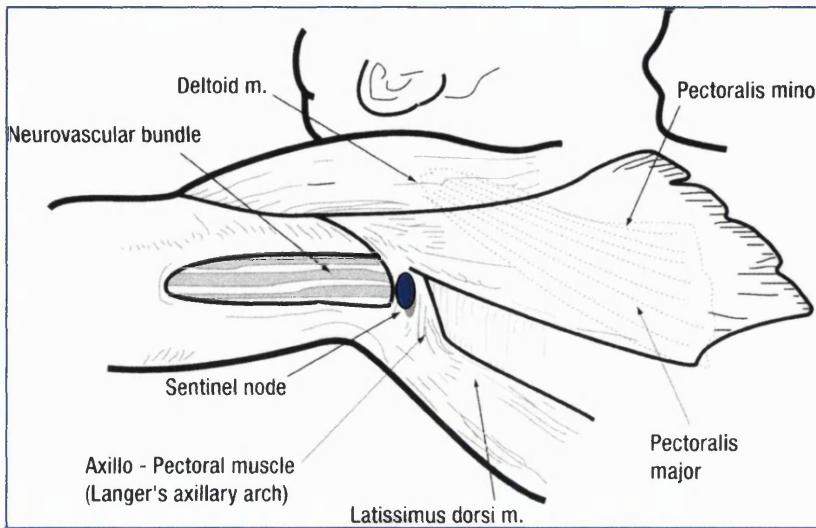


Fig 3.34 Langer's axillary arch. An anatomical variation of latissimus dorsi muscle insertion

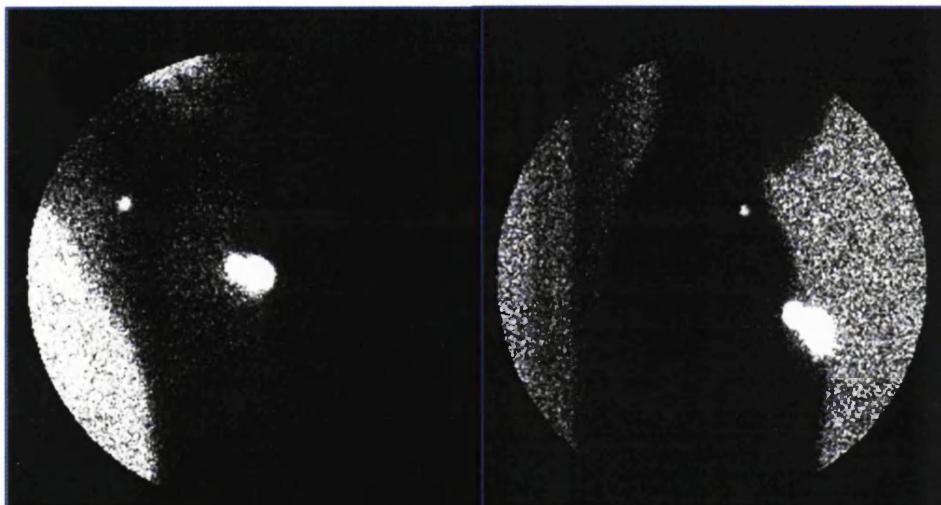


Fig 3.35 Lymphoscintigram showing a hot spot located high in axilla.

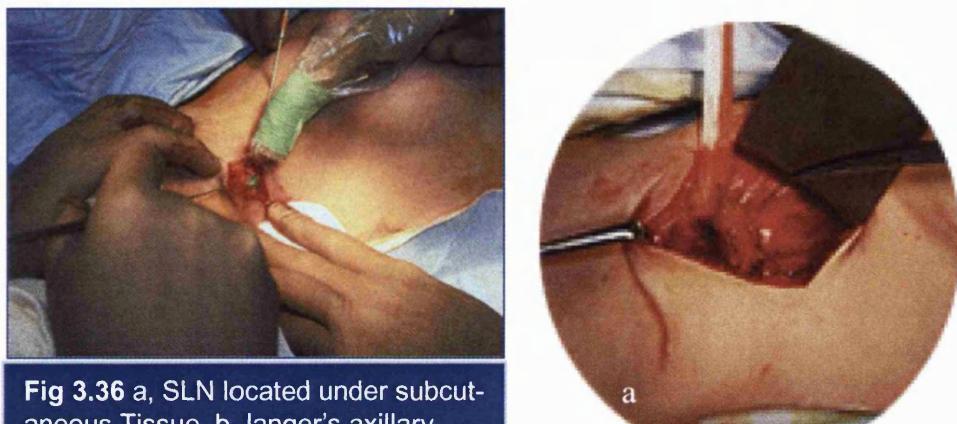


Fig 3.36 a, SLN located under subcutaneous Tissue b, langer's axillary arch

blue dye and gamma detection probe guided surgery with the Neoprobe-1500. A blue and hot SLN was located at level 1 in the axilla, just beneath the subcutaneous fat which was excised (fig. 3.36). As dissection progressed, a well developed axillo-pectoral muscular band was encountered underneath the SLN bed which had to be excised to get access to the axilla for ALND.

Case 2

A 45 year old lady presented with a 1 cm lump in the infra-mammary sulcus of the right breast which proved to be a breast carcinoma on triple assessment. She underwent a wide local excision, SLN biopsy and ALND. On lymphoscintigraphy the sentinel node was evident in the axilla. At operation, the sentinel node identification proved to be difficult. As the dissection progressed, a Langer's axillary arch was encountered and after dividing the muscular band, the sentinel node could be localised underneath the arch which was blue and hot.

3.8.9.2 Discussion

The reported incidence of Langer's axillary arch is about 7%. In the aforementioned cases the presence of a Langer's axillary arch affected the SLN biopsy. In the first case the SLN localisation was extremely easy due to the superficial location of the node as it was lying over the arch. The arch is fully stretched when the arm is abducted and as the arm was in abducted position during imaging, the pre-operative scan was misleading in showing the high location of the SLN.

In the second case the SLN was underneath the Langer's axillary arch and hence its detection was difficult. It was possible to localise the SLN after the

muscle band was excised. Excision of Langer's axillary arch is also recommended by others to get access to the axilla.

These two cases represent a relatively rare anatomical variation, encountered during sentinel node biopsy. This resulted in a misleading lymphoscintigraphic finding in one case and difficult SLN localisation in the other. Division of the band is essential for adequate access to the axilla. This anatomical variation should be kept in mind during the sentinel node biopsy and axillary lymph node dissection.

3.9 Histological Analysis of the Sentinel Node

The aim of our study was to evaluate the role of frozen section and paraffin section histology, serial sectioning and immunohistochemistry in detecting the histological status of the sentinel lymph node.

3.9.1 Study Protocol

Our research protocol involves the use of frozen section followed by confirmatory paraffin section. The axillary dissection at present is dealt with routinely. If the sentinel lymph node is negative on frozen sections and on paraffin sections, a pan cytokeratin marker (MNF116) is used to check for micrometastasis. The data from the sentinel lymph node is then correlated with the axillary dissection.

3.9.1.1 Frozen section

The sentinel node after excision and acquisition of imprint cytology sample is sent separately to the pathology laboratory. The SLN is processed within 30

minutes of excision and is kept on ice during the transport as our laboratory is located at a different site. The gross measurements and appearance of the lymph node are recorded and the node is bisected into two pieces. The node is subsequently frozen using cryo-spray. This is followed by sectioning at three levels (hence total of 6 levels) and stained with H&E. All frozen sections are examined by one histopathologist with an interest in breast disease. The findings of the frozen section are recorded without knowledge of paraffin section histology.

3.9.1.2 Paraffin sections

Confirmatory paraffin section is done following the frozen sectioning on the lymph node. These are assessed independently of the frozen section diagnosis. The SLN is therefore examined at 6 levels plus a paraffin section.

3.9.1.3 Immunohistochemistry

Lymph nodes which are negative both on frozen and on paraffin H&E section, are also investigated with immunohistochemistry using a pan-cytokeratin antibody (MNF116). It is anticipated that the combination of the three techniques on the sentinel lymph node would identify more than 99% of the micrometastasis. The axillary dissection is currently dealt with routinely with only a single H&E section examination of all the lymph nodes. Ideally all lymph nodes of the axilla should also be examined at 6 levels, also with immunohistochemistry technique. It however is not a feasible proposition in our laboratory at the present time.

The final axillary status is matched up with the diagnosis from the frozen

sections and the confirmatory H&E paraffin sections.

3.9.2 Discussion

The histological status of the axillary lymph node remains the most important prognostic indicator in breast cancer^{37,38}. Other important prognostic indicators include tumour type, size and grade^{39,40}.

These four prognostic features are routinely assessed during the histopathological examination of a breast specimen. These criteria have its limitations as there is inter-observer variability in the assessment of the histopathological parameters and some deficiencies in the techniques used for the examination. Inter-observer variability is unavoidable but the second aspect needs some elaboration. Breast cancer is a heterogeneous disease, both clinically and morphologically. It is not surprising therefore that factors such as the number of sections examined per case could change the typing and the grading of the tumour. By examining a limited amount of tissue, there is a possibility of missing important histological findings. This can in turn lead to inadequate adjuvant treatment. The analysis of lymph nodes is in most laboratories is at present confined to the examination of a single haematoxylin & eosin stained (H&E) section from each node.

The introduction of sentinel node biopsy in breast carcinoma, has challenged the pathologist to perform a more detailed examination on a limited number of lymph nodes as pre-selected by the surgeon.

Over 50 years ago Saphir and Amromin⁴¹ demonstrated that serial sectioning of lymph nodes resulted in an improvement in the detection of metastatic disease.

In their study, 33% of patient who were initially diagnosed on routine histology as being lymph node negative were converted to lymph node positive by the examination of multiple serial sections. Subsequent studies confirmed Saphir and Amromin findings. In 1961 Pickren⁴² demonstrated occult metastasis in 22% of node negative cases and a number of studies followed. All highlighted the importance of serial sectioning in detecting small volume disease and micrometastases⁴³⁻⁵⁰. The number of cases has ranged from 5 to 92 and the type of examination has ranged from a limited number of sections, to serial sections of the entire lymph node. The detection rate for occult metastasis has ranged from 7% to 31%. The follow-up period for many studies was only 2-3 years. In one study however, it was greater than 16 years⁴⁸.

The prognostic significance of the detection of micrometastases by serial sectioning was first described by Trojani et al⁴⁹. These authors demonstrated a negative impact of micrometastasis on disease-free and overall survival and this was statistically significant. The next important study that confirmed a correlation between the detection of micrometastases and prognosis came from the Ludwig Breast Cancer Study Group⁵⁰. The micrometastasis were present in 9% of patients. At 5 years, a significant difference in the disease-free survival and the overall survival of these patients was seen! The authors concluded that the pathological examination of a single H&E section is 'probably no longer clinically tenable'. But they also acknowledged the enormity of the task as far as processing of the specimen is concerned. Since then a large number of studies have demonstrated that the detection of occult metastasis within the lymph node has prognostic significance for the patient.

The question that remains unanswered is how extensive should node sectioning be? In the studies reported to date, there is a wide variability in the level of sectioning of the lymph node, ranging from a few cuts through the node to complete examination of the whole node using serial sectioning.

Additional immunohistochemical analysis of the lymph node, improves the detection of micrometastasis as compared to H&E staining. This technique is particularly useful in certain types of breast carcinoma. Invasive lobular carcinoma is a good example, as it tends to infiltrate either with single cells or via a small group of cells⁵¹. Tumour cells from invasive lobular carcinoma are very difficult to differentiate from histiocytes, found as a normal reaction within lymph nodes. There have been a large number of studies in the last 20 years reporting the use of immunohistochemistry in the diagnosis of occult metastasis within lymph nodes⁵¹⁻⁵⁸. Monoclonal antibodies for the detection of cytokeratins have been used either alone or in combination with H&E in these studies.

Over the last five years, advances in molecular biology have been translated into diagnostic tests for the detection of altered genes or gene products in tumour samples. The advancement with the polymerase chain reaction (PCR) based technology has meant that analysis can also be carried out on paraffin embedded tissues. It has been demonstrated that reverse transcriptase polymerase chain reaction (RT-PCR) is a sensitive method for the detection of specific gene products in cancer cells⁵⁹⁻⁶¹. The technology is extremely sensitive and capable of detecting one cancer cell within a population of 10^6 normal lymph node cells. Initial results from such molecular studies look promising.

It is clear that molecular techniques are more sensitive at detecting occult lymph node metastasis compared to either H&E sectioning alone or in combination with immunohistochemistry.

The question about the prognostic significance of detecting micrometastases based on a molecular technique remains nevertheless unresolved.

3.10 Results

Before commencement of our trial, we made a decision to conduct this study in two phases. A 'learning phase' in which the first 30 patients were to be included. After analysis of results and review of the experience gained, we would proceed to the 'recruitment phase'.

3.10.1 Results of the learning phase

A Total of 30 consecutive patients were recruited. The patient's characteristics are outlined in table-3.3.

		No. of Patients
Sex		
Female		28
Male		2
Age		
Range 35-86 (median=60)		30
Laterality		
Right		13
Left		17
Initial presentation		
Palpable mass		29
Non-palpable abnormality		1
Tumor location		
Upper outer quadrant		15
Lower outer quadrant		11
Upper inner quadrant		1
Lower inner quadrant		2
Central		1
Treated by		
Mastectomy		7
Breast conservation		23

Table 3.3- Patients characteristics

No side effects were observed after injection of the radiopharmaceutical

(Albures). The tumour size was <2.0 cm (T1) in 10 patients and 2.0-5.0 cm (T2) in 19 patients and >4.0 cm (T3) in one patient. The location of the primary tumour was: upper outer quadrant (UOQ) in 15 patients, upper inner quadrant (UIQ) in 1 patient, lower outer quadrant (LOQ) in 11 patients and lower inner quadrant (LIQ) in 2 patients. In one patient, the tumour was located centrally.

Dynamic, early and delayed static imaging was performed in all 30 patients as described earlier. Static imaging comprised of combination of emission and transmission acquisition.

Lymphoscintigraphy was successful in delineating a focal uptake in 29 patients. In one patient with the primary carcinoma located high in upper outer quadrant (axillary tail), imaging failed to reveal a hot spot.

In another patient with a UIQ carcinoma, no focal accumulation of the tracer was detected after lymphoscintigraphy. As this patient's surgery was postponed due to lack of operating time, lymphoscintigraphy was repeated after five days. We employed the same injection technique. On this occasion, lymphoscintigraphy was successful in revealing a hot spot in the axilla.

Axillary drainage was noted in 27 patients on lymphoscintigraphy. In two patients there were exclusive drainage in to the internal mammary chain.

In 21 patients (70%), tracer preferentially accumulated in one focus and in 7 patients (20%) hot spots were seen in two foci. In one patient 3 hot spots were visualized (3%).

Table-3.4 summarizes the results of lymphoscintigraphy.

	No.	%
Total number of patients	30	100
Number of procedures performed	31	-
Successful mapping procedure	28	93.6
Failed mapping procedures	2	6.4
Route of administration of the isotope		
Intra-dermal	31*	100
Tumor size		
T1	10	33
T2	19	63
T3	1	4
Anatomic location of sentinel nodes		
Level 1	26	93
Level 2	1	3.5
Level 1 and 2	1	3.5
Positive lymphoscintigram sites		
Axilla only	27	93
Internal mammary only	2	7
Axilla + internal mammary	0	0
No. of hot spots on lymphoscintigraphy		
one	21	70
two	7	23
three	1	3

Table-3.4 Results of lymphoscintigraphy

*One patient had lymphoscintigraphy twice

A comparison of early (within the first hour after injection) and late (12-18 hours), data acquisition, did not reveal any variation in the intensity or the number of hot spots in 29 out of 30 patients. In one patient, early acquisition revealed two areas of focal tracer uptake. One, was near the axillary tail of the breast and the other, located higher up within the axilla. Late imaging only revealed the higher hot spot in the axilla. Analysis of dynamic data including region of interest and time activity study, did not indicate progressive accumulation of tracer, which is a characteristic finding for the SLN with this technique.

Three patients refused to undergo ALND but consented to SLNB. One patient had undergone a modified radical mastectomy on the contralateral side 4 years earlier. She had an unpleasant experience from the earlier ALND, due to post-operative complication of lymphoedema. The other patient was disabled as a result of polio during childhood and had severe limitations in shoulder movement. The third patient was a 68 year old artist who was concerned about the functional consequences of ALND.

In two patients, we noted exclusive drainage of tracer into the ipsilateral internal mammary chain (7%). In one of these patients, the primary tumour was located in the upper outer quadrant of the breast (fig.3.37).

At operation, a combination of probe guided surgery and blue dye technique failed to detect a hot or a blue node in the axilla. There was gross evidence of disease within the axilla. The internal mammary chain was not explored (it is not our policy to explore these nodes). Histology of the primary tumour revealed a grade 3 invasive ductal carcinoma, with intravascular invasion (fig. 3.38).

Analysis of axillary lymph nodes confirmed evidence of

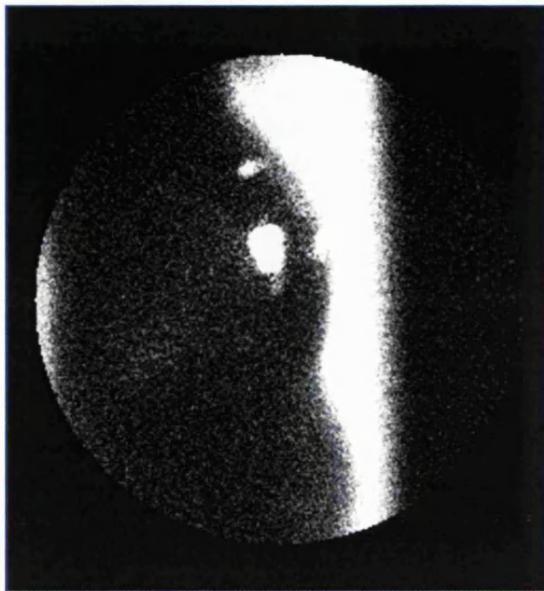


Fig 3.37 Exclusive drainage of radiotracer to internal mammary node

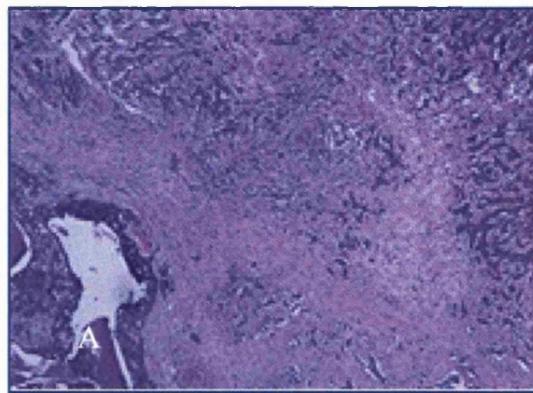


Fig 3.38 A,Grade 3 invasive ductal carcinoma (H & E)

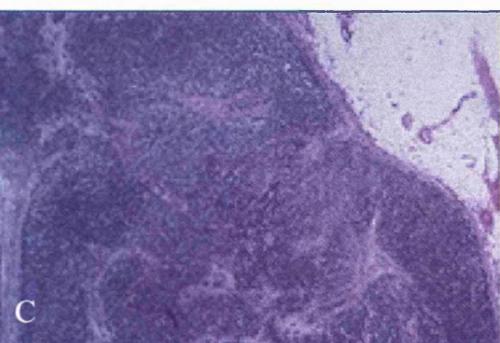
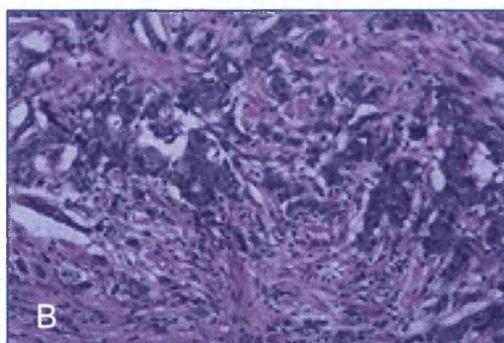


Fig 3.38 B higher magnification of the tumour **C**, Axillary lymph node completely replaced with metastatic carcinoma.

metastatic carcinoma in six nodes, two of which were completely replaced by the tumour (fig.3.38). There was also evidence of extra-nodal spread of the cancer into axillary fat.

The other patient was a 54 year old man who presented with a central breast carcinoma. Lymphoscintigraphy in this patient also demonstrated exclusive internal mammary nodes

In this patient there was also evidence of extensive disease within the axilla. This case is presented in detail in section 3.8.4.1 of this chapter.

A total of 41 SLN were biopsied in 27 Patients (1.5 nodes per patient). Axillary specimen contained 10 ± 3 lymph nodes. In 24 patients we compared the histological status of the SLN with that of the axillary lymph nodes.

In those patients who underwent combined SLNB and ALND, histology revealed evidence of axillary nodal metastasis in 7 patients (29%). The sentinel node correctly predicted the histological status of the axilla in 22 patients out of 24 (91%).

In two patients SLN was negative for metastases but histological examination of the ALND specimen revealed axillary involvement, leading to a false negative rate of (28%).

3.10.1.1 *False negative cases*

Case-1

A 76 years old female with a 6.5 cm mass in the upper inner quadrant of the left breast. Clinically, there was clinical evidence of “peau d'orange”. There were no palpable axillary lymph nodes. A mammogram confirmed the diagnosis of breast cancer and also revealed a multifocal tumour (fig. 3.39).

On dynamic imaging, there were evidence of several lymphatic tracts, with slow progression of the tracer. A focal area of accumulation was noted in the axilla after 17 minutes post tracer administration and this was confirmed by a static acquisition with the gamma camera (fig.3. 40).

At operation, using the combination of patent blue dye and intraoperative probe, a hot node was identified which was not stained with the blue dye. The node was biopsied and sent separately for histological examination and a complete ALND performed. The histology of the primary tumour revealed a multifocal grade 2 invasive ductal carcinoma. SLN did not contain any metastatic deposits as judged by H & E staining and immunohistochemistry. Three of the other axillary non-sentinel nodes showed involvement by metastatic cells (fig. 3.41).

Case-2

A 42 year old female with carcinoma in the upper outer quadrant of the breast. Pre-operative lymphoscintigraphy revealed a hot spot in the axilla. At operation, the gamma probe did not function due to a technical failure. An intraoperative detection of the SLN was therefore performed by the blue dye

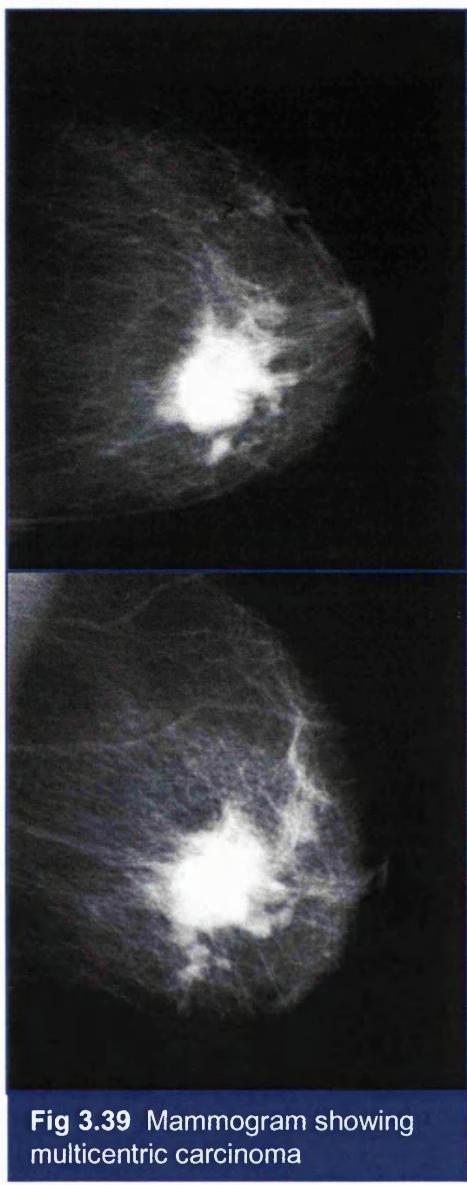


Fig 3.39 Mammogram showing multicentric carcinoma

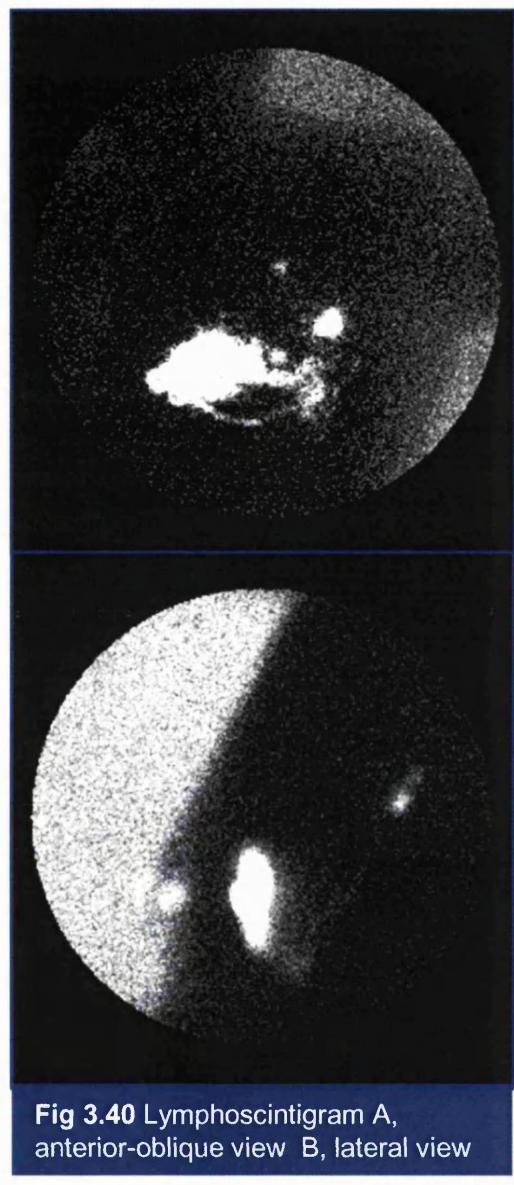


Fig 3.40 Lymphoscintigram A, anterior-oblique view B, lateral view

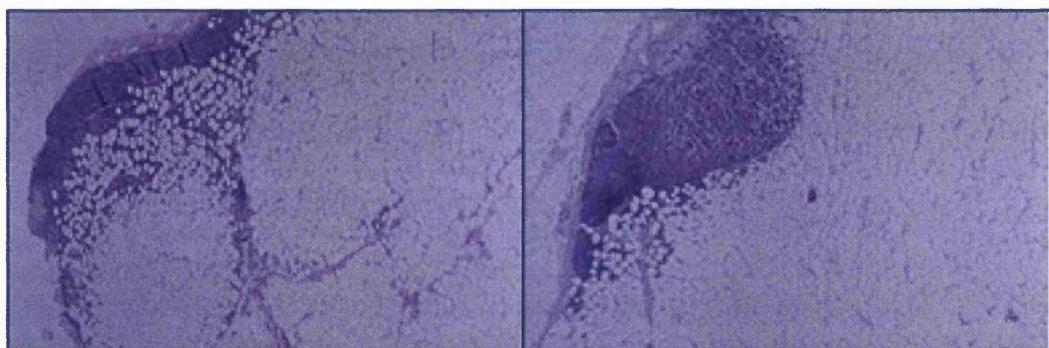


Fig 3.41 A, Sentinel node not involved with cancer and mostly replaced by fat B, Non-sentinel node involved with cancer and also shows fatty replacement

technique alone. With some difficulty a blue node was detected, but a blue tract leading to it was not evident. The node was harvested and considered as the sentinel node. The histology of this node did not detect any evidence of metastatic disease. Histology of the rest of the axilla confirmed presence of metastases in one lymph node out of 15 nodes which were dissected.

In one patient, probe guided surgery assisted in localizing a hot but dye negative node, located close to the chest wall. The histological analysis of this node confirmed evidence of metastatic disease and this was the only involved lymph node.

After harvesting the lymph node and preparing the imprint cytology samples, the SLN was sent for frozen section histology. Because of logistical reasons and considering that the histological status of the SLN did not influence the extent of axillary surgery, frozen section slides were reported by the pathologist subsequently. Technical difficulties were experienced with sectioning of the SLN on frozen section histology due to fat replacement of the node.

As far as histological analysis of the SLN is concerned, multiple sectioning of the SLN helped in detecting a small metastatic deposit in one SLN which was evident in one section only. This was located at the periphery of the node, within the subcapsular sinus region.

Immunohistochemistry did not reveal any micrometastases in the sentinel nodes which were negative on definitive H & E histology.

3.11.1.2 Discussion

The main objective of this phase of our study was to gain experience on the technical aspects of the procedure and learn from the difficulties encountered.

The following observations were made.

Lymphoscintigraphy was successful in detecting a hot spot in 28 patients (93.3%). In one case, lymphoscintigraphy failed to detect a hot spot in a patient where the primary carcinoma was located high in the axillary tail of the breast. There are two possible explanations. This could have been caused by the 'shine through phenomenon' whereby the activity from the injection site overshadows much weaker activity from the SLN. Retraction of breast downward and medially can help to overcome this problem (see figure 3.8). Lateral acquisition of image is also helpful in this situation but in this particular case, it did not show any focal uptake. The other possibility is that the tracer was delivered in a deeper plane into the subcutaneous fat which has very scanty lymphatic supply and particle kinetics are very slow.

It was interesting to note that in one patient, lymphoscintigraphy failed to detect a focal uptake in the first instance. As this procedure was repeated with the same dosage of the tracer and the same injection technique delivered by the same operator; a focal hot spot in the axilla became evident. We noted that the only variable in this case was the fact that a different batch of Albures was used for each injection. As we were aware of problems with batch to batch variation of this product, we abandoned the use of this colloid and decided to use Nanocolloid in the second phase of the trial.

In two patients, lymphoscintigraphy revealed exclusive drainage to the internal mammary chain, with no uptake in the axilla. As it is not the policy of our unit to explore the nodes in the internal mammary chain, these were left alone during surgery. It is unusual to see exclusive internal mammary drainage in a patient with the primary tumour located in the upper outer quadrant. Both patients with internal mammary drainage showed evidence of extensive disease within the axilla. Most of the level I lymph nodes were completely replaced by metastatic carcinoma. There was evidence of extra-nodal spread in both cases.

It is now recognised that the internal mammary chain is rarely depicted by an intradermal injection technique. The only possible explanation in these two cases is a 'skip phenomenon' as a result of directional flow changes due to mechanical obstruction of the true sentinel node.

In one patient, there was a transient hot spot detected in early imaging. This was not evident in the late acquisition data set. Time activity study of the dynamic data also failed to demonstrate progressive accumulation of the tracer which indicated that this was not a lymph node. Transient hot spots can be visualized due to accumulation of the tracer around the lymphatic valves.

Three patients refused ALND during this phase. In one patient, this was due to an unpleasant past experience of this procedure on the contra-lateral side. The other two patients refused ALND on the basis of their anxiety about the functional consequences of this procedure. These cases highlight patient's desire for a less invasive staging procedure.

One of the false negative cases in this series was a patient who had clinical

evidence of peau d'orange and a multifocal primary breast cancer. It is now also recognised that there is a clear association between multifocal carcinoma and a false negative SLN result³⁻⁶. The other confounding factor in this case was the presence of peau d'orange. This condition is the manifestation of obstruction to dermal lymphatic flow. This may have led to the opening of collateral channels leading to alternate pathways of lymph drainage.

A technical failure of the gamma detection probe resulted in one false negative SLN biopsy. Lymphatic mapping in this case was performed by the injection of patent blue dye only. Blue dye lymphatic mapping alone is a difficult technique to master and requires extensive experience. It has limitations and the reported success rate is not as high as with the combined technique. This case also highlights the importance of the regular maintenance and check up of the gamma probe prior to commencement of surgery.

In one patient, the addition of probe guided surgery detected a SLN which was hot but not blue. This node showed evidence of metastatic carcinoma and it was the only involved lymph node. This case highlights the importance of using a combination of blue dye lymphatic mapping and probe guided surgery for best SLN detection^{2,6,28}. The overall success rate of SLN localization is maximized with a lower incidence of false negative results and a shorter learning period. (see Table 3.1).

Table-3.5 summarizes the lessons derived from the learning phase of this trial.

Summary of lessons learned from first 30 cases (learning phase)

- Appropriate patient selection is key to the success of the SLNB.
- Extensive disease in the axilla is a significant potential pitfall which can lead to false negative results.
- Multifocal primary breast cancer can lead to false negative results.
- Patients with clinical sign of peau d'orange should not be considered for intradermal injection of the tracer.
- Pre-operative lymphoscintigraphy, in addition to localizing the SLN, is a good predictor of the success of the procedure.
- Combination of transmission and emission imaging is optimal.
- Particle size is important parameter in SLNB. Batch to batch variation in size of the particle must be avoided.
- Upper outer quadrant lesions of breast can pose problems during imaging and surgical detection. Retraction of the breast downward and medially can overcome this problem during imaging. Additional use of external collimator can be helpful during surgery.
- Combination of probe guided surgery and blue dye mapping are complementary and deliver the highest detection rate.

Table-3.5 Summary of the lessons learnt in learning phase

3.10.2 Results of Recruitment Phase

A total of 51 patients were recruited in this phase. The patient's characteristics are presented in table-3.6.

		No. of Patients
Sex		
Female		51
Male		0
Age		
Range 35-86 (median=60)		51
Laterality		
Right		30
Left		20
Bilateral		1
Initial presentation		
Palpable mass		49
Non-palpable abnormality		2
Tumor location		
Upper outer quadrant		29
Lower outer quadrant		12
Upper inner quadrant		3
Lower inner quadrant		6
Central		2
Treated by		
Mastectomy		14
Breast conservation		37

Table 3.6- Patients characteristics

Table-3.7 presents the summary of the results of lymphatic mapping in these patients.

	No. of patients	%
Total number of patients	51	100
Number of procedures performed	52*	100
Successful lymphatic mapping procedure	52	100
Failed lymphatic mapping procedures	0	0
Route of administration of the isotope		
Intradermal	52	100
Tumor size		
T1	28	54
T2	24	46
Anatomic location of sentinel nodes		
Level 1	47	90
Level 2	1	2
Level 1 and 2	2	4
Intra-mammary	2	4

Table-3.7 Results of lymphoscintigraphy. * one patient had bilateral mapping procedure.

There were no side effects as a result of the injection of the radiopharmaceutical (nanocol). The tumour size was 2.0 cm (T1) in 28 patients and 2.0-4.0 cm (T2) in 24 patients. The location of the primary tumour was: upper outer quadrant (UOQ) in 29 cases, upper inner quadrant (UIQ) in 12 patient, lower outer quadrant (LOQ) in 3 patients and lower inner quadrant (LIQ) in 6 patients. In two patient, the tumour was located centrally .

3.10.2.1 Lymphoscintigraphy data

Lymphoscintigraphy was successful in delineating a hot spot in all patients.

It revealed a total of 125 hot spots in 52 procedures. (2.4 hot spot per patient).

One patient underwent bilateral SLN localization.

Careful analysis of preoperative lymphoscintigraphy helped us to differentiate between the true sentinel node and the second tier or secondary echelon nodes. At operation, the additional use of blue dye also proved helpful.

3.10.2.2 Dynamic and static imaging

All data were acquired using a LEHR collimator with 140 keV, 20% width, 3% offset energy window, and 256 x 256 W pixel matrix.

Anterior oblique (30°) dynamic imaging commenced immediately following injection, acquired in an over-sampled format to allow for maximum flexibility when reformatting for later analysis. This comprised 90 x 10 sec. frames, followed by 30 x 60 sec. frames.

For each patient, the early dynamic image dataset was assessed specifically for

its additional value in identifying SLN(s) and interpreting patterns of uptake. Using the information gathered here, minimum requirements for the collection of early dynamic data were also determined.

Data analysis was achieved by reformatting the raw dynamic dataset to generate the following additional image files:

- i) 3 x 5 min. frames (15 min total).
- ii) 15 x 1 min. frames (15 min total).
- iii) 90 x 10 sec. frames (15 min total).
- iv) 9 x 5 min. frames (45 min total).
- v) 45 x 1 min. frames (45 min total).

Image data for each patient was reviewed blindly by an experienced observer (fig. 3.42).

A critical analysis of data generated from the acquired dynamic dataset showed that all early anterior oblique image information is present within the first 15 minutes p.i. We noted that in 46 patients (90%), the first 5 minutes data were adequate in providing the required information. We found dynamic imaging helpful, only in those situations where we were unsure whether a hot spot was a transient one or a true SLN. This was achieved through plotting a time-activity curve, which has a characteristic feature in each situation (fig. 3.43). Dynamic acquisition was not adding any other additional information.

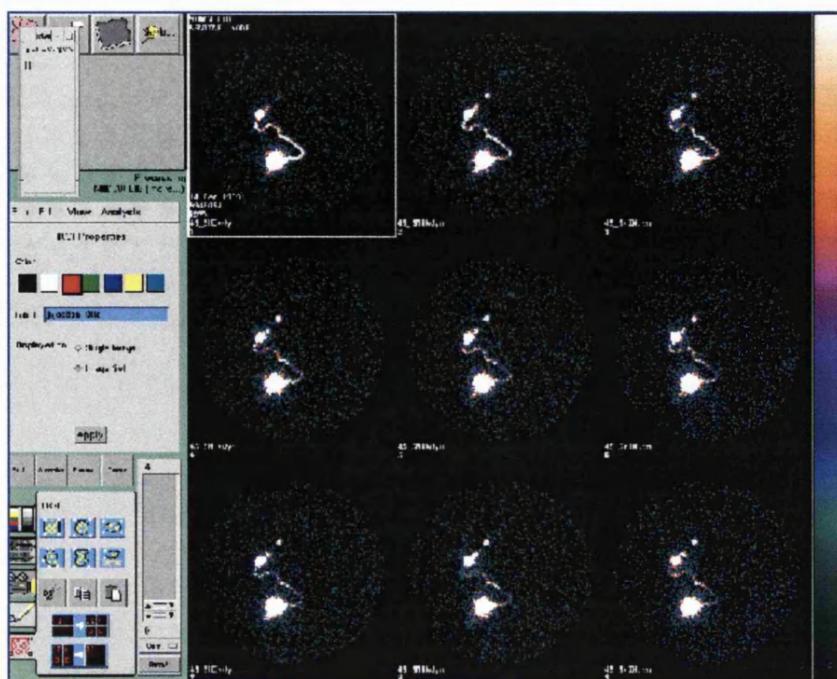


Fig 3.42 Analysis of dynamic data. Forty five minutes data set is compressed into nine frames of five minutes data

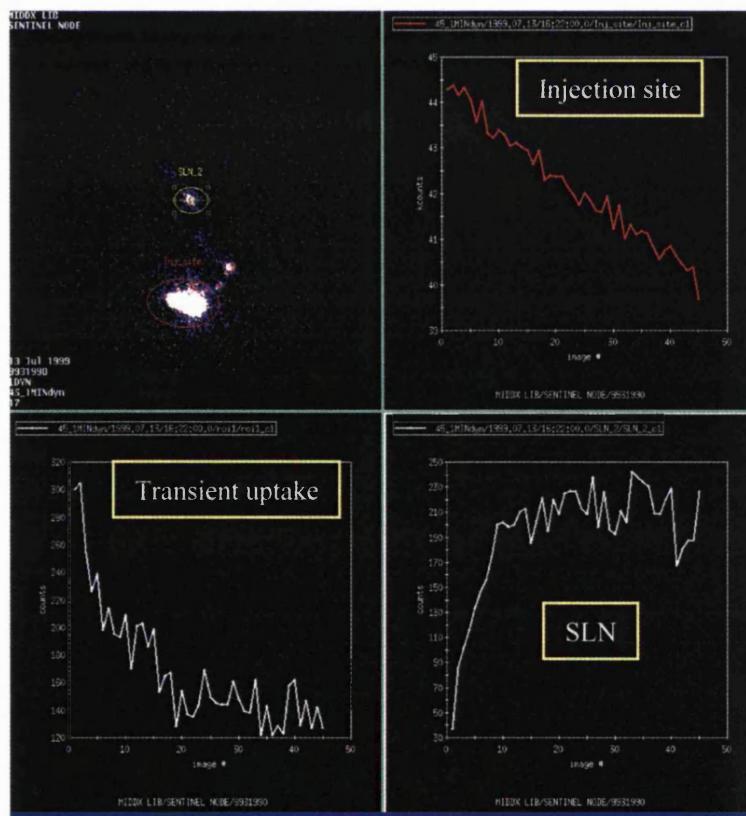


Fig 3.43 A, Time activity curve in a, an SLN b, a transient hot spot

In 11 / 52 studies lymph ducts were seen to lead *directly* to the SLN, this being seen solely in the early dynamic data.

We did not find the 10 seconds framing data acquisition sequence useful. A one minute acquisition of dynamic data was much more informative. As far as delayed imaging is concerned (12-18 hours post-injection), this too, did not add any information as that available from the early acquired data.

One patient underwent bilateral sentinel node localization. On the left side she had 40 mm invasive ductal carcinoma and on the right, extensive DCIS.

Preoperative lymphoscintigraphy revealed an almost identical pattern of drainage on each side.

Figure 3.44 illustrates the importance of dynamic imaging in distinguishing between the true sentinel node and second tier lymph node. In presence of more than one hot spot on a lymphoscintigram, visualization of lymphatic tract leading to the hot spot can help to differentiate between the two.

3.10.2.3 Intra-operative lymphatic mapping

A total of 92 SLN were harvested in 52 procedures (1.8 SLN per patient). Thirty three hot spots were considered to be second echelon nodes due to the spill over of the radiopharmaceutical to the second tier nodes and these were labeled separately. 13 patients (25%) had a single SLN, in 38 procedures (73%) two SLN were harvested and one patient (2%) underwent three SLN biopsies. Radioactive nodal uptake was sufficiently high to allow easy detection with the gamma detecting probe. During surgery, gamma probe assisted in localizing the SLN in three patients. In these patients the node was hot but not blue. One of these (intramammary SLN) did show evidence of metastatic carcinoma, which would have been missed otherwise.

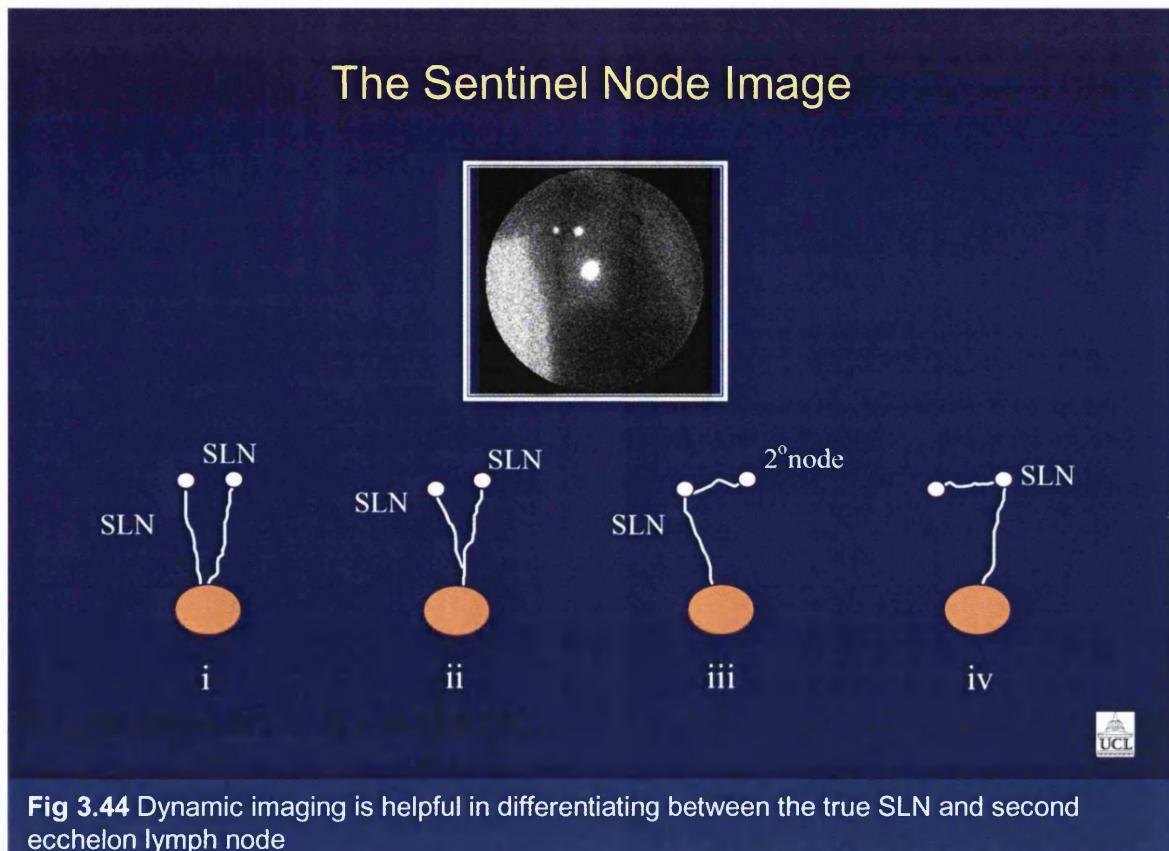


Fig 3.44 Dynamic imaging is helpful in differentiating between the true SLN and second ecchelon lymph node

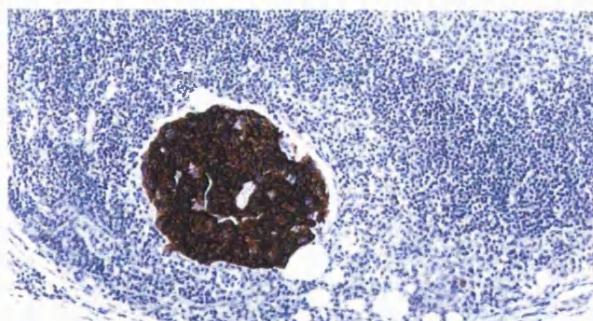


Fig 3.45 A, Positive immunohistochemistry (MNF116) in an SLN

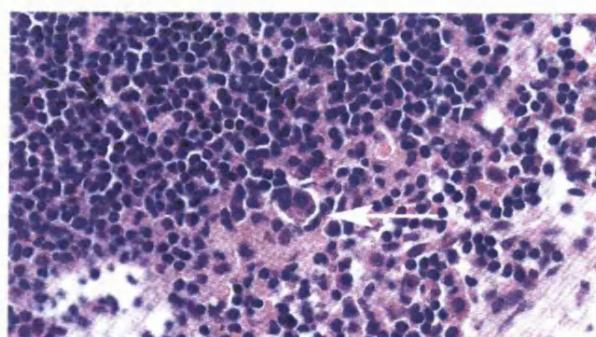


Fig 3.45 B, Corresponding micro-metastases which was discovered on reviewing the H & E Slides (arrow)

ALND confirmed the presence of axillary metastases in 15 of these patients (28.8%). The sentinel node correctly reflected the histological status of the axilla in all patients. There were no false negatives in this series, giving a 100% sensitivity. The SLN was the only involved node in 4 patients (26.6%).

In two patients, lymphoscintigraphy revealed a hot spot within the breast tissue. At operation, with the help of gamma probe these hot spots were identified. These were intra-mammary lymph nodes. In one of these patients, the SLN had not taken up the blue dye and localization was successfully performed with the aid of the probe. Histology of this node revealed evidence of metastatic deposit.

Eighty nine SLN's (97%) were located at level 1, within the axilla. One SLN was found at level 2 and we came across intramammary SLN's (2%), in two patients in this series. We did not see any drainage to the internal mammary chain.

In two patients we found a Langer's axillary arch, during excision of the sentinel node. This is an anatomical variation of the latissimus dorsi insertion. These two cases are presented in section 3.8 of this chapter.

3.10.2.4 *Histopathology*

Frozen section histology revealed evidence of metastases in 12 patients (23%). However in two occasions frozen section was falsely negative (14.2%). H & E staining confirmed presence of metastases in 13 patients (26.9%). In one patient, with medullary carcinoma of breast, both frozen section and paraffin sections were reported as negative, but on immunohistochemical staining, there was evidence of micrometastases (fig. 3.45).

On reviewing the H & E slides, a very small area of micrometastases was detected, which was missed on original reporting.

3.10.3 SLNB in Special Histological type Breast Cancer

Our aim, in this part of the study was to evaluate the role of SLNB in DCIS and in tumours with good prognosis. Table-3.8 presents the patients characteristics.

		No. of Patients
Sex		
Female		20
Male		0
Age		
Range 34-68 (median=49)		20
Laterality		
Right		12
Left		8
Initial presentation		
Palpable mass		9
Non-palpable abnormality		11
Primary tumour		
DCIS		11
Special histological type carcinoma		7
- Medullary carcinoma		4
- Mucinous carcinoma		2
- Papillary carcinoma		1
T1a invasive carcinoma (<0.5 cm)		2
Treated by		
Mastectomy		5
Breast conservation		15

Table 3.8- Patients characteristics

Twenty patients were recruited in this group. Eleven patients were diagnosed as having extensive DCIS. The diagnosis was made on the basis of mammography and core cut biopsy. The remaining 9 patients had either good

prognosis, special histologic type carcinoma (7 patients), or small invasive ductal carcinoma (T1a) (2 patients) in whom the clinicians had specifically requested SLNB only.

Table-3.9 presents the summary of the results of lymphatic mapping in these patients.

	No. of patients	%
Total number of patients	20	100
Number of procedures performed	20	100
Successful lymphatic mapping procedure	20	100
Failed lymphatic mapping procedures	0	0
Route of administration of the isotope Intradermal	20	100
Anatomic location of sentinel nodes Level 1	20	100
Level 2	0	0

Table-3.9 Results of lymphoscintigraphy in special histological tumour group

In the DCIS group, 5 out of 11 patients underwent simple mastectomy (45%).

The remaining 6 patients had segmental excision, after wire localization. A total of 18 SLN's were harvested in these patients (1.6 node per patient). In two patients, histological analysis of primary tumour , revealed presence of micro-invasion. Histology of the SLN, revealed presence of metastatic carcinoma in both of these patients (18%). One of these was evident on step serial sectioning and the other, on immunohistochemistry (MNF116). After a median follow up of 8 months (range 4-16 months), we have not encountered any patient with evidence of regional recurrence.

3.10.4 Discussion

We were successful in localizing the SLN in all 51 patients in this study. We did not find any correlation between the pathological features such as tumour type, grade or size and the success rate of SLN identification. Location of the primary tumour within the breast, also did not affect the identification rate.

Dynamic imaging was not very useful. Although it is an essential aspect of SLN localization in malignant melanoma (in particular in trunk lesions), in breast cancer, this is not so.

By omitting dynamic imaging, significant camera time can be saved. This is particularly relevant, as the demand for performing SLNB in breast cancer is increasing. We also noted that in 90% of patients, the first 5 minutes of the data acquisition, provided adequate information about the drainage pattern and the hot spots. In 100% of patients, a hot spot was visualised within 15 minutes. The fact that we used intradermal injection technique for the delivery of the radiopharmaceutical is the possible explanation for this. It is well established that the particle kinetics is much faster with this technique.

The average number of hot spots visualized on lymphoscintigraphy, was higher than the learning phase (1.4 vs 2.4 SLN per patient). We believe this was due to switching to a different radiocolloid in this phase (Nanocolloid instead of Albures). Due to the smaller particle size of nanocolloid, spill-over of the radiotracer into the second tier node is more frequent. This subject is discussed in detail in the radiopharmaceutical section of this chapter.

On reviewing the delayed imaging data set (12-18 hours post-injection), there was no evidence that new and significant information was obtained.

Frozen section histology of the SLN was associated with a 14.2% false negative rate. Although this is not as high as some of reported cases in the literature, the procedure is time consuming and processing of the SLN can pose some difficulty and freezing artifact can interfere with interpretation of slides. This subject is discussed in the histology section of this chapter.

Immunohistochemistry diagnosed micrometastasis in one patient with the primary medullary carcinoma of the breast. This was missed on paraffin section histology. After a positive IHC in this case, the original slides were reviewed and the area of micrometastases was detected subsequently. In another patient with widespread DCIS and micro-invasion, IHC detected micrometastases in the SLN. These two cases illustrate the importance of performing immunohistochemistry. As it is not practical to perform this test on every single lymph node, SLN biopsy seems to be a logical approach in selecting the most susceptible node for the pathologist.

The fact that the SLN was the only involved node in 4 patients (28.5%) provides additional support that this concept is valid in breast cancer.

Further histologic support for this concept is reported by Turner et al⁶². These authors used cytokeratin immunohistochemical staining to examine the sentinel and non sentinel nodes in 103 patients. In 60 patients whose SLN were metastases free by H & E staining and immunohistochemistry, only one additional tumour positive lymph node was identified in 1087 non-sentinel nodes.

Our data also highlight the importance of performing SLNB in patients with extensive DCIS. In two out of 11 patients with extensive DCIS, microinvasion was detected and SLN's were involved with metastatic deposits.

In a study performed by Silverstein et al⁶³, in 1031 patients with breast carcinoma, investigated axillary node positivity, disease free survival and breast cancer specific survival in six breast cancer subgroups. These included different T categories: Tis (DCIS), T1a, T1b, T1c, T2 and T3. It was noted that the nodal positivity for DCIS was 0%; T1a 3%; T1b 17%; T1c 32%; T2 44%; T3 60%. There was reduction in the disease-free and breast cancer specific survival with every increment in T value. The authors make a case for the elimination of routine ALND in patients with T1a breast carcinoma as only 3% are likely to be positive. "How can we justify 100 node dissections in an attempt to find three patients with positive nodes to treat with chemotherapy, one patient of which, at most, will be helped. The cost of such procedure was estimated to be in a region of 1 million US dollars"⁶¹.

Table-3.10 presents the frequency of tumour positive lymph nodes in breast cancer related to tumour size.

Author	Group size	T1a (<0.5 cm)	T1b (cm)	T1c (cm)	T2 (cm)
Silverstein	1031	3	17	32	44
McGee	3077	12	23	33	54
Giuliano	259	10	13	30	-
Cady	570	-	17	31	44
Weighted Average	4937	7%	19%	32%	51%

Table-3.10 Frequency of tumour positive lymph nodes in breast cancer related to tumour size

We believe, the reason for such a high positive rate of SLN involvement in our study is the fact that we chose a subgroup with extensive high grade DCIS which has the highest chance of microinvasion.

Breast cancers with specific histology and with good prognosis (e.g. tubular, papillary and colloid) are another group which have an extremely low rate of axillary lymph node spread. These would also not benefit from routine ALND.

It seems logical to perform SLN biopsy only, in patients with screen detected breast cancers, small breast carcinoma (less than 1 cm (T1a)), patients with specific histological type cancers. These patients which has a very low probability of nodal metastases will avoid morbidity associated with the ALND without compromising the staging information.

3.10.5 *Conclusions*

1. The sentinel node concept is valid in the management of patients with breast cancer.
2. The intradermal injection technique of the radioisotope is associated with high success rate for delineation of the sentinel node.
3. A reduced number of internal mammary node uptake is noted with intradermal injection technique.
4. Dynamic imaging is not an essential component of lymphoscintigraphy in breast carcinoma. Time-activity curve analysis of the tracer uptake can help to differentiate a transient uptake from the true sentinel node.
5. Imaging can commence immediately after the injection of the radiotracer and all hot spots are visualized within 15 minutes of injection after intradermal injection technique.
6. Delayed imaging does not add any additional information.
7. Lymphoscintigraphy is helpful in delineating the hot spot in unusual locations, i.e. intramammary, high axillary or internal mammary.
8. Combination of imaging, probe guided surgery and blue dye lymphatic mapping is associated with a high success rate of SLN identification and low false negative rate.
9. Frozen section histology is associated with a high false negative rate.
10. Immunohistochemical staining of the SLN is helpful in diagnosing micrometastases.

11. SLNB is a staging procedure of choice in extensive DCIS when micro-invasion is suspected. It seems logical to perform SLN biopsy only in patients with screen detected breast cancers, small breast carcinoma (less than 1 cm (T1a), patients with specific histological type cancers. These patients which has a very low probability of nodal metastases will avoid morbidity associated with the ALND without compromising the staging information.

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4.1 Imprint Cytology in Sentinel Node Biopsy for Breast Cancer

4.1.1 *Introduction*

The histological status of the axillary lymph node remains one of the most important prognostic indicators in breast cancer patients. It also has therapeutic implications as the knowledge of axillary nodal status has a significant bearing on the decision about adjuvant therapy.

With the introduction of sentinel node biopsy as a minimally invasive staging procedure in breast cancer immediate and reliable intraoperative information on the histological status of the sentinel node remains a key issue to be solved for this technique to be used more widely. This is because the decision to proceed to total axillary lymph node dissection (ALND) depends on the knowledge of the SLN histological status at the time of surgery. If the diagnosis of SLN status has to await paraffin section histology (definitive histology), those patients with involved sentinel node will have to undergo a second operation of completion lymphadenectomy. In addition to cost implications, this would have a negative psychological impact on the patient.

Traditionally frozen section histology has been used in the past as an intraoperative diagnostic tool.

Frozen sections are technically difficult to prepare. The procedure is time consuming and unreliable in detecting micrometastases. Freezing artifact limits the pathologist's ability to interpret the specimen^{1,2}. Moreover, it is associated with an unacceptably high false negative rate. There are serious questions raised about the reliability of frozen section in SLNB.

Intraoperative touch imprint cytology (TIC) provides an opportunity to overcome this limitation. It is one of the oldest methods in cytopathology and is probably under-utilised at present. It is a simple, rapid and reliable technique that offers

many advantages over the traditional frozen sections for pathological examination. TIC can provide clear cytological detail and it is possible to identify micrometastases. The other potential advantage of this procedure is that a larger volume of the lymph node can be examined in a short span of time with possible increase in accuracy.

Intra-operative TIC was first described by Dudgeon and Patrick³ in 1927 and its usefulness has been confirmed in different clinical settings^{1,4-6}. TIC has proven useful in the intra-operative diagnosis of lymphadenopathy and has been used as an alternative to frozen section^{2,4,5}. This technique has also been used in determining intraoperative margins of breast lesions to confirm that the completeness of the excision⁷.

It was the aim of this study to evaluate the accuracy of touch imprint cytology as an intra-operative diagnostic tool to determine the histological status of the sentinel lymph node in patients with invasive breast cancer.

4.1.2 Patients and Methods

Between September 1997 and February 2000, a total of 117 patients were enrolled in this study. This work was done in collaboration with the Norfolk and Norwich NHS Trust. The study was approved by the local ethics committees. After explaining the SLNB procedure to the patients, informed consent was obtained. Patients with T1, T2 breast cancer diagnosed on triple assessment i.e. clinical examination, imaging (mammogram and/or ultrasound scan) and tissue diagnosis (FNAC or core cut biopsy) were eligible to take part in this clinical trial. Pregnant and lactating women, patients with multicentric tumours,

patients with clinically involved axilla and those who had previous axillary or breast surgery were excluded from the trial. The median age of the patients was 58 years (range, 34 to 83 years). All patients underwent either breast-conserving surgery or mastectomy. As all patients had completion ALND after SLNB and the result of TIC did not influence the extent of surgical intervention, these were reported on the following day.

At operation, the SLN was localised by a combination of probe guided surgery and blue dye lymphatic mapping. After harvesting, the SLN was bisected and a clean glass slide touched onto the cut surface, resulting in cell imprints (fig 4.1). During this process, care was taken to avoid rubbing of the node on to the slide and to ensure that all surface area of the SLN was represented on the imprint. Three imprints were prepared on each slide (fig 4.2) although in large nodes, this was limited to two.

We prepared three slides per each sentinel node that was harvested. One slide was stained with the rapid staining technique (fig 4.3), one with standard (May Grunwald Giemsa) staining and the final slide was left for immunocytochemical (anticytokeratin) staining. Slides were subsequently examined by an experienced cytologist under the light microscope (fig 4.4). The SLN was labelled and sent separately for histological analysis.

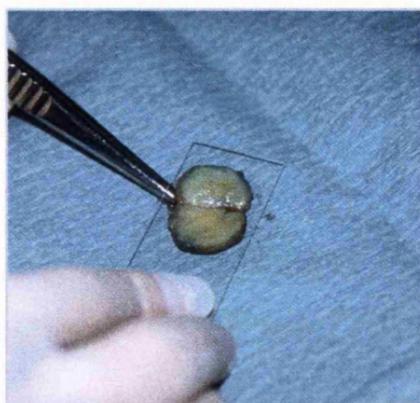


Fig. 4.1 Preparation of touch imprint slides



Fig. 4.2 Rapid staining of slides

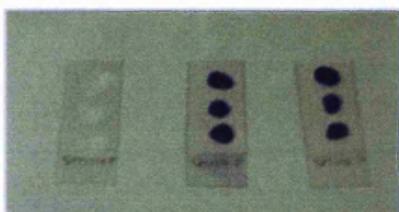


Fig. 4.3 Three slides are prepared per sentinel node.



Fig. 4.4 Slides are viewed by experienced cytologist

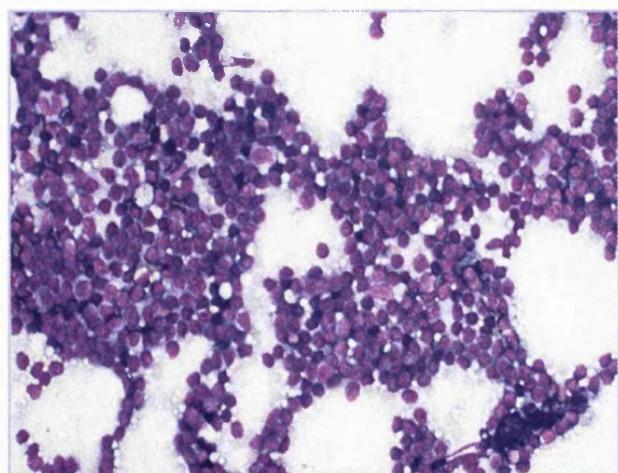


Fig. 4.5 Low power view of lymph node imprint. Numerous lymphoid cells are present

4.1.3 Microscopic Findings

Cytological details are well preserved and clearly visible. Typical findings from a reactive sentinel lymph node, show numerous lymphoid cells. These appear in large aggregates (fig 4.5) and single cells including a variety of follicle centre cells. With routine use of patent blue dye for detection of the SLN during surgery, we increasingly see tingible body macrophages containing blue pigments (fig 4.6).

In case of metastatic disease, the picture is different. Large aggregates of epithelial cells are seen. The cells are much larger than lymphoid cells and show cytological features of malignancy (fig 4.7, 4.8). Immunocytochemical investigation shows these cells to be positive for cytokeratin thus confirming the diagnosis of metastatic disease (fig 4.9).

4.1.4 Pitfalls

It is important that the cytologist is experienced and familiar with imprint cytology. The potential *pitfalls* of sentinel TIC are two folds:

1. Those due to technical preparation
2. Those due to interpretation

The technical preparation of imprints, although not very demanding and producing abundance of cells, creates layers of various cell thickness and unpredictable pattern of cell distribution on the slide. Some of the thicker cell layers take longer to air dry (and are therefore subject to drying artifacts) and make it difficult for the rapid stains to penetrate, appear pale on screening and may on low power appear to be non lymphoid in origin (fig 4.10).

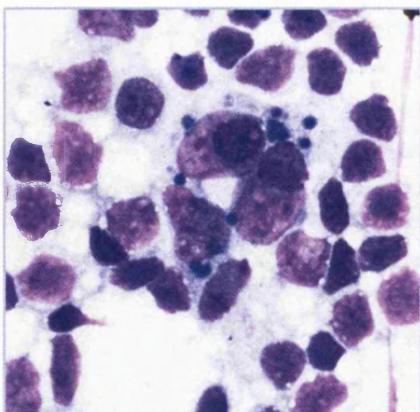


Fig. 4.6 High power view of the tingible body macrophage containing blue pigment.

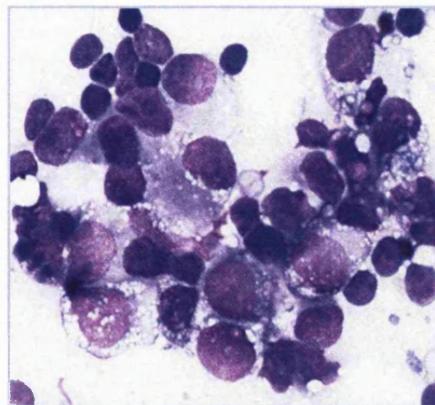


Fig 4.7 Aggregate of malignant epithelial cells in a lymph node imprint.

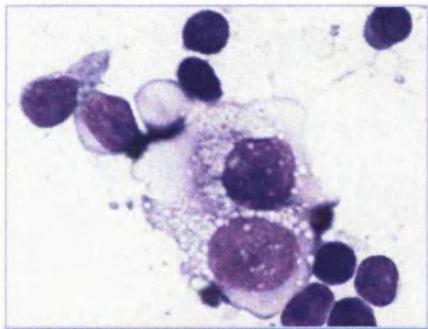


Fig 4.8 High power view of malignant epithelial cells in the lymph node imprint, showing irregular nuclei and prominent nucleoli.

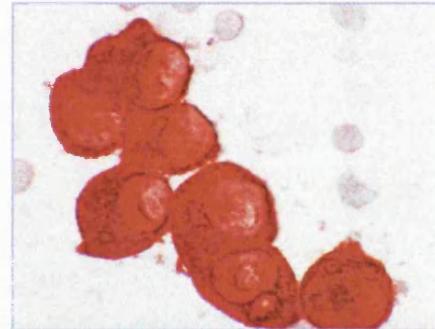


Fig 4.9 Immunocytochemical markers for cytokeratins confirm the epithelial nature of large cells and confirm metastases (MNF 116).

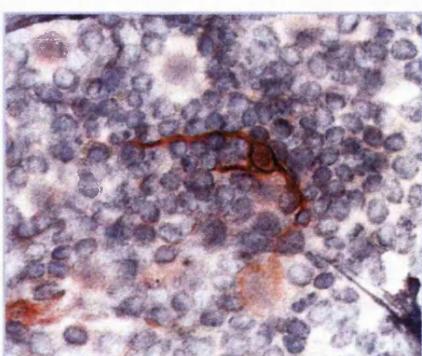


Fig. 4.10. Follicular dendritic cells can be confused with metastatic cells.

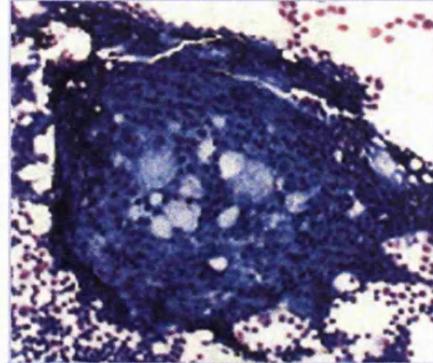


Fig. 4.11. Presence of large aggregates of lymphoid cells, may appear to be epithelial.

Although it was our finding that the spreading of smear from FNA by experienced hand to be superior, producing a mono-layer of cells of similar density but as this was operator dependent and not reproducible, it was abandoned.

The interpretation pitfalls are illustrated by the presence of a single malignant cell in a sea of lymphocytes. This can be mistaken with a follicular dendritic cells from a reactive lymph node. Another interpretation pitfall is when follicular dendritic cells are mistaken for metastatic cells after immunocytochemical staining with anti-cytokeratin marker (fig 4.11).

4.1.5 Results

A total of 141 SLN were biopsied from 117 patients (1.2 nodes per patient). Touch imprint cytology was performed on all of these nodes. The pathological diagnosis by TIC was accurate compared with permanent sections in 115 patients (98.2%). In thirty patients there was evidence of metastatic deposit on definitive histology (25.6%) and TIC correctly diagnosed evidence of metastases in 28 patients. In two patients with involved SLN on definitive histology, imprint cytology failed to detect these and were reported as negative (false negative rate of 6.6%). In both false negative cases there was evidence of metastatic deposits on the periphery of the lymph node (subcapsular sinus) and imprint cytology failed to detect these. In another patient TIC and paraffin section histology were reported as negative but on routine immunohistochemical staining a single malignant cell was evident . The primary tumour in this patient was an invasive lobular carcinoma.

In one patient TIC did show evidence of malignant cells but definitive histology

was reported as negative for metastatic carcinoma. On review of histology slides presence of a small metastatic focus became evident. This was confirmed by further sectioning and immunohistochemistry. There were no false positive cases in this series. With two false negative cases, the sensitivity of this technique was 93.7%, the specificity was 100% and the positive predictive accuracy, 100% and negative predictive accuracy, 97.7%.

4.1.6 Discussion

Lymphatic mapping, SLNB and selective axillary lymph node dissection represent a logical approach in the management of breast cancer patients.

Frozen section histology as an intra-operative diagnostic tool has not been proven to be accurate. The procedure is time consuming and needs expensive equipment and experienced technical staff. Interpretations of slides become difficult due to artifacts as a result of freezing of the node. This problem is more pronounced in fatty lymph nodes as sectioning is very difficult. Moreover, small volume of tumour is difficult to detect with frozen section histology.

Studies published on frozen section histology of the SLN have shown a significant false negative rate and are no longer considered sufficiently reliable to guide a surgical management decision. Veronesi et al⁸ reported a sensitivity of 64% when using standard intraoperative frozen section of the SLN. Authors examined frozen section of sentinel nodes in 107 cases and they found that in 18 patients (17%) the intra-operative histological diagnosis was falsely negative and micrometastatic foci were missed. In another study Giuliano et al⁹ reported 89% success rate of identifying metastatic deposit on the SLN.

A study performed by Gentry⁴, comparing the reliability of frozen sections and TIC in determining the histological status of the pelvic lymph nodes in patients with prostate carcinoma, demonstrated the superior quality of TIC. In another study Handjiminas et al¹⁰ used TIC for assessment of axillary nodal status in 114 breast cancer patients and compared it with definitive histology. Forty three patients had positive TIC result and histological examination confirmed presence of metastases in 40 patients only. Therefore TIC identified 3 extra patients with metastatic deposit. In one patient in our series, the definitive histology of the SLN was reported as negative but TIC showed malignant cells and review of histology slides confirmed evidence of small metastatic deposit which was overlooked previously.

Klimberg and co-workers⁷ have reported a diagnostic sensitivity of 96.3% and a specificity of 100 when using TIC in determining completeness of resection margins in patients with early breast cancer who underwent breast conserving surgery.

The role of TIC in patients undergoing ALND was studied by Fisher et al¹¹. Of the 50 patients who underwent axillary lymph node dissection, 21 patients had histologically confirmed metastatic disease; intraoperative TIC detected 18/21 positive lymph nodes. It was established that, should the technique of imprint cytology have been used intraoperatively, 29 out of 50 patients would have avoided the ALND operation.

Another study by Quill et al¹² who performed TIC on 86 nodes from 13 patients with breast cancer, correctly diagnosed the nodal status in 82 of the 86 sampled lymph nodes (95%) with a sensitivity of 93%.

The other argument in favor of TIC is the fact that immunocytochemical staining is feasible and improves its diagnostic accuracy. In the study of 109 sentinel lymph node imprints from 86 patients, 26 of which harbored micrometastases, Ahmad et al¹³ found that 14 were not detected by imprint cytology after standard staining and 3 were missed by permanent histopathology. They found that immunocytochemistry performed on cytological preparations detected micrometastases in 23 out of 26 cases thus upstaging the nodal status previously identified on either imprint cytology or histology. They conclude that lymph node imprints and histology findings can be improved by the use of intraoperative immunocytochemistry (anticytokeratin). We believe that although immunocytochemistry is helpful in increasing the diagnostic accuracy of the technique, the findings can sometimes be misleading. The choice of immunocytochemical markers and the knowledge of their cross reactivity with normal cells is important. Our case illustrates how a commonly used anticytokeratin antibody stains some normal constituents of the lymph node, which can be recognised in most instances by their morphology (long cytoplasmic processes). Like frozen section histology, TIC is also operator dependent and an experienced cytologist is vital for success of this technique. The study of inter-observer agreement on the diagnostic accuracy of lymph node imprint cytodiagnosis found it to have a 91.2% positive predictive value for detection of secondary malignancy¹⁴.

In a study by Usman et al¹⁵, a total of 55 patient with invasive breast cancer underwent axillary lymph node dissection. 157 nodes were examined by TIC prior to histological examination. The authors correctly diagnosed the histological status of the nodes with 100% concordance with no false negative result.

Rubio et al¹⁶ using TIC in 55 patients after SLNB, reported a sensitivity of 95.7% and a specificity of 100%, with a positive and a negative predictive value of 100% and 99%, respectively.

There were no false positive cases in our study. There were two SLN that were negative on touch imprint cytology and positive on permanent sections (two false negative cases). In clinical practice, this would have meant that no patient with a negative SLN would have undergone ALND and only 2 out of 117 patients would have returned to the operation room for a second procedure.

In both of the false negative cases, metastatic deposits were located at the periphery of the lymph node (subcapsular sinus) and imprint cytology failed to identify this. We believe this was due to inadequate bivalving of the SLN, therefore peripheral aspect of the node was not represented on the imprint. After this case, we took extra care in bisecting the lymph node and ensured that the peripheral aspect of the node was represented on the imprint. In clinical practice, this would have meant that no patient with a negative SLN would have undergone ALND and only 2 out of 117 patients would have returned to the operation room for a second procedure. In summary TIC offers many advantages as an intraoperative diagnostic tool in a sentinel node biopsy setting.

1. It is a simple, cheap and rapid technique.
2. Slides can be prepared and read within 5 minutes.
3. It provides a clear cytological detail.
4. It is possible to perform immunocytochemical staining within 15 minutes to

identify micrometastases; a further improvement in the diagnostic accuracy of the technique.

4.1.7 Conclusion

This study demonstrates that touch imprint cytology is a rapid and reliable intra-operative method for determining the histological status of the sentinel lymph node in patients with breast carcinoma . It will enable the surgeon to decide on performing ALND at the time of initial surgery with acceptable accuracy. Immunocytochemistry may be used to improve diagnostic accuracy of this method. Nevertheless, both the surgeon and the pathologist should be aware of the potential pitfalls in diagnosis.

4.2. Optical Biopsy in SLNB for Breast Cancer

4.2.1. Introduction

The absence of a rapid and reliable intra-operative diagnostic tool to determine the histological status of the sentinel lymph node is a major limiting factor in its clinical application. In an attempt to overcome this limitation, for the first time, we examined the role of 'optical biopsy' in determining the histological status of the sentinel lymph node. The instrumentation for this project has been developed at the Los Alamos National laboratory in USA and this clinical program is being undertaken in collaboration with the University of Arkansas for Medical Sciences in the USA and the National Medical Laser Centre, Department of Surgery and Institute of Nuclear Medicine, University College London.

4.2.2 Theoretical advantages of an optical biopsy system

1. The system is portable and can easily be used intraoperatively to determine the sentinel node status
2. The result can be produced in few seconds after the sentinel node excision which, if proven reliable, will enable the surgeon to decide whether to proceed with axillary lymph node dissection (ALND). In addition to avoiding the morbidity of ALND in a large number of patients, this technique will have potential cost saving advantages.

4.2.3. Background

The intensity of scattered light varies with the wavelength and with the tissue being tested. When light enters the tissue, three main interactions may occur: it may be elastically scattered, inelastically scattered, or absorbed. The scattering properties of the tissue depend on two factors:

1. the cellular structure of the tissue
2. the wavelength of the light

Tissues with different cellular structure are expected to have different elastic-scatter spectra than the optical signals from normal tissue where cells and cell components are of roughly uniform shape and size and are arranged regularly.

The hypothesis is that scattering occurs primarily from components of the highest relative refractive index, such as nuclear and subnuclear structures, and bilipid membranes.

A technique which is sensitive to the wavelength dependence of scattering may therefore be able to detect changes in pathological tissues, particularly cancers, (e.g. cell size, shape, cytoarchitecture, nucleocytoplasmic ratio, the form of the

cell membrane, clustering patterns etc.).

The purpose of this study was to assess the potential of the optical biopsy technique based on these principles, in establishing the histological status of the sentinel lymph node. As part of another ongoing trial, experiments on fresh ex-vivo specimens of human breast tissue were performed and differences in the spectra between normal and malignant tissue were evident.

4.2.4. Objectives

1. To evaluate the potential of an optical probe based on the principle of Elastic Scattering Spectroscopy (ESS) in determining the sentinel lymph node (SLN) histological status. Optical spectral measurements were obtained from a number of SLN's and the results correlated with conventional histology findings.
2. Ultimately to develop an optical biopsy instrument capable of diagnosing the status of the sentinel lymph node pre or intraoperatively.

4.2.5. Patients and Methods

All patients scheduled to undergo sentinel node biopsy procedure were eligible. The day prior to surgery, patients received subdermal 15 MBq of ^{99m}Tc -nanocolloid at the skin site overlying the primary breast tumour. This was followed by two view lymphoscintigraphy. At operation the SLN was harvested by a combination of probe guided surgery and blue dye lymphatic mapping. After harvesting, the SLN was bi-valved and the optical biopsy probe applied over the surface of the lymph node (fig 4.1) at either a single or multiple sites. A short pulse of white light was then applied through the fibre. Light which is

elastically scattered is transmitted back through a second fibre with its tip close to that of the first and is then analysed spectroscopically (fig 4.12,4.13). The effective wavelength range of the system is 300-750nm, and the delivered power and energy are low enough to avoid any effect on the SLN. The complete optical probe, including outer jacket, is less than 2mm in diameter. Only the fibre optic probe touches the patient and this is sterilised for intra-operative in-vivo use. It is essential that tissue for conventional histology is available from every site where an optical measurement is made, therefore the optical biopsy site was marked. The marking technique included injection of a small amount of Indian ink or carbon particles or marking with sutures or metal clips. The spectra from the optical measurements were analysed by the Los Alamos team and correlated with the conventional histology which was examined in London and Little Rock.

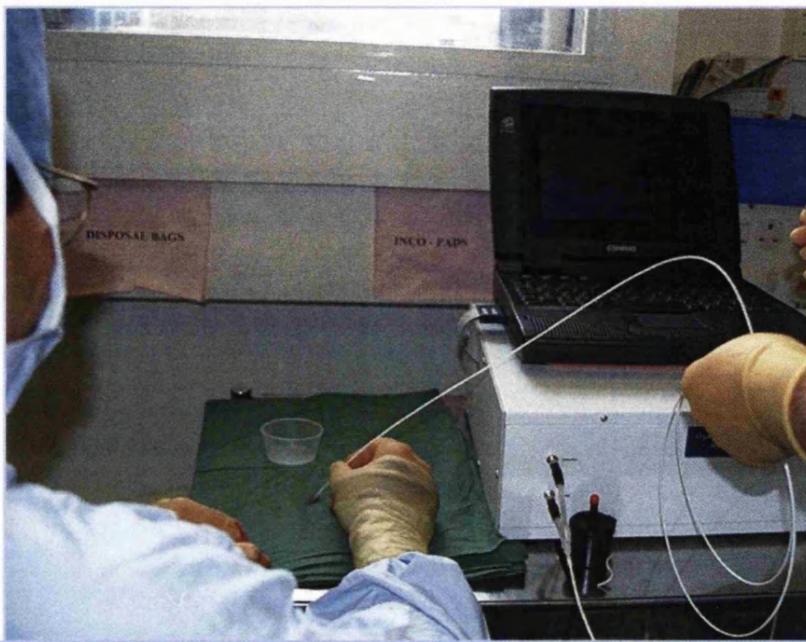


Fig 4.12 Optical biopsy of the sentinel lymph node in progress

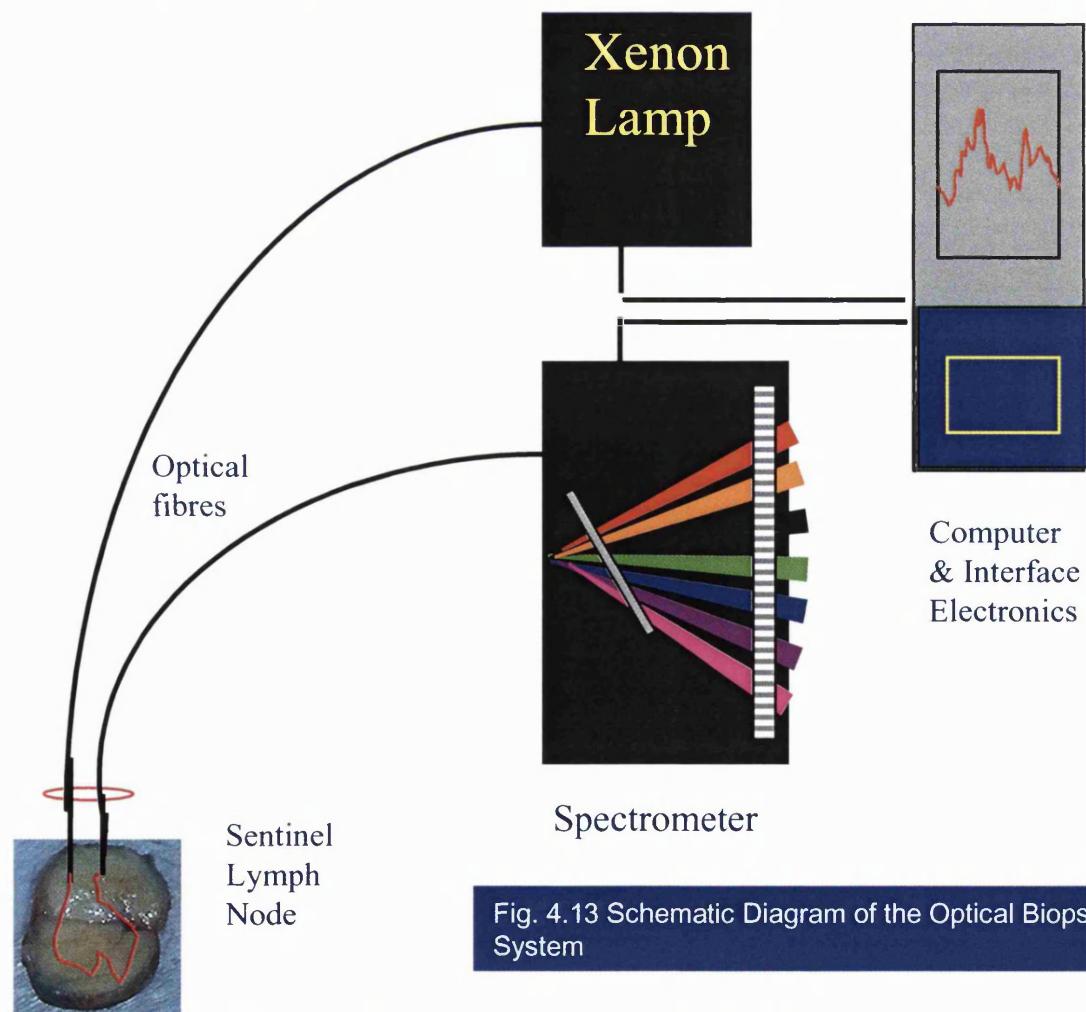


Fig. 4.13 Schematic Diagram of the Optical Biopsy System

It is essential to obtain as many individual spectral measurements as possible to establish a reference library of signals from various types of lymph nodes i.e. negative, positive and reactive nodes (fig 4.14). Artificial-intelligence pattern-recognition (AI-PR) methods are used for spectral classification and analysis.

Two different AI-PR methods of spectral classification have been employed to assess the degree of correlation between pathology and spectral pattern differences: artificial neural networks (ANN) and hierarchical cluster analysis (HCA). Although both ANN and HCA methods are intrinsically statistical-based classification methods, some preprocessing or weighting of input parameters can be implemented to "assist" classification when some properties about the underlying tissue optical properties are known. (Such preprocessing can, in effect, combine benefits of both statistical-based analysis and model-based analysis.) In its simplest form this means that the input parameters for training the AI methods, which are derived from the raw spectra, should be structured so as to include the spectral information known to have diagnostic relevance. Careful computer analysis of the spectra will identify features, which can be reliably correlated with normal or malignant tissue.

It is far too difficult at our present level of understanding to try and correlate individual spectral features with particular features of normal or malignant cells or regions of normal and malignant tissue, so we have to look for more general features. The more data which become available, the easier this is likely to be.

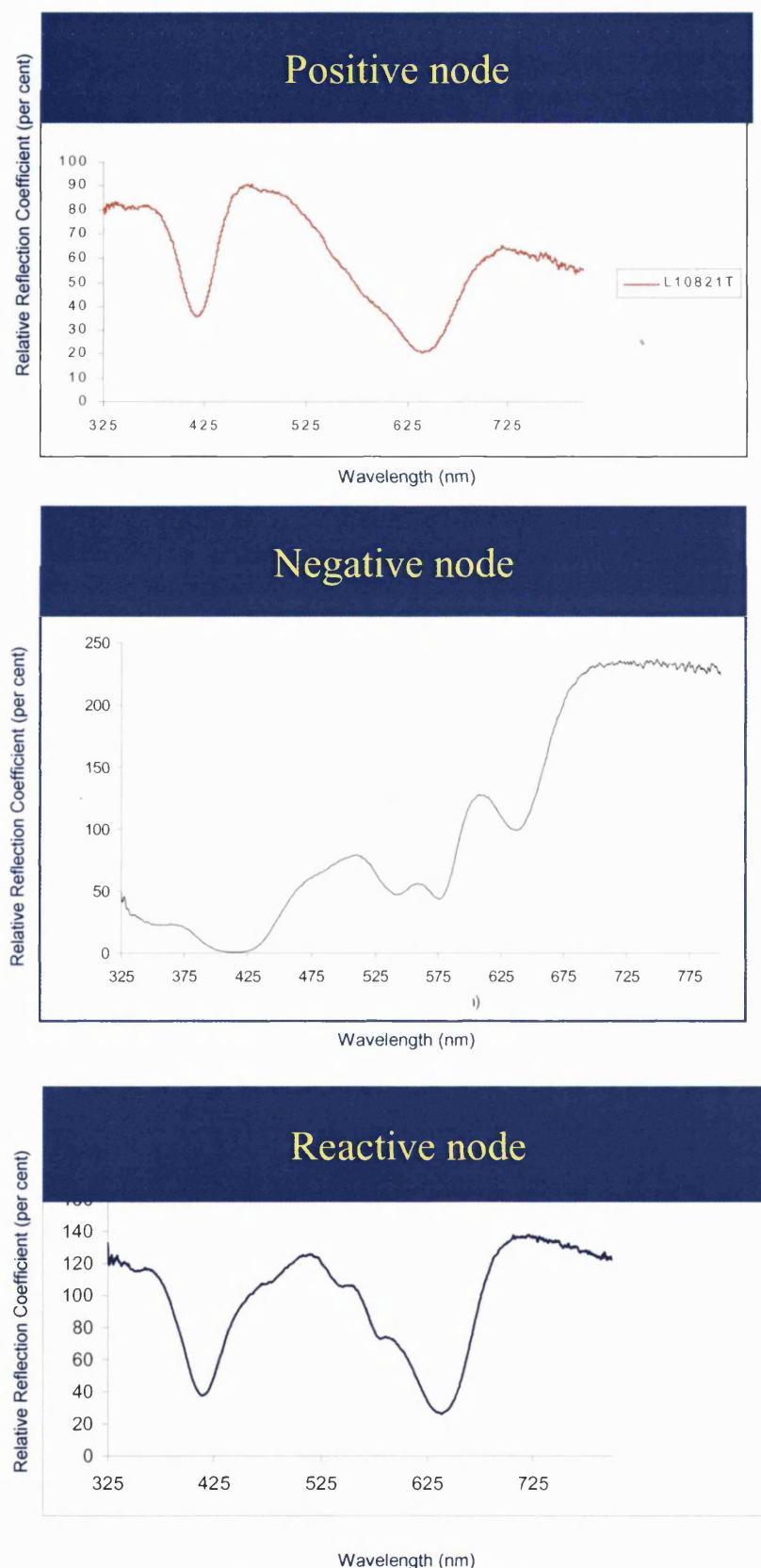


Fig. 4.14 Different weave forms for various histological status of the sentinel lymph node

4.2.6 Results

Thirty optical biopsy data sets were obtained from 24 sentinel nodes in 18 patients with breast carcinoma. The patients median age was 46 years (range 37-74). The location of the primary tumour and their size and histological grade, definitive histology of the sentinel nodes and the optical biopsy findings are summarized in table- 4.1.

Pt. No	Age	SLN No	SLN Histology	Optical Biopsy	Primary location	Laterality	Tumour size (mm)	Primary Tumour Histology
1	45	2	Negative	Negative	UOQ	LEFT	15	ILC + DCIS
2	58	2	Negative	Negative	UOQ	RIGHT	2	DCIS
3	37	1	Negative	Negative	LIQ	LEFT	13	IDC
4	74	1	Positive	Positive	LOQ	RIGHT	25	ILC
5	46	1	Negative	Negative	LIQ	RIGHT	15	DCIS
6	46	1	Negative	Negative	Central	LEFT	40	IDC
7	46	1	Negative	Negative	LOQ	RIGHT	20	IDC
8	56	2	Negative	Negative	UOQ	RIGHT	16	ILC
9	51	2	Negative	Negative	LIQ	LEFT	3	IDC + DCIS
10	49	1	Negative	Negative	UOQ	LEFT	10	IDC
11	63	1	Negative	Negative	LOQ	LEFT	18	MIXED ILC/IDC
12	69	3	Negative	Negative	UOQ	LEFT	12	ILC
13	33	1	Positive	Positive	IOQ	RIGHT	50	IDC+DCIS
14	62	1	Positive	Positive	UOQ	LEFT	20	IDC
15	62	1	Positive	Negative	UOQ	LEFT	20	IDC
16	88	1	Positive	Positive	LOQ	RIGHT	30	ILC
17	77	1	Positive	Positive	UIQ	LEFT	35	IDC
18	71	1	Positive	Positive	UOQ	LEFT	40	IDC

Table 4.1 Results of optical biopsy on sentinel lymph node ID= Invasive Ductal Carcinoma
IL= Invasive Lobular Carcinoma DCIS= Ductal Carcinoma in Situ

All of the biopsied sentinel lymph nodes were in an axillary location. Twenty two SLN in 12 patients did not show any evidence of metastatic involvement on definitive histology. Optical biopsy correctly determined the histological status of these nodes and there were no false positive cases. There were eight lymph nodes in six patients which were involved with metastatic carcinoma on

definitive histology. Optical biopsy correctly predicted the histological status in 7 nodes. There was one false negative report, in a patient with a grade 3 invasive ductal carcinoma who had involvement of the SLN on definitive histology. Optical biopsy signals were interpreted as that of an uninvolved lymph node by the computer. With one false negative case, the sensitivity of this technique in this very limited series was 88% with 100% specificity.

Presence of blue dye within the node has not caused problem in analysing the spectra as the characteristic wavelength of malignant versus normal tissue are remote from the interference caused by the blue dye.

4.2.7. Discussion

Preliminary studies have been performed on the surface of tissues in the gastrointestinal tract by the optical biopsy probe¹⁷ and shown differences between normal and malignant and dysplastic tissue. In this study, the authors conclude that the spectral data from colon, rectum and stomach appear promising for detecting dysplasia. They also state that with improved detector stability and standardization of probes and more sophisticated spectral data analysis may improve the reliability of this technique.

The optical biopsy device has also been used in 10 patients with a suspected bladder carcinoma¹⁸ and In vivo measurements were made. Elastic-scatter spectra over the wavelength range 250-800 nm were obtained using a fiber-optic probe through one of the lumens of a urological cystoscope. Measurements were done on normal areas and areas of uncertain abnormality, as well as those suspected to be cancerous. After measurements were made with the optical biopsy system, biopsy samples were taken at the measurement sites. Comparisons of the histopathology and the optical spectra were then

made and the authors provided a diagnostic algorithm for distinguishing malignant from benign tissue. They report a sensitivity of 100% and specificity of 97% for the limited number of patients investigated in this study.

4.2.8 Conclusion

Early spectral data from optical biopsy of the sentinel lymph node is encouraging as a 'real time' diagnostic tool for determining the histological status of the nodes. Potential applications include intra-operative use of the device to determine the status of the SLN without any delay and at the same time the tumour margin can be examined to confirm the completeness of excision of the primary tumour.

4.3 Autoradiography and Electron Microscopy of the Sentinel Node

4.3.1 Introduction

The distribution of radionuclides can be detected in tissue sections by autoradiography using either a radiation-sensitive film or emulsion technique.

In vitro autoradiography was first described by Young and Kuhar in 1979¹⁹, describing the distribution of binding sites (potential pharmacological receptors) both in the CNS and in peripheral tissues.

Since these methods have been devised to identify regions exhibiting radioligand binding, they are potentially useful for studying the distribution and cellular localisation of radionuclides (such as ^{99m}Tc often used in oncology).

The techniques described here are based on established methods for the *in vitro*

identification of receptors on slide-mounted tissue sections²⁰. Since the half life of ^{99m}Tc is relatively short (6 hours), successful autoradiography requires a concerted effort of a multidisciplinary team.

We evaluated the role of autoradiography in the sentinel node setting with the following aims:

1. To assess the distribution of radioactive tracer within the sentinel lymph node in correlation with definitive histology.
2. To study the uptake of the radiotracer at a cellular level.

To our knowledge, there is no report in the literature studying this aspect of the SLN.

4.3.2 Patients and Methods

We performed autoradiography in 15 sentinel lymph nodes from 15 patients. These included 13 patients with breast carcinoma and 2 patients with malignant melanoma.

After harvesting the sentinel lymph node with a combination of probe guided surgery and blue dye technique, frozen sections were prepared. Ten micron sections were cut in a cryostat at approximately -25°C. They were subsequently thaw-mounted onto gelatinised microscope slides. After drying at room temperature, tissue sections were fixed (acetone at 4°C for 30 min) before being exposed to Hyperfilm 3H (Amersham International) in x-ray cassettes. These are special radiation sensitive films, designed for this purpose. These were kept overnight at room temperature.

Films were subsequently processed using undiluted D19 high contrast developer

(Kodak Ltd) and fixed in Hypam (Ilford Ltd), diluted 1:4 with tap water. After a 20 minute wash in tap water, films were dried before viewing autoradiographs on a Nikon macro system and photographed where appropriate.

Slide-mounted tissue was subsequently stained with haematoxylin and eosin for histology. This method of autoradiography is easy to perform and gives an indication of the distribution of injected $^{99\text{-m}}\text{Tc}$ in sections of biopsy material. The resultant autoradiograms are also suitable for quantitative analysis by computer-assisted densitometry. However, radiation-sensitive films are no longer in register with the underlying tissue sections after processing. This, in combination with the relatively large silver grain size of the film emulsion, limits the resolution of the images generated in this way²¹.

To localise the radiation activity at the cellular level (high-resolution autoradiography) we performed emulsion autoradiography in 9 patients who also had, contact autoradiography. Nuclear emulsion (Ilford K2 emulsion or Amersham Hypercoat) was used. Slide-mounted tissue was prepared as described above and coated by immersing in molten emulsion (45°C in a water bath), under dark-room conditions. Once the emulsion had dried, sections were stored at 4°C in sealed light-proof boxes containing desiccant, overnight. After this brief exposure period, slides were allowed to equilibrate at room temperature and the emulsion processed (essentially as above, in D19 developer, followed by fixation in Hypam). Tissue sections were then stained with haematoxylin and eosin for histology for comparative analysis.

Such autoradiographs are best viewed under dark-field illumination, where

radioactivity is evident as white grain accumulations against a dark background. Underlying tissue histology may be viewed under bright-field illumination and distribution of ^{99m}Tc determined by superimposition of autoradiograph and tissue histology.

4.3.3 Results

Contact autoradiography was successful in mapping the distribution of the radiotracer in 14 patients (93%). Definitive histology confirmed presence of metastases in 4 patients with breast carcinoma. In the patient in whom autoradiography failed, there was extensive fatty infiltration of the sentinel lymph node on the definitive histology which revealed only a thin rim of functioning lymphoid tissue. This node did not show evidence of metastases (fig. 4.15).

In analysing the distribution of the radiocolloid, there was no indication of a specific dispersion pattern.

In one patient with extensive metastatic deposit from breast carcinoma, the uptake was confined to the periphery of the node (subcapsular sinus) (fig. 4.16).

The emulsion technique was not successful in delineating the distribution pattern at the cellular level despite our efforts in 9 patients (fig. 4.17).

We also performed conventional electron microscopy in three sentinel nodes in an attempt to visualise the colloidal particles. This was also unsuccessful as colloidal albumin particles are not electron dense (fig. 4.18).

We subsequently decided to superimpose the autoradiography image against the histology slides to correlate the area of increased activity with the underlying cells in three patients. In one patient this correlated with the location of a macrophage

in the histology slides .

4.3.4 Discussion

We did not find any specific pattern of distribution of the radiocolloid in our series.

In one patient with breast carcinoma, the SLN revealed heavy infiltration with metastatic carcinoma. Contact autoradiography revealed limited uptake confined to the periphery of the lymph node along the subcapsular sinus. In a study performed by Zeidman and Buss²², it was demonstrated that the tumour emboli are immediately trapped in the subcapsular sinus on entering the lymph node through afferent lymphatics. This may represent reduced functional capacity of the node due to the metastatic infiltration.

In another patient with failed autoradiography using the contact technique, the node was heavily replaced by fat . This also suggested reduced functional capacity and limited uptake of the radiocolloid. These two aspects of the SLN detection are

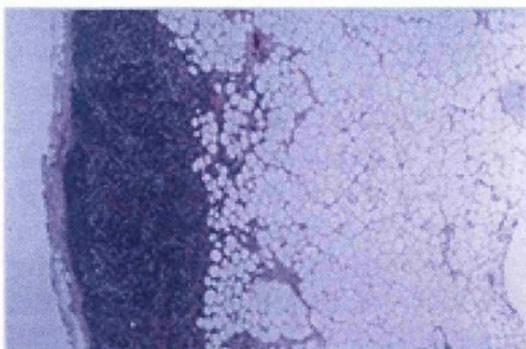


Fig. 4.15 Extensive fatty infiltration of the SLN in a patient in whom contact autoradiography failed

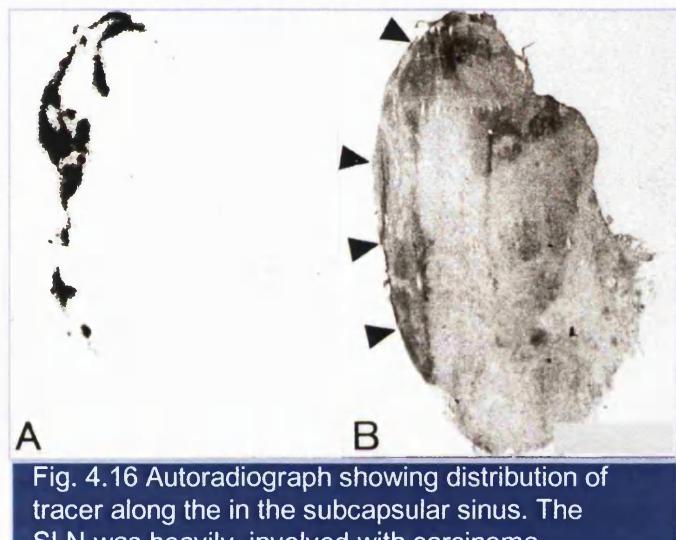


Fig. 4.16 Autoradiograph showing distribution of tracer along the in the subcapsular sinus. The SLN was heavily involved with carcinoma.

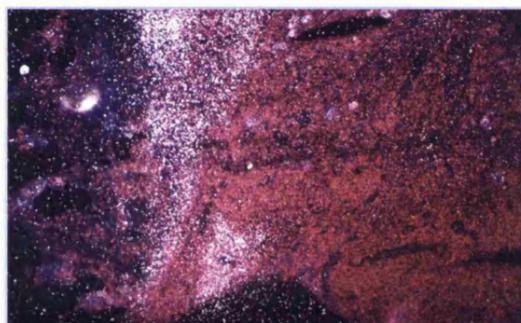


Fig. 4.17 Emulsion autoradiography unhelpful in delineating the radiotracers at cellular level.

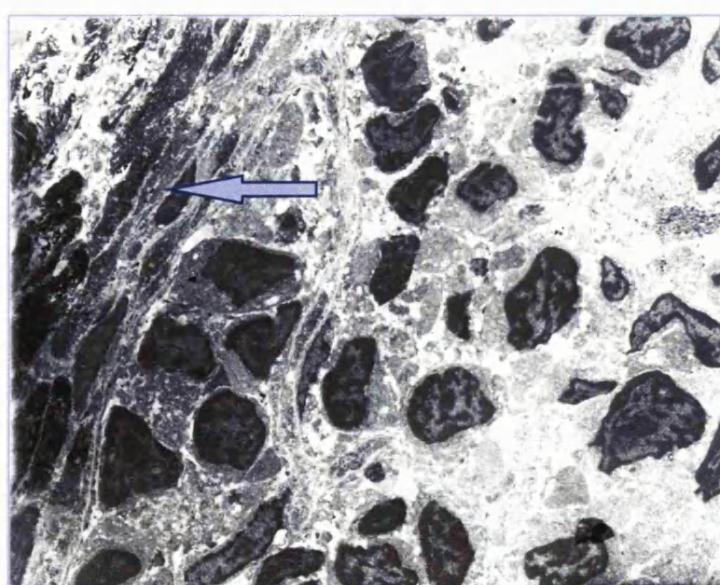


Fig. 4.18 Frozen section of a sentinel node prepared for the electron microscopy. The nodal capsule and the subcapsular sinus is clearly visible

discussed in detail in the pitfalls section of this chapter.

At the cellular level, emulsion autoradiography was not successful in all 9 patients. We feel that this was due to some technical error which we hope to overcome as the study continues.

In our imprint cytology study we have demonstrated the presence of blue pigments within the tingible body macrophage (fig 4.6). We believe that the radiocolloids have the same fate but we were unable to demonstrate this by emulsion autoradiography. With the help of superimposition technique, we could demonstrate a hot spot superimposing a macrophage. Further studies are required to confirm these needs findings.

4.3.5 References

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Chapter-5

A NEEDLE-FREE VEHICLE FOR THE ADMINISTRATION OF TRACER FOR SENTINEL NODE BIOPSY

5.1 Introduction

There have been reported cases of patients' refusal to undergo SLN localisation. This is because of needle phobia and the fear of pain experienced during the injection of the radionuclide in vulvar and penile carcinoma¹. Needle phobia is a recognised condition and needs to be considered in clinical practice.

We were prompted to evaluate the feasibility of the administration of the radionuclide with a needle-free injection system, as we encountered patients in our sentinel lymph node biopsy (SLNB) project who wished to participate in the trial but were needle phobic.

5.2 Aims

The aims of this study were:

1. To determine the feasibility of delivery of the radiopharmaceutical using new and less invasive needle free injection system
2. To establish its success in sentinel lymph node localisation.

5.3 Needle Free Injection System

The J-Tip® needle-free injection system (National Medical Products, Irvine, USA) represents a novel, single-use, disposable device for the delivery of (liquid) diagnostic or therapeutic agents. The injectate is delivered into the

dermis and the subcutaneous space. It has certification from both the Food and Drug Administration (USA) and the European Commission (by award of a CE mark).

The device is approximately 10 cm in length and weighs 9 g (fig 5.1). The injectate is loaded into the syringe with the help of a special adopter and transporter, which are supplied together (fig 5.2).

Two sizes are available with 0.25 ml and 0.5 ml capacity. “Medication” delivery is under high pressure from a compressed carbon dioxide gas cartridge which is situated on the proximal end of the device. The gas is released (following pressing a trigger button) which releases the safety catch, thereby snapping open the gas cartridge. This allows the gas to push a plunger onto a piston and hence the medication is propelled from the sterile syringe. Combination of the driving force behind the liquid column and the critical diameter of the opening at the tip of the syringe forces the liquid through the natural pores of skin into the dermal and subcutaneous space. This is achieved in about 0.2 s, with an approximate penetration depth of 3-8 mm. Because delivery is via a micro-orifice in the J-Tip device at such high velocity, it is virtually pain-free. Once used, the system cannot be re-filled. It is disposed of into an ordinary clinical waste bin. There is no need for a “sharps bin” as no needles are used at any time either for syringe filling or for its use².

The depth of penetration of the injectate by J-Tip syringe is between 3-8 mm (fig 5.3). This was measured by injecting the patent blue dye in patients

undergoing mastectomy and SLNB for widespread ductal carcinoma in situ (DCIS). After the specimen was retrieved, the depth of penetration was determined by dissecting the injection site and measuring the spread of the blue stained area (fig. 5.4).

The dermal layer of skin was heavily stained with the blue dye and there was evidence of blue staining of subcutaneous fat and superficial breast parenchyma. Therefore in SLN mapping for breast carcinoma this injection technique is equivalent to a combination of intradermal and parenchymal injection techniques (fig 5.5).

The main advantages of a needle-free delivery tool are two-fold. First, the device eliminates anxiety associated with needles. Second, and of importance, is the fact that “needle-stick” injuries no longer occur.

5.4 Patients and Methods

Informed consent was obtained from all patients. 18 patients were studied (Table-5.1).

One day prior to surgery, patients received 15 MBq of 99m Tc-labelled colloidal albumin in a volume of 0.2 ml.

To administer the radiopharmaceutical, the syringe was applied firmly and perpendicular to the skin surface (fig 5.6). This was to safeguard against the spillage of the radiopharmaceutical during administration of the tracer.

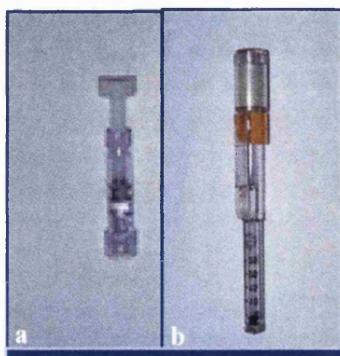


Fig 5.1 Needle-free injection system a, adopter & transporter, b, syringe

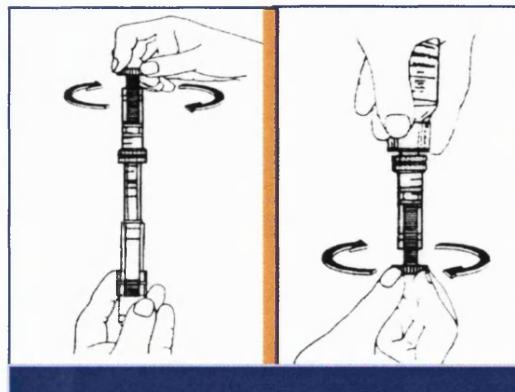


Fig 5.2 Diagrammatic representation of the loading of the needle free syringe

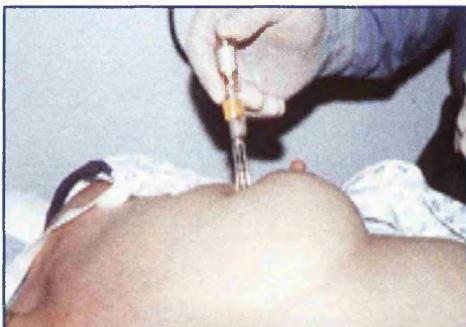


Fig 5.3A Injection of radio-pharmaceutical in a patient with breast carcinoma

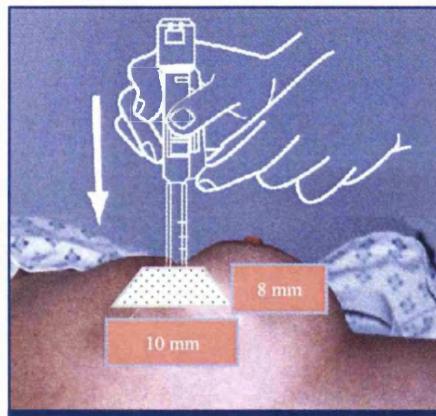


Fig 5.3B Representation of the penetration depth

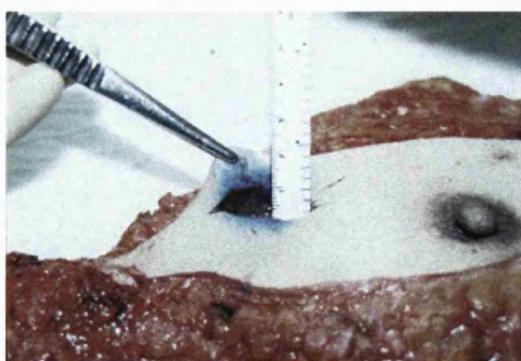


Fig. 5.4 Depth of penetration after delivery of blue dye in a patient who underwent mastectomy.

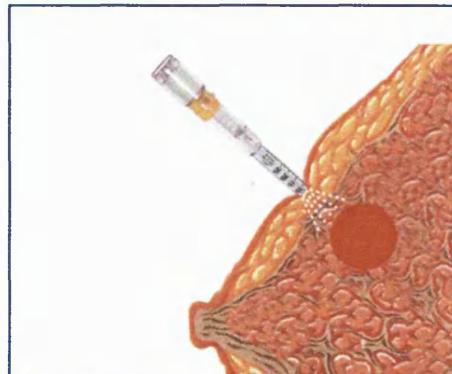


Fig 5.5 The delivery of the injectate is combination of intra-dermal and peritumoural technique

Patients and site of primary malignancy	
Breast carcinoma	7
Malignant melanoma	3
Anal Carcinoma	1
Penile carcinoma	2
Oral carcinoma	1
Volunteers	4
Total	18

Table-5.1

Spillage can result in contamination artefact which makes data interpretation difficult and can also raise radiation safety concerns (fig 5.7). The injection was therefore administered away from the camera face. We also ensured that a limited area of skin at the injection site was exposed and the remaining area was covered with an absorbent pad (fig 5.8).

Patients were warned about a hissing noise that is heard as soon as the trigger button is activated.

Dynamic and static imaging commenced immediately after the delivery of the radiopharmaceutical as described in the imaging section 3.6.

5.5 Results

In 17 individuals, the injection of the radiotracer led to visualisation of a hot spot on lymphoscintigraphy (94.4%). The injection site, lymphatic duct and the SLN were clearly visible on lymphoscintigraphy (fig 5.9)

In one patient, spillage of the radiopharmaceutical occurred which resulted in contamination artefact which made data interpretation extremely difficult (fig 5.8). This patient received conventional subdermal injection using a 25G needle after the skin was decontaminated. Subsequent imaging led to successful localisation of the SLN.

Thirteen patients underwent SLN biopsy as 4 studies were performed on volunteers. At operation combination of blue dye lymphatic mapping and probe guided surgery were employed. Sentinel node was successfully identified in all patients without any difficulty. Total of 24 SLN were biopsied (1.8 SLN per patient). In a patient with anal carcinoma and in one patient with penile carcinoma, the tracer injection demonstrated drainage to both inguinal regions. These cases are discussed separately in section 6 of this thesis.

SLN was involved with metastatic tumour in 4 patients (2 melanoma and 2 breast carcinoma).

Patients did not report any pain as a result of injection. They mostly complained about the hissing noise which is heard as soon as the trigger button is pressed. Careful analysis of the time-activity curve of dynamic acquisition showed slower particle kinetics as compared with the conventional injection technique. A

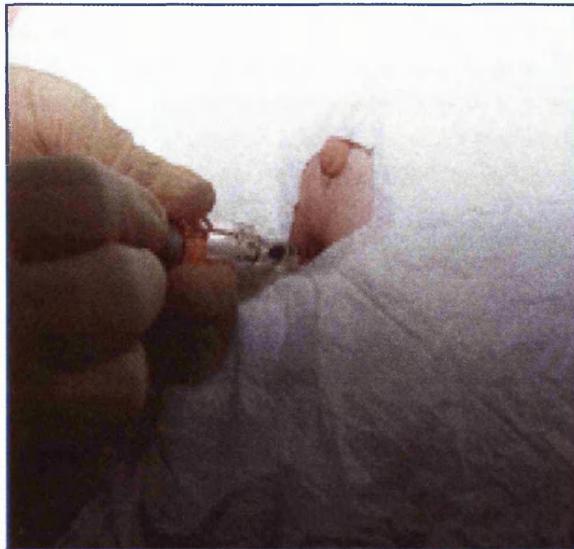


Fig 5.6 Limited exposure of skin to safeguard against accidental contamination artefact.

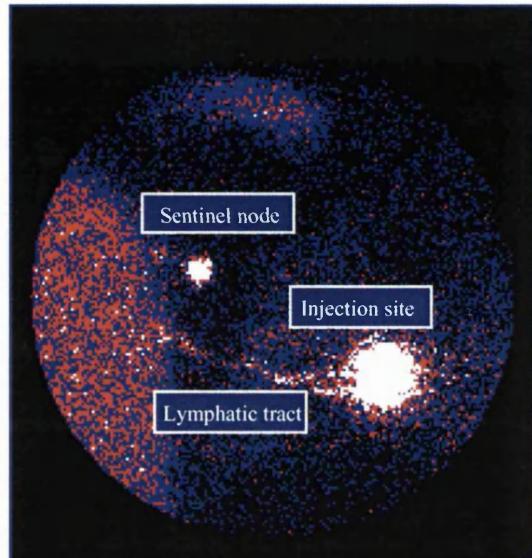


Fig 5.7 A composite static lymphoscintigram showing the injection site, the lymphatic tract and the sentinel node

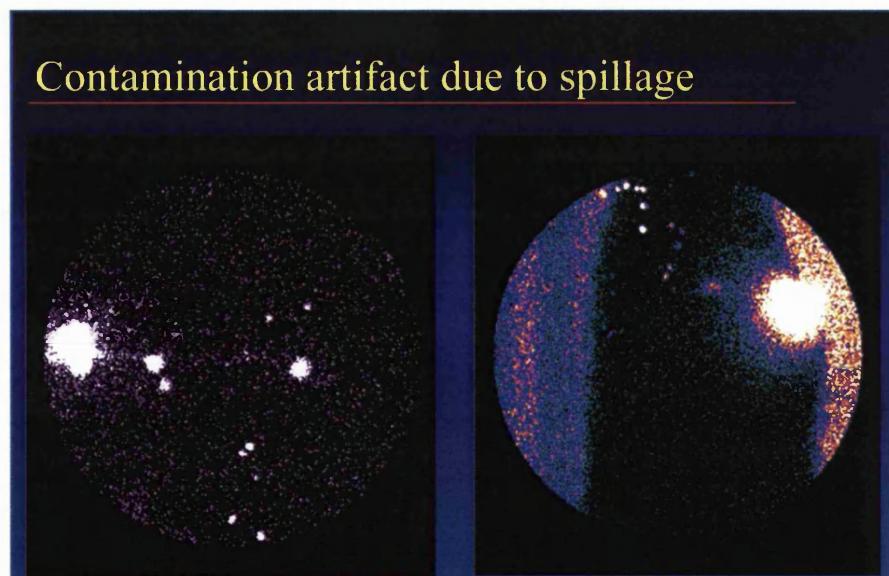


Fig 5.8 Spillage of the radiopharmaceutical resulting contamination artefact which makes data interpretation extremely difficult

region of interest analysis showed that the area of dispersion of the tracer was also larger than was achieved by the conventional technique; however, image quality was not affected.

5.6 Discussion

As the concept of SLNB is being evaluated in various aspects of surgical oncology, there is potential for an increasing demand for this technique. As injection at some particular sites (around genitalia) can be extremely painful, it is desirable to offer these patients a less invasive injection technique. This is illustrated by the number of patients refusing to undergo SLNB due to genuine fear of pain¹. There are patients who are needle phobic and suffer from carcinoma. Inflicting additional pain to these patients can have negative psychological impact.

We have used the needle-free injection device in our triple assessment breast clinic. In this study we evaluated the feasibility of delivering local anaesthetic agents prior to fine needle aspiration cytology (FNAC) in over 100 patients. Patients were asked to score their pain experience after the injection of local anaesthetic with the J-tip syringe and after FNAC procedure respectively. These were recorded on a visual analogue scale. Over 90% of patients reported no pain or minimal discomfort after injection with the needle free syringe³.

This study demonstrates that the radiopharmaceutical can be successfully delivered for SLN localisation in different pathologies. In using this technique,

in 18 individuals, there was one complication of spillage which resulted in a contamination artefact. This complication occurred earlier in our series. Lack of experience was responsible for this as, sufficient pressure was not applied prior to pressing the trigger button. The fact that the operator loses control of the injection process as soon as the trigger button is pressed highlights the need to observe extra care.

In a separate study, we have demonstrated a positive perception of patients from this device as their pain experience was minimal. Moreover, the added advantage of elimination of risk of sharp injuries makes it a very desirable device for clinical use. The price of the entire system (including the syringe, transporter and adopter) is around £3.00.

5.7 Conclusions

- Our feasibility study indicates that a new and less invasive technique of delivery of the radiopharmaceutical is possible and it can lead to successful SLN localisation.
- The needle-free injection system is a very suitable device for SLN biopsy in needle-phobic patients.
- Alternatively the device can be used to administer a local anaesthetic before administration of the radiopharmaceutical in a conventional manner to make it a pain free experience for patients.
- Further studies are necessary in order to test this technique's sensitivity in localisation of the SLN. If applicable routinely in this context, it would have a major impact on patients acceptance of SLN biopsy.

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Chapter-6

THE SENTINEL NODE IN MALIGNANT MELANOMA, PENILE, COLORECTAL, ANAL & ORAL CARCINOMA

6.1 The Sentinel Node in Malignant Melanoma

6.1.1 Introduction

There has been a dramatic increase in the incidence of cutaneous malignant melanoma over the last decades¹. Patients tend to seek early medical advice as a result of public awareness programs. Hence the majority of patients at the time of presentation have no clinical evidence of regional lymph node metastases (i.e. stage I disease)^{2,3}. The prognosis is directly related to Breslow thickness, measured in millimeter (mm)⁴. A higher thickness is associated with more lymphatic and distant metastases. Tumours thinner than 0.75 mm do not seem to metastasize and are localized in almost 100% of cases. In thicker melanomas the incidence of recurrences increases from 6-27% (for 0.76-1.49 mm Breslow thickness) to 23-43% (2.5-3.99 mm Breslow thickness) within the first 2 years after presentation⁵. When nodal metastases are present, the 5-year survival is decreased by 25-50%, depending on the volume of disease in the regional basin. Other prognostic factors include ulceration and location of the primary tumour.

Most melanomas are seen to be superficial (65%), but can present as nodular (25%), as lentigo maligna (5%) and as acral lentiginous forms in a further 5% of cases.

6.1.2 Materials and Methods

In a feasibility study, we performed sentinel node biopsy in patients with localised malignant melanoma and a Breslow thickness of more than 1 mm. Informed consent was obtained from all patients. We performed sentinel node mapping on 15 patients (6 men 9 women). The age range was 34-80 years (median=46 years).

The primary tumours were located in the lower limb (n=9), upper limb (n=2), shoulder (n=1), trunk (n=2) and head and neck (n=1). Prior to SLN procedure all patients had undergone excision biopsy to establish the diagnosis.

6.1.2.1 Injection of radiopharmaceutical

On the day before surgery or on the same day of the operation, all patients underwent lymphoscintigraphy to locate the SLN. A dose of 15 MBq of 99m Tc-nanocolloid was injected in a volume of 0.2 ml using a 25-G needle. The injection was administered on each side of the biopsy scar, approximately 5mm away from the wound edge, using also a 25 G needle (fig 6.1). The syringe was held parallel to the skin surface and the needle made to enter at an angle of 10-20° to the horizontal plane.

6.1.2.2 Dynamic and Static Imaging

After injection, dynamic images were immediately obtained to visualise the lymphatic drainage. Ten seconds dynamic acquisition was continued for 15 minutes followed by 1 minute acquisition for a further 30 minutes (fig 6.2). This was followed by static imaging of the draining lymphatic basin, in standard anterior and lateral views for 5 minutes (fig 6.3). Static emission scanning was

followed by transmission imaging using a ^{57}Co flood source to include the body outline in the image for easier interpretation. By using the electronic marking facility, fixed anatomic landmarks were also recorded to make image interpretation easier. The location of the SLN was marked with indelible ink on the patient's skin, using a ^{57}Co point source marker and the camera in persistence mode (fig 6.4).

6.1.2.3 Intraoperative Detection Technique

At operation, the lymphatic basin was prepared in a standard fashion and 1.5-2 ml of patent blue dye, injected at approximately the same points as the colloid around the excision biopsy wound (fig 6.5). The location of the SLN was subsequently determined through the intact skin using a gamma detection probe (Neoprobe 1500, Columbus, OH). A small incision was then made over the hot spot. The probe constantly guided the surgeon to the SLN. When a blue-stained lymphatic duct was detected, it was traced to the first draining lymph node (fig 6.6).

After the SLN was excised, the ex-vivo radiation count was recorded. The probe was reinserted into the wound to determine the residual radiation count to ensure complete excision. The excised SLN was submitted for histopathological examination including paraffin hematoxylin-eosin (H&E) sections and immunohistochemical staining with S100 protein and HMB-45 antigen. Only when there was histological evidence of nodal metastases, a formal regional lymph node dissection was performed. The average follow up of these patients was 12 months (range 5-22 months).



Fig 6.1 Injection of radiopharmaceutical in a patient with malignant melanoma right leg

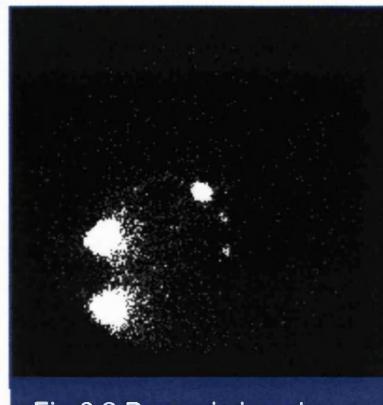


Fig 6.2 Dynamic imaging

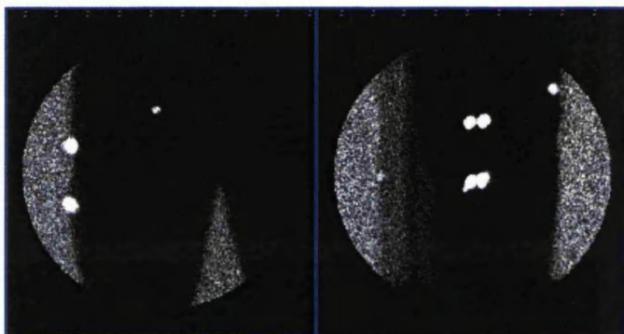


Fig. 6.3 Static anterior and lateral composite image



Fig. 6.4 Skin marking with the help of ^{57}Co point source marker



Fig. 6.5 Injection of Patent Blue dye at operation

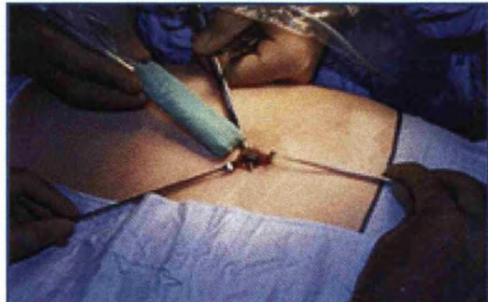


Fig. 6.6 Intraoperative detection of the SLN using gamma detection probe. A blue and hot SLN is evident

6.1.3 Results

Lymphoscintigraphy was successful in 14/15 patients (93.3%). In one patient with a malignant melanoma of left shoulder, lymphoscintigraphy showed very faint uptake in the ipsilateral axilla and subsequent delayed imaging failed to show any focal uptake and therefore the sentinel node biopsy was not performed on this patient.

In the remaining 14 patients, sentinel node biopsy was successful in all and a total of 24 sentinel node were biopsied from fourteen lymphatic basins (1.7 lymph node per patient). The sentinel nodes were hot in all and blue in 13 patients. In one patient with malignant melanoma of the foot, there was no blue lymphatic duct or a blue node and the SLN was identified with the help of gamma detection probe only.

Marking of the skin proved to be accurate in the groin where frequently more than one hot node was present. In the axilla, skin marking was less accurate. The SLN's were positive in 3 patients on histological examination (21.4%). One of these was an 80 year old female with malignant melanoma in the upper arm with Breslow thickness of 7.5 mm. One sentinel node was detected on lymphoscintigraphy and after it was biopsied, histology revealed complete replacement of the lymph node with metastatic melanoma. This patient underwent therapeutic axillary lymph node dissection and on histology, there was evidence of extensive nodal involvement (6/15 lymph nodes involved) with extracapsular spread of metastatic tumour in the soft tissue. This patient developed satellite skin metastases five months after surgery.

In one patient with a positive sentinel lymph node, the SLN was the only involved lymph node (33%).

Immunohistochemistry did not detect any evidence of micrometastases in the remaining 11 patients with negative SLN on H & E staining.

Two patients with lower limb melanoma had undergone wide excision of their primary site and lymphoscintigraphy and sentinel node biopsy was successfully performed (fig 6.7).

In one patient the primary tumour was located on the right cheek (fig 6.8). This was a 32 year old male with a 6.5 mm nodular melanoma of the right cheek. On lymphoscintigraphy two hot lymph nodes were evident in the lower neck on anterior view. On lateral acquisition two additional hot nodes became obvious in the submandibular group of lymph nodes. These nodes were overshadowed by the activity from the injection site (fig. 6.8). At operation, an external collimator was used on the probe due to the proximity of the SLN to the primary injection site. During dissection a blue lymphatic tract leading to two blue and hot nodes were evident. These corresponded to the two submandibular nodes seen on the lateral view. These were biopsied and histology did not show any evidence of metastatic or micrometastatic deposits.

6.1.4 Discussion

The development of sentinel lymph node biopsy (SLNB) has provided an attractive alternative to routine ELND for all patients at risk of metastasis and delayed regional lymph node dissection, once there is clinical evidence of



Fig. 6.7 Injection of radiotracer and blue dye in a patient who had undergone wide excision and skin grafting

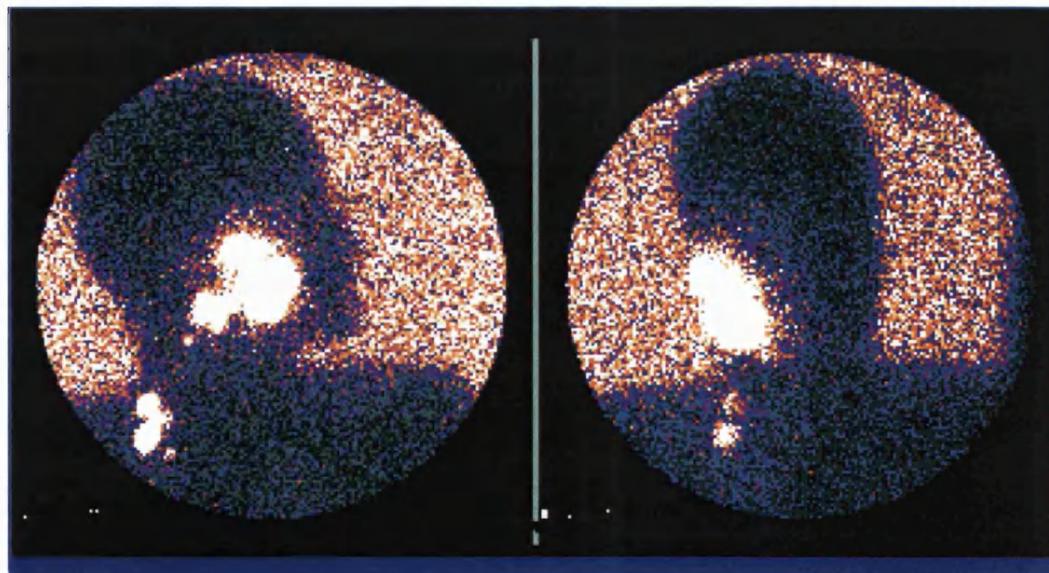


Fig. 6.8 Lymphoscintigram in a patient with cheek melanoma

regional lymph node involvement. It has been proven that the histological status of sentinel lymph node is an accurate predictor of the status of the regional lymph node basin. The ability to perform lymph node staging with only a lymph node biopsy type of procedure inevitably results in lower morbidity for the patient²⁷.

This procedure can allow the surgeon to select patients who would potentially benefit from regional lymphadenectomy. Although it remains to be seen whether the application of this concept will translate into a survival advantage for stage I and II patients, the low morbidity and technical ease of the procedure has led to its increasing application in the management of patients with intermediate thickness malignant melanoma. Indeed, the introduction of lymphatic mapping and SLNB is described as an enormous advance in the treatment of the patient with malignant melanoma⁶.

Lymphoscintigraphy is an essential first step in lymphatic mapping. It serves several important purposes including: providing a road map for the surgeon to identify the draining basin; helping to indicate the number of sentinel nodes; helping to distinguish first-tier nodes from secondary nodes; facilitating identification of sentinel nodes in unexpected locations and finally to enable the clinician to mark the location of the sentinel node on the skin. The importance of pre-operative lymphoscintigraphy has been emphasized by many authors^{7,11}. In addition to dynamic imaging, static acquisition needs to be performed in at least two views. In one patient with a melanoma of the cheek, true sentinel nodes were obscured due to overshadowing from the injection site on the anterior view. They became evident only after obtaining a lateral projection

image. In head and neck melanoma lymphoscintigraphy and sentinel node biopsy can be a technically demanding procedure¹²⁻¹³.

As far as intra-operative technique is concerned, a combination of probe guided surgery and blue dye mapping is the recommended approach which complement each other¹⁴. In one patient in our series the SLN was found only by gamma detection probe. This was in a patient with a melanoma of the foot. It is possible that it takes longer for the blue dye to reach to the groin when the primary is located far away from the basin and one needs to wait longer after injection of the blue dye for successful detection.

The challenge facing most investigators at this time is to evaluate the clinical significance of upstaging patients with a more detailed examination of the SLN. There is no adjuvant treatment that has convincingly shown to be effective in patients with an involved sentinel node and this issue needs to be addressed.

6.1.5 Conclusions

Lymphatic mapping and sentinel node biopsy for the management of patients with melanoma is now well established. Preoperative lymphoscintigraphy is an essential part of the procedure. The combination of probe guided surgery and blue dye mapping improves the success of SLN detection intraoperatively. The histological status of the sentinel node is an important prognostic factor. There is currently no evidence that incorporating sentinel node biopsy in the routine management of melanoma improves survival or regional tumor control.

6.2 The Sentinel Node in Penile Carcinoma

6.2.1 Introduction

The incidence of penile carcinoma approximates 1/100 000 in developed countries but in other parts of the world the incidence may be as high as 7.9/100 000^{15,16}. Almost 95% of penile cancers are squamous cell carcinomas. Tumour dissemination occur mainly through the lymphatic system.

The histological status of the lymph node is one of the most important prognostic indicators in penile carcinoma^{17,18}. The sentinel lymph node concept, was introduced by Cabanas¹⁹ over 20 years ago. In an 8 year period, the lymphangiograms, anatomic dissections and microscopic reports of 100 patients including 80 patients with penile carcinoma were studied. Cabanas performed lymphangiography by canulating the dorsal lymphatics of the penis and an X-ray was then obtained after injection of contrast media. He demonstrated the existence of a specific lymph node station which he called 'sentinel lymph node'. This was located in the superficial group of inguinal lymph nodes close to the superficial epigastric vein. He reported that anatomically, clinically and pathologically, the sentinel node was the first site of metastasis and often the only site⁴. He recommended preliminary bilateral sentinel lymph node biopsy and provided there was evidence of nodal involvement in these, to proceed with inguino-femoral lymph node dissection.

6.2.2 Patients and Methods

To extend our experience of SLN detection, we performed sentinel node biopsy in three patients presenting with penile carcinoma.

Case-1

A 42 year old male was diagnosed with a squamous cell carcinoma of the penis four years before. He was treated by circumcision and cauterization. He subsequently developed metastasis to the right groin after three years for which he underwent radical groin dissection. This was complicated by developing lymphoedema of the right lower limb. He was referred for sentinel node biopsy to assess the nodal status on the left groin. Injection of the radionuclide was preceded by application of local anaesthetic cream on the dorsum of the penis just proximal to the site of previous cauterization. This was followed by subdermal injection of 15 MBq of 99m Tc -nanocolloid. The patient experienced significant pain and discomfort during the injection.

Imaging confirmed focal uptake of tracer in the left groin. Through a 2.5 cm incision, the left groin was explored and two hot and blue lymph nodes were identified. Both nodes were biopsied and submitted for histological examination. There was no evidence of metastatic deposits on either lymph nodes on serial sectioning, H & E staining as well as immunohistochemistry (MNF116).

Case-2

A 67 year old homosexual man who had syphilis 30 years ago, presented with a 2 months history of a painless ulcer on the dorsum of the foreskin. There was no clinical evidence of inguinal lymph node involvement.

After circumcision, histology revealed a grade 2 squamous cell carcinoma of the penis. As the injection of the tracer was poorly tolerated by the first patient, we decided to use the needle free syringe for administration of the radiopharmaceutical in this patient. One day prior to surgery, after obtaining informed consent, the patient received 15 MBq of 99m Tc-labelled colloidal

albumin in a volume of 0.2 ml. To successfully administer the radiopharmaceutical and safeguard against spillage, the syringe was applied firmly and perpendicular to the skin surface and the surrounding skin was covered with a protective sheet before the injection (fig 6.9). The patient was asked to score their pain experience on a visual analogue scale from 1 to 10, one being no pain and 10, the worse imaginable pain. He was also warned about a hissing sound that is heard as soon as the trigger button is activated. The injection was well tolerated and the patient reported a pain score of 2/10.

After injection, dynamic and static imaging was performed. Lymphoscintigraphy revealed focal accumulation of the radionuclide in both groins (fig 6.10) and the patient underwent bilateral groin exploration. The sentinel nodes were successfully identified and biopsied using the combination of probe guided surgery and the blue dye lymphatic mapping (fig 6.11). Histology did not reveal any evidence of metastatic deposits in either sentinel lymph nodes.

Case-3

A 62 year old man presented with a fleshy lesion arising from the inner aspect of his foreskin, which had been present for about five years and had grown progressively. Biopsies confirmed squamous cell carcinoma. Clinically there was no evidence of inguinal lymphadenopathy and the CT scan did not show any evidence of distant metastasis. He underwent radical excision of the glans and penile skin with preservation of the external meatus.

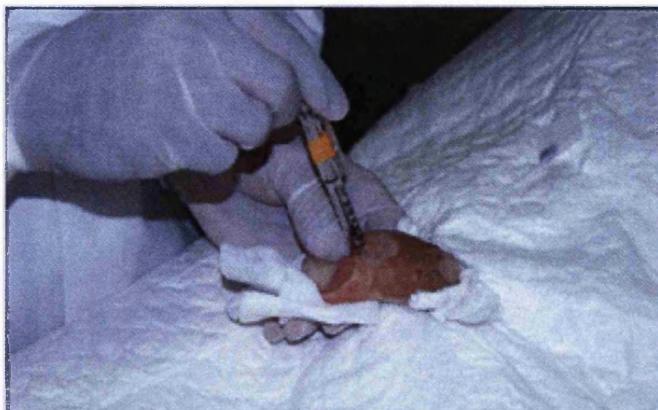


Fig 6.9 Injection of radiocolloid with the needle free syringe

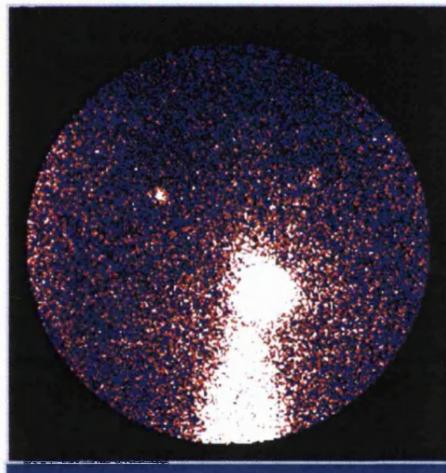


Fig 6.10 Static composite image showing the injection site and bilateral inguinal hot spots



Fig 6.11 A blue and hot node detected at operation.

This was followed by lymphoscintigraphy by injecting the radiotracer with the needle free syringe as described above. On lymphoscintigraphy there was focal accumulation of the radiopharmaceutical in both groins. The patient scored his pain experience as 2/10. At operation the sentinel nodes in both groins were identified with combination of probe guided surgery and blue dye technique. Histology did not reveal any evidence of metastatic deposits in either sentinel lymph nodes.

6.2.3 Discussion

It is interesting to note that although the sentinel node concept was introduced over two decade ago in penile carcinoma, its clinical significance was not realized until pioneering work was reported by Morton and co-workers in malignant melanoma¹⁰.

The first patient in our study experienced severe pain after the injection of the tracer. There have been reported cases of patient's refusal to undergo SLNB because of the fear of pain experienced during the injection²⁰. In the subsequent two patients we used the needle free syringe for delivery of the radiopharmaceutical. Both patients tolerated the injection very well and this lead to successful localisation of the sentinel node.

We have reported the feasibility of delivering the radiopharmaceutical with the J-tip needle-free injection system in patients with breast carcinoma and malignant melanoma²¹. The administration of the radiopharmaceutical is virtually pain free.

Despite the fact that the sentinel node concept is validated in the malignant melanoma and breast carcinoma, it is not widely accepted in penile carcinoma.

6.2.4 Conclusion

This very small series of patients demonstrate that the sentinel lymph node biopsy in penile carcinoma is feasible with a combination of probe guided surgery and blue dye lymphatic mapping. A new and less invasive technique of delivery of the radiopharmaceutical is possible. It can improve the patient's acceptance of the procedure and lead to successful SLN localization.

6.3 The Sentinel Node in Colorectal Carcinoma

6.3.1 Background

The status of the lymph node in colorectal cancer is an important prognostic indicator. It is known that the five-year survival drops from 80 percent in patients without nodal involvement (TNM stage II) to 45- 50 percent in patients with metastatic disease in their lymph nodes, (TNM stage III)²². Currently patients with stage III disease, receive adjuvant chemotherapy, which can reduce the risk of local recurrence with some survival advantages. This is not the case in patients with stage II disease and the majority of these patients do not receive adjuvant therapy except as part of clinical trials^{23,24}. Nevertheless, more than 30% of such patients subsequently go on to develop recurrent disease. It seems that patients with TNM stage II cancer are a heterogeneous group. Fifty percent of these patients have excellent prognosis whereas the prognosis in the remaining half is similar to that of patients with stage III disease²⁵. It is likely that these patients have microscopically undetectable tumor cells (micrometastases) in their lymph nodes that are missed during routine histology. These could be identified by analysis of micrometastatic disease in their lymph nodes.

As the sentinel lymph node (SLN) has the highest chance of harbouring metastatic carcinoma, lymphatic mapping and sentinel node biopsy (SLNB) can potentially improve staging in colorectal cancer and provide important prognostic information. This would have a direct impact on clinical management of these patients. By pre-selecting the most likely node(s) to contain metastasis a pathologist can perform a more detailed examination of the lymph node including serial sectioning, immunohistochemistry and reverse transcriptase polymerase chain reaction (RT-PCR).

Unlike in patients with malignant melanoma and breast carcinoma, SLNB in colorectal cancer does not affect the extent of lymphadenectomy. Curative resection for colorectal cancer should include removal of the lymphatic drainage of the tumour-bearing segment of bowel. The exact extent of the lymphadenectomy required for colorectal cancer, however, remains a matter of debate^{26,27}.

As regards histopathological examination of the resected specimen, there are no exclusive criteria for the minimum number of lymph nodes to be examined per specimen²⁸. Since the chance of finding tumour-positive nodes rises as the total number of lymph nodes examined increases, the trend has been to harvest as many lymph nodes as possible from the specimen²⁹.

6.3.2 Aims

The aims of this study were:

1. To determine the feasibility of blue dye lymphatic mapping in colorectal cancer.
2. To compare histological status of SLN with the remaining regional (non-sentinel) nodes, to determine its accuracy.

6.3.3 Patients and Methods

Patients with primary adenocarcinoma of colon and rectum were eligible to take part in this study. In patients with colon carcinoma, intraoperative lymphatic mapping was performed by sub-serosal injection of 2 ml of patent blue dye around the tumour at 2-4 sites (fig 6.12). Care was taken to avoid spillage of the dye during injection, as this could lead to blue discolouration of surrounding tissue and cause difficulty in locating the SLN.

Mobilization of colon was continued after injection and usually within five minutes, blue node(s) could be identified. The first node that took up the blue dye was marked with a stitch. It is important to do this, immediately after the first blue node is visualized, since the dye moves progressively from one node to another leading to blue discolouration of most of the mesenteric lymph nodes as the procedure continues³⁰. After resection of the primary tumour and segmental lymph nodes, the blue node which was marked separately was sent to the laboratory for histological examination. This included standard H & E staining as well as immunohistochemistry of the blue node using a pancytokeratin marker (MNF116).

We performed blue dye lymphatic mapping in 22 patients with colorectal carcinoma. The median age was 65 (range 43-88 years). There were eighteen colon and four rectal carcinomas. The location of primary tumour and tumour grade is summarized in table-6.1.

In rectal carcinoma, we found it difficult to inject the blue dye sub-serosally, due to limited access and high risk of spillage of the dye during injection. After the first case, we decided to inject the patent blue dye submucosally around the tumour using a proctoscope whilst the patient was under general anaesthesia

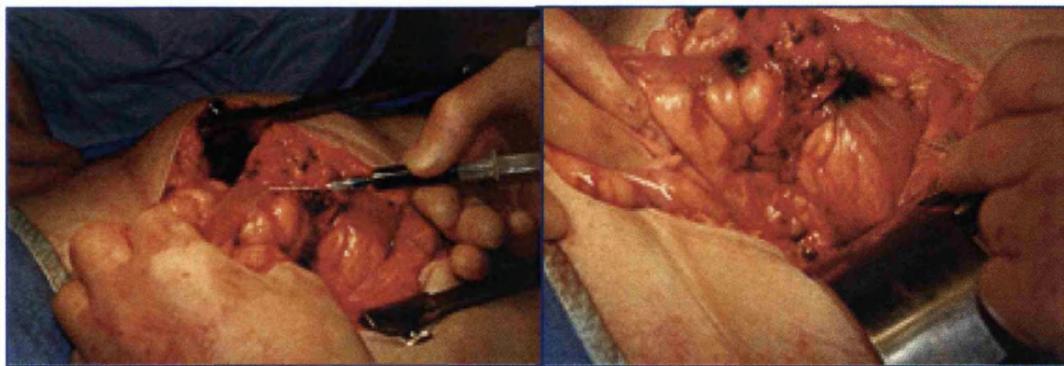


Fig 6.12 sub-serosal injection of 2 ml of patent blue dye



Fig 6.13 Submucosal injection of the patent blue dye using the proctoscope in rectal cancer

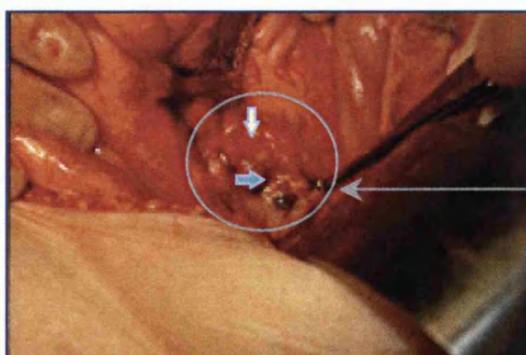
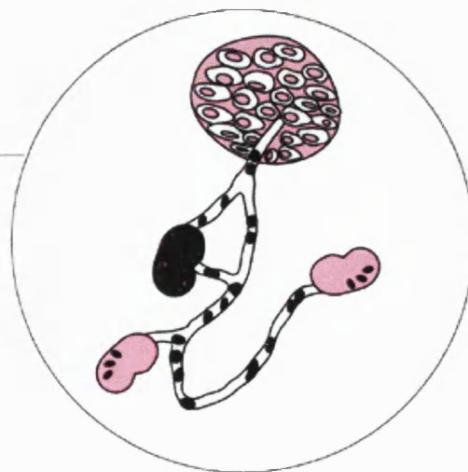


Fig 6.14 True sentinel node is replaced by metastatic carcinoma. This can lead to a false-negative SLNB result



Tumour site	
Right colon	7
Transverse colon	1
left colon	3
Sigmoid	7
Rectum	4
Tumour differentiation	
Well	2
Moderate	18
Poor	2

Table 6.1

The only drawback of this technique is rapid progression of the dye, leading to blue discolouration of most of the mesorectal lymph nodes by the time the specimen is retrieved³¹. After anterior resection was complete, careful ex-vivo examination of the mesorectum was performed to identify the closest blue node to the primary tumour which was then sent for histological examination separately.

6.3.4 Results

Total of 30 SLN (1.36 per patient) and 239 non-SLN (10.8 per patient) were harvested. Lymphatic mapping was successful in 21 patients (95.4%). There were eight patients with metastatic nodal disease on definitive histology (36.3%). In one of these node positive patients lymphatic mapping failed. In the remaining seven patients, the diagnosis of nodal metastases was made on H & E histology in six patients (85.7%) and on immunohistochemistry in one

(14.2%). The SLN method thus correctly diagnosed the true histological status of the mesenteric lymph nodes in only two patients and failed to determine the nodal status in the remaining five patients. This gives a false negative rate of 71.4%, with a sensitivity of 58.3% and 100% specificity (as there were no false positive cases). The positive predictive accuracy is 100% with a negative predictive accuracy of 73.6%, in this limited series.

In three patients with false negative results gross involvement of the mesenteric lymph nodes at the time of surgery was evident. None of the pathological nodes took up the blue dye and a small distal node was stained with the blue dye (fig 6.14).

In two patients the SLN was the only lymph node involved(28.5%). One of these was diagnosed by immunohistochemical staining after a negative H & E and the other positive node revealed a small focus of metastases at its periphery, in the subcapsular sinus.

6.3.5 Discussion

The sentinel node concept has proven reliable in diagnosing occult metastasis in melanoma and breast cancer. The concept is also gaining increasing acceptance in penile, vulvar and head and neck malignancies. The success rate of identifying the sentinel node in our limited series was 95.4%. We failed to localise the blue node in one patient with low rectal carcinoma, after finding it difficult to inject the dye into the subserosal plane. After this failure we changed our practice and performed the injection submucosally as mentioned earlier. Localisation of the blue node was successful in the remaining 3 patients with rectal carcinoma.

We observed a high false negative rate in our series. In five out of seven patients with positive lymph nodes, the SLN failed to diagnose the true nodal status. These results are in sharp contrast with the low false-negative rates described in other sentinel node studies, with values ranging from 0 to a maximum of 12.5 per cent³².

A number of reasons may explain this high rate of tumour-negative blue nodes in node-positive patients. In three out of five false positive cases, there were pathologically enlarged lymph nodes that seemed to be completely involved with metastatic tumour. A likely explanation for this skip phenomenon in these patients is that the blue dye bypassed the first draining node as this was replaced and blocked by the metastatic tumour. Blockage of afferent lymphatic channel leads to directional change in the lymph flow, bypassing the true sentinel node (fig 6.14). This is one of the potential pitfalls in SLNB in breast carcinoma and malignant melanoma; therefore patients with palpable nodes suspicious of clinical involvement are excluded from SLNB. In colorectal carcinoma, it is not possible to accurately diagnose lymph node enlargement pre-operatively and even during surgery this is difficult.

The other possible explanation for skip metastases is that injection of the blue dye around the tumour may not be representative of the actual tumour lymphatic drainage pattern. Intra-tumoural injection of the blue dye does not lead to blue staining of the lymph nodes⁶¹. It is also possible that the route of lymph flow could have been altered with the tumour growth into the bowel wall. It is conceivable that several alternative lymphatics are involved in draining the tumour and that these routes alter during tumour progression, especially for large growths.

Lymphatic mapping using a blue dye only, is technically demanding and is associated with a longer learning period. As the number of patients in this series are small and there is a definite learning period associated with the procedure and taking in to consideration the fact that four surgeons were involved in performing the procedure, it is possible that the high false negative rate in this series could also be due to the lack of sufficient experience of the surgeons involved. Addition of radio-colloid and probe guided surgery in SLNB for colorectal cancer, may reduce the learning period and also increase the sensitivity of the method in this context.

Immunohistochemical staining of the SLN in one patient revealed micrometastases. Routine H & E staining failed to identify this. The prognostic relevance of detection of micrometastasis in colorectal cancer is unclear. There are studies that confirm the occurrence of micrometastases but fail to demonstrate its effect on survival³³. A study performed by Cutait et al³⁴, evaluated the prognostic significance of micrometastasis in stage II colorectal cancer. These authors re-evaluated 603 lymph nodes from 46 lesions which were reported negative on H & E histology. The study was based on the detection of carcinoembryonic antigen (CEA) and cytokeratins in neoplastic epithelial cells. Micrometastasis were detected in 22 nodes from 12 patients. However 5 year survival failed to show statistically significant difference between this group and the one without micrometastasis.

However, Liefers et al²⁵ looked at the correlation of micro metastasis and survival in the node negative colorectal cancer. The authors used a carcinoembryonic antigen-specific nested RT-PCR amplification of

carcinoembryonic antigen mRNA in lymph node from stage II colorectal cancer. They were able to show a significant survival difference between the two groups after analyzing 192 lymph nodes from 26 consecutive patients with stage II colorectal cancer. Five-year follow-up information was obtained in all patients. Observed and adjusted survival rates were assessed in patients with and without micrometastases. Micrometastases were present in one or more lymph nodes in 14 of 26 patients (54%). The adjusted five-year survival was 50% in this group as compared to 91% in 12 patients who did not have any evidence of micrometastasis. The groups were similar with respect to age, sex, tumor location, degree of tumor differentiation and size of the primary tumour. They conclude that molecular detection of micrometastases is a prognostic tool in stage II colorectal cancer.

There are conflicting reports on the success and accuracy of SLNB in colorectal cancer. Joosten et al²⁹, performed SLNB in 50 patients with colorectal cancer using patent blue lymphatic mapping. They reported a success rate of 70% in localizing the blue lymph node. Twenty patients showed evidence of nodal involvement on definitive histology (20%). In twelve patients, blue lymph nodes failed to detect metastases leading to a false negative rate of 60%. It was concluded that, blue stained nodes do not accurately predict the histological status of the remaining nodes in the lymphatic basin, giving rise to an unacceptably high false negative rate. The authors questioned the validity of the SLN concept in colorectal carcinoma. Our limited data seems to support Joosten et al findings.

On the other hand Saha et al³⁵ were clearly more optimistic. Lymphatic mapping was performed in 76 consecutive patients with colorectal carcinoma by injecting 1 ml of lumphazurin, subserosally around the tumour. After retrieval of the blue node, routine H & E histology and immunohistochemistry was performed. They report a success rate of 98.7% in identifying the blue node and diagnostic accuracy of 96%. The reported incidence of skip metastases was 2.6%. In 13 patients (17%) micrometastasis were only found on immunohistochemistry of the SLN with silent H & E staining. The authors concluded that SLNB in colorectal carcinoma is highly successful and cost-effective. The added advantage of detection of micrometastasis had increased the accuracy of staging in 17% of patients who may benefit from adjuvant chemotherapy.

6.3.5 Conclusion

Lymphatic mapping in colorectal cancer, with the blue dye is feasible. It is associated with a high false negative rate and with poor sensitivity. Further studies are required to validate these findings but the addition of the radionuclide technique and probe guided surgery may change our viewpoint.

6.4 The Sentinel Node in Anal Carcinoma

6.4.1 Introduction

Anal carcinoma is a rare cancer, comprising 1-2% of all large bowel cancers³⁶.

The peak incidence is during the sixth decade, but recent epidemiological data suggest an increase in males under 45 years old³⁷. There is an association between benign anorectal conditions and anal carcinoma.

Surgical excision by abdominoperineal resection (APR) has been the standard treatment. In the 1920's and 1930's inguinal lymph node dissection was included in the surgical management of these patients^{38,39}. In the 1950's it was evident that the morbidity associated with lymph node dissection was much greater than any survival benefit and this procedure was abandoned⁴⁰. Since 1974 'multimodality treatment' with a combination of radiation and chemotherapy has become the standard⁴¹. This approach has the obvious advantage of sphincter preservation and a substantial survival benefit has been reported compared to surgery alone.

Historically, overall 5-year survival rates following abdominoperineal resection (APR) was 38-71%, with an associated postoperative mortality rate of 3-6%⁴². With multimodality therapy an overall 5-year survival of 65-90% have been reported with a treatment-related mortality of 0-4%.

The status of the inguinal lymph node is an important prognostic indicator and the presence of lymph node metastases is an independent prognostic factor for local failure and overall mortality⁴³. Depending on primary tumor size and

histologic differentiation, metastases to the superficial inguinal lymph nodes occur in 15-25% of cases⁴⁰.

Since initial treatment of anal canal cancer is predominantly medical and does not lead to a surgical specimen for pathologic staging, it is difficult to assess the true lymph node status in these patients. This is further impaired by the unpredictable pattern of lymphatic drainage as a result of the extensive lymphatic connections between the inguinal and pelvic lymph node basins⁴⁴. Lesions in the perianal skin and distal anal canal metastasize predominantly to the superficial inguinal and femoral lymph nodes⁴⁵. Cancers from the dentate line and proximal anal canal will metastasize mostly along the inferior and middle hemorrhoidal vessels to the hypogastric, internal pudendal, and obturator nodes. The most proximal lesions can spread along the superior hemorrhoidal vessels to the inferior mesenteric lymph nodes.

Even with modern imaging techniques such as endo-anal ultrasound and magnetic resonance imaging, nodal involvement cannot be reliably identified. In a study performed by Wade et al, using a nodal "clearing" technique as part of surgical treatment of anal carcinoma, 44% of all lymph node metastases were less than 5 mm in diameter⁴⁴. Clearly lymph node size is an unreliable parameter and histological nodal status remains the gold standard.

The sentinel node concept can be applied to the management of patients with anal carcinoma. By injecting the radiopharmaceutical followed by imaging, the first draining lymph node(s) from the anal region can be identified. As this node has the highest possibility of harbouring metastatic carcinoma, the pathologist can perform detailed examination of the lymph node and should be able to detect a small volume of metastases. This may well have management

implications. We have performed SLNB in our patient with anal carcinoma.

6.4.2 Patient and Method

A 49 year old male with a long standing history of anal warts presented with a mass around the anal verge. His presenting symptoms were pain and occasional bleeding. On clinical examination, there was a polypoid mass on the anterior aspect of the anal verge and associated anal warts. There was no evidence of palpable inguinal lymph nodes. The patient underwent examination under general anaesthesia and biopsy of the lesion was performed. Histology confirmed the presence of a well differentiated squamous cell carcinoma. After obtaining informed consent from the patient, a day prior to SLNB the patient received 5 MBq of 99m Tc-nanocolloid in a volume of 0.2 ml at four sites around the tumour. We used the needle free injection system for the delivery of the radionuclide to minimise patient discomfort (fig 6.15). The patient underwent dynamic and static imaging (fig 6.16). Anterior and lateral views were obtained. On lymphoscintigraphy, there was evidence of bilateral drainage to both groins with focal accumulation of radioactivity. The hot spots were marked on the patient's skin using an indelible marking pen. This was followed by SLNB which was performed under general anaesthesia with a combination of probe guided surgery and blue dye lymphatic mapping (fig 6.17). As the probe was facing the injection site, an external collimator was used at all times during surgery to minimise interference from the site of injection.

A blue and hot lymph node was identified in each groin. These were biopsied and after harvesting the nodes were bivalved and touch imprint cytology slides were prepared and the nodes sent for histological examination.



Fig 6.15 Injection of the radiocolloid with the needle-free syringe

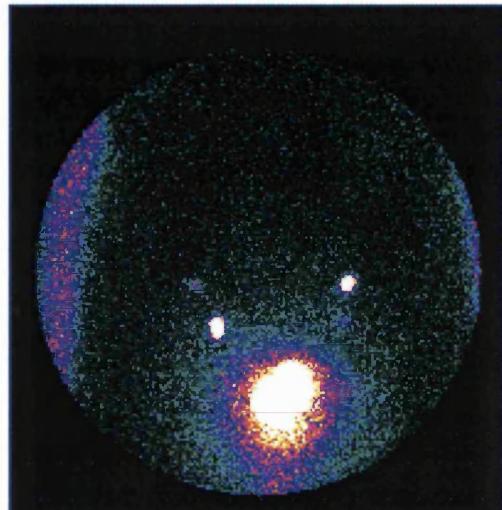


Fig 6.16 A Anterior static composite image

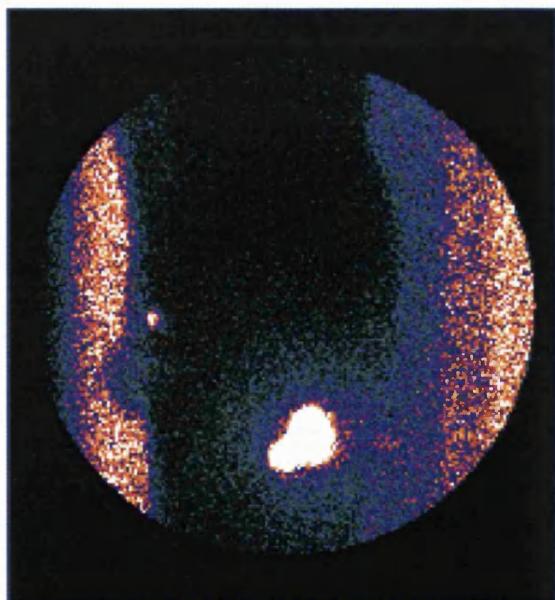


Fig 6.16B Lateral composite static image

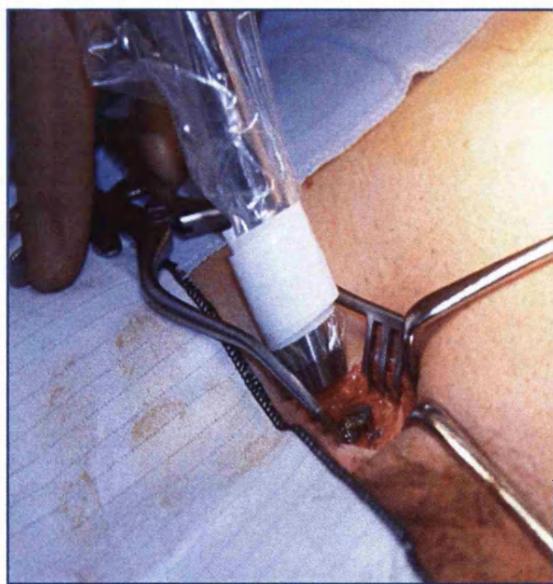


Fig 6.17 A blue and hot SLN was detected at operation

Imprint cytology did not show any malignant cells in either nodes. There was no evidence of metastatic deposit on both H & E staining and immunohistochemistry using a pancytokeratin marker (MNF116). The patient made an uneventful post-operative recovery and was scheduled to receive multimodality treatment without radiotherapy to the groin region.

6.4.3 Discussion

To our knowledge, this is the first time that SLNB has been performed in anal carcinoma. As there is a strong correlation between prognosis and nodal involvement, this technique can potentially improve the detection of nodal status which would improve staging and thus have potential management implications.

Control of synchronous inguinal lymph node metastases can be achieved in 90% of patients with chemoradiation^{46,47} versus 65% with radiation alone⁴⁸ and 15% with surgery alone⁴⁰. Five-year survival ranges between 0% and 20% with surgical treatment alone⁴⁸, compared to 43% after lymph node dissection combined with radiation⁴⁹. Retrospective studies comparing surgery, radiation alone and combined radiation and chemotherapy for control of inguinal lymph node metastases demonstrate an advantage for the nonsurgical treatment. By performing SLNB, treatment can be planned for individual patients according to their nodal status.

The incidence of perianal carcinoma has been increasing in young HIV positive males. Administration of the radiopharmaceutical with the needle free syringe has two advantages in this condition. It minimizes the discomfort experienced by the patient which makes the procedure more acceptable and it also protects the operator against needle stick injuries in high risk patients.

6.4.4 Conclusion

In conclusion, the SLNB is feasible to perform in patients with anal squamous cell carcinoma. This minimally invasive procedure can potentially improve staging of these patients. The accuracy of this technique needs to be tested in a large multicenter trial.

6.5 The Sentinel Node in Oral Squamous Cell Carcinoma

6.5.1 Introduction

The accurate clinical assessment of the status of cervical lymph nodes in patients with oral squamous cell carcinoma (SCC) is fraught with difficulty. Clinical examination remains inaccurate and false negative results of up to 40% have been reported⁵⁰ in clinically node negative patients (N0). Imaging with ultrasound, computerised tomography (CT) and magnetic resonance (MRI) are helpful but the reported false negative rates are 32%, 34%, and 25% respectively. This is unacceptably high for their routine use⁵¹. Positron emission tomography (PET) has also been used as a staging investigation in this setting and the reported false negative rate is of the order of 22%⁵².

The presence of metastatic disease in the cervical lymph nodes reduces the five year survival rate by 50%, irrespective of T stage at presentation⁵³. The effect on survival depends on the level of nodal involvement, total number of nodes involved, and the presence or absence of extra capsular spread. It is essential to stage these patients accurately for adequate treatment. This may be difficult, particularly when trying to detect small tumour deposits in the clinically N0 neck.

Controversy remains about the best means to determine the nodal status of patients at presentation. Although only one third of such patients will have cervical lymph node metastases, the gold standard procedure for their accurate staging is formal block dissection of the cervical lymph nodes⁵⁴. By performing this procedure, two thirds of the patients are overtreated and subjected to significant morbidity, prolonged hospital stay and unnecessary cost of this major surgical procedure.

There is, therefore, a need to identify a minimally invasive technique to accurately diagnose the nodal status in these patients.

The sentinel node biopsy (SLNB) can potentially overcome this management dilemma. It has been accepted as an accurate staging procedure in the management of patients with malignant melanoma, where it is shown that patients without clinically palpable lymph nodes and a SLN clear of metastatic disease, are highly unlikely to have metastases elsewhere within the nodal basin. There is also growing evidence that this is an accurate staging procedure in patients with breast carcinoma. The clinical application of SLNB has been extended to other areas of surgical oncology including vulval⁵⁵ and penile carcinoma, dermatological malignancies⁵⁶ and bowel cancer³⁵.

We decided to evaluate the accuracy of the SLNB as a staging procedure in patients with clinically N0 SCC of the oral cavity. This work is done in collaboration between the Institute of Nuclear Medicine and the Department of Maxillo-facial Surgery at the UCL.

6.5.2 Hypothesis

Sentinel lymph node detection and biopsy will reliably identify those N0 patients with oral SCC who have progressed to metastatic disease in the neck.

6.5.3 Outcome Measure

Histological validation of the accuracy of lymphoscintigraphy and sentinel lymph node biopsy in predicting the presence or absence of malignant disease in the cervical lymph nodes.

6.5.4 Inclusion Criteria

All patients with biopsy proven squamous cell carcinoma, with clinically N0 necks who are scheduled to undergo surgical resection of their primary disease in conjunction with some form of cervical lymph node dissection.

6.5.5 Exclusion Criteria

Patients with previous surgery or radiotherapy to the neck

Patients with multifocal carcinomas of the oral cavity

Patients with palpable lymph nodes

Pregnant and lactating women

6.5.6 Patients and Methods

Following ethical approval from the local Ethics Committee, patients with histologically proven T1-T4, oral SCC, but with clinically N0 necks, who were scheduled to undergo surgical resection of their primary disease and block dissection of the cervical lymph nodes were enrolled for this study.

Patients underwent lymphoscintigraphy approximately 18 hours prior to surgery.

They received a total of 40 MBq of ^{99m}Tc -colloidal albumin (Nanocolloid) at four

sites around the primary tumour in a volume of 0.2 ml per site in divided doses. The injection was administered in the submucosal plane at 12, 3, 6 and 9 o'clock position respectively. Care was taken to avoid inadvertent spillage of radiocolloid into the mouth during injection. Patients were asked to rinse their mouth with a mouthwash after each injection, to prevent artifact as a result of the swallowing the radioactive tracer (fig 6.18).

Dynamic imaging was started within 2 minutes of the injection for the duration of 45 minutes, (90 X 10-sec frames and 30 X 60 sec frames) in the antero-posterior (AP) projection, with the patient lying supine. This was followed by static imaging in both AP and lateral projections (right and left lateral in patients with bilateral drainage). A ^{57}Co flood source was used to project the patients outlines. A gamma camera fitted with a low-energy, general purpose (LEGP) collimator was used. By using the electronic marking facilities, fixed anatomical landmarks were marked. These include the suprasternal notch, angles of the jaw and the chin.

The location of the sentinel node(s) was marked on the patients' skin. The position of a ^{57}Co solid source pen was observed on the cameras' persistence display, and the pen moved until its position overlaid that of a radioactive node. This position was then marked using an indelible pen.

In one patient with anterior SCC of the tongue, who had positive serology for hepatitis C, we administered the radiopharmaceutical with the needle free injection system at four sites around the primary tumour (fig 6.19).

At operation, 1-2 ml of Patent Blue V dye (Laboratoire Guerbet, Aulnay-Sous-

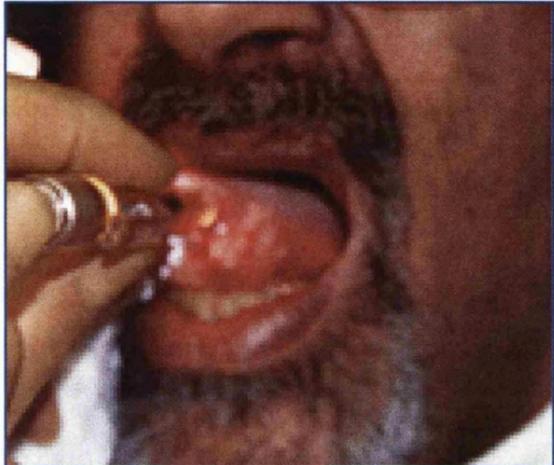


Fig 6.18 Injection of the radiocolloid in a patient with a tongue SCC

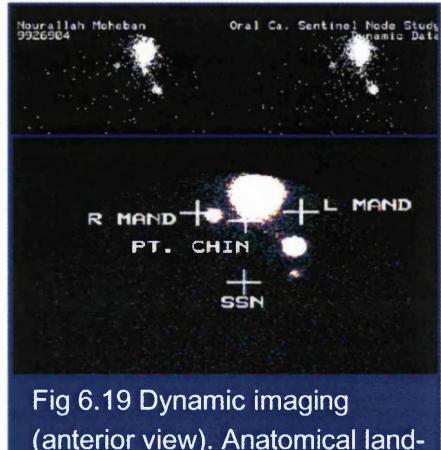


Fig 6.19 Dynamic imaging (anterior view). Anatomical landmarks are marked using the electronic marking facility

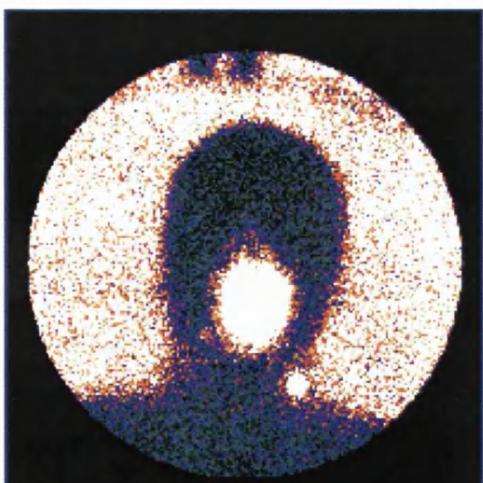


Fig 6.20A Anterior composite static image

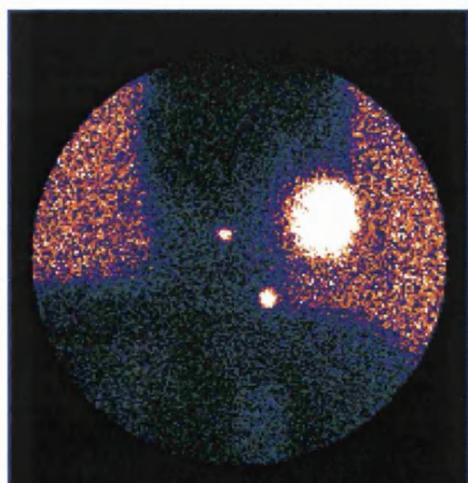


Fig 6.20B Lateral composite static image

Bois, France) was injected at the corresponding sites of radionuclide injection. The skin flaps were raised and by careful dissection, the deep cervical chain of lymph nodes were exposed. The Neoprobe-1500 hand-held gamma probe (Neoprobe Corp., Dublin, OH), fitted with a 12-mm diameter straight collimated probe, was used to identify radioactive sentinel nodes. Due to the proximity of the SLN to the injection site the probe was always used along with the external collimator to reduce interference from the injection site.

After identification of the SLN, with a combination of probe guided surgery and blue dye lymphatic mapping, it was excised and ex-vivo radiation count was recorded. The probe was then re-inserted in to the wound for measurement of the 'residual radioactivity' to ensure that no radioactive node was left behind. The remainder of the neck dissection then continued as normal.

After harvesting, the SLN was bisected and imprint cytology slides were prepared. The SLN was then sent to the histopathology department. The block dissection specimen was also sent to the laboratory separately. The histological examination included routine H & E staining as well as immunohistochemistry using pancytokeratin marker (MNF 116). The histological status of the SLN was compared to that of remaining cervical lymph nodes to determine the overall accuracy of SLNB in oral SCC patients.

6.5.7 Results

We performed SLNB on seven patients with confirmed diagnosis of oral SCC. Ten neck dissections were performed on these seven patients, three patients had their tumours crossing or close to the midline which showed bilateral

drainage on lymphoscintigraphy. These patients underwent bilateral SLNB and block dissection of the neck nodes.

The SLNB was successful in all patients (100% success rate). Eighteen SLN were biopsied in seven patients (2.5 SLN per patient). Sixteen nodes were hot and blue (88.8%) and two nodes were only hot but not blue (11.1%). In two patients, lymphoscintigraphy revealed drainage to a level III lymph node.

Lymphoscintigraphy and SLNB was successful in the patient who received the radionuclide injection with the needle free syringe.

In three patients there were evidence of cervical lymph node metastases on histological examination. The SLN correctly diagnosed the nodal status in two patients. In one patient the SLN was reported as negative both on H & E staining and immunohistochemistry but examination of the rest of the block dissection nodes revealed one lymph node in proximity to the SLN at level I, with metastases giving rise to one false negative rate (33.3%). The SLN correctly predicted the nodal status of the remaining four patients without nodal metastases.

6.5.8 Discussion

This ongoing study was performed to determine the feasibility and accuracy of the SLNB in oral SCC. Our early results suggest that the SLNB is feasible when using a combination of preoperative lymphoscintigraphy and intraoperative identification of radioactive nodes with the gamma detection probe and blue dye lymphatic mapping.

There were three patients who had evidence of nodal metastases on definitive histology and the SLN correctly predicted the nodal status in two of these patients. In both patients the SLN was the only involved node. There has been

one false negative case in this small series. This was in a 70 year old Indian male with a carcinoma of buccal mucosa who gave a history of tobacco chewing for several years. There was significant mucosal fibrosis with limited mouth opening. Lymphoscintigraphy revealed drainage to the ipsilateral submental group of lymph nodes at level one. During surgery a weakly radioactive and blue node was harvested which was uninvolved but there was another node at the same level which revealed metastasis.

Spillage of blue dye can also cause difficulty in identifying the SLN and to safeguard against this problem, use of a syringe with a luer lock is recommended for the blue dye administration.

The SLNB procedure for head and neck carcinoma is a technically demanding procedure. This is partly due to the proximity of the injection site to the SLN which may cause some difficulty in its intraoperative identification and partly due to the unpredictable nature of the lymphatic drainage of head and neck lesions. Our false negative case was one of the earlier cases in this series and lack of sufficient experience in performing the procedure could be the likely explanation for this failure.

In one patient with anterior SCC of the tongue and positive hepatitis C serology, we successfully administered the radiopharmaceutical with the needle free injection system. This led to identification of the SLN without any difficulty. This is the first time that the J-tip needle free syringe was used for radionuclide administration in a patient with oral carcinoma. The advantages of this system include pain free delivery of the radiotracer as well as protection against needle

stick injury in this high risk patient.

In two patients with SCC of the tongue, lymphoscintigraphy revealed direct drainage to level III group of lymph nodes. This highlights the importance of careful imaging preoperatively as the head and neck lymphatic drainage is highly unpredictable.

The SLNs were hot and blue in over 88% of cases and in the remaining patients gamma detection probe was successful in identifying the hot node. The combined use of probe guided surgery and the blue dye technique improves the success of the procedure^{57,58}. A study performed by Shoaib et al⁵⁷ highlights this point. In this study all patients undergoing prophylactic or therapeutic neck dissections were divided into two groups. The first group underwent SLNB using lymphatic mapping with the blue dye only and the second group underwent preoperative lymphoscintigraphy and SLNB with combination of blue dye and probe guided surgery. During surgery, blue stained lymphatics were followed to blue nodes, and a gamma detection probe was used to identify radioactive nodes. In the first group, 5 out of 13 patients (38.4%) with blue lymph nodes were identified and none of these contained tumor. Metastases were identified in other neck nodes in 3 of 5 (60%).

On the contrary, the identification of the SLN was successful in 15 of 16 (93.7%) patients receiving combination of the blue dye, and radiocolloid. The SLN was accurate in diagnosing metastatic involvement in all seven patients (100%) who had nodal metastases. The authors conclude that sentinel node biopsy using a combination of the blue dye and radiocolloid improves the

success rate and sensitivity of the procedure in the N0 neck.

6.5.9 Conclusion

We conclude that it is feasible to perform SLNB in patients with oral squamous cell carcinoma. The accuracy of this technique needs to be established before it is introduced as a routine use as a staging investigation.

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Chapter-7

RADIATION SAFETY ASPECTS IN SENTINEL NODE BIOPSY

7.1 Introduction

With increasing acceptance of sentinel node as a staging procedure in patients with breast cancer and malignant melanoma, there is a potential for significant increase in the use of this technology. As the administration of a radiopharmaceutical is key to the success of this procedure; this brings with it the need to address best practice and radiation safety .

Sentinel lymph node biopsy (SLNB) differs somewhat from other nuclear medicine investigations in that, it involves an even larger multidisciplinary team. The technique requires the use of radioactive materials in the operating theatre with active samples taken to the pathology laboratory, and the generation of radioactive waste as a result. Although nuclear medicine staff and physicists are fully aware of radiation protection issues, this may not be true in respect to other clinical staff involved in the care of these patients.

The radiation dose to the patient should also be determined to provide appropriate information to the patient whilst obtaining informed consent. It would also be helpful to offer reassurance based on medical evidence.

7.2 Aims

The aims of this study were:

1. To determine the radiation dose to patients and staff groups.
2. To measure the radioactive clinical waste generated as a result of SLNB.
3. To propose guidelines for clinical and best practice, based on these data.

7.3 Patients and Methods

The day preceding surgery, a single dose of 10-15 MBq of Tc-99m labeled Albures or Nanocolloid (both Nycomed Amersham) was injected intradermally at the tumour site, using a 25 G needle and a volume of 0.2 ml. The Albures preparation we have used was filtered to a particle size of less than 400 nm, whilst Nanocolloid has an average particle size of less than 80 nm. This was followed by dynamic, early and late static imaging.

7.3.1 Patient Dosimetry

We analysed dynamic imaging data by the region of interest method and time-activity curves were recorded to determine the extent of the clearance of tracer from the injection site. Critical analysis of images for any evidence of tracer uptake in the liver, spleen or bone marrow was performed.

Blood samples were also obtained from the patients (a total of 18 samples from 14 patients) at 1-48 hours post injection (p.i.). These were primarily withdrawn between 1 - 2 hours and 24 - 48 hours p.i. From each sample, 2 ml whole blood was extracted and assayed in a gamma well counter which had been previously calibrated (fig. 7.1). At surgery, 18 - 24 hours p.i., after harvesting the SLN, it was carefully weighed and its activity content was measured in the 'well counter'.

A total of 30 nodes were taken from 22 patients, and these comprised both sentinel and second echelon nodes.

7.3.2 Surgical Staff Dosimetry

The radiation dose to surgical staff was determined by issuing personal radiation dosimeters to the two surgeons performing the surgical procedure. One surgeon conducted the sentinel node biopsy and another the excision of the primary tumour and axillary lymph node dissection. A 'bleeper' type Geiger-Muller whole body dosimeter (Gothic Crellon Ltd., Wokingham, Berks UK) was worn by each surgeon in the chest pocket underneath their sterile gown (fig 7.2).

Additionally, extremity dosimeters were worn by each surgeon on the index finger of their dominant hand, placed underneath their sterile surgical glove (fig 7.3). These consist of a small (10 mm) lithium fluoride (LiF) disc which exhibits thermoluminescence and make it possible to measure the radiation dose retrospectively. Both form of dosimeters were worn by each of the two surgeons for 19 surgical procedures.

The reading from Geiger-Muller dosimeter was recorded at the end of the operation and the thermoluminescent dosimeter (TLD) was sent to determine its radiation exposure.



Fig 7.1 Analysis of blood in a gamma well counter



Fig 7.2 Geiger-Muller whole body dosimeter (Gothic Crellon Ltd., Wokingham, Berks UK) worn by the surgeon



Fig 7.3 Extremity (TLD) dosimeters were worn by the surgeon on the index finger of the dominant hand

7.3.3 Radioactive Clinical Waste

The radioactive waste generated within a nuclear medicine department will be subject to a program of radiation monitoring with appropriate storage until physical decay of its constituent radionuclide make it safe for disposal.

Radioactive waste generated in the operating theatre was examined by monitoring the waste material for the presence of radioactivity using a suitable calibrated scintillation contamination monitor. This demonstrated an uptake of measurable levels of radioactivity confined to the surgical swabs used during surgery (fig. 7.4).

All swabs used during surgery were transferred to the nuclear medicine department and further analysed by quantitative measurement of their radioactive content. This was accomplished by placing the waste swabs directly onto the collimator face of the camera together with a reference counting tube containing a known activity of ^{99m}Tc (fig 7.5). An Image was acquired for 30 minutes, outside working hours. Swab outlines were marked with a $^{57}\text{Cobalt}$ marker (fig. 7.6). Figure 7.7 illustrates the swab imaging after mastectomy.

Region of interest analysis of this image data for both the swabs and counting tube yielded a decay-corrected activity estimate for the swabs.

We obtained waste swabs data for a total of 16 surgical procedures. Eleven samples were obtained from a wide local excision procedure and 5 samples originated from patients undergoing mastectomy when the entire tissue of the breast is removed, incorporating the injection site *en-bloc*.

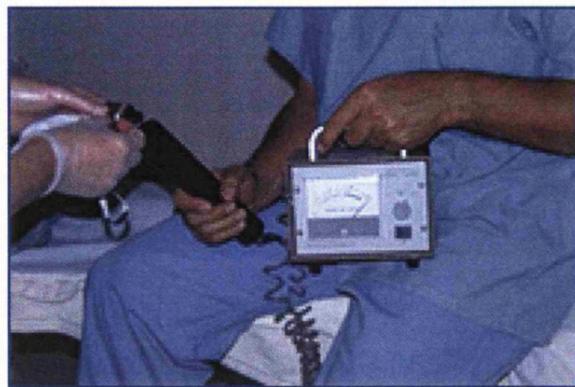


Fig 7.4 Checking operative swabs with scintillation contamination monitor



Fig 7.5 Imaging of surgical swab for quantitative analysis

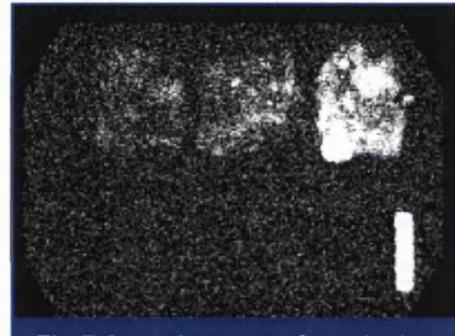


Fig 7.6 static mage of surgical swab after wide local excision of breast carcinoma



Fig 7.7A Imaging of surgical swab after mastectomy

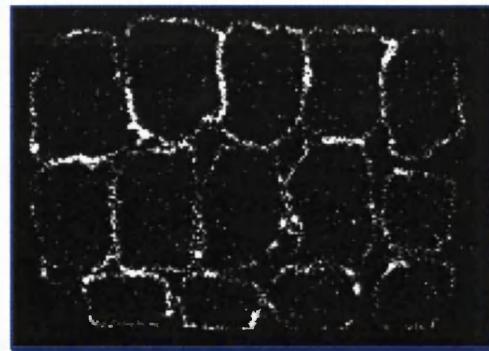


Fig 7.7B Static image of surgical swabs after mastectomy

7.4 Results

7.4.1 Analysis of Imaging Data

Early imaging data analysis, clearly demonstrated that at least 95%, and frequently close to 99%, of the administered radioactivity is initially retained as a localised source at the injection site. On analysing delayed imaging data, it was also evident that a very high proportion of this tracer activity remained at the injection site for at least 24 hours p.i.

There was no evidence of uptake of radiopharmaceutical in the liver, spleen and bone marrow on scrutiny of the image data obtained for any of the patients studied. This finding was observed when both Nanocolloid and Albures were used as the radiolabelled tracer.

7.4.2 Peripheral Blood Assay

Data obtained from the direct assay of 18 blood samples in a gamma well counter revealed that (decay-corrected) mean uptake of colloidal-tracer was determined as 0.73 ± 0.37 % of injected dose (i.d.) per total blood volume (actual range 0.07-2.46%).

(fig 7.8)

7.4.3 Radiation Activity of the SLN

Gamma well counting of the SLN indicates that between 0.0038 % and 5.14 % injected dose was present within the sentinel nodes examined at the time of excision (referenced to the time of injection), with a mean uptake of 0.96 ± 1.33 % injected dose. Figure 8 details the results for all 30 nodes examined. The weight of the nodes ranged between 0.29 - 1.98 g, (mean 0.801 ± 0.812 g). (fig 7.9).

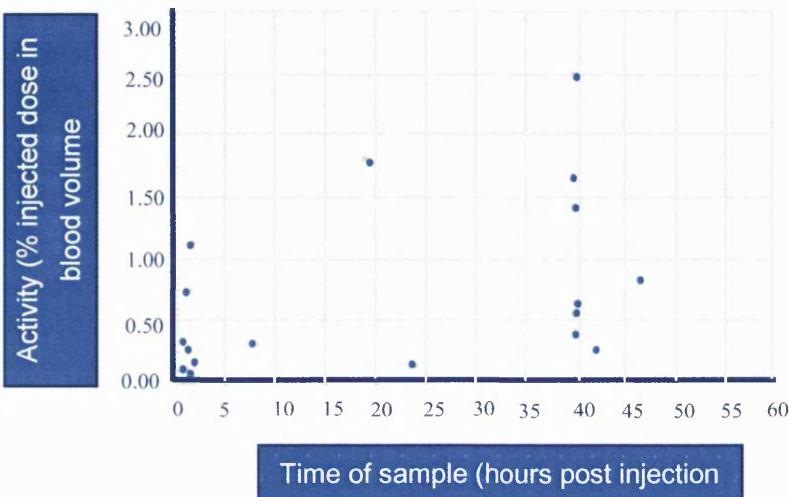


Fig 7.8 Tracer activity present in whole blood sample expressed as % injected dose in whole blood volume

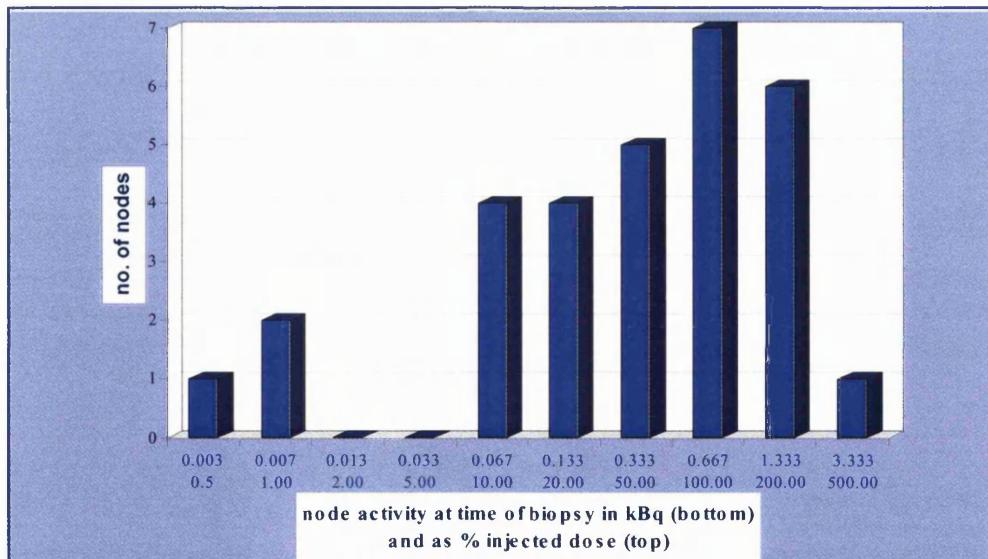


Fig 7.9 Uptake of radiotracer in the SLN

7.4.4 Radiation Dose to Surgical Staff

The whole body Geiger-Muller dosimeters used, display the integrated dose to which they are exposed in μSv (1×10^{-6} Sievert). With the exception of the one patient who underwent surgery four hours after injection of the tracer (injected dose 15 MBq) and attended by one surgeon only, the whole body dose recorded by each of the two surgeons was in all cases less than 2 μSv per procedure, and in 20/38 cases less than 1 μSv per procedure. The radiation dose resulting to the one surgeon who conducted the operation scheduled at 4 hours p.i. was 4 μSv . The mean value of the doses recorded by the surgeon performing the sentinel node biopsy was $0.21 (\pm 0.37) \mu\text{Sv}$, and $0.47 (\pm 0.96) \mu\text{Sv}$ for the surgeon performing the tumour excision (by mastectomy or wide local excision) and axillary node dissection. The mean value of all readings was $0.34 (\pm 0.73) \mu\text{Sv}$.

The mean recorded finger dose with TLD dosimeter for the surgeon performing the sentinel node biopsy was $0.06 (\pm 0.04) \text{ mSv}$, and $0.12 (\pm 0.23) \text{ mSv}$ for the surgeon performing the tumour excision with a mean dose for all readings of 0.09 mSv .

7.4.5 Radioactive Clinical Waste

Waste swabs from 16 surgical procedures were measured for their radioactive content, and the result referenced to the % injected dose at the time of injection (for 15 MBq administered activity). The mean activity content for all 16 samples was $4.89 (\pm 6.14) \%$ of injected dose, but there was a significant difference between the activity content of those swabs used during wide local excision and

mastectomy procedures (see fig. 7.6 & 7.7). The mean activity content for the 11 swab samples used during a wide local excision was 7.10(\pm 6.39) % injected dose, compared with 0.03 (\pm 0.08) % injected dose after mastectomy ($P = 0.004$). The most active sample from a wide local excision contained 21.89 % injected dose with six other samples containing between 5 and 10 % i.d. This maximum recorded figure corresponds to an activity content of 205 kBq at 24 hours p.i.

7.5 Discussion

It is clear from the biodistribution data that after intradermal injection of the radiotracer, there is no obvious migration of the colloidal beyond the sentinel node. The SLN on average receives a mean uptake of only 0.96 % of the injected dose. It is also important to note that there was no visual evidence of any uptake via the reticuloendothelial system into the liver, spleen and bone marrow.

The concentration of tracer in circulating blood samples is very low, with a maximum of 2.46 % of i.d. found in the total blood volume at 40 hours p.i. Direct measurement of tracer uptake into the sentinel node itself is also low, ranging from an extremely low figure of 0.0038 % to just over 5 % for one sample only, with a mean uptake of just under 1 % injected dose. On the whole these data and findings confirm that the tracer is almost entirely retained within interstitial tissue spaces at the injection site which is excised the following day. It is also evident that only a small fraction of the tracer migrates to the sentinel lymph node, reaching further only in a small proportion of cases to second echelon nodes.

These findings were observed when both Nanocolloid and Albures were used as the colloidal tracer. It has been stated for Nanocolloid that 95 % of the colloidal particles have a diameter of less than 80 nm¹, and for Albures that 90 % of particles lie in the range between 200 - 1000 nm diameter^{1,2} with a mean diameter of 500 nm³ and, although spill over of tracer into second echelon nodes has been noted for Nanocolloid¹ it is regarded as minimal. It would seem from the observations obtained here that the variation in particle size represented by their different size range does not lead to a noticeable difference in the overall biodistribution of the two tracers.

The estimated mean breast radiation dose of 10.8 mGy for a tracer administration of 15 MBq is relatively low when compared with the range of radiation doses resulting from typical nuclear medicine procedures⁹.

Low level radiation forms part of our natural environment. On earth, are all exposed to radiation from cosmic and natural background sources. It is probably more informative to compare the radiation dose resulting from a sentinel lymph node study more generally against the radiation dose resulting from a number of natural causes⁶.

It is of comparable magnitude to that experienced from cosmic rays during a long-haul airflight (London-New York return trip : 0.06 mSv), or due to living at high altitude (Denver, USA : 0.88 mSv/year), or in an area overlying granite bed-rock (two weeks residence in Cornwall, UK: 0.25 mSv).

Investigation	Effective Dose (mSV)
Sentinel node biopsy (breast)	0.32
Technetium-99-m bone scan	3.6
Technetium-99-m lung scan	1.0
Iodine-123 thyroid scan	4.4
Mamography (four films)	0.4
Chest X-ray	0.04
Abdominal CT	7.2
Chest CT	8.3
Brain CT	1.8
Intravenous urography (IVU)	4.6
Abdominal X-ray	1.5

Table 7.1 Effective Dose for a number of commonly performed nuclear medicine and radiographic procedures (from data published by the RCR⁴, Perkins⁵ and ARSAC⁶).

The annual dose limit for a member of the public as recommended by the International Commission on Radiological Protection (ICRP) is 1 mSv. The value for a designated radiation worker is 20 mSv. The dose limit of 1mSv per annum also applies to staff members who are not designated radiation worker (table-7.2).

Source of radiation exposure/relevant legislative limits	Radiation dose
	(mSv)
Sentinel node technique (Breast carcinoma)	0.32
Return flight: London-New York (cosmic radiation dose)	0.06
Two weeks residence in Cornwall, UK	0.25
One year's residence in Denver, USA	0.88
Average UK annual radiation dose to public (all causes)	2.6
Proposed annual UK dose limit to public (ICRP 60,1990)	1.0
Existing annual UK dose limit to the public (IRR, 1985)	5.0
Proposed dose limit to radiation worker (ICRP 60, 1990)	20
Existing annual dose limit for radiation worker (IRR 1995)	50

Table-7.2 Radiation dose of SLN technique compared with range of various sources and statutory dose limits (data obtained from the National Radiological Protection Board (NRPB))

The mean whole body dose to surgical staff performing SLNB procedure is 0.34 µSv, with the maximum recorded dose below 2 µSv. This represents a very small fraction of the dose limit to a member of the public and, based upon the mean whole body dose, up to 3000 procedures could be performed per year before even this dose limit is exceeded. Use of the maximum recorded dose would suggest a ceiling of 500 procedures per year. This is clearly unlikely to occur in all but the busiest surgical practices.

The mean finger dose of 0.09 mSv recorded here represents less than 1/5000th of the relevant dose limit - for a surgeon who has been designated as a radiation worker. Again, this is not deemed likely to occur in practice.

Considering that the pathologist spends less time than the surgeon in handling the tissues and also keeping in mind the short half life of ^{99m}Tc (6 hours), radiation risk to pathologist is minimal and some studies suggested that there should be no delay in pathology examination of the specimen⁷. Others advocate storing the radioactive specimen for 24-72 hours in formalin behind shielding before it is processed⁸. However there is no risk assessment study here backing such a procedure with evidence!

Immediate analysis of sentinel node in the form of frozen section histology or imprint cytology upon completion of surgery may cause some concern amongst the histopathology staff. Inferring from the data collected, we can estimate that total body doses are very low. An analysis of the primary specimen immediately upon completion of surgery scheduled at 4 hours p.i. leads to an estimated dose of a magnitude likely to impact upon occupational dose limits, at approximately $1/600^{\text{th}}$ of the annual dose limit to a member of the public, or $1/12000^{\text{th}}$ of the maximum dose limit to a radiation worker. Moreover, analysis at this point is usually only ever performed upon sentinel lymph node specimens, where the equivalent dose is approximately $1/12000^{\text{th}}$ of the dose limit to a member of the public. The radiation dose to the fingers is perhaps the more relevant risk to this staff group, but fifteen minutes close contact with the primary specimen at 4 and 24 hours p.i. is seen to lead to estimated doses representing approximately $1/1250^{\text{th}}$ and $1/12500^{\text{th}}$ of the dose limit to a radiation worker respectively. Moreover, the radiation dose to the lens of the eye resulting from prompt analysis of slides prepared immediately following surgery at 4 hours p.i. is estimated to be less than $1/300000^{\text{th}}$ of the specified dose limit to this organ for a radiation worker⁹. However it is important to obtain

direct evidence of radiation exposure for histopathology staff and it is our intention to monitor radiation dose to this staff group in a prospective manner.

As far as clinical waste is concerned, in the operating theatre, surgical swabs and gloves represent the only detectable source of radioactive waste arising from procedures for the management of breast cancer. Data obtained from measurement of their radioactive content indicate that significant levels of contamination are present in swabs specially after wide local excision procedure.

We noted that swabs which were stained with the blue dye during surgery had most contamination with radioactivity. It was also interesting to note a relatively low levels of activity observed from swabs used at a mastectomy procedure, where tissue comprising the injection site is left intact within the excised specimen. This is an important finding, as it highlights the mechanisms by which significantly contaminated waste may be generated at other surgical procedures.

It should also be noted that radioactively contaminated swabs, have the capability to affect intra-operative detector measurements if they are placed in close proximity to the probe while it is used to detect the relatively low level of uptake observed in sentinel nodes. Thus care should be taken to ensure that they are kept clear of the immediate vicinity of the probe while it is in use.

From a knowledge of the activity present in pathology specimens taken from the sentinel node patient it is clear that their radioactive content may be in excess of that mandated for disposal as non-radioactive waste, and thus specific arrangements for the storage of radioactive waste prior to disposal may be required if disposal of declared radioactive waste is not permitted. Legal

requirements for disposal of 'dustbin-level' radioactive waste differ between countries, but as an example United Kingdom legislation¹⁰ and its associated guidance specifies as 'very low level waste' (VLLW) placed in sealed bags or bins and with an activity concentration below 400 kBq/0.1m³ (and below 40 kBq per article) and as being exempt from the need for further processing, provided that it is consigned specifically to landfill¹¹. However, radioactive waste requiring incineration due to the nature of its contents (e.g. clinical waste) must meet a much lower maximum activity concentration of below 400 Bq/kg for it to be regarded as 'non-radioactive' and therefore suitable for disposal via this route¹². This latter category in particular is a stringent requirement, and necessitates that the primary specimen be stored for around three days from the time of surgery before its radioactivity concentration falls to meet this level. The routine storage of pathology specimens beyond this time period may however be existing practice.

7.6 Conclusions

1. The radiation risk to the patient is very low relative to that from many other medical exposures.
2. The radiation dose to staff groups involved in all aspects of the technique are also very low. Under normal circumstances and levels of workload routine radiation monitoring will not be required.
3. Radioactive waste is created in the operating theatre, and may be generated in the pathology laboratory. Waste material from surgery and pathology should be held for decay-in-storage before disposal as a biohazard waste.

Table-7.1 Summary of Recommendations for Good Practice

- Inject the tracer in the Nuclear Medicine Department if at all possible.
- Follow recommended injection techniques closely with respect to radiation protection advice.
- If possible obtain dose-rate readings in the operating theatre to verify occupational exposure levels for key staff, for an initial representative number of sentinel node procedures performed according to local protocols.
- Take account of the appropriate occupational radiation dose limits in the light of the currently prevailing sentinel node workload.
- Follow routine sterile precautions in the operating theatre whenever tracer-bearing tissue or clinical waste are handled.
- Exercise caution in the continued use of sterile swabs once these have been directly exposed to the injection site.
- Store clinical waste in a safe manner, suitably labeled and located, and for not less than three days prior to disposal.
- Mark all histological specimens as arising from a sentinel node procedure.
- Follow storage and disposal guidelines for the pathology laboratory, decontaminating such equipment after use as is considered necessary.

7.7 References

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Chapter-8

A PRELIMINARY STUDY OF THE COST

8.1 Introduction

Breast cancer is the most common malignancy in women, and comprises 18% of all female cancers. Most patients that are diagnosed with breast cancer presently undergo surgery as an inpatient. This consists of a Wide Local Excision (WLE) of the tumour, and an Axillary Nodes Clearance (ALND) in order to stage the disease. The patient will then stay in hospital for, typically, about 5 days, and will then be followed up as an outpatient.

This chapter is exploring the resource implications of an alternative treatment, described in more detail in other chapters. Essentially, the patient would still undergo the WLE of the tumour, but would also have a Sentinel Lymph Node Biopsy (SLNB), which would determine the status of the primary lymphatic drainage node. The status of the sentinel node would determine whether the patient would proceed to an ALND. From an economic point of view, this could generate savings over the present treatment, as the procedure without ALND should particularly reduce post operative inpatient stay and complications. This chapter attempts to define the workings of a model that can be used in calculating this cost. Due to the extremely small sample sizes of the three groups, this data can only be regarded as truly preliminary findings.

8.2 Resource Use In Breast Cancer Treatment

Patients will consume hospital resources when they receive either form of treatment. Therefore, for this particular study, five categories of resource use were defined. The first category is operating theatre usage. In any surgical

treatment, the length of the operation will be a significant factor in the total level of resource consumed by the patient.

The second category of hospital resource use is the length of stay in hospital. Obviously, the more invasive the procedure, the longer the recovery time in hospital will be. There is also the risk of a higher level of complications with more invasive procedures, and this has been dealt with separately.

The third resource use is the identification of the sentinel node, involving the use of both the radiopharmaceutical, and the time spent in Nuclear Medicine mapping the sentinel lymph node. This relates only to those patients who have SLNB.

Fourthly, histopathology tests are undertaken on all patients who undergo breast surgery, but patients who have SLNB will have different tests to those that have the standard treatment. Finally, an attempt has been made to cost the complications that arise from surgery, and to determine if there is a different level of post operative complication in the patients who have ALND, and the level of cost associated with this difference.

Throughout the model, both overheads and depreciation of capital equipment have been excluded, as their method of inclusion is to some extent subjective, and by focusing on costs, and not charges, it may also be easier to make comparisons with other centres.

8.3 Categories Of Patients In The Study

The data from the UCLH trial has three sets of patients:

1. patients who have undergone WLE and SLNB only, with no axillary clearance (n=7)
2. patients who have undergone WLE, SLNB and ALND (n=17)
3. patients who have had conventional treatment with WLE and ALND (n=30)

The number of patients in each of the groups is small, and as such the data is used only for the purpose of illustration of the model. Often, one patient has severely affected the mean. As will be seen, some of the standard deviations prove that the spread of the data is very large, and this makes reliable estimates of the population mean difficult. In terms of using averages for length of stay and operating time to estimate the resource use this data is also of limited value, but this will improve as more patients are included in the study.

8.4 Differences In Resource Use

8.4.1 Operation costs

The resources used in the operating theatre can be divided into two; staff time, and consumables used.

Using this principle, data was obtained from the UCLH theatres monitoring computer, which details the time at which a patient is anaesthetised, the start time of the operation, entering and leaving recovery, and so on. Using this information it is possible to calculate the time spent at each stage, and the staff time associated with it. It has been assumed that the same numbers of personnel attend at each stage, and a cost per hour was developed for each, by using an average grade of each staff member, using payroll data.

Three aspects of the time that the patient has been in theatre have been costed:

- I) anaesthetic time - time of the anaesthetist and nursing staff in the anaesthetic room
- II) operation time - cost of the surgeons' actual operating time
- III) time in recovery - cost of the recovery nurse

The results of this for the three groups are tabled below:

	WLE & SLNB		WLE, SLNB & ALND		WLE & ALND	
	Mean	1 Standard Deviation	Mean	1 Standard Deviation	Mean	1 Standard Deviation
Anaesthetic time	10	5.39	9	3.22	12	4.96
Operation time	79	29.82	100	23.30	63	26.84
Recovery time	69	16.69	63	17.60	57	15.82

Table-8.1 Result of operation cost in the three groups

As can be seen, the standard deviations are large. This makes the task of drawing conclusions from the data difficult. There is also an anomaly that needs explaining. Whilst the operation time for and WLE & ALND seems reasonable at 63 minutes, it would be expected that the WLE & SLNB operation would take less time. The fact that it takes just over 10 minutes longer can only be due to the learning process of detecting the sentinel node. Clinical estimates of the time it takes to find a node would suggest that it should take about 15 minutes, and the overall time taken for this operation should therefore be nearer 50 minutes than 80. Some studies would suggest that, over time, the technique of the surgeon will improve¹, and some reduction of this time should therefore result. The time should also therefore fall for the group of patients that have WLE, SLNB & ALND.

Whatever the restrictions of the data, it can be readily converted into average mean cost by using proportions of the hourly rate, and is shown below.

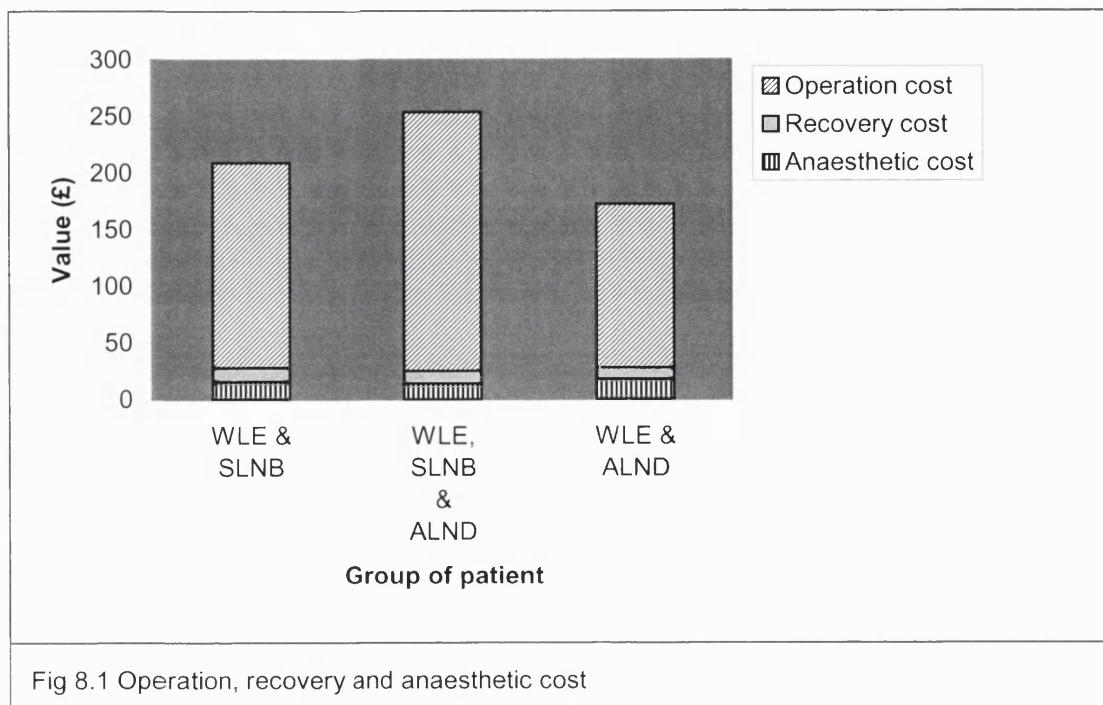


Fig 8.1 Operation, recovery and anaesthetic cost

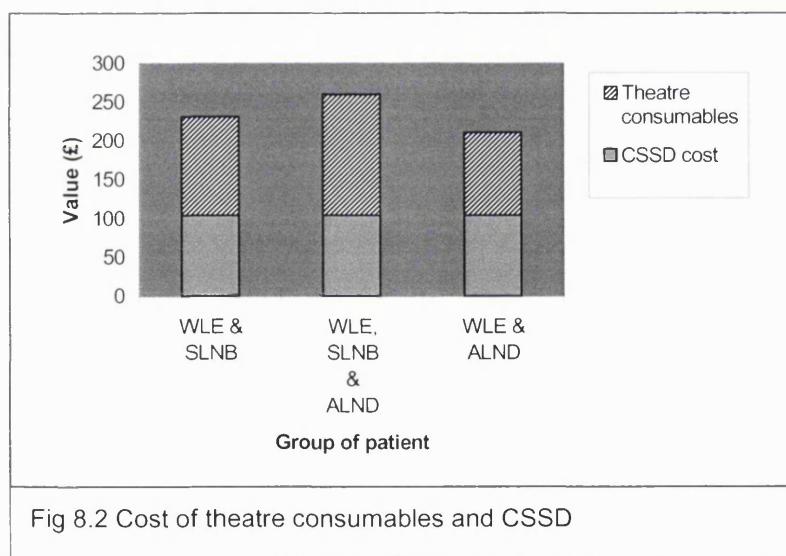
As can be seen from the graph and table above, the majority of the time, and therefore cost, is made up of the operating time. Although the recovery time varies noticeably, it makes up a tiny proportion of the total cost, as the staff costs are relatively low.

From this data, we can calculate three significant costs. Firstly, the cost of the SLNB can be derived from subtracting the WLE & ALND group from the group that underwent WLE, ALND & SLNB, which is £81. Secondly, the ALND cost can be derived by subtracting the group that underwent WLE & SLNB from those who had WLE, ALND & SLNB, which is £45. Finally, if we subtract the WLE & ALND group from those who had WLE & SLNB, then we derive the cost the extra cost of doing the SLNB even when the ALND is not undertaken, and this is £37.

This result suggests that there is a substantial 47% rise in costs by performing a SLNB over the conventional treatment. By comparison the ALND is relatively cheap at only 12% of the costs of conventional treatment.

The other result of note is that if the SLNB is performed per-operatively, and is found to be negative, avoiding an ALND, it will still be £37 more costly than the conventional treatment.

There are also other costs of operating that need quantifying. Firstly, there is the cost of the instruments that are used, in terms of their sterilisation (CSSD) cost. As the equipment used for each operation is the same regardless of the type, the cost for this was calculated at £104 per patient. The drugs and other consumables of theatres were assumed to vary with the length of the operation, and thus have been calculated by multiplying the hourly rate for these consumables, £86, by the length of time of operation. This breaks down for the three groups of patient as:



There is relatively little variation between the three groups, and reflects the same pattern as the staff costs. Overall, therefore, the combination of all the costs for theatres is therefore as follows:

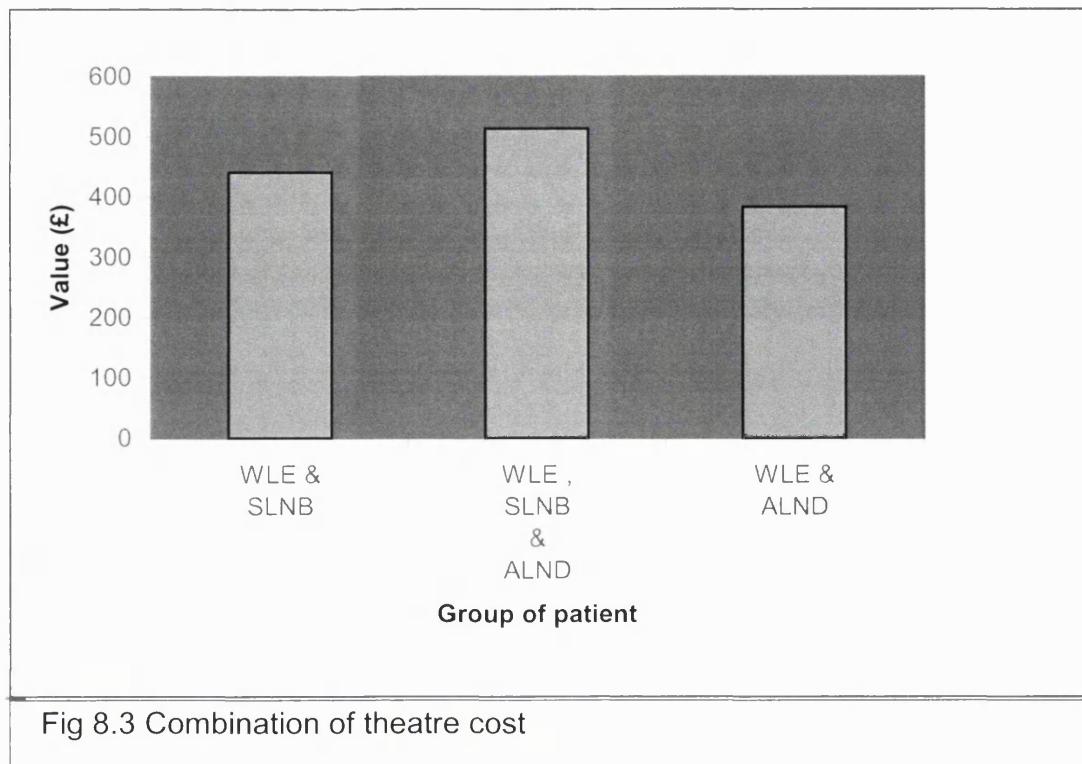
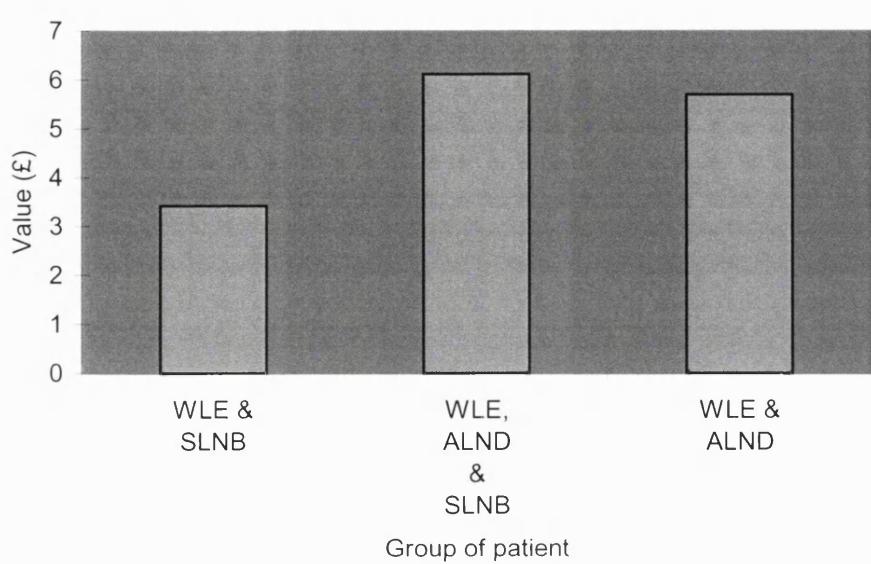


Fig 8.3 Combination of theatre cost

8.4.2 Length Of Post Operative Hospital Stay

For all the importance of these operation costs, it is important to remember that the length of time that a patient stays in hospital costs up to three times the amount. The cost of the hospital stay has been calculated using an average daily rate, and multiplying by the length of stay (LOS) of each patient. The daily rate included the cost of drugs, nursing time, dressings, linen and other miscellaneous expenditure of the breast cancer ward. This revealed a cost of just under £103 per patient per day. The mean average length of stay for each group is shown below:



This is an interesting finding. Whereas the groups which have axillary clearance, WLE, ALND & SLNB and WLE & ALND, have mean length of stays (LOS) of 6.12 and 5.77 respectively, with associated cost of £627 and £591, the group WLE & SLNB (no axillary clearance) had a LOS of just 3.43 days (£352).

This translates into a saving of between £239 and £275 of avoiding axillary clearance in this study, which is a significant saving.

At this stage, it is worth noting again, the small sample sizes of the three groups. The table below shows the mean average length of stay, and standard deviation of the three groups.

Group of patients	Mean	1 Standard Deviation
WLE & SLNB	3.43	1.49
WLE, ALND, SLNB	6.12	2.70
WLE & ALND	5.77	4.70

Table-8.2 Length of stay in the three groups and its associated cost

Given the small sample size of the three groups, it is not possible to estimate the population mean with any confidence, as some of the estimates would have a negative length of stay which is clearly not possible. There does seem to be, however, a clear distinction between those patients who had ALND, and those who did not.

8.4.3 Nuclear Medicine Cost

In order to locate the sentinel node, the patients are injected with a radiopharmaceutical the day before the operation and undergo mapping of the sentinel node. The injection of a blue dye just before operation helps to identify the sentinel node during the operation. As well as looking at the savings that might be made from the SLNB method of treatment, we must be careful to include costs such as these, which represent an area of increased expenditure for these patients. Obviously the expenditure above relates only to those patients who undergo SLNB, and is broken down into:

- hourly rate for the operator of the camera
- cost of the radiopharmaceutical

The cost of operator is approximately £17 per hour, and a clinical judgement was taken to give an assumption that patients used, on average, about an hour of camera time in nuclear medicine. The cost of the radiopharmaceutical is £20 per patient.

Thus, for each of the patients who have undergone SLNB a cost of £37 is incurred.

8.4.4 *Histopathology Cost*

With conventional treatment, the nodes from the axillary clearance are sent for histopathology. Using the paraffin method, they are sectioned and stained, which costs about £20 per patient.

For the WLE & SLNB patient, just one node will have histopathology, but as frozen sections are taken, it is a more labour intensive process, and so the cost is £16 per patient.

The main change as far as histopathology is concerned is the change to peri-operative testing, as this will be more significant than the change in costs outlined above.

8.4.5 *Complications Cost*

There are significant advantages in moving away from ALND, as the detractors can point to the potential morbidity of, nerve injury, increased risk of a frozen shoulder, and additional recovery time. The removal of level III nodes carries up to a 37% risk of lymphoedema of the arm². In favour of SLNB, however, is the fact that up to 80% of patients with early breast cancer have pathologically non-involved lymph nodes, and would therefore not need ALND of any form.

This study has looked at the complications post-operatively for all the patients, and the results for the three groups are as follows. The costing of the complications has followed exactly the same reasoning as above for operation time and hospital length of stay and, in addition, an attendance at outpatients has been costed as £42 with the cost of consumables and staff in the clinic.

8.4.5.1 WLE & SLNB

Of this group of 7 patients, only 1 developed a complication.

Complications	Elements of cost	Actual cost (£)
Wound infection	1 out-patient attendance	42

Table-8.3 complication cost in WLE & SLNB group

The total cost of complications for the group is £42, which gives a mean average of £6 per patient.

8.4.5.2 WLE, ALND & SLNB

Of the 17 patients in this group, 5 had complications

Complication	Element of cost	Actual cost
Axillary seroma	2 out-patient attendance	84
Bleeding breast site	1 out-patient visit	42
Seroma mastectomy site	1 out-patient visit	42
Infected prosthesis	5 days LOS, 1 out-patient visit, theatre time	637
Post op axillary bleed	6 days LOS, 1 out-patient visit, theatre time	721

Table-8.4 Complication cost in WLE & ALND & SLNB group

The total cost of the complications for this group was £1526, with a mean average of £90 per patient.

8.4.5.3 WLE & ALND

Of this group of 30 patients, 5 had complications

Complications	Elements of cost	Actual cost (£)
Axillary wound	1 out-patient attendance	42
Axillary seroma	1 out-patient attendance	42
Major wound infection	24 days LOS, 1 out-patient visit, theatre time	2560
Axillary haematoma	2 days LOS, 1 out-patient visit	247
Breast wound cellulitis	4 days length of stay, 1 out-patient visit	452

Table-8.5 complication cost in WLE & ALND group

The total cost for this group is £3343, and a mean average of £111 per patient.

8.5 Summary

The summary cost table from the preceding sections is:

	WLE & SLNB	WLE, ANC, SLNB	WLE & ANC
Operation cost	440.51	513.06	383.29
Length of stay cost	351.55	627.28	591.29
Nuclear medicine cost	37.36	37.36	0
Histopathology cost	16.00	36.00	20.00
Complications cost	6.00	90.00	111.00
TOTAL COST	851.42	1283.70	1105.58

Table-8.6 Summary of all costs

8.6 Conclusions

In drawing conclusions from this data, we have two comparisons to make. The first comparison is the WLE & ALND group versus the group that had WLE & SLNB only, and were thus saved the ALND and associated higher length of stay and complications costs. The second is the WLE & ALND versus the WLE, ALND & SLNB group to determine the extra costs of the sentinel node biopsy in the patients that have to have an ALND after the sentinel node tests positive.

The first comparison, which is the saving resulting from not clearing the axillary basin in the conventional treatment, is £254 per patient. The second comparison, which is the additional cost relating to dissecting the axillary node after the sentinel biopsy, reveals that the additional cost per patient is £178 per patient.

Before drawing conclusions from this, there is a further area of investigation that is needed which has not been costed in this chapter and is worthy of mention. That is the issue of the false negative result of the sentinel node. Potentially, the patient's disease will not be detected for some time after the admission, and may well incur more costs than would have been the case if the node had been detected as positive and an axillary clearance performed. This has not been covered in this chapter, as a costing of this kind would need long term data collection of patients who had not had ALND. This is outside the scope of the trial presently being undertaken.

However, we can still draw conclusions using the costing results of this study, it is possible to estimate the savings from the results of other trials. The literature would suggest that 70% to 80% of lymph nodes in early breast cancer are negative. Taking a sample size of 100 patients, between 70 and 80 would have a saving of £254 each, which would generate a total saving of between £17,780

and £20,320. The additional cost of the 20 or 30 patients that would have to undergo the axillary clearance, at £178, is between £3,560 and £5,340.

Taking the 70% lymph node negative figure, the savings would total £17,791 and the additional costs would be £5,521. Thus the net saving would be £12,269 per 100 patients. With the 80% lymph node negative figure, savings would total £16,592 with additional costs £3,740, and the saving of £20,332 per 100 patients.

The conventional cost of treatment on these patients would be £124,714, and thus the sentinel node treatment would save between 10% and 13% of total costs. If the selection of patients for sentinel node biopsy could be improved then the number of patients that would need an axillary clearance as well should fall towards zero, and thus the savings of sentinel node biopsy treatment could rise as high as 20%. These savings are meaningful, and will also result in a significantly better system of care for patients with early breast cancer.

8.7 References

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CHAPTER-9

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The sentinel node being the lymph node at greatest risk of harbouring metastatic deposits indicates that the lymphatic dissemination of cancer is not only orderly and sequential but predictable. Retrieving this node requires a concerted effort from a team of experts. This approach is minimally invasive and appears to allow the same information (if not more) to be gathered as with axillary node dissection but with less morbidity. Sentinel lymph node biopsy has been described as the most significant revolution in surgical oncology of the last decade.

There have been two international conferences on this subject to date. These were held in Amsterdam (1999) and Santa Monica (2000), which is considered the birthplace of lymphatic mapping. Encouraging data from all over the world have been presented and numerous randomised controlled trials are well on the way. A third international sentinel node will be held in Yokohama in 2002.

9.1 Breast Carcinoma

The sentinel node concept is validated in breast carcinoma. It has the advantage of being a less invasive procedure with less morbidity as compared with axillary lymph node dissection. This point is illustrated by the randomised study performed by Schrenk et al¹.

There are significant variations in the technique of the SLN localization in breast cancer. There are several techniques available for detection of the sentinel lymph node, several radiopharmaceuticals available for injection; there is controversy as to the injection site, dose and volume of the injectate. Despite

these variations, various investigators report encouraging results.

As far as tracers are concerned, it is important to realise that the tracers that are currently used for lymphatic mapping and sentinel node localisation, were not developed for this purpose and are not ideal. The ideal tracer is one, which is easy to prepare and remains stable. It should accumulate in the lymphatic system and migrate to the first draining lymph node rapidly without leaving significant residual activity at the injection site.

There is a need for developing new tracers but this is difficult and costly. In the area of tracer development, there is progress and a new tracer $^{99m}\text{TcDTPA}$ -mannosyl-dextran² has been tried on animal models. This tracer exhibits rapid injection site clearance and low secondary node accumulation. The preliminary data on animal studies are encouraging and we await the results from clinical studies. There is work by Valdes Olmos³, in progress in increasing the purity of radiochemical by preparing ^{99m}Tc -colloid in vacuum vials, which resulted in improved labelling and SLN detection rate.

As far as the injection technique is concerned, there is no consensus but it seems that a correct SLN is detected within the axilla no matter how the tracer is administered in the breast. A study by Dr. Martin et al⁴ from the Memorial Sloan Kettering cancer centre comparing intradermal and intraparenchymal administration of the radiotracer in 298 patients indicate that the success rate of identifying the SLN is higher with intradermal technique (98% vs 89%). The false negative rate and SLN to background ratio were also better with

intradermal injection. It seems that detection of internal mammary lymph nodes are less common with this technique and this has been our observation too.

As far as multicentric breast cancers are concerned there have been several early trials indicating a higher false negative rate if SLNB is performed in these patients. On the basis of these early reports, multicentric tumours were considered as an exclusion criterion in our study. Recent work by Schrenk et al⁵ reports 100% accuracy in identification of the SLN, using subareolar injection technique. There were no false negative cases in 19 patients with multicentric breast cancer who were studied in this trial.

Patient selection is very important factor in success of SLNB procedure in breast carcinoma. Recent paper by Silverstein et al⁶ on axillary lymph node positivity based on tumour size and palpability reveals that there is significantly reduced risk of lymph node involvement in non-palpable tumours as compared to the palpable tumours of the same size. High-grade ductal carcinoma in situ (DCIS) and DCIS with microinvasion is another group of patients who will potentially benefit from this procedure. Recent work by Klauber-Demore et al⁷ on patients with high grade DCIS and DCISM revealed 12% and 10% nodal positivity rate respectively. Over 90% of these nodes showed evidence of micrometastases only. At present, there is no clear guidelines on how to proceed with further management of these patients. Table 9.1 summarises tumours that are suitable for sentinel node biopsy based on current evidence available. The only definite exclusion criterion is presence of clinically involved axillary lymph node.

Pre-operative lymphoscintigraphy is important to identify true sentinel lymph node, those that are located in unusual position and SLN's in different lymphatic basins. Lymphoscintigraphy is also considered a very good predictor

of the success of SLNB procedure. There are some concerns regarding the workload implications to Nuclear Medicine departments if SLN biopsy is accepted as standard of care in breast cancer management. Use of imaging probes (small portable cameras) in operating theatre is an area, which is developing fast and can potentially address this issue. This is another example of close collaboration between surgeons and nuclear medicine physicians. The probe can be used in operating theatre and gives the surgeon a real time image in addition to audiovisual signal to locate the SLN (fig 9.1). If successful, this will obviate the need for pre-operative lymphoscintigraphy.

As far as intraoperative detection technique is concerned, there is no doubt that combined approach of blue dye lymphatic mapping and probe guided surgery is superior to using each of these modalities alone. Recent survey of surgical practice in the United States⁸, reflects that over 90% of surgeons employ combined technique for sentinel node detection.

Training is an important factor in the success of the procedure as there is no doubt that the procedure is operator dependent and there is a definite learning curve. Current recommendation by the American College of Surgeons is to perform 30 SLNB procedures accompanied by completion ALND, before accreditation.

It is also clear from the literature that as the sophistication of the methods used to gather pathological evidence from sentinel lymph node increases, so does the sensitivity of detection of micrometastases. The significance of detecting a



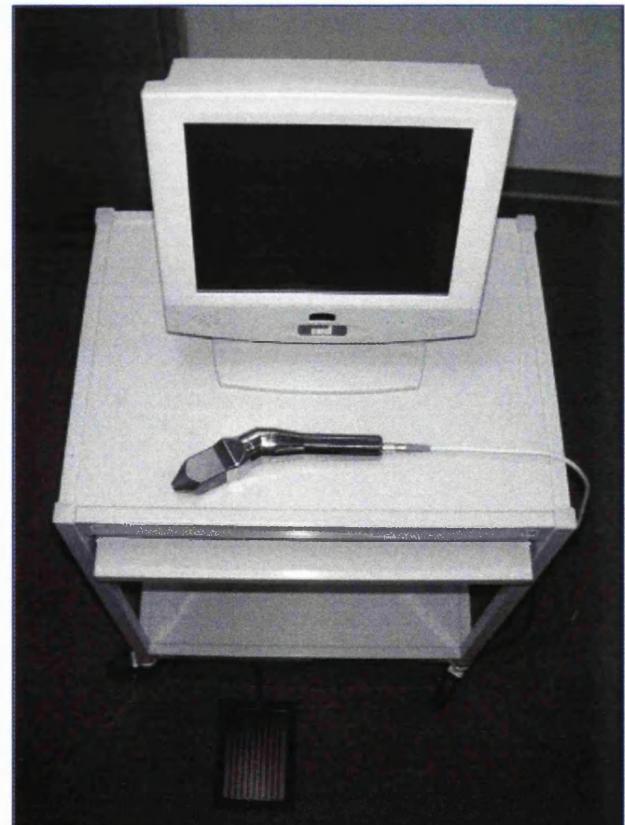
Patient Selection

- High Risk DCIS/DCISM
- Non-palpable breast carcinoma
- T1, T2 carcinomas
- Special good prognosis tumours
 - **Mucinous**
 - **Papillary**
 - **Colloid**
 - **Adenoid cystic**

Table –9.1 Patient selection



Fig. 9.1 Hand-held imaging probe



few if not single micrometastases in a lymph node is still unknown. The absence of reliable intra-operative tools for the detection of the histological status of the sentinel node is a limiting factor. Our result on imprint cytology and optical biopsy are encouraging.

The critical issue in SLNB is the false negative rate as this can lead to inadequate treatment decision. We need to balance the advantage of a less invasive staging investigation against the risk of a false negative rate. SLNB can be a useful method of nodal staging, if it can be performed with a similarly low rate of false negative results.

The question of whether SLNB is ready to replace conventional ALND in breast cancer remains unanswered. As far as staging is concerned, there is enough evidence in the literature to support that this technique is a reliable staging investigation. As far as regional control and long term survival is concerned, we simply do not know the answer at present as long-term follow up data on risk of axillary recurrence and success of salvage surgery are lacking. We need to await the results of multicenter randomised controlled trials before abandoning the ALND in patients with breast cancer and accepting the SLNB as standard of care.

9.2 Malignant Melanoma

The development of sentinel lymph node biopsy has provided an attractive alternative to routine elective lymph node dissection (ELND) for all patients at risk of metastasis.

This procedure can allow the surgeon to select patients who would potentially benefit from regional lymphadenectomy. Although it remains to be seen whether the application of this concept will translate into a survival advantage for stage I and II patients, the low morbidity and technical ease of the procedure has led to its increasing application in the management of patients with intermediate thickness malignant melanoma. The other advantage of lymphatic mapping in malignant melanoma patients is that it provides important prognostic information. Donald Morton and colleagues have shown that five-year survival to be 90-95% if the sentinel node is free of metastatic disease and around 65% if the node contains metastases².

Lymphoscintigraphy is an essential first step in lymphatic mapping. Dynamic imaging is considered an important aspect of lymphoscintigraphy. It can help to distinguish the first-ecchelon from the second tier nodes, which need not be removed. Lymphoscintigraphy serves several important purposes including: providing a road map for the surgeon to identify the draining basin; helping to indicate the number of sentinel nodes; helping to distinguish first-tier nodes from secondary nodes; facilitating identification of sentinel nodes in unexpected locations and finally to enable the clinician to mark the location of the sentinel node on the skin.

As far as therapeutic implications of detection of micrometastases are concerned, Donald Morton suggests that adjuvant immunotherapy is one of the

most promising approaches. Moreover therapeutic cancer vaccine with minimal toxicity and good quality of life for patients is another alternative. CancerVax, which is the polyvalent allogeneic vaccine, is one such vaccine that is extensively studied melanoma vaccine since 1984.

In a study of one hundred and fourteen patients with stage 1 and 2 melanoma who underwent SLN biopsy at H. Lee Moffitt Cancer Centre² with a follow up period of 6-48 months (mean equal 28 months), it was noted that twenty three out of one hundred and fourteen patients (20.2%) had histological positive SLN (histo+) with H&E and immunohistochemistry. All of these nodes were RT-PCR positive (PCR+). H&E histology identified 73% of metastasis and HMB-45 and S-100 stained detected additional 27% of patients with nodule metastasis.

Out of remaining ninety-one patients with histological negative (histo-) SLN, 47 (51.6%) showed evidence of disease on RT-PCR (PCR +). Of patients who were histo - but PCR+, four out of forty seven (12.9%) recurred. On the other hand patients who's SLN was histo- and PCR-, only one of forty-four patients (2.3% recurred). The difference in the rate of recurrence between the histo-/PCR+ and histo-/PCR- group was statistically significant ($P=0.02$). The authors conclude that sentinel node biopsy provides a more accurate staging of patients with melanoma and has prognostic significance. Detection of micrometastasis has the potential of sub grouping the histological negative population and allowing the adjuvant therapy to be applied selectively for the patients. Although the long term outcome is not clear at present but this has potential therapeutic implications.

Although the concept of SLNB is validated in malignant melanoma, there is currently no evidence that incorporating sentinel node biopsy in the routine management of melanoma improves survival or regional tumour control. There is a sixteen centre randomised trial conducted by Dr. Morton investigating the impact of lymphatic mapping on regional control and survival. Total of 1784 patients have been recruited in that trial from various centres so far we await the outcome of this trial.

As far as intra-operative technique is concerned, a combination of probe guided surgery and blue dye mapping is the recommended approach which complements each other.

9.3 Sentinel node in other areas of surgical oncology

Sentinel node biopsy technique is being used in other areas of surgical oncology and encouraging results are reported in head and neck squamous carcinoma, papillary thyroid carcinoma, penile carcinoma, anal carcinoma, Merckel cell carcinoma and colorectal, vulvar and cervical carcinoma.

Delivery of radiopharmaceutical with needle free syringe can be helpful in administering the radiotracer in penile, anal and vulvar carcinoma where insertion of needle can be extremely painful. It can also be used for the delivery of radiopharmaceutical in needle phobic patients.

Gastrointestinal cancer is the main new focus in sentinel node biopsy at present. The sentinel node technique is being used in pancreatic carcinoma, small bowel carcinoma. It has also been reported in oesophageal and gastric carcinoma where

the radiopharmaceutical is injected endoscopically and lymphoscintigraphy is performed. Fujimora et al from Kanazawa University in Japan² have reported their results on intraoperative endoscopic lymphatic mapping (IELM) in identifying SLN of patients with early gastric cancer. A total of 184 patients with an early stage gastric cancer underwent IELM. They successfully identified the SLN in 176 patients (96%). The authors report 90% sensitivity (28/31), 100% specificity (145/145) and 98% predictive accuracy (173/176). They conclude that limited nodal dissection could be applicable in early gastric cancer.

Kitawaga et al from Kieo University in Japan² report their experience of SLN biopsy in GI cancer. They recruited 188 patients with GI malignancies. These included 33 oesophageal cancer, 106 gastric and 49 colorectal carcinomas. On this occasion they injected radioisotope (99m-technetium) through an endoscope and the radioactive SLN was identified with the help of gamma detection probe. They report success rate of identifying the SLN at 91%. The sensitivity varied between 83%-88% depending on the primary site of carcinoma. They report an overall diagnostic accuracy of 90%. The authors conclude that sentinel node biopsy is a viable alternative to excessive lymph node dissection and can potentially be more accurate. Micrometastatic deposit can be identified. Laparoscopic probes are also being used which can facilitate minimally invasive sentinel node biopsy in GI malignancies.

Image fusion technology is another area that is making its impact in sentinel node biopsy. With this technique, scintigraphic tomographic images can be fused to the corresponding X-ray tomographic image. This combines functional and anatomical imaging which can be helpful in difficult circumstances.

9.4 Summary of Conclusions of the Thesis

We tested the hypotheses 1-7 presented in the section 1.9 of this thesis and our conclusions are as follows:

1. The sentinel node concept is valid in the management of patients with breast cancer.
2. The SLN concept holds true in other areas of Surgical Oncology with the exception of colorectal carcinoma where it is associated with a high false negative rate and with poor sensitivity.
3. Touch imprint cytology (TIC) is a rapid and reliable intra-operative method for determining the histological status of the sentinel lymph node in patients with breast carcinoma . It will enable the surgeon to decide on performing ALND at the time of initial surgery with acceptable accuracy.
4. Early spectral data from optical biopsy of the sentinel lymph node is encouraging as a 'real time' diagnostic tool for determining the histological status of the nodes. Potential applications include intra-operative use of the device to determine the status of the SLN without any delay and at the same time the tumour margin can be examined to confirm the completeness of excision of the primary tumour.
5. A new and less invasive technique of delivery of the radiopharmaceutical is possible and it can lead to successful SLN localisation.
6. The radiation risk to the patient and staff groups involved in all aspects of the technique is very low.
7. SLN biopsy in breast carcinoma is associated with minimal morbidity and is cost-effective.

9.5 References

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